The Botanical Safety Consortium: Evaluating NAMs for Use with Complex Mixtures

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NIEHS and BSC Steering Committee

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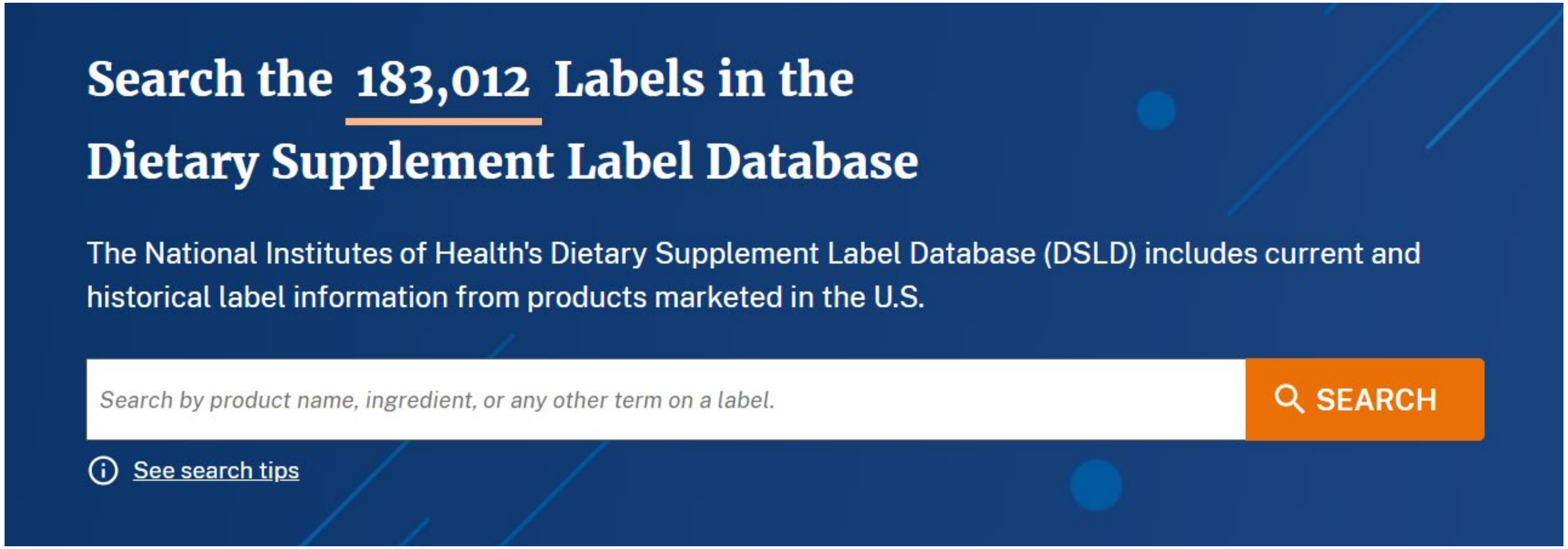
There are many botanical products in the market



Over 31,000 botanical products currently on the market

Few botanical ingredients have comprehensive safety data available

Completed:

- Aloe vera
- Bitter orange
- Black cohosh
- Ephedra
- *Ginkgo biloba*
- Ginseng
- Goldenseal
- Gum guggul
- Kava kava
- Milk thistle
- Senna
- Usnea lichen

Ongoing:

- *Echinacea purpurea*
- Garcinia cambogia
- Valerian root

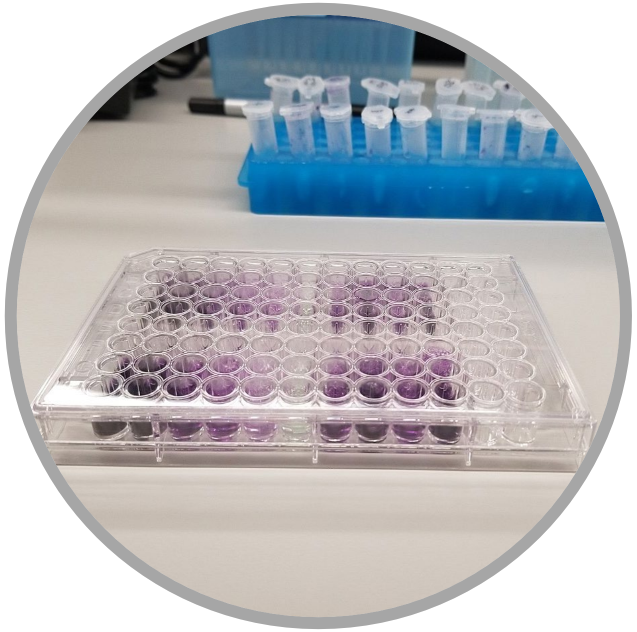


https://ntp.niehs.nih.gov/whatwestudy/topics/botanical

New Approach Methodologies

- Methods for assessing toxicity, hazard, and risk that do not include traditional mammalian bioassays
  - *In vitro* assays – Ames assay, cell-free receptor binding assays, skin irritation test with reconstructed human epidermis (OECD 439),
  - *In silico* models – Derek Nexus, OECD QSAR Toolbox, Leadscope
  - Whole animal assays – *C. elegans,* developmental zebrafish
- NAM application can:
  - Improve risk assessment by providing more information on the mechanisms of toxicity
  - Incorporate human-based models for translational purposes
  - Lower the cost of assessing risk from exposure to chemicals in commerce and the environment
  - Make data more rapidly available to decisionmakers

Challenges



- NAMs are simplified models of complex systems
  - Lack of human relevant exposure routes (e.g., direct application to cells or aquatic exposures)
  - Incomplete or different biotransformation and transport machinery
  - Uncertainty in the transition from an activity signal to a truly adverse response
  - Presence of artifacts based on certain physicochemical properties
    - Autofluorescence of ringed structures
    - Limitations of testing volatile chemicals
    - Stickiness to plastic of highly lipophilic compounds

Challenges

- NAM testing platforms have been developed and optimized for use with single chemicals (e.g., drugs, pesticides)

- For complex mixtures testing…
  - determining the concentration is not straightforward
  - the active constituent is often unknown
  - multiple constituents could contribute to toxicity
  - a minor constituent could be the toxicity driver
  - the potential for matrix interference is increased with some complex mixtures
  - some constituents may not go into solution or may precipitate in the testing media
  - in vitro to in vivo extrapolation is complicated

Major efforts in NAM development and testing

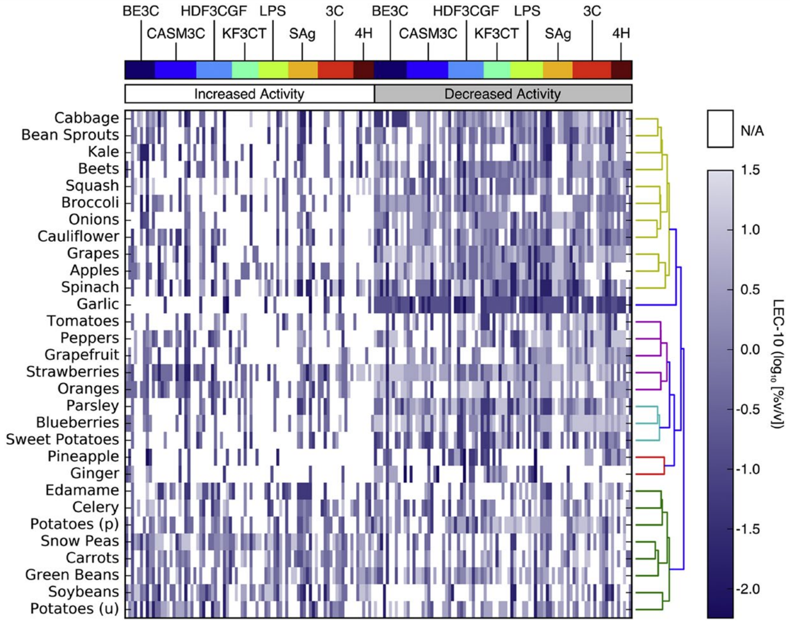
- Toxicology in the 21st Century (Tox21)
  - Federal collaboration between EPA, NIH (National Center for Advancing Translational Sciences and the National Toxicology Program) and the Food and Drug Administration
  - Phase 2 involved evaluating the 10k chemical library (8193 unique chemicals) in over 75 quantitative high throughput assays measuring stress response and nuclear receptor activity
  - Mostly focused on single chemicals, some defined mixtures included
- Toxicity Forecasting (ToxCast) at EPA
  - ToxCast includes Tox21 data plus additional assays (20+ assay sources)
  - Provides tools for storing, managing, curve-fitting, and visualizing data

Evaluating complex mixtures in HTS assays

Toxicology in Vitro
Volume 54, February 2019, Pages 41-57

Assessing bioactivity-exposure profiles of fruit and vegetable extracts in the BioMAP profiling system

Barbara A. Wetmore [a d], Rebecca A. Clewell [a], Brian Cholewa [a], Bethany Parks [a], Salil N. Pendse [a], Michael B. Black [a], Kamel Mansouri [a], Saad Haider [a], Ellen L. Berg [c], Richard S. Judson [b], Keith A. Houck [b], Matthew Martin [b], Harvey J. Clewell III [a], Melvin E. Andersen [a], Russell S. Thomas [b], Patrick D. McMullen [a]



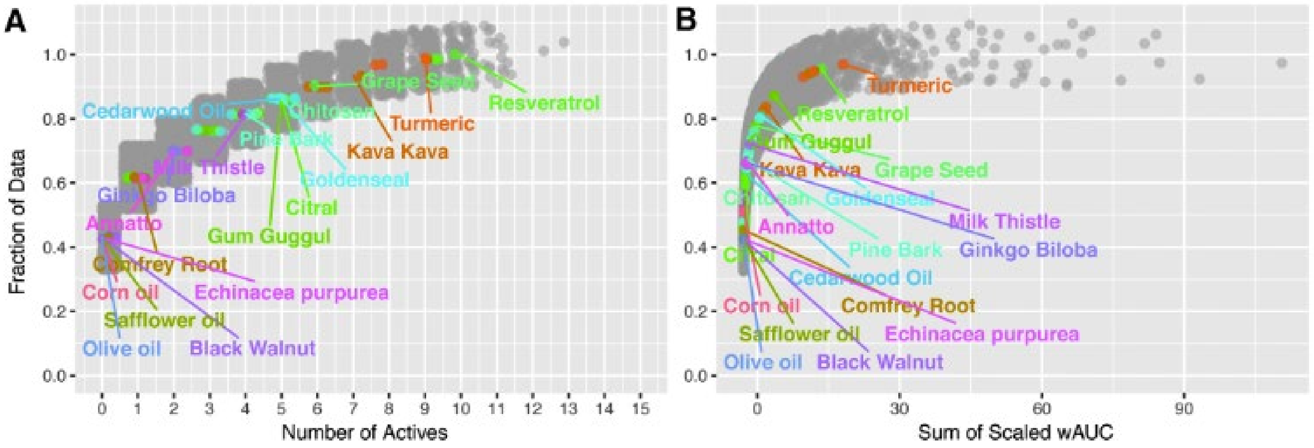
Evaluating complex mixtures in HTS assays

Applied In Vitro Toxicology > Vol. 5, No. 1 > Original Articles

Using Tox21 High-Throughput Screening Assays for the Evaluation of Botanical and Dietary Supplements

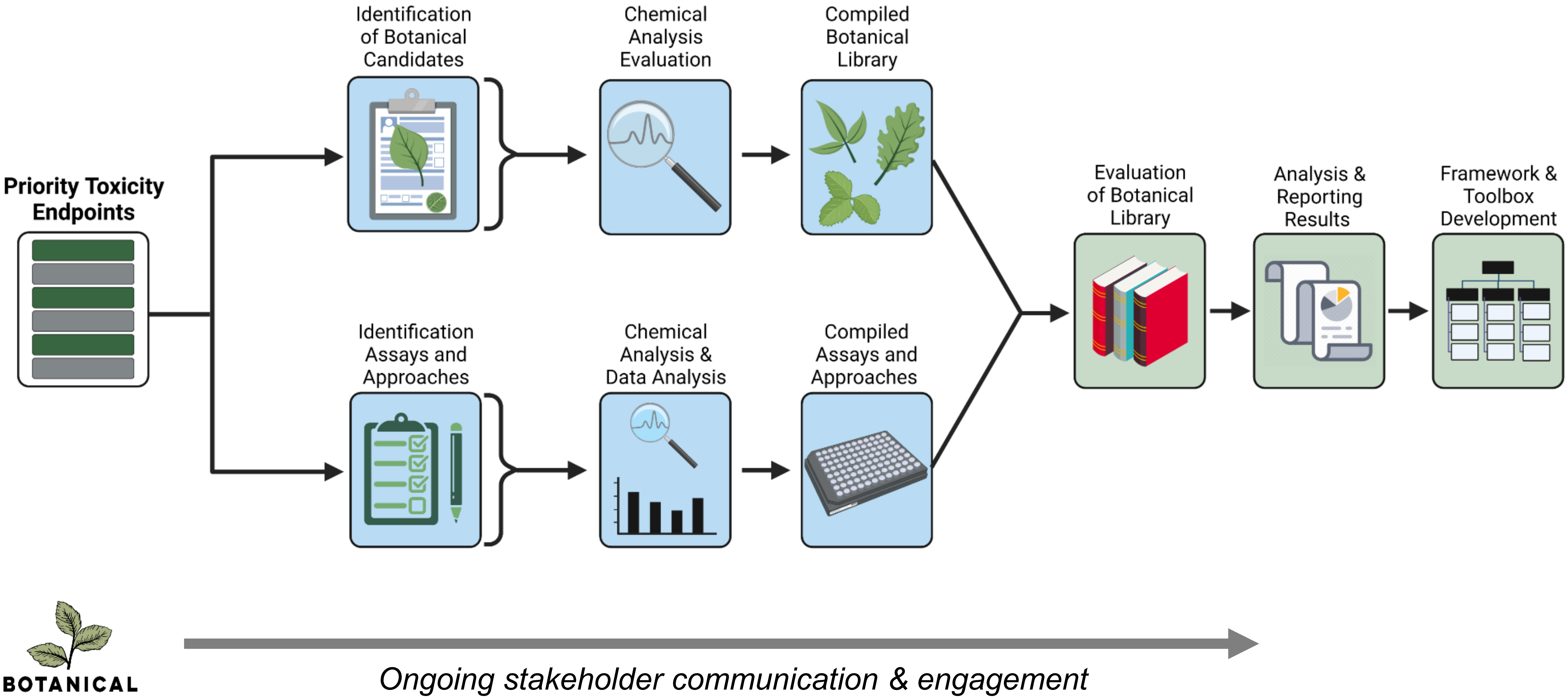
Troy D. Hubbard, Jui-Hua Hsieh, Cynthia V. Rider, Nisha S. Sipes, Alexander Sedykh, Bradley J. Collins, Scott S. Auerbach, Menghang Xia, Ruili Huang, Nigel J. Walker, and Michael J. DeVito

Published Online: 13 Mar 2019 | https://doi.org/10.1089/aivt.2018.0020



Mission: Evaluate the suitability of assays for testing botanicals as complex mixtures

Botanical Safety Consortium Framework



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Technical Working Groups

- Nominate botanicals based on:
  - Toxicity information
    - Preclinical evidence (e.g., rodent, dog)
    - Human evidence (clinical evidence, adverse event reporting)
    - *In vitro* evidence
  - Known toxic constituents
  - Availability via reputable source
  - Robust analytical method(s)

- Identify assays based on:
  - Biological coverage of important endpoints/processes
  - Reliability and reproducibility
  - Sensitivity (minimize false negatives)
  - Human relevance
  - Commercial availability

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Botanical library

Standardized Common Name	Scientific Name	Plant part(s)
Aconite	*Aconitum napellus* L.	Mixed parts
Aristolochia fangchi	*Aristolochia fangchi* Y.C. Wu ex L.D. Chou & S.M. Hwang	Root
Ashwagandha	*Withania somnifera* (L.) Dunal	Root
Asian Ginseng	*Panax ginseng* C.A. Mey.	Root
Blue cohosh	*Caulophyllum thalictroides* (L.) Michx.	Root and Rhizome
Comfrey	*Symphytum officinale* L.	Root or leaf
Ephedra	*Ephedra sinica* Stapf	Aerial parts
Green Tea	*Camellia sinensis*	Leaf
Goldenseal	*Hydrastis canadensis* L.	Root and Rhizome
Kava	*Piper methysticum* G. Forst.	Root and Rhizome
Kratom	*Mitragyna speciosa* (Korth.) Havil.	Leaf
Milk thistle	*Silybum marianum* (L.) Gaertn.	Seed
Oleander	*Nerium oleander* L.	
Usnea lichen	*Usnea* spp.	Whole Plant
Thunder God Vine	*Tripterygium wilfordii* Hook. F.	Root
Yohimbe	*Pausinystalia johimbe* (K. Schum.) Pierre ex Beille	Bark

ELSEVIER

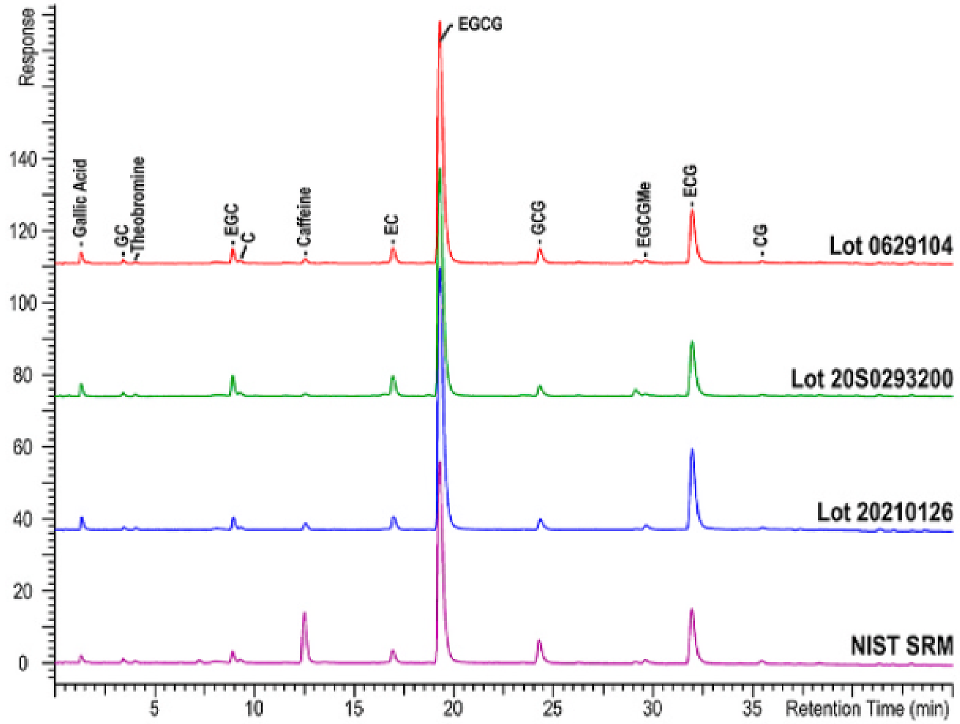
Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

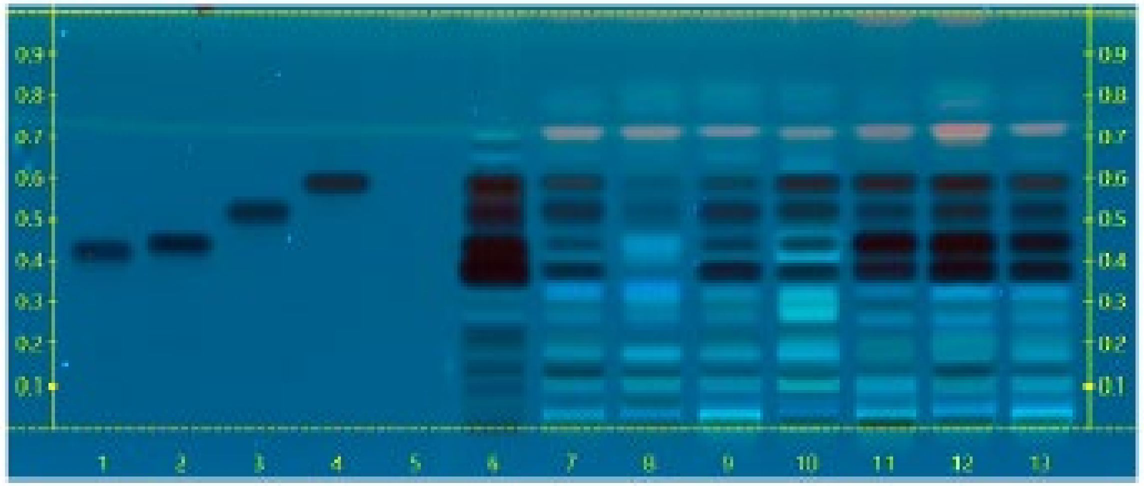
Advancing botanical safety: A strategy for selecting, sourcing, and characterizing botanicals for developing toxicological tools

Suramya Waidyanatha [a], Bradley J. Collins [a], Tim Cristy [b], Michelle Embry [c], Stefan Gafner [d], Holly Johnson [e], Josh Kellogg [f], Julie Krzykwa [c], Siheng Li [g], Constance A. Mitchell [c,*], Esra Mutlu [a,a,1], Sarah Pickett [h], Hong You [i], Richard Van Breemen [j], Timothy R. Baker [k]



Key steps

- Purchase of extracts or ground material extraction in 95% ethanol
- Evaluation of solubility in vehicle (DMSO)
- Characterization (e.g., HPTLC, LC-MS, UV-CAD-HRMS)
  - Authentic standards used when available
- Quantitation of constituents
- Analysis for contamination or adulteration



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Data availability

https://cebs-ext.niehs.nih.gov/cebs/paper/15717

Botanical Constituents and Quantification

- Botanical Constituents and Quantification (41 KB)

Solubility

- DMSO (15 KB)
- Ethanol (15 KB)

Aconite *(Aconitum napellus)*

- Chemical Analysis - Aconite (Aconitum napellus) (341 KB)

Aristolochia *(Aristolochia fangchi)*

- Chemical Analysis - Aristolochia (Aristolochia fangchi) (550 KB)
- HPTLC - Aristolochia (Aristolochia fangchi) (726 KB)

Ashwagandha *(Withania somnifera)*

- Chemical Analysis - Ashwagandha (Withania somnifera) (717 KB)
- HPTLC - Ashwagandha (Withania somnifera) (167 KB)

Asian Ginseng *(Panax ginseng)*

- Chemical Analysis - Asian Ginseng (Panax ginseng) (2 MB)
- HPTLC - Asian Ginseng (Panax ginseng) (448 KB)

Blue Cohosh *(Caulophyllum thalictroides)*

- Certificate of Analysis - Blue Cohosh (Caulophyllum thalictroides) (71 KB)
- Chemical Analysis - Blue Cohosh (Caulophyllum thalictroides) (435 KB)
- HPTLC - Blue Cohosh (Caulophyllum thalictroides) (902 KB)
- Macroscopic - Blue Cohosh (Caulophyllum thalictroides) (2 MB)
- Microscopic - Blue Cohosh (Caulophyllum thalictroides) (377 KB)

Comfrey *(Symphytum officinale)*

- Certificate of Analysis - Comfrey (Symphytum officinale) (71 KB)
- Chemical Analysis - Comfrey (Symphytum officinale) (599 KB)
- HPTLC - Comfrey (Symphytum officinale) (401 KB)
- Microscopic - Comfrey (Symphytum officinale) (371 KB)

Ephedra *(Ephedra sinica)*

- Chemical Analysis - Ephedra (Ephedra sinica) (2 MB)

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Nominated assays

Genotoxicity

- Ames test for mutagenicity
- In vitro micronucleus test
- ToxTracker

Neurotoxicity

- Zebrafish embryos – also for DART
- C. Elegans – also for DART
- Multi electrode arrays in neuronal cells

Dermal Toxicity

- TBD

Developmental and Repro Tox

- Transcriptomics in human cell lines - will provide info for other endpoints
- Zebrafish embryos – also for neuro
- C. Elegans – also for neuro
- devTOX quickPredict assay

Hepatotoxicity

- Transcriptomics in human cell lines (will provide info for other endpoints including BDI)
- ROS glo assay
- LDH release
- Cytotoxic Reactive Metabolites
- Cyp3A4 induction and inhibition

Cardiotoxicity

- Seahorse (O2 assay)
- Multi electrode arrays in cardiomyocyte cells
- Voltage sensitive dyes
- Transient Calcium measurements
- Safety Pharmacology Screen
- Direct Contractility

ADME

- GastroPlus Modeling
- ADMET Predictor

Green = draft data completed

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Botanical testing

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

ELSEVIER

Neuroactivity screening of botanical extracts using microelectrode array (MEA) recordings

Regina G.D.M. van Kleef[a], Michelle R. Embry[b], Constance A. Mitchell[b], Remco H. S. Westerink[a,*]

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Utrecht University

Remco Westerink
Utrecht University

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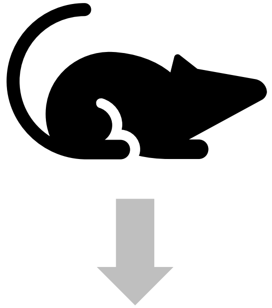
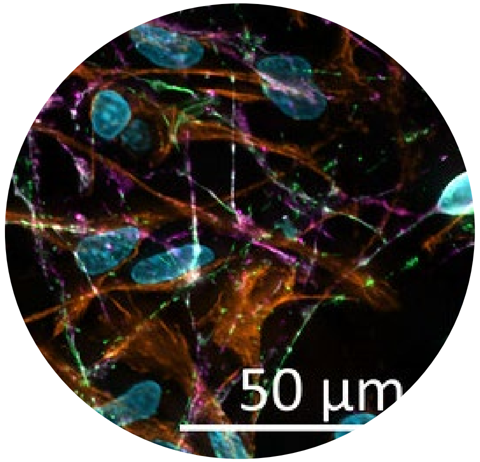
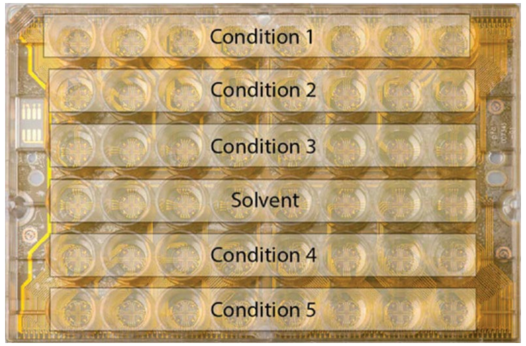
Microelectrode Array (MEA)





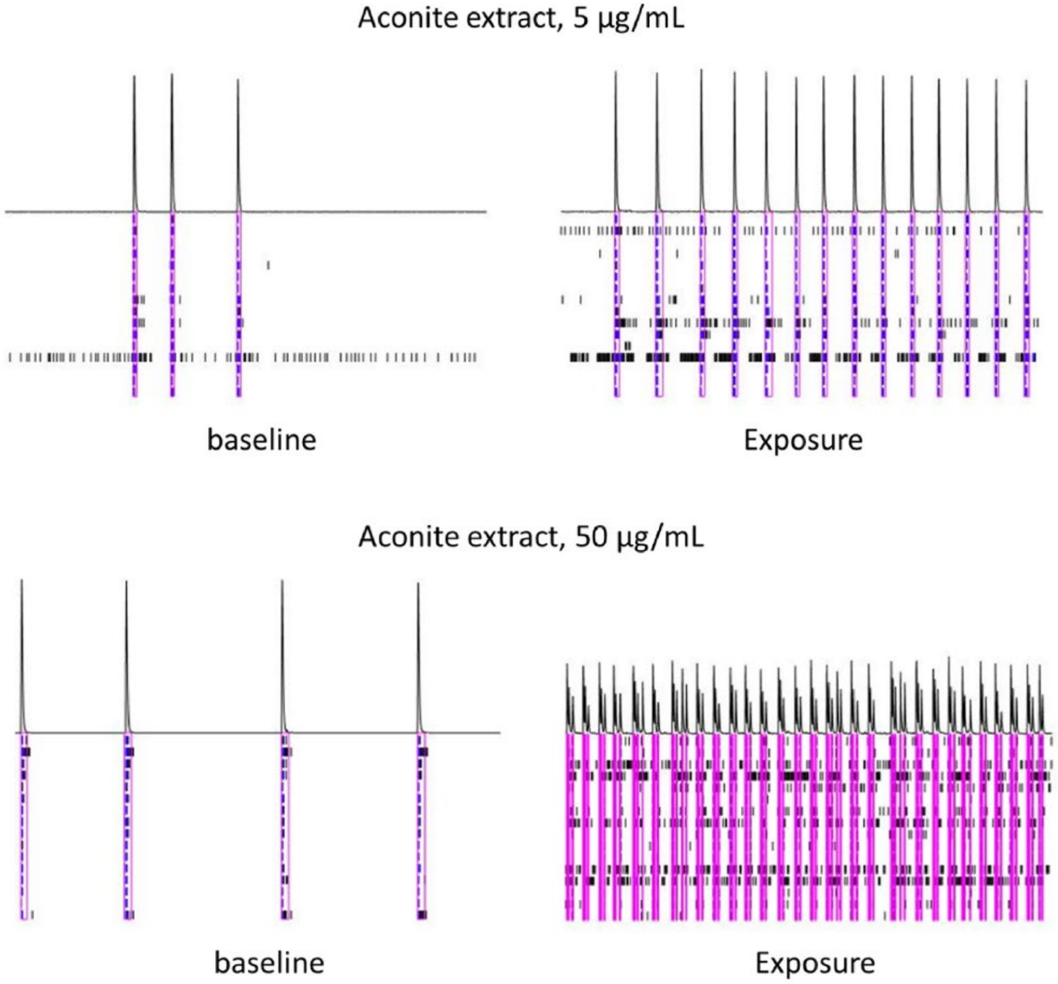
Primary cortical neurons

7-day exposure



Electrodes record neuronal activity

Measure mitochondrial activity to evaluate cell viability



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Microelectrode Array (MEA)

- What are the possible responses?
  - Directionality
    - Increase (Excitation)
    - Decrease (Inhibition)
    - Both (Biphasic)
  - Strength
    - Some change in activity
    - Strong change in activity

Botanical	Effect on neuronal activity
Aconite	Hyperexcitation
Oleander	Biphasic
Kava	Strong inhibition
Kratom	Strong inhibition
Thunder god vine	Strong inhibition
Yohimbe	Strong inhibition
Ginseng	None
Milk thistle	Inhibition
Aristolochia fangchi	Inhibition
Ashwagandha	None
Blue cohosh	Excitation
Comfrey	None
Ephedra	Inhibition
Goldenseal	Biphasic
Green tea	Inhibition
Usnea lichen	Inhibition

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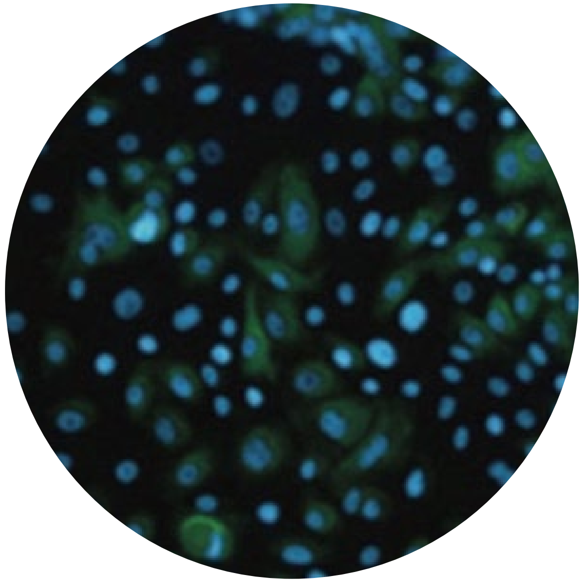
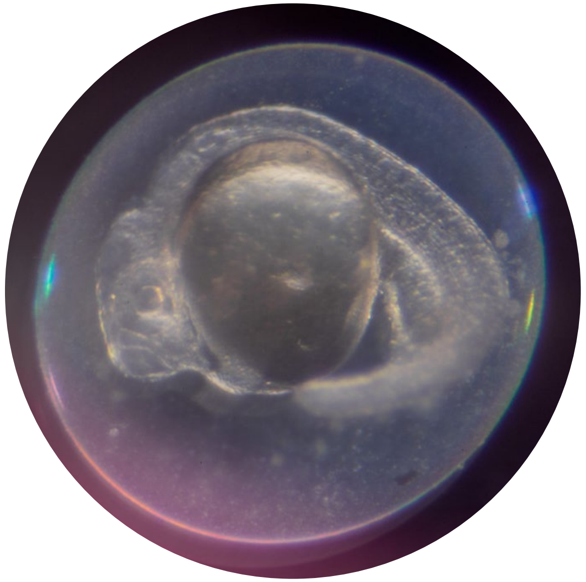
Building case studies

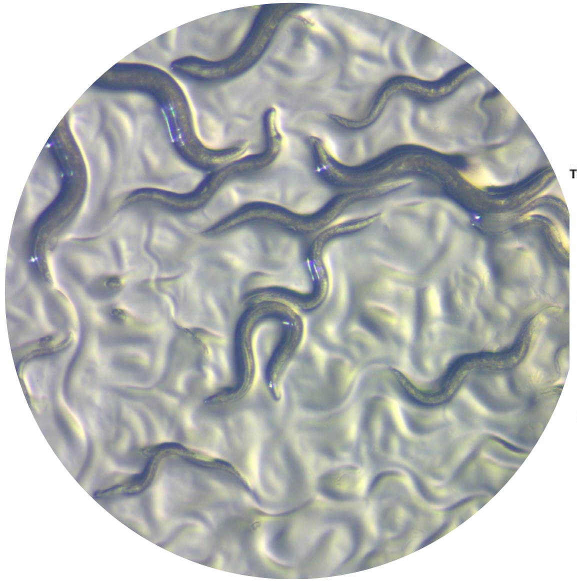
Developmental and Reproductive Toxicity (DART)



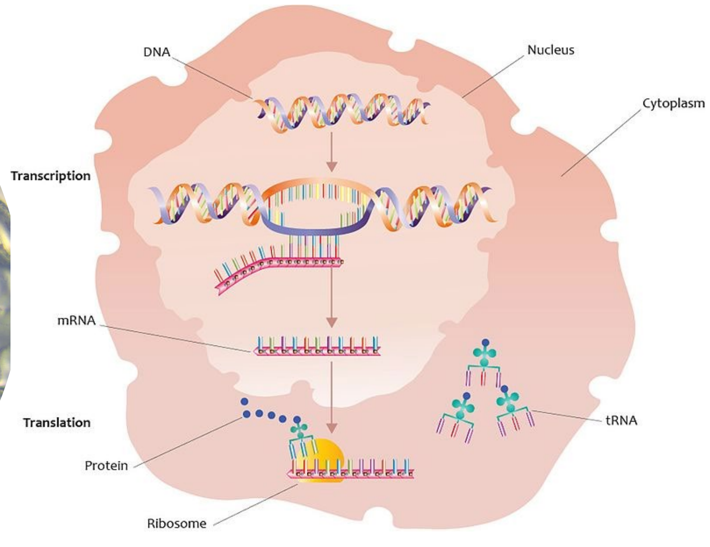
DevTox Quick Predict
Ornithine/cystine ratio
in iPS cells



Zebrafish
developmental assay



C. Elegans assay



Gene expression in 4
cell lines: MCF7,
A549, HepG2, iCAR

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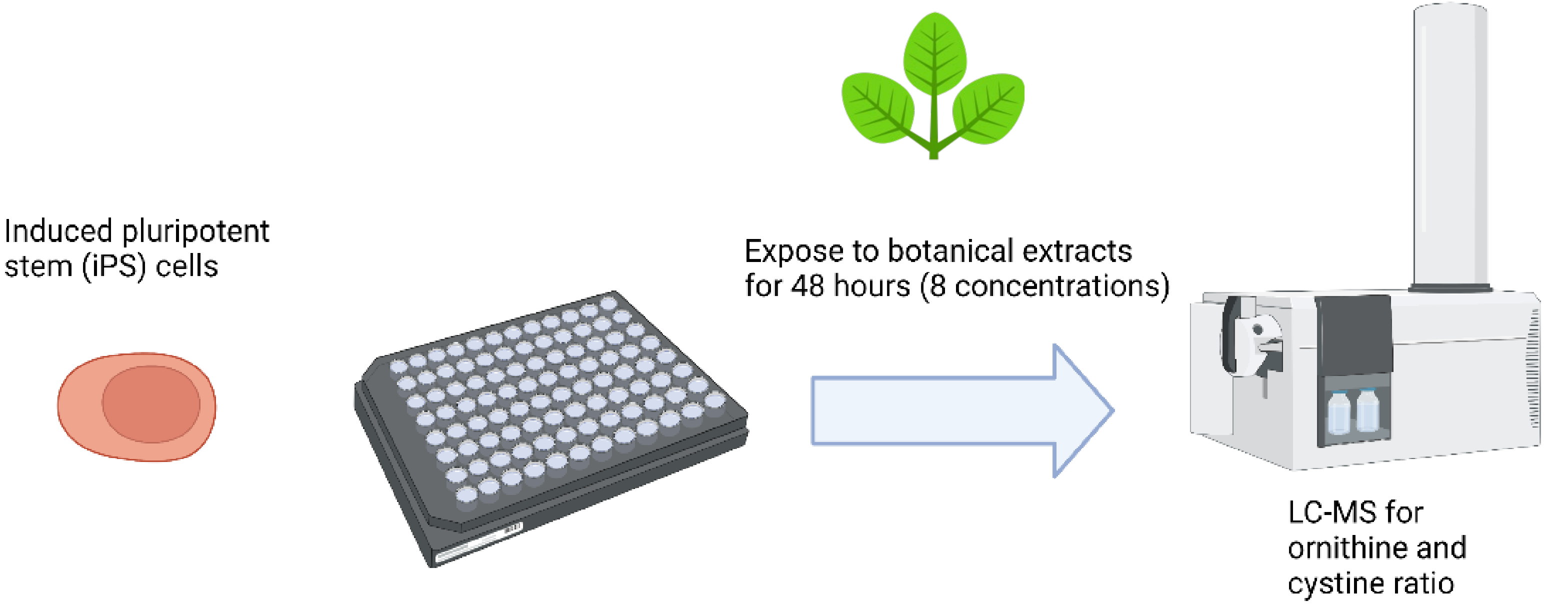
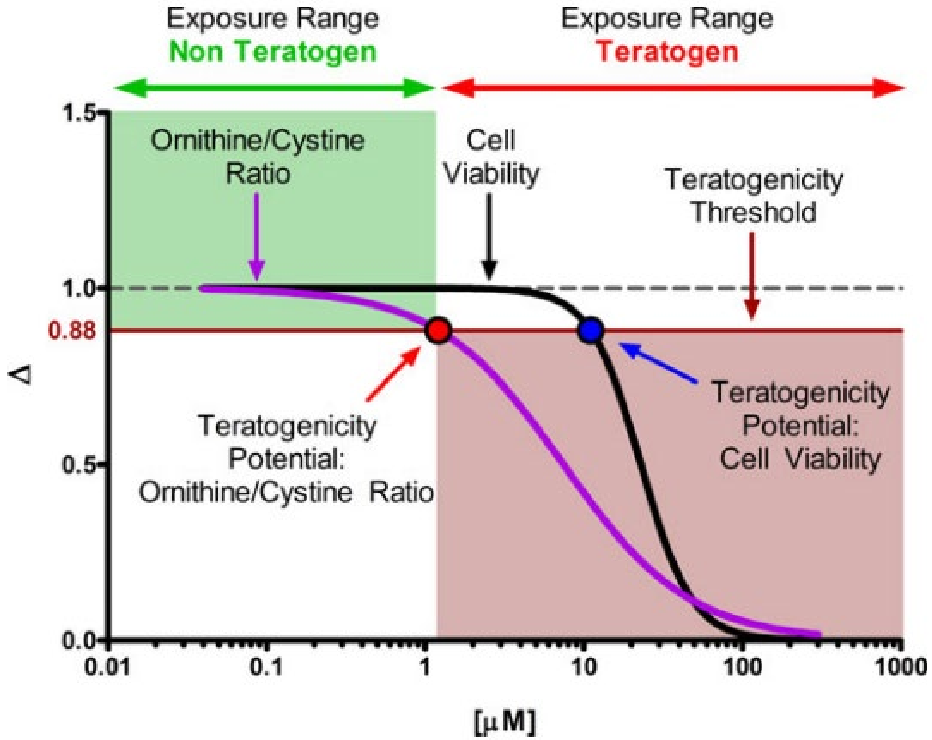
Building case studies

DevTox Quick Predict





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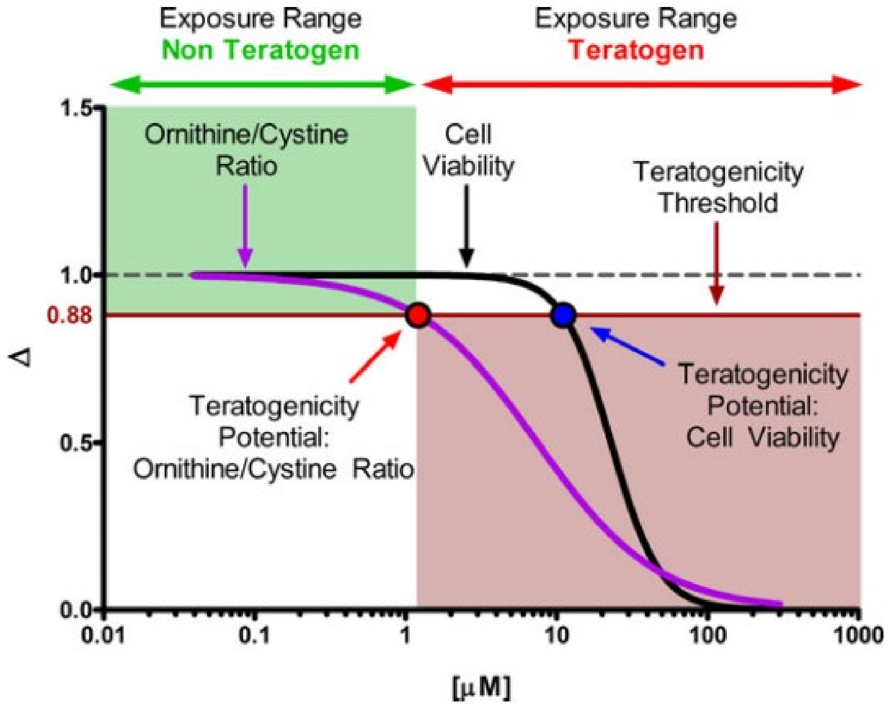
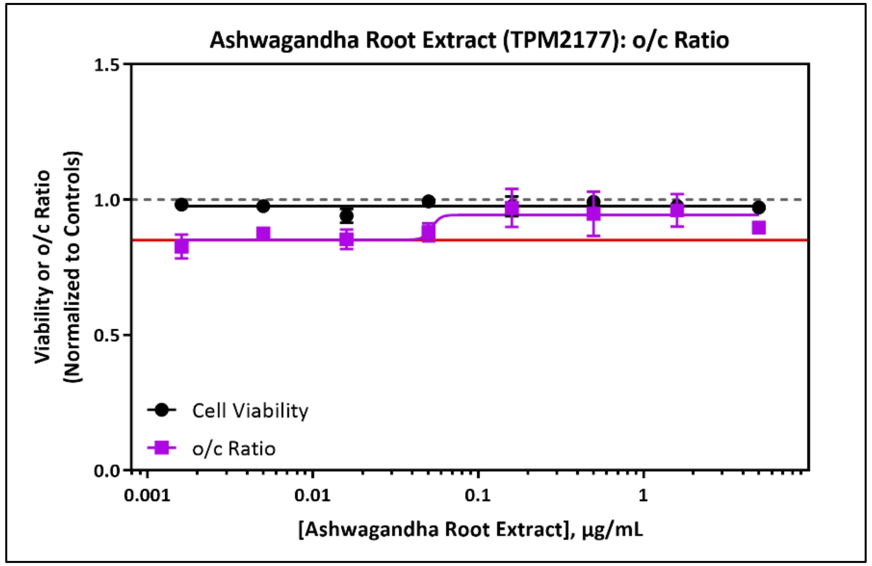
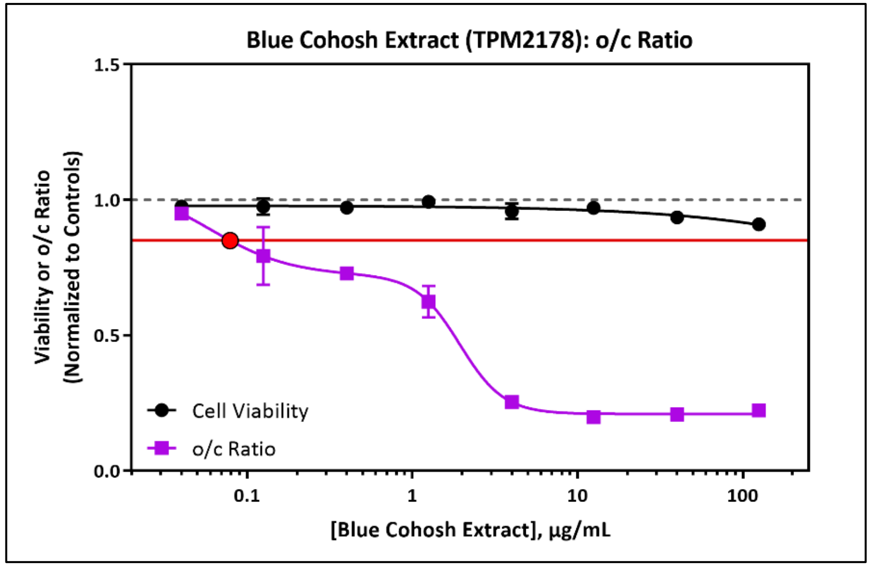
Laboratory: Stemina (J. Palmer); slide: Connie Mitchell

Building case studies

DevTox Quick Predict







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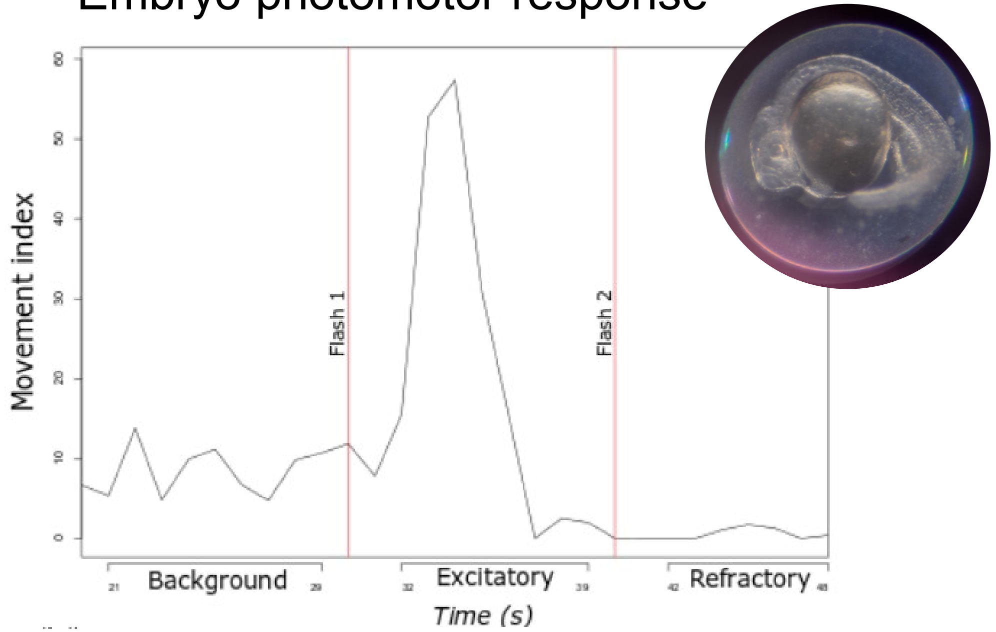
Laboratory: Stemina (J. Palmer); slide: Connie Mitchell

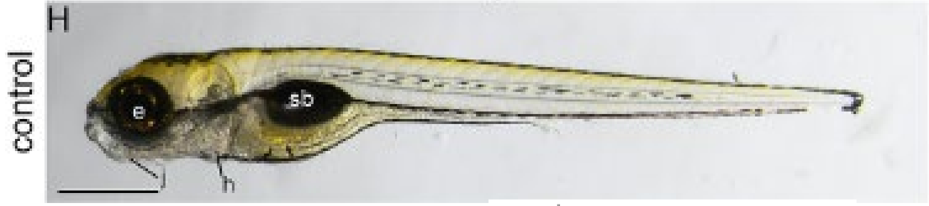
Building case studies

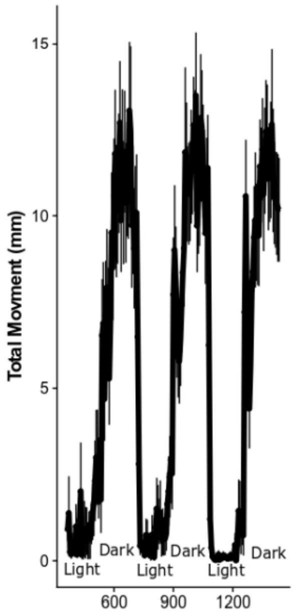
Developmental Zebrafish Assay

Embryo photomotor response

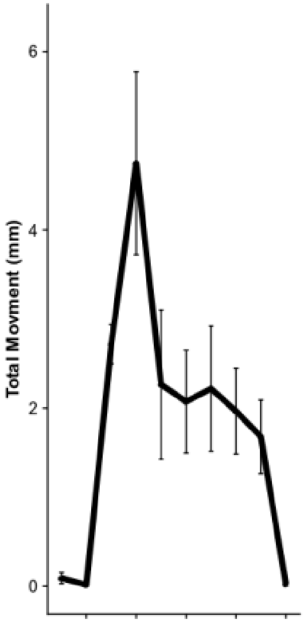


5 dpf



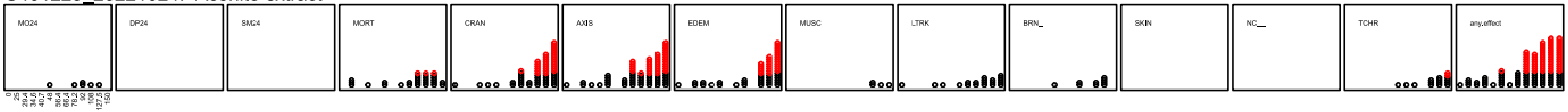


Larval photomotor response



Larval startle response

C401229_20221024: Aconite extract



24h

5d

Laboratory: Robyn Tanguay (Oregon State University)

Building case studies (preliminary data evaluation)

Botanical	DevTox	C. elegans	Zebrafish malformations	Transcriptomics
Blue		Positive	Negative	
Usne			Negative	
Ashw				
Ginseng				
Milk thistle	Negative	Positi		
		Positi		NA
		Positive	Positive	NA
		Positive	Negative	NA
Ephedra	Positive	Positive	Negative	NA
Goldenseal	Positive	Positive	Negative	NA
Green tea	Negative	Positive	Negative	NA
Kava	Positive	Positive	Negative	NA
Kratom	Positive	Positive	Negative	NA
Oleander	NA	Positive	Positive	NA
Thunder god vine	NA	Positive	Positive	NA
Yohimbe	Positive	Positive	Negative	NA

Assay is too sens
need to evaluate
apply an activit

Multiple mismatches. Is the endpoint or assay misaligned to the e toxicity?

Additional work is needed to distill results from multiple cell lines into a single yes/no call for DART potential

Is there an ADME-related explanation for the positive ginseng result?

Conclusions (so far)

- NAMs were developed and refined for use with single chemicals and require careful evaluation for application to complex mixtures
- Botanical ingredients offer an excellent opportunity to compare NAM-based data to in vivo and human data
- The Botanical Safety Consortium is a public-private partnership that focuses on evaluating the performance of NAMs with complex botanical mixtures
- Initial results highlight the need to integrate across assays and better distinguish activity from adversity in NAM platforms

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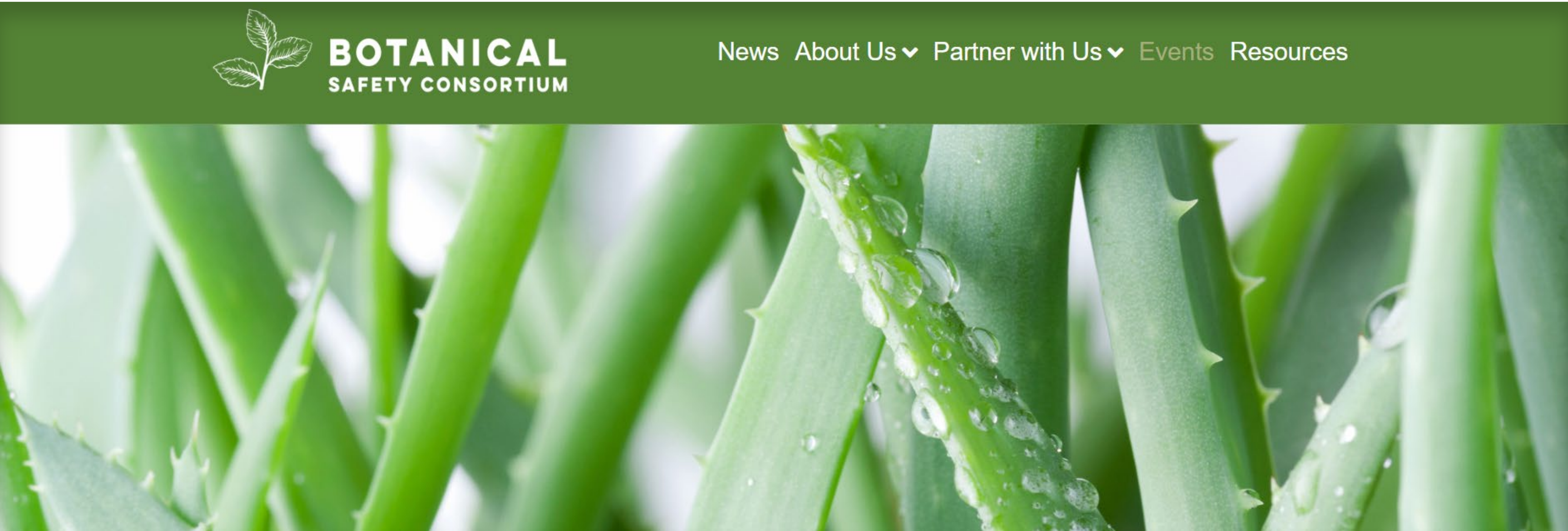
2024 Botanical Safety Consortium Summit

October 10-11
Durham, NC
In person and virtual options

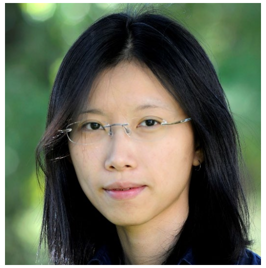


Acknowledgements

DTT Botanicals in Tox21


Mike DeVito


Troy Hubbard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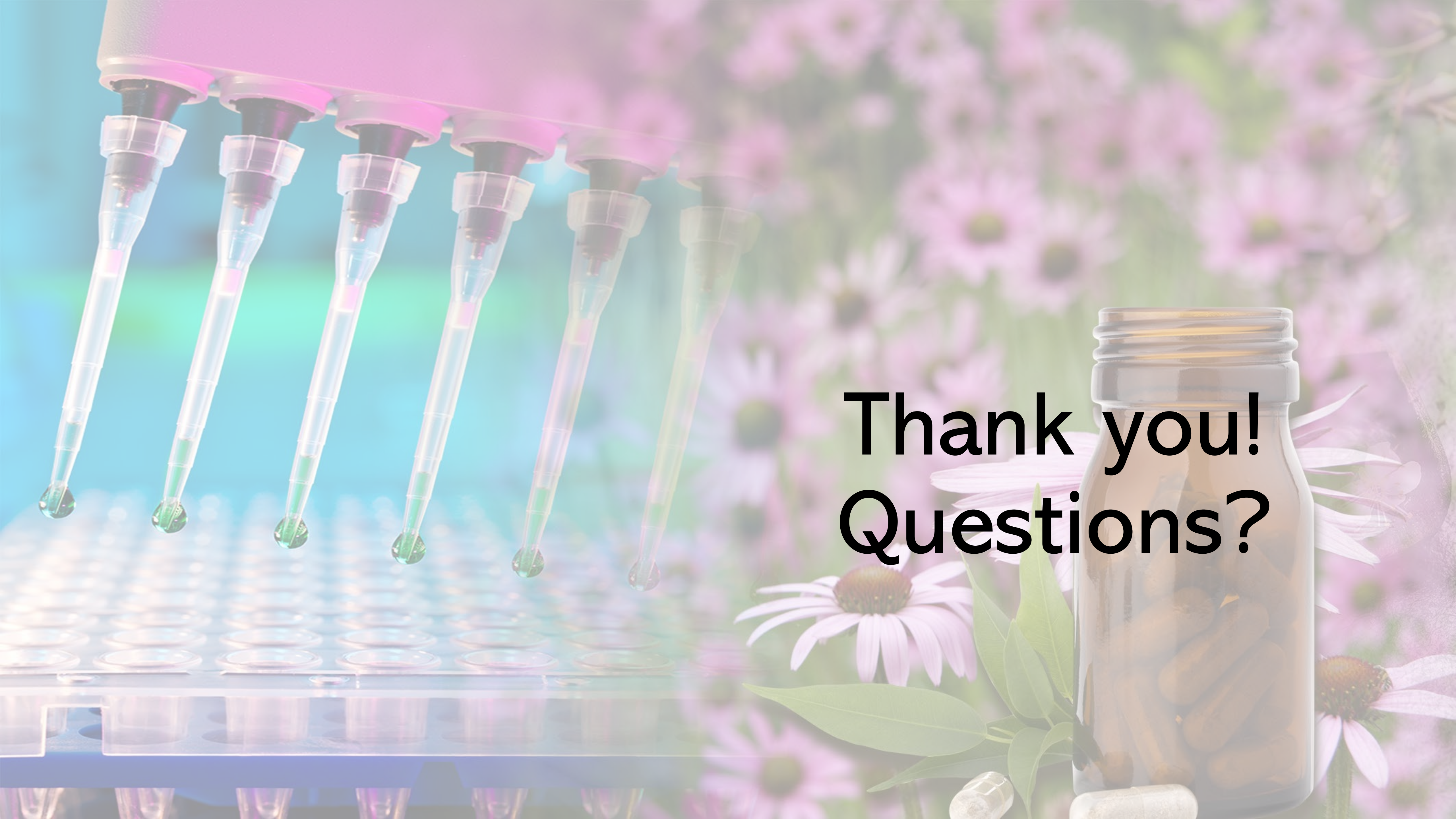

Thank you!
Questions?