Applying the Latest Toxicology Tools to Botanical Safety Evaluation

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Overview

- The National Toxicology Program
- Rationale for evaluating botanical safety
- Use of innovative methods and technologies for understanding botanical safety
- The Botanical Safety Consortium

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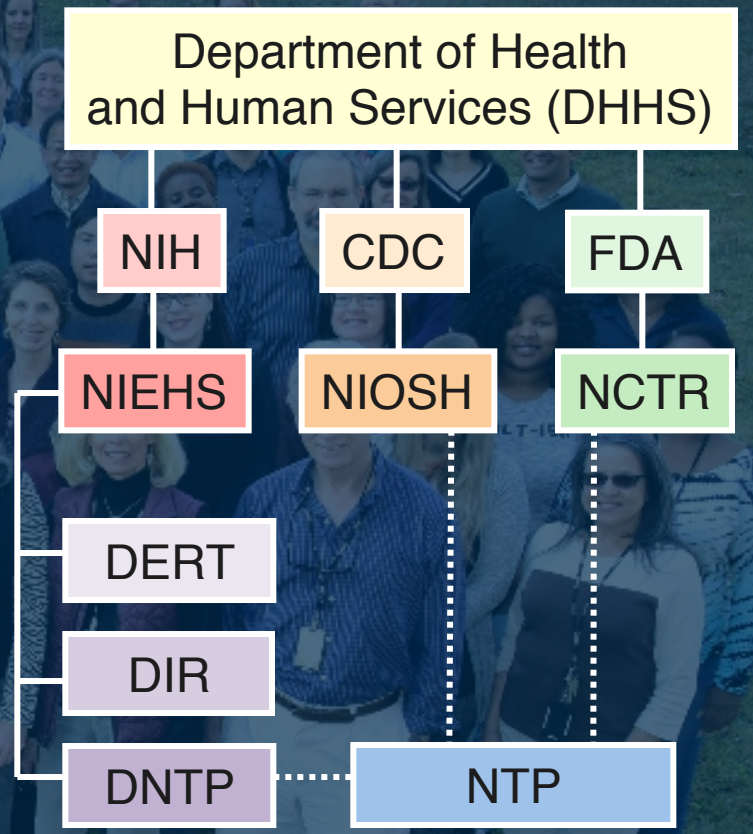
National Toxicology Program

- Interagency program
  - Headquartered at NIEHS
- Research on nominated test articles
  - Thousands of agents evaluated in comprehensive toxicology studies
  - GLP compliant testing through government contracts
- Analysis activities
  - Report on Carcinogens (RoC)
  - Health Assessment and Translation Reports
  - NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)



Mission: To evaluate agents of public health concern by developing and applying tools of modern toxicology and molecular biology.

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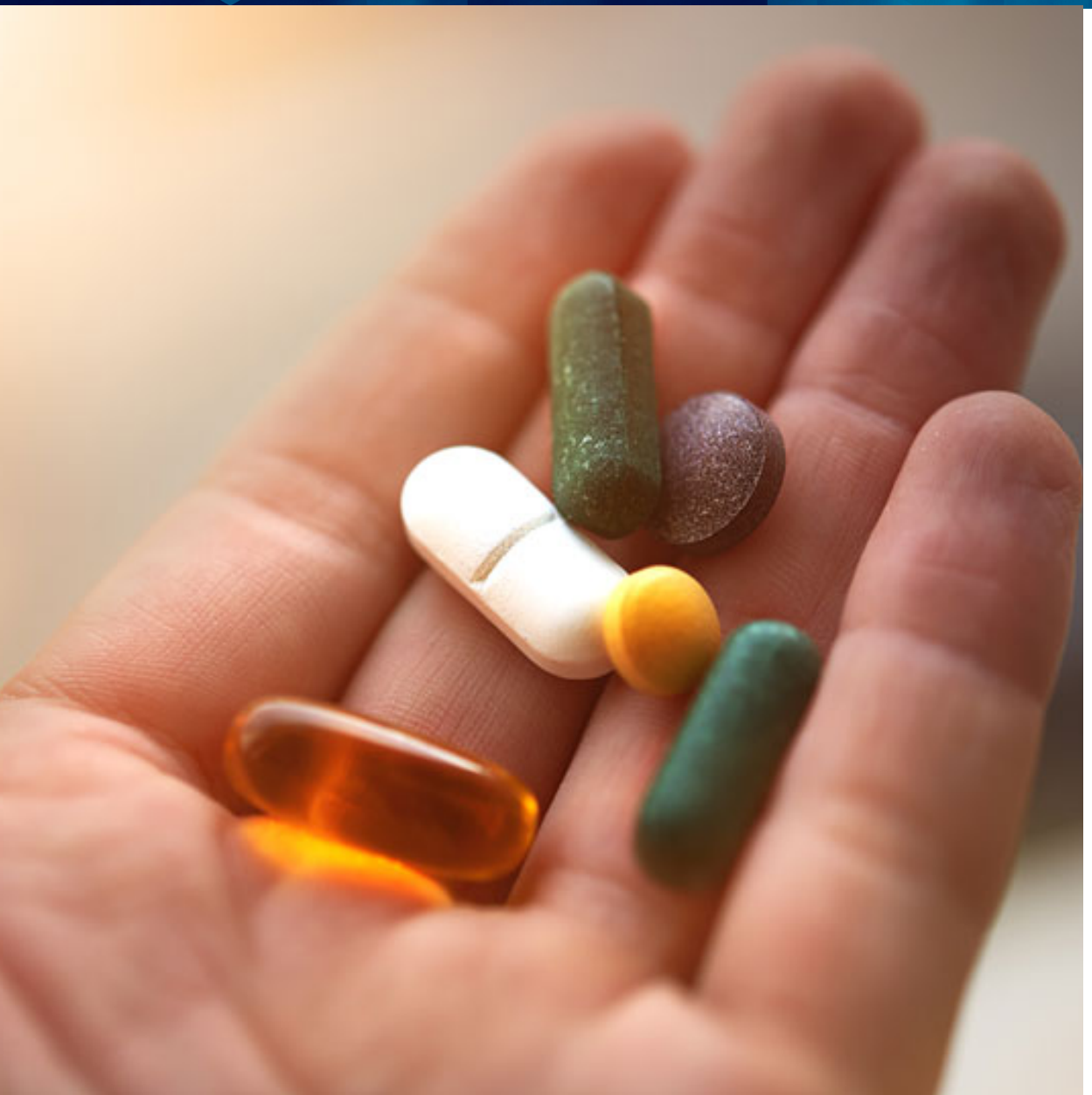
Why study botanicals?



- Many people take botanical dietary supplements – about 18% of US population
- Recommended doses can be high (100s - 1000s mg per day)
- Adverse events following botanical use have been reported
- Safety data are often inadequate
- Concerns about quality and integrity of botanical products

Toxicity and carcinogenicity

Completed:

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

Ongoing:

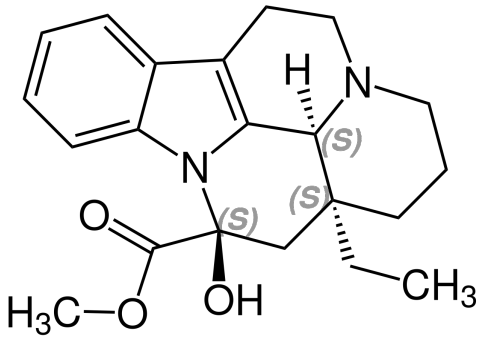
- Black cohosh
- Dong quai
- *Echinacea purpurea*
- Garcinia cambogia
- Usnea lichen
- Valerian root

Strategy

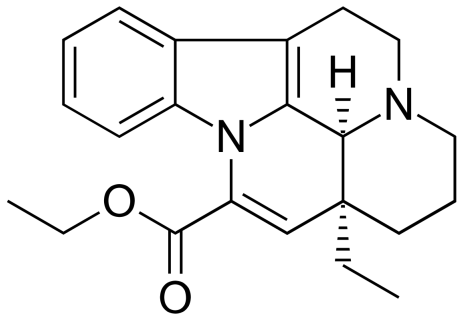
- Identify knowledge gaps
  - Specific concern: Ephedra and cardiotoxicity
  - General: Lack of toxicity and carcinogenicity data
- Test article selection
  - Authentic and representative of marketplace
- Fit for purpose study design
  - *In vivo* (e.g., mice and rats) to characterize hazard
  - Complementary New Approach Methodologies (NAMs) to elucidate mechanism of action or to translate from rodent to human

Vinpocetine

Vincamine



Vinpocetine



Scoping the safety issues:

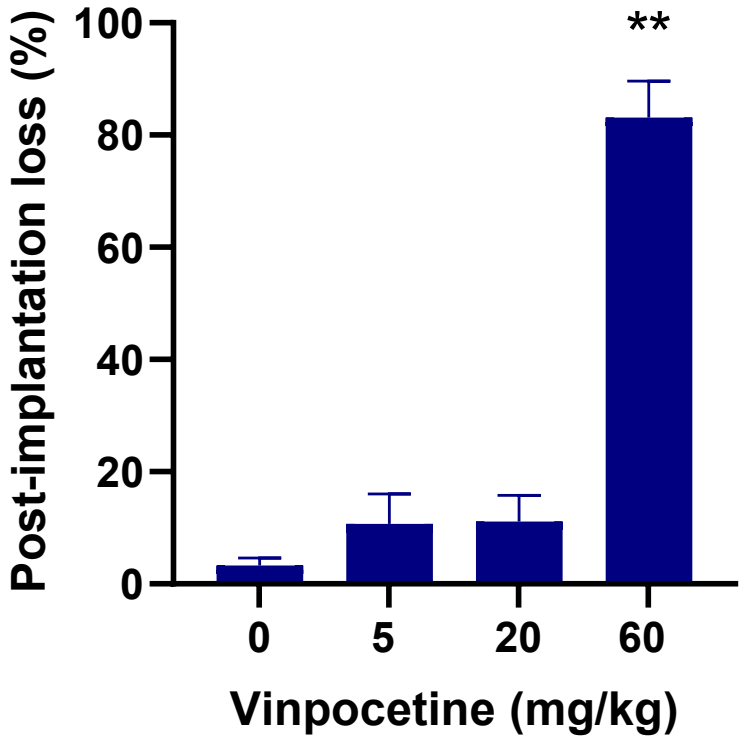
- Vinpocetine is a pure synthetic chemical (not found in nature)
- Lack of comprehensive toxicity data
- Some signs of potential developmental toxicity (summarized in Cholnoky and Dömök 1976)

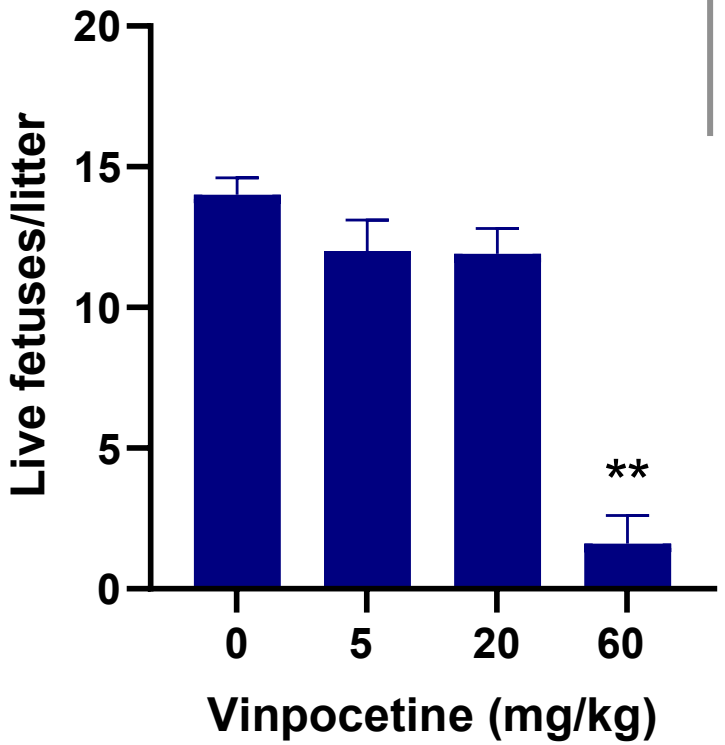
Vinpocetine

GD0 GD6 GD20

Dosing period

GD21
Evaluation of dams and fetuses





Catlin et al., (2018). Embryo-fetal development studies with the dietary supplement vinpocetine in the rat and rabbit. Birth Defects Res. 110: 883-896.

FDA STATEMENT

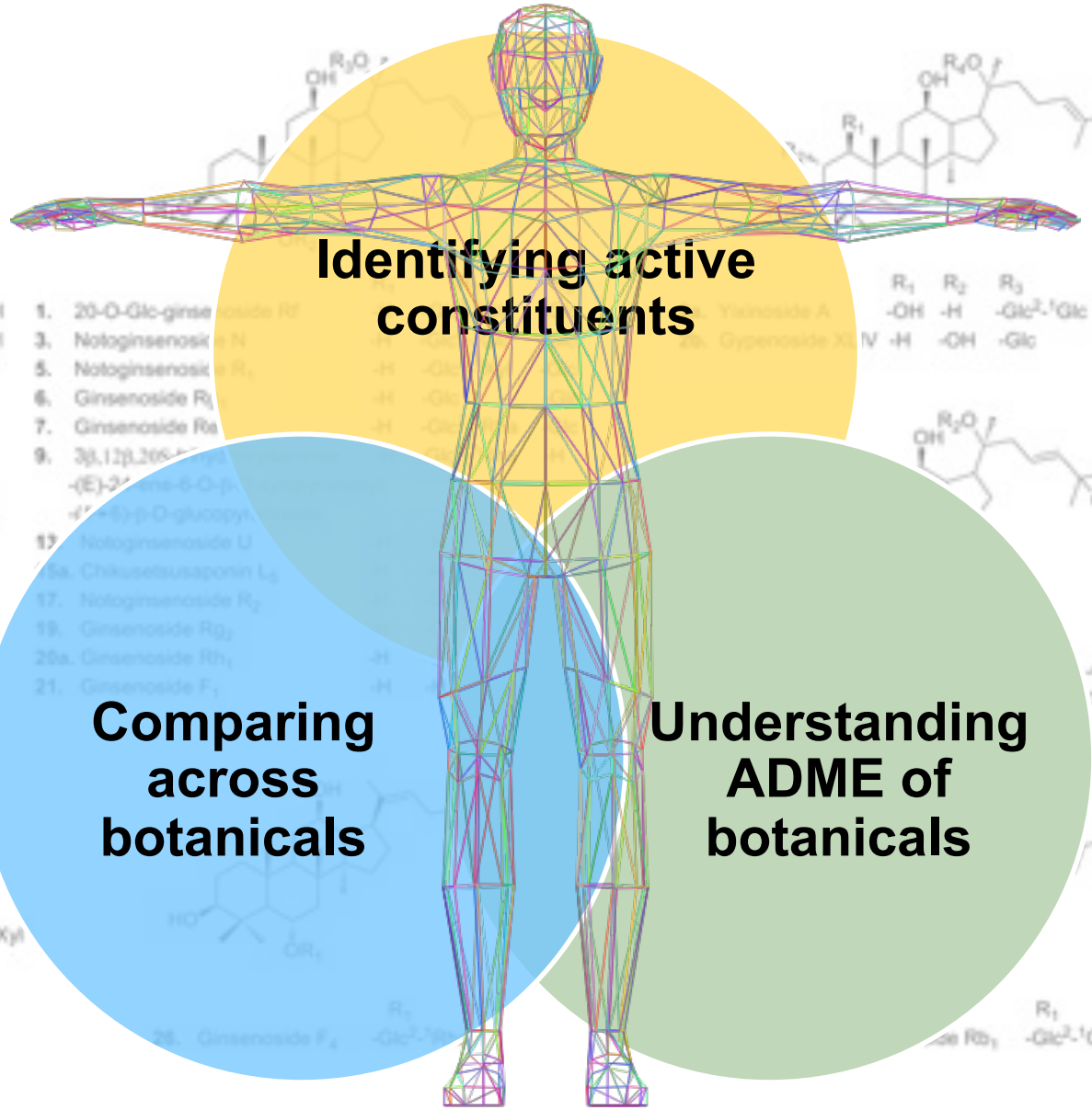
Statement on warning for women of childbearing age about possible safety risks of dietary supplements containing vinpocetine

For Immediate Release: June 03, 2019

Statement From: Amy Abernethy, MD, PhD.
Principal Deputy Commissioner - Office of the Commissioner

Frank Yiannas
Deputy Commissioner for Food Policy and Response - Food and Drug Administration

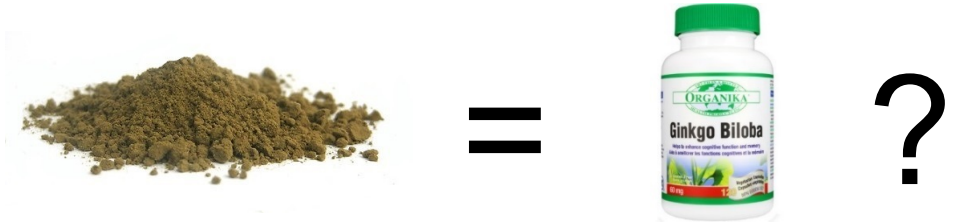
Challenges


Identifying active constituents
Comparing across botanicals
Understanding ADME of botanicals

Comparing across botanicals

Sufficient similarity = phytoequivalence

Two mixtures are similar enough that data from one mixture (*reference mixture*) can be used to estimate safety or risk from exposure to another (*mixture of interest*)



Why is this important?

There are thousands of products in the marketplace and we are not going to test all of them

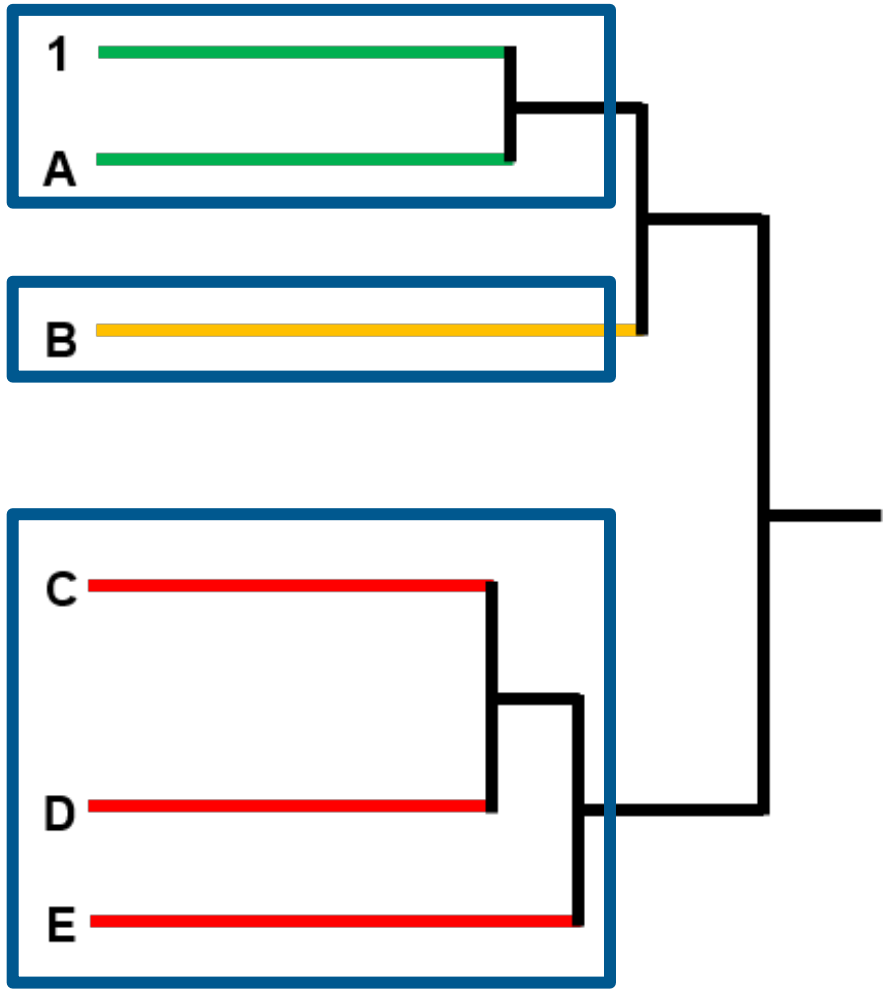
Ginkgo biloba extract

- Has been used in traditional medicine for a very long time
- Current use of *Ginkgo biloba* is often as a leaf-based extract to promote circulation and brain function
  - Large epidemiological studies did not find an improvement in memory with *Ginkgo biloba* extract use
- Typically taken in tablet or capsule form with recommended doses of 120-240 mg per day
- Selected for testing based on a lack of toxicity and carcinogenicity data
  - Liver and thyroid identified as targets of *Ginkgo biloba* in mice and rats

Key question: Can we use data from the test article to evaluate the safety of other *Gingko biloba* products?

Sufficient similarity

1. Generate data (any kind of data – chemistry, *in vitro*, *in vivo*) on the reference and mixtures of interest
2. Apply multivariate statistical approaches to analyze data (PCA, hierarchical clustering)
3. Make similarity judgment for each mixture
   a) Mixtures in the same group as the reference are considered “similar”
   b) Mixtures in the most different group are considered “different”
   c) Mixtures in neither the most similar or the most different groups are considered “maybe similar”



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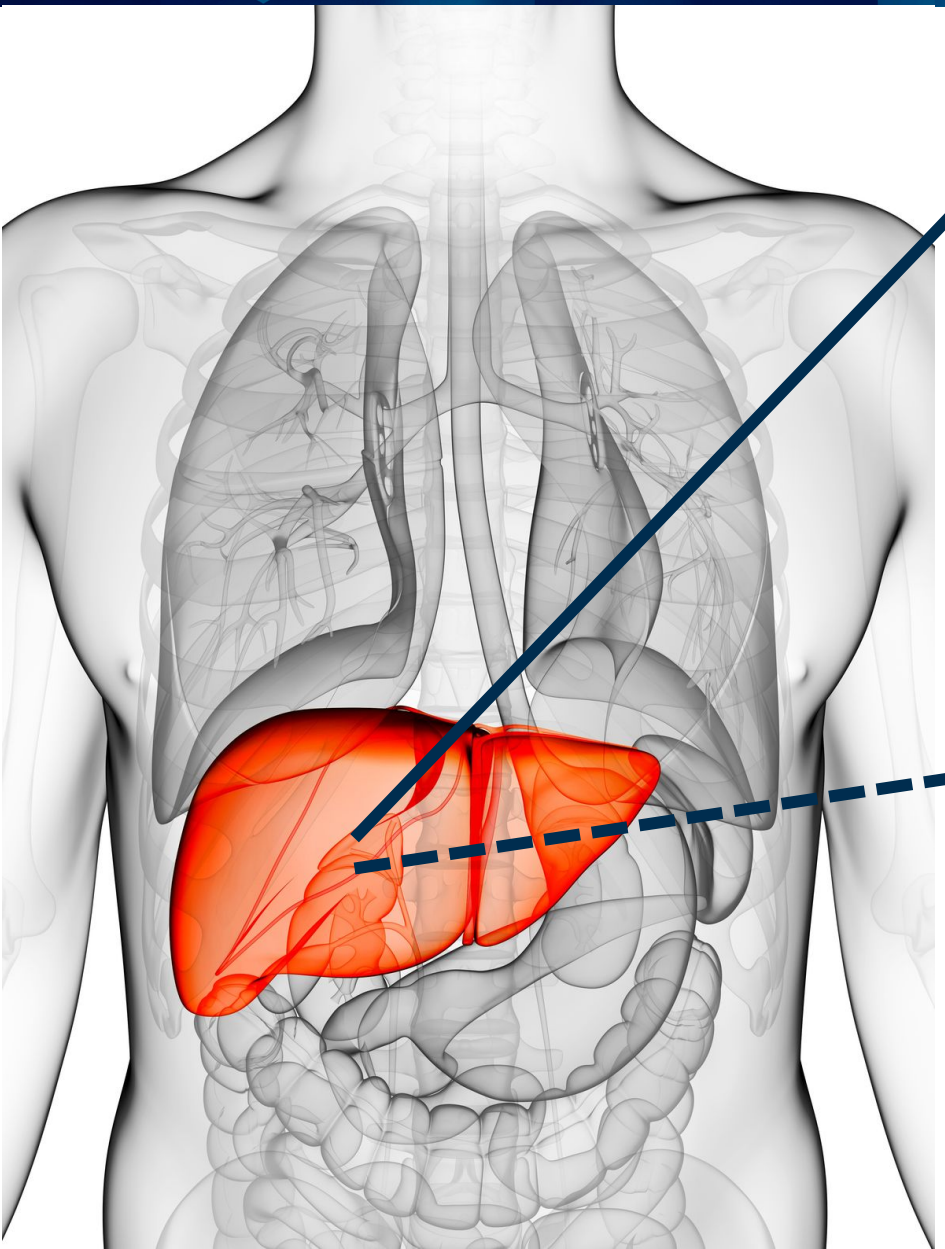
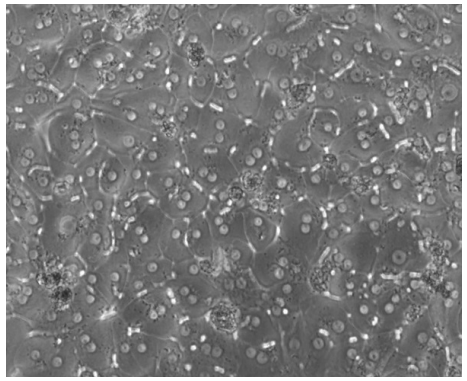
Sufficient similarity



Model system

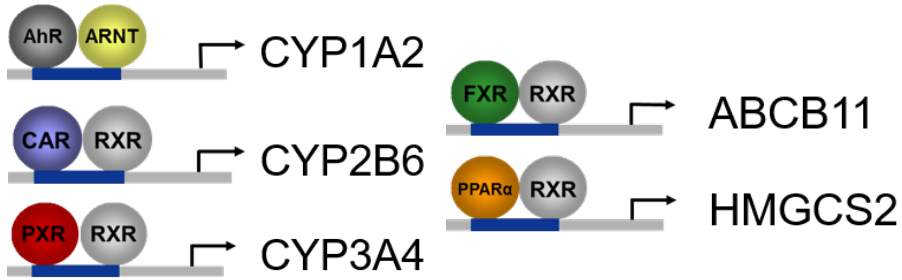
Human primary hepatocytes



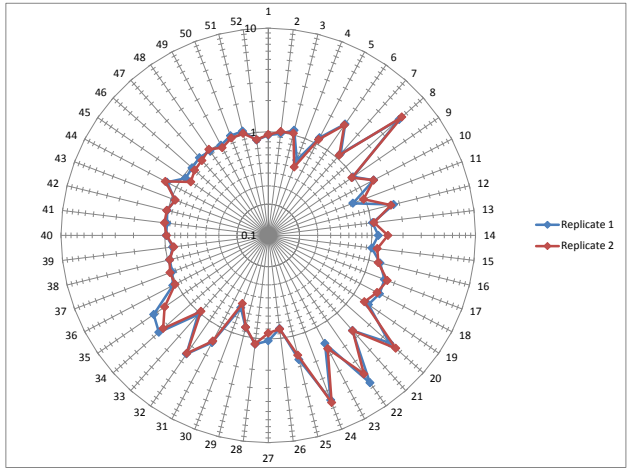
Attagene Factorial™ Assays in HepG2 cells - immortalized cell line derived from human liver carcinoma cells

Endpoints

Expression of genes indicating activity of key metabolizing enzymes



Numerous transcription factors and nuclear receptors



Sufficient similarity

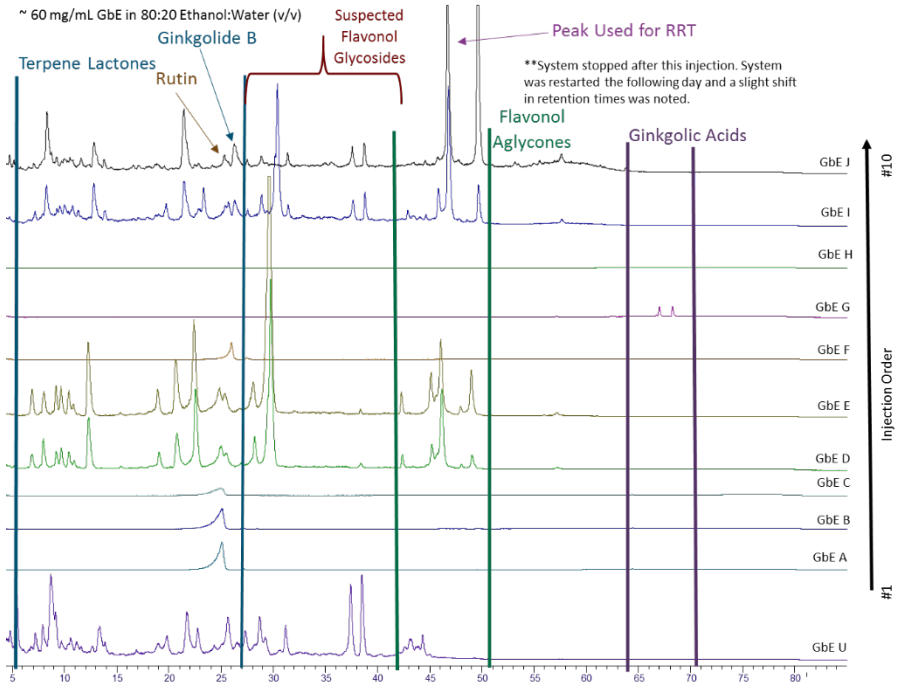
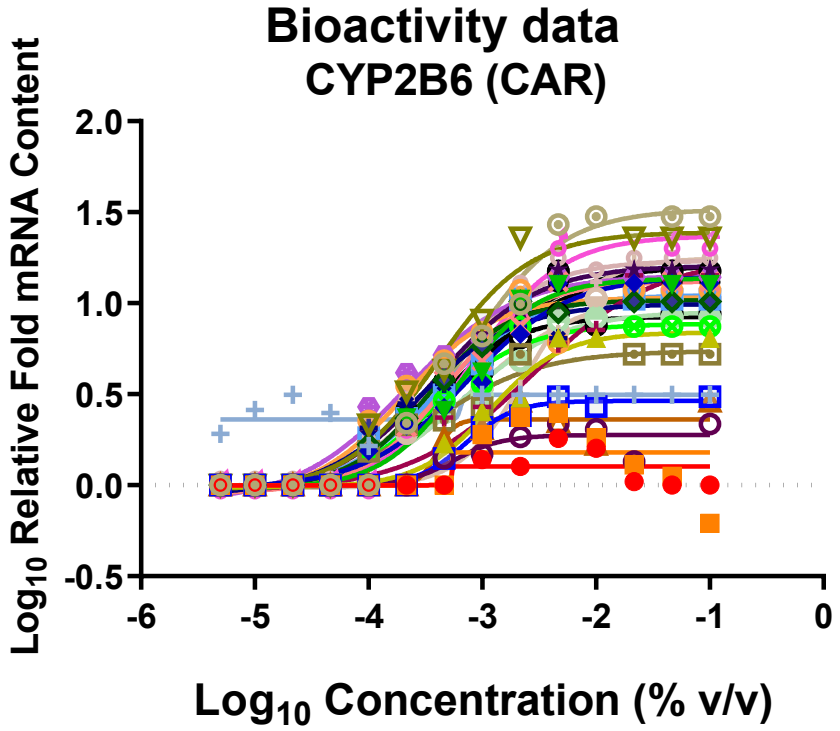
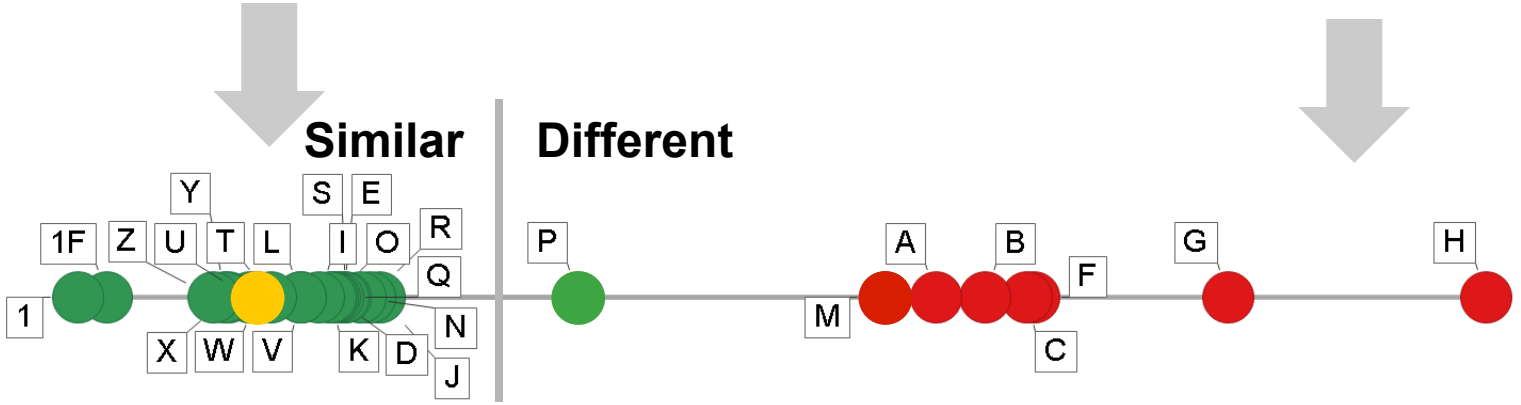


Figure 2. Non-Targeted Fingerprint Chromatograms of First Set of GbE Samples (Not Hydrolyzed), HPLC-ELSD





Catlin et al., (2018). How similar is similar enough? A sufficient similarity case study with *Ginkgo biloba* extract. Food Chem Toxicol. 118: 328-339.

Bioassay Guided Fractionation

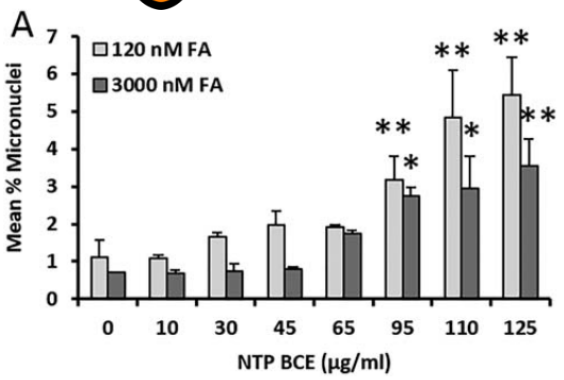
Bioassay guided fractionation

Extraction

Bioassay

Active extract

Separation



Bioassay

Isolation/
Identification

Chemical Structure

Active fraction

Roberts et al., 2019. Food and Chemical Toxicology. 124: 431-438.
Smith-Roe et al., 2018. Environmental and Molecular Mutagenesis 59:416-426.

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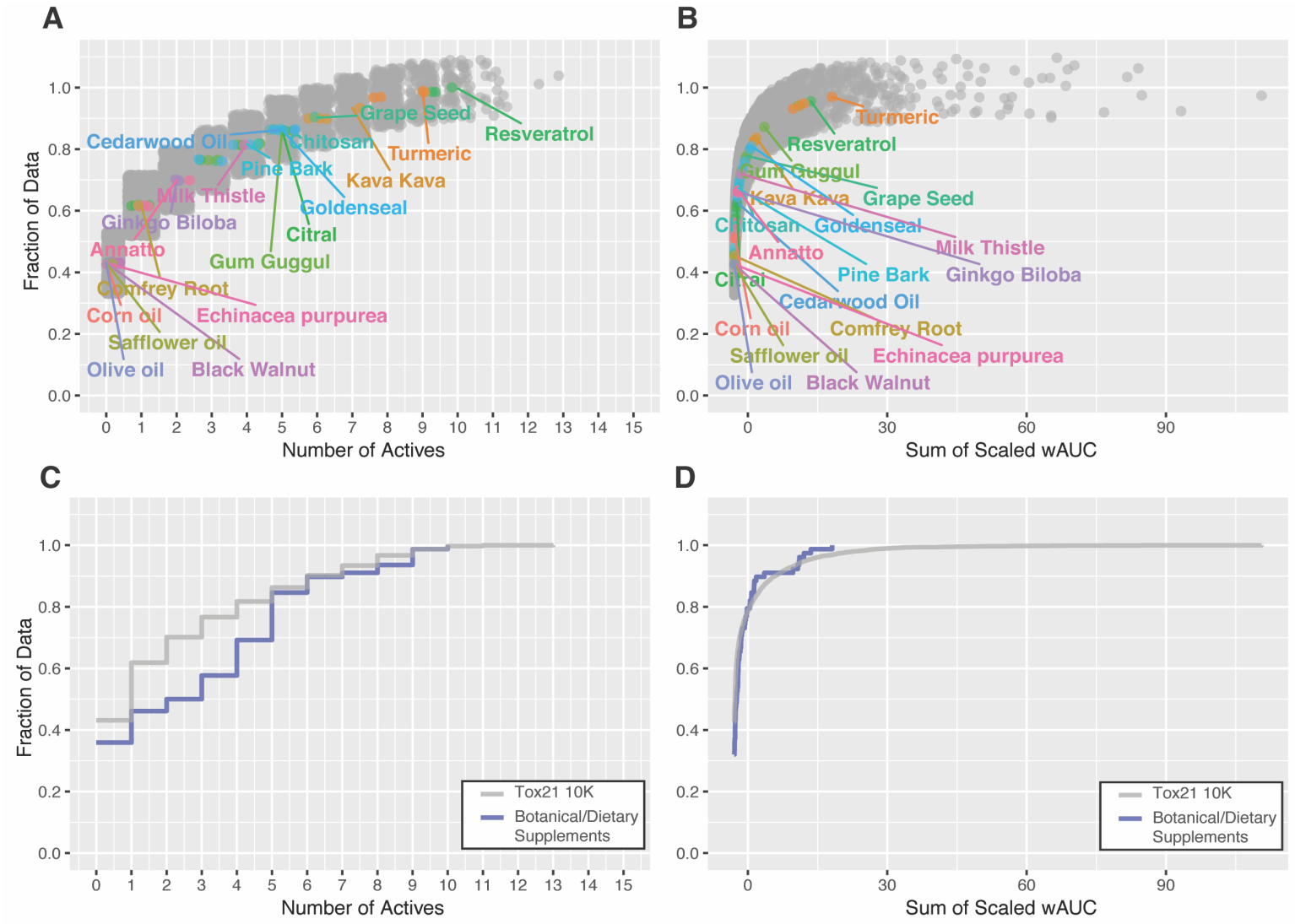
Botanicals in Tox21



Hubbard et al., (2019). Using Tox21 High-Throughput Screening Assays for the Evaluation of Botanical and Dietary Supplements. Appl In Vitro Toxicol. 5(1):10-25.

A public-private partnership to improve botanical safety

BOTANICAL SAFETY CONSORTIUM

The Botanical Safety Consortium (BSC) was officially convened in November 2019, as the result of a Memorandum of Understanding between the US Food and Drug Administration (FDA), the National Institutes of Health's National Institute of Environmental Health Sciences (NIEHS), and the non-profit Health and Environmental Sciences Institute (HESI).

Get Involved

At a Glance

Learn More

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Objectives

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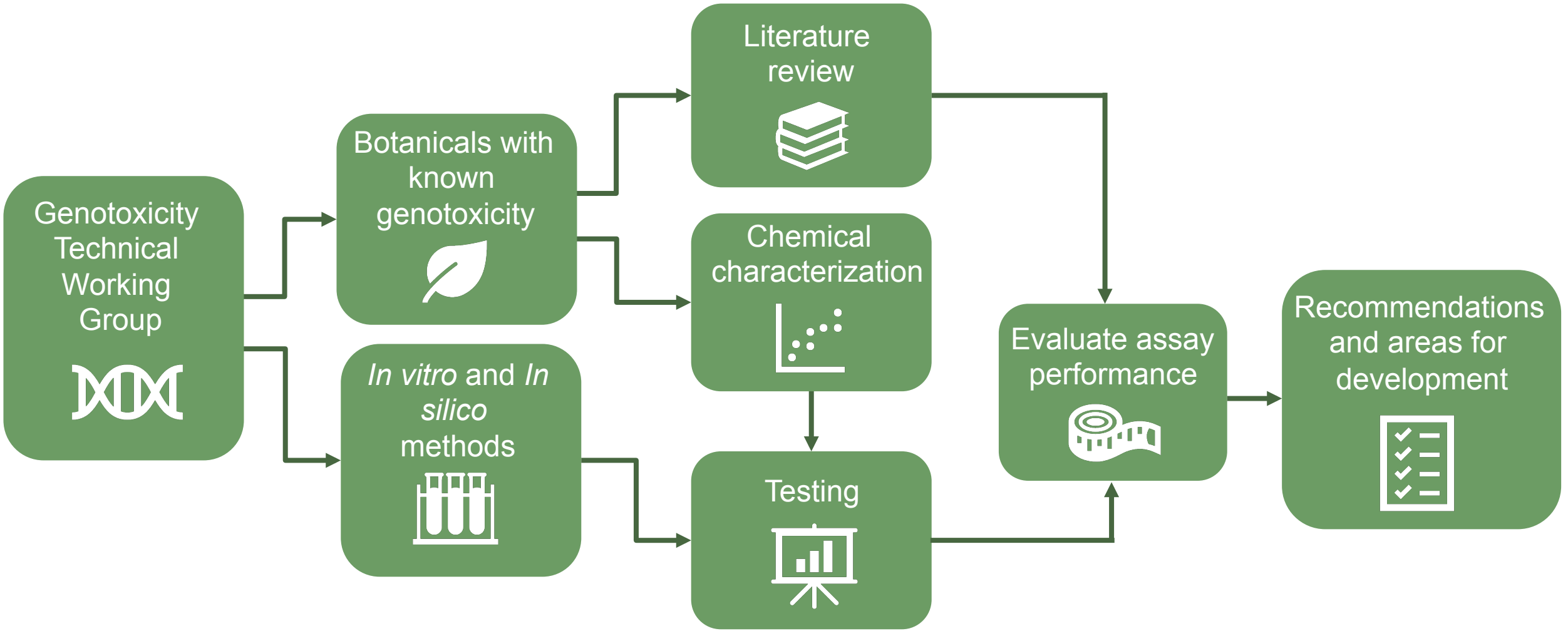
Workflow



Key points

- Botanicals present an important public health challenge due to their widespread use, high doses, and complex chemistry
- While animal studies represent an important tool for evaluating the safety of botanical ingredients, predictive new approach methodologies are needed for more rapid and cost-effective screening purposes
- *In vitro* assays have been successfully applied to determine sufficient similarity of complex botanical mixtures and identify active constituents through bioassay guided fractionation
  - Correlation between *in vivo* findings and responses in human cells
  - Allow for testing of numerous samples
- The Botanical Safety Consortium is dedicated to expanding the toolbox of methods available for botanical assessment and providing a recommended framework for evaluating botanical safety

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Acknowledgements

DNTP *Ginkgo biloba* project


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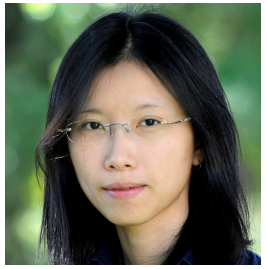
DNTP Black cohosh project


Stephanie Smith-Roe

DNTP Botanicals in Tox21


Troy Hubbard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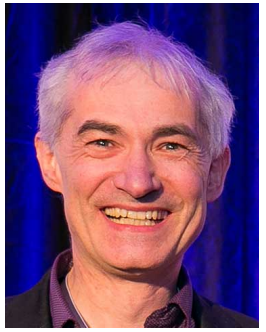
Cara Welch USFDA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Elan Sudberg Alkemist


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Thank you!