NTP
National Toxicology Program

Research on Botanical Safety at the National Toxicology Program

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May 29, 2020

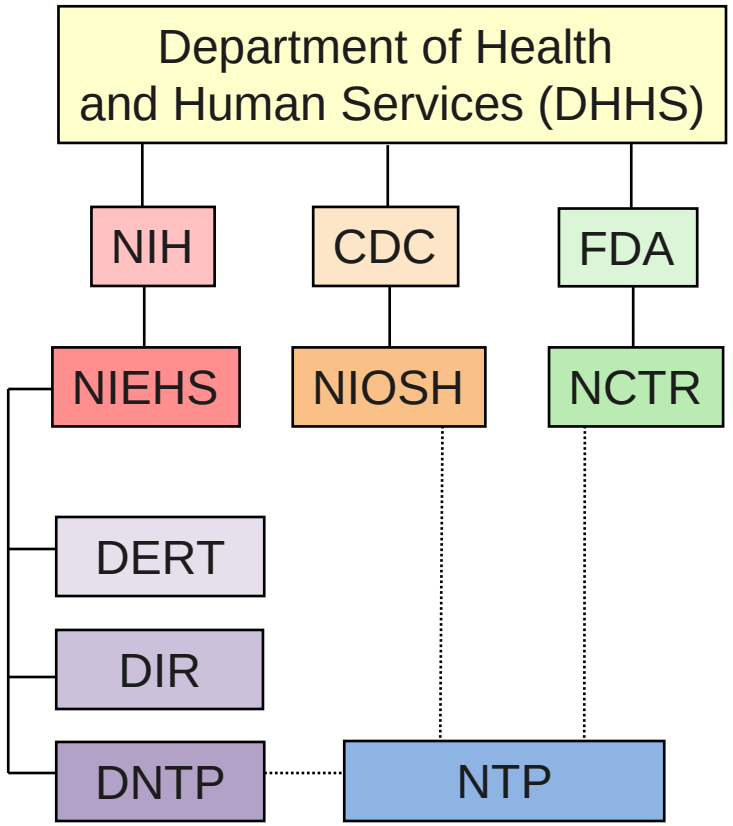
Outline

- Background on the National Toxicology Program and botanical testing
- Projects to address challenges in botanicals safety assessment
  - Methods to compare across botanicals
  - Identification of active constituents
  - Approaches for ADME of botanicals
  - High throughput screening of botanicals for activity

Milk thistle
Silybum marianum

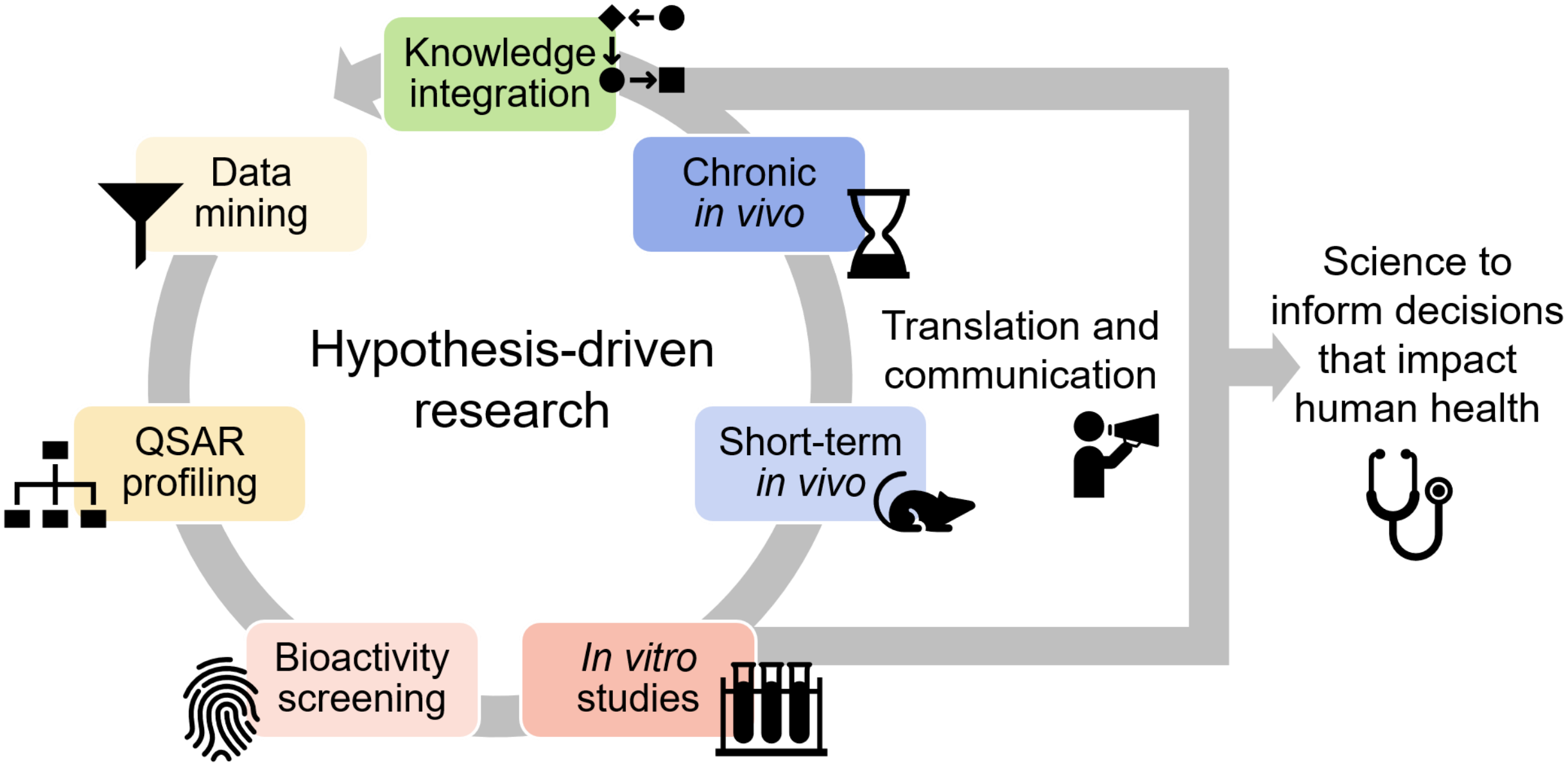
National Toxicology Program

- 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)
  - Office of Health Assessment and Translation (OHAT)
  - 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.

Tools and strategy



Strategic areas of focus

Health Effects Innovation Programs

- Cardiovascular Health Effects Assessment
- Carcinogenicity Testing for the 21st Century
- Developmental Neurotoxicity Modeling

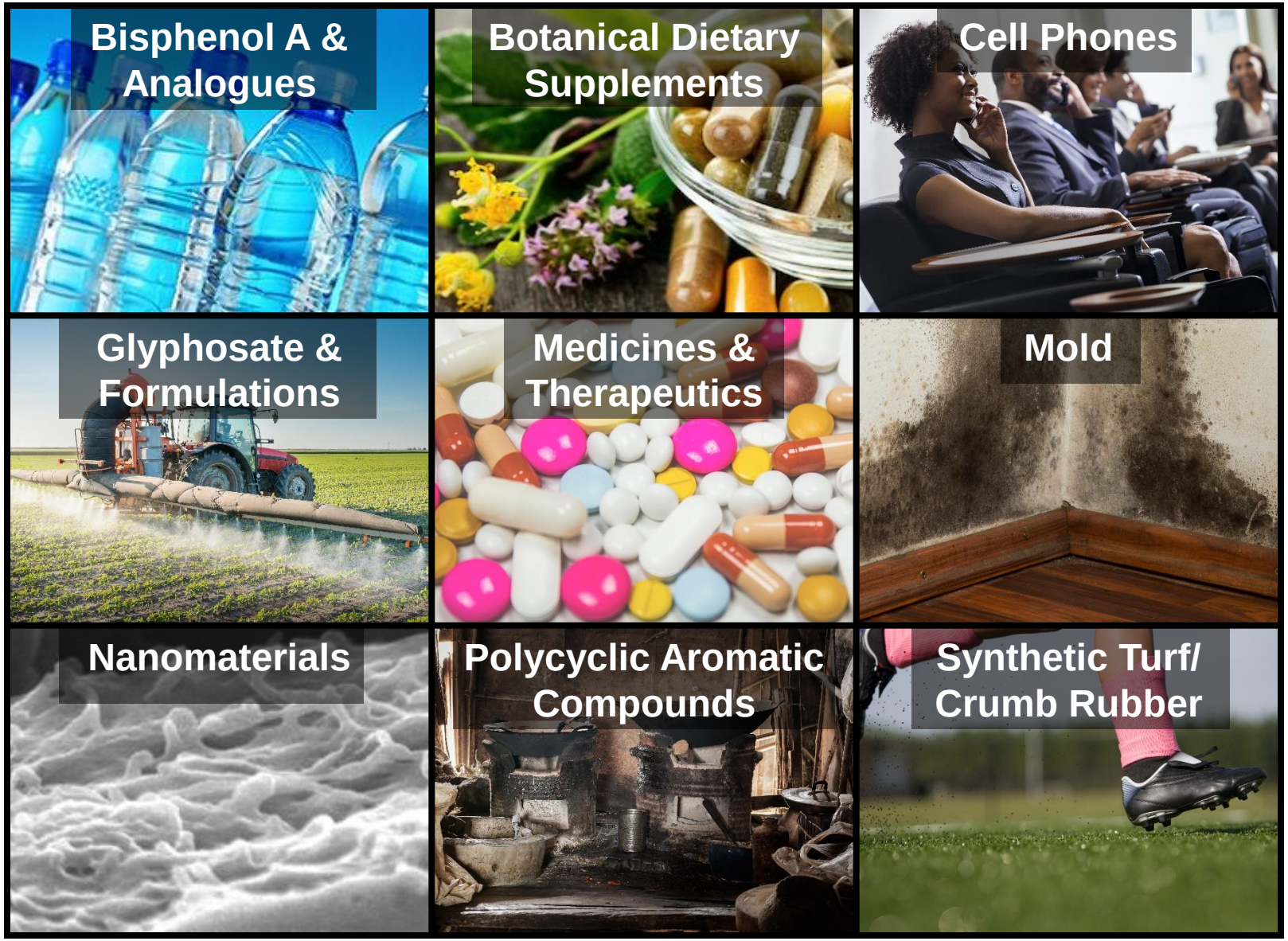
Exposure-Based Research Themes

- Combined Exposures and Mixtures
- Consumer Products and Therapeutics
- Occupational and Inhalation Exposures

Responsive Research

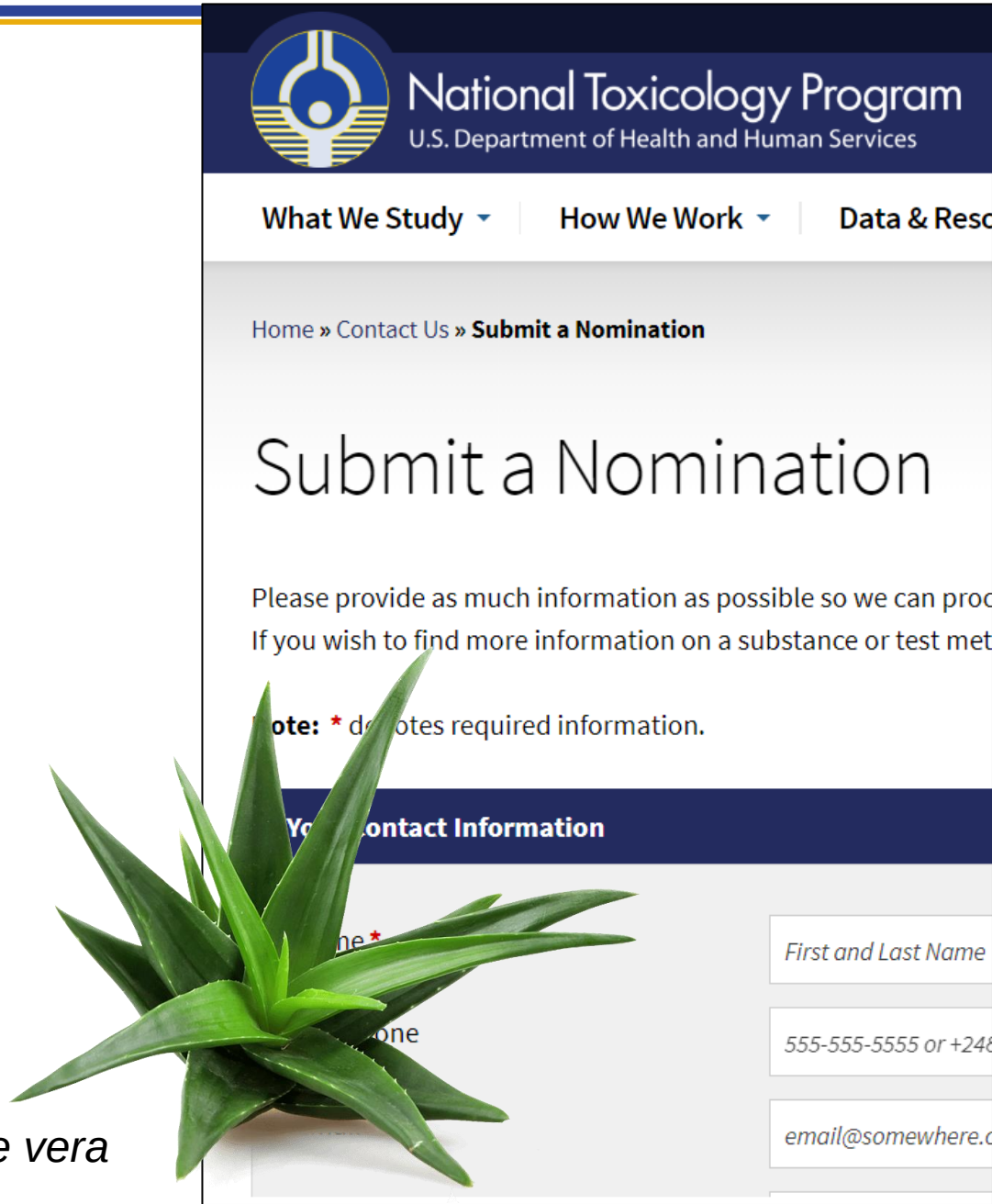
- Per- and Polyfluoroalkyl Substances (PFAS)
- Emerging Contaminants & Issues of Concern
- Safe and Sustainable Alternatives

Current research



NTP interest in botanicals

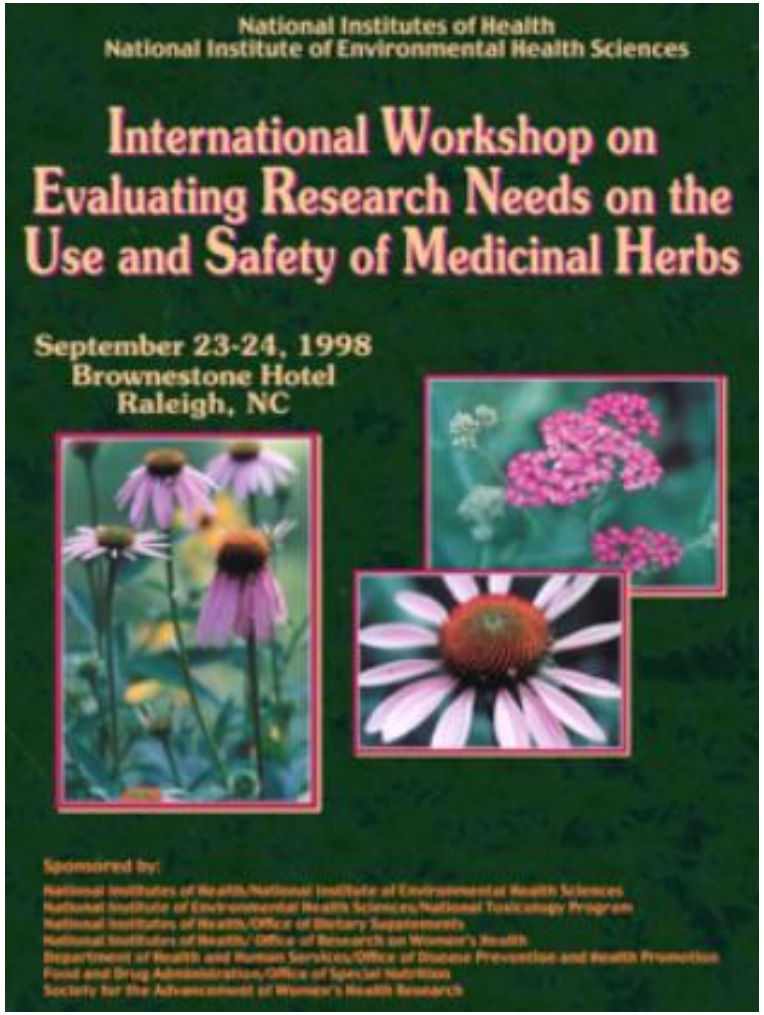
- NTP evaluates substances that are of public health concern
  - Widespread exposure in potentially vulnerable populations (people with underlying conditions, elderly, pregnant and lactating women, children)
  - Relatively high doses
- There is little safety data on most botanicals
- Public concern about the quality and integrity of botanicals available in the marketplace
- NTP has received a number of nominations to study botanical dietary supplements
  - National Cancer Institute (9), NIEHS (5), Private Individuals (3), FDA (2)



Aloe vera

Origin of NTP work in botanicals

1998 NTP Workshop



- Recommendations from the workshop:
  - Research on potential toxicity associated with high dose or prolonged use
  - Identification and standardization of product ingredients by industry
  - Increased consumer education through package inserts
  - Identification of herb-drug and herb-herb interactions
  - Research on risk to sensitive subpopulations

Mathews et al., 1999. EHP. 107(10): 773–778.

Testing approach

Ginkgo biloba

- Identify knowledge gaps
  - Specific concern: Ephedra and cardiotoxicity
  - General: Lack of toxicity and carcinogenicity data
- Test article selection
- Study design (general)
  - Animals: Male and female B6C3F1/N mice and Sprague Dawley rats (previously F344)
  - Exposure duration: 2-week, 3-month, 2-year
  - Dosing paradigm: typically oral gavage for botanical dietary supplements
  - Endpoints: clinical chemistry, hematology, genotoxicity, sperm motility and vaginal cytology, histopathology

Current botanical portfolio

Completed

- *Aloe vera* nondecolorized whole leaf extract
- Bitter orange extract
- *Ephedra* (ma huang)
- Ginseng root extract
- *Ginkgo biloba* extract
- Goldenseal root powder
- Green tea extract
- Kava kava extract
- Milk thistle extract
- *Senna*

Ongoing

- Black cohosh extract
- Dong quai (root powder or extract)
- *Echinacea purpurea* extract
- Garcinia cambogia
- Gum guggul extract
- *Usnea* lichen
- Valerian root extract

Coneflower
Echinacea purpurea

History of NTP botanical research

Botanical	Male Rats	Female Rats	Male Mice	Female Mice
Aloe vera	Clear	Clear	No	No
Ginkgo biloba	Some	Some	Clear	Clear
Ginseng	No	No	No	No
Goldenseal	Clear	Clear	Some	No
Green tea	No	No	No	No
Kava Kava	Equivocal	No	Clear	Clear
Milk thistle	No	No	No	No
Senna	Not tested	Not tested	No	No
Bitter orange	Increased heart rate and blood pressure			
Ephedra	Cardiotoxicity			

Green tea
Camellia sinensis

NTP botanical workshop

April 26-27, 2016, NIH Campus, Bethesda, MD

Calendar & Events | News & Media | Get Involved | Support

National Toxicology Program
U.S. Department of Health and Human Services

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Home » About NTP » Workshop: Addressing Challenges in the Assessment of Botanical Dietary Supplement Safety

2016

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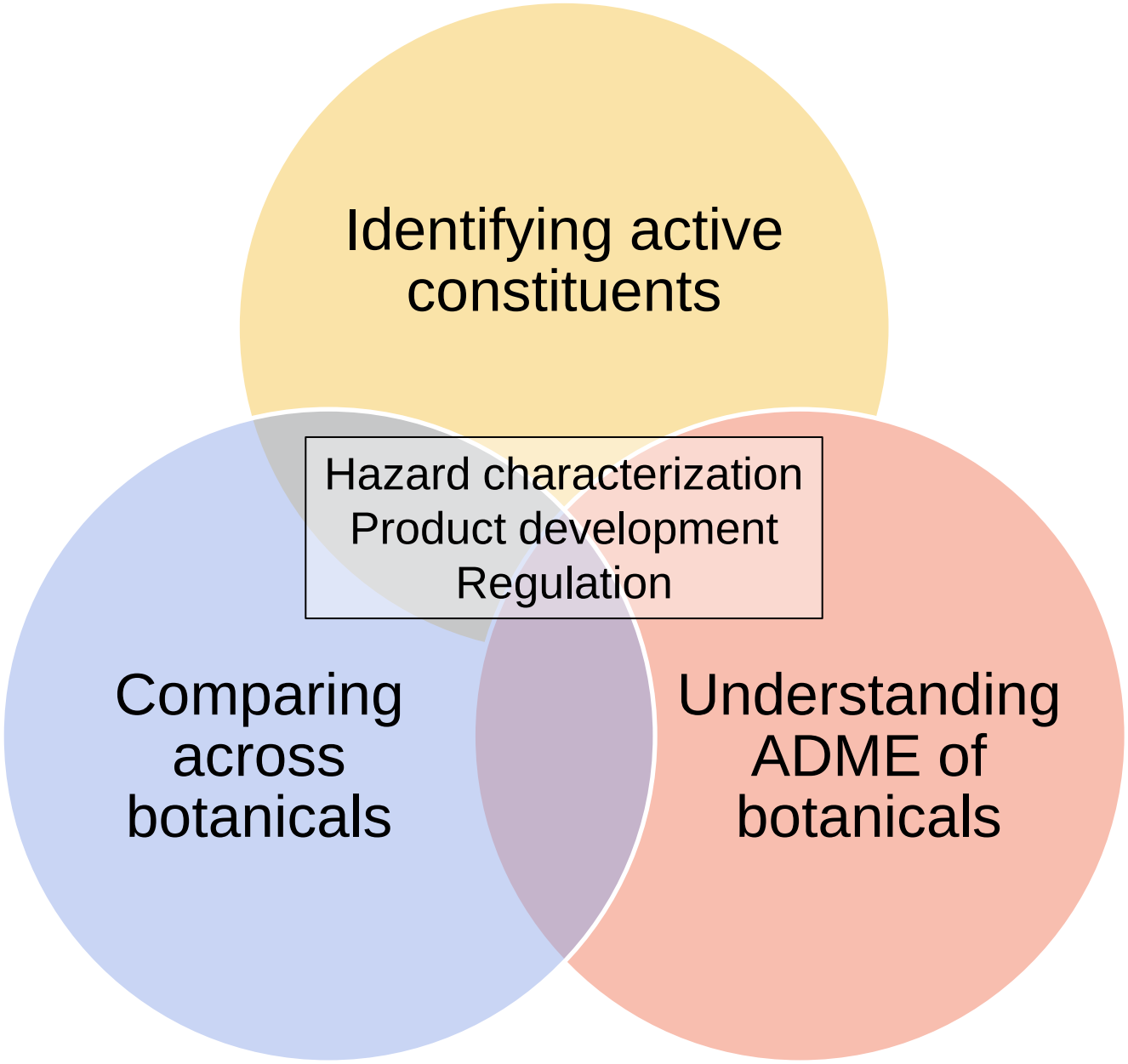
https://ntp.niehs.nih.gov/go/workshop_botanicals

Workshop: Addressing Challenges in the Assessment of Botanical Dietary Supplement Safety

April 26-27, 2016
9 a.m. - 5 p.m. EDT
Location: Lister Hill Auditorium
National Institutes of Health (NIH), Bethesda, Maryland

http://ntp.niehs.nih.gov/about/presscenter/events/2016/index.html

Key challenges in assessing safety



Developing methods for comparing complex botanicals

Food and Chemical Toxicology 118 (2018) 328–339

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

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,*]

TOXICOLOGICAL SCIENCES, 172(2), 2019, 316–329

doi: 10.1093/toxsci/kfz189
Advance Access Publication Date: August 27, 2019
Research Article

Evaluating Sufficient Similarity of Botanical Dietary Supplements: Combining Chemical and *In Vitro* Biological Data

Kristen R. Ryan,*,1 Madelyn C. Huang,*,1 Stephen S. Ferguson ,* Suramya Waidyanatha,* Sreenivasa Ramaiahgari,* Julie R. Rice,* Paul E. Dunlap,* Scott S. Auerbach,* Esra Mutlu,* Tim Cristy,† Jessica Peirfelice,† Michael J. DeVito,* Stephanie L. Smith-Roe,* and Cynthia V. Rider*,2

Determining sufficient similarity

Chemical data

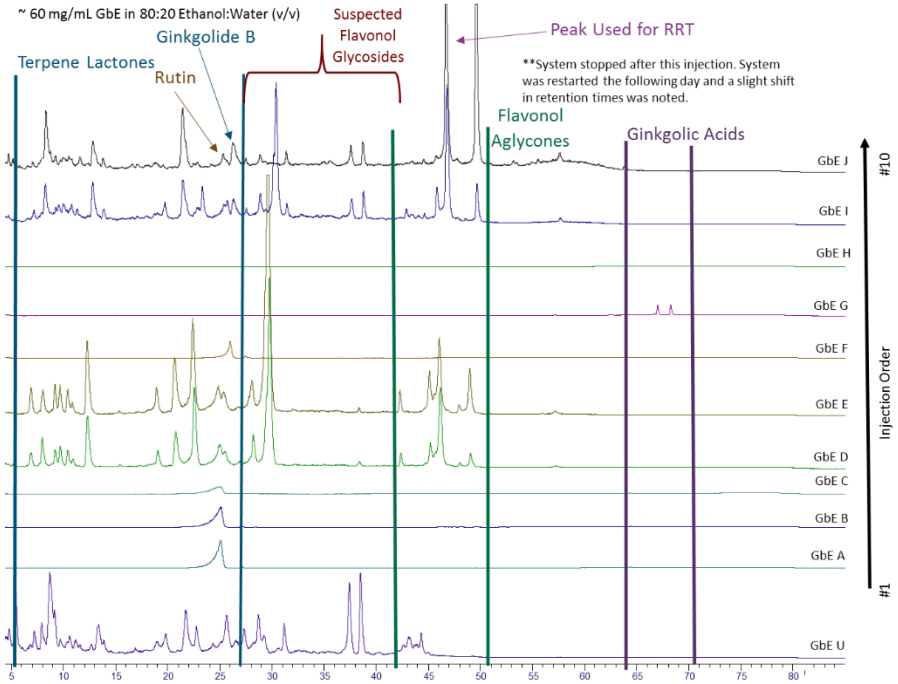
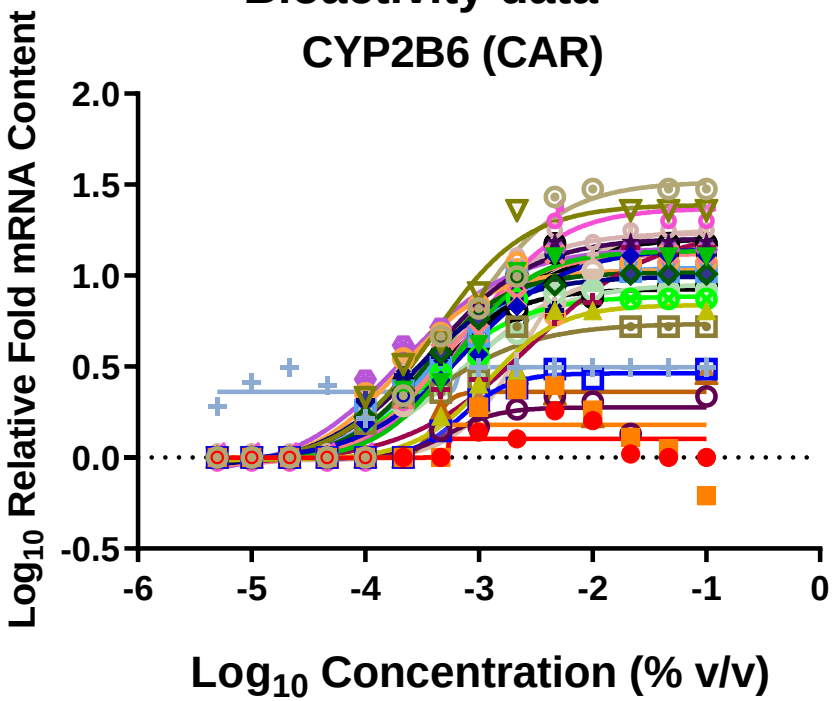
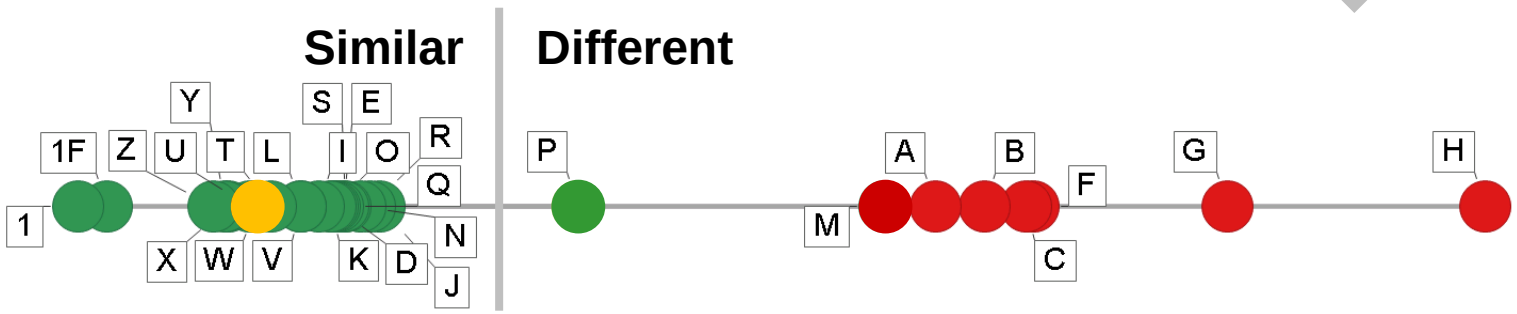


Figure 2. Non-Targeted Fingerprint Chromatograms of First Set of GbE Samples (Not Hydrolyzed), HPLC-ELSD

Bioactivity data





Identifying active constituents

Licorice root
Glycyrrhiza glabra

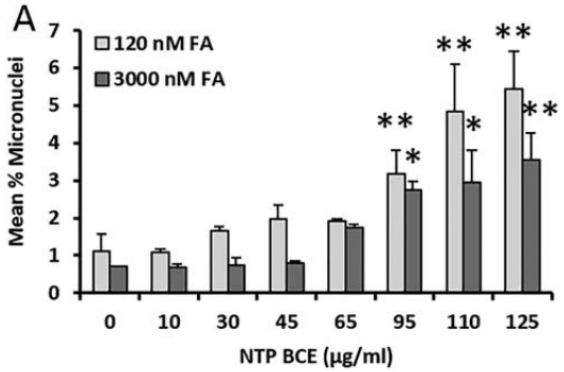
Extraction

Bioassay

Active extract

Separation

Bioassay



Active fraction

Isolation/
Identification

Chemical Structure

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

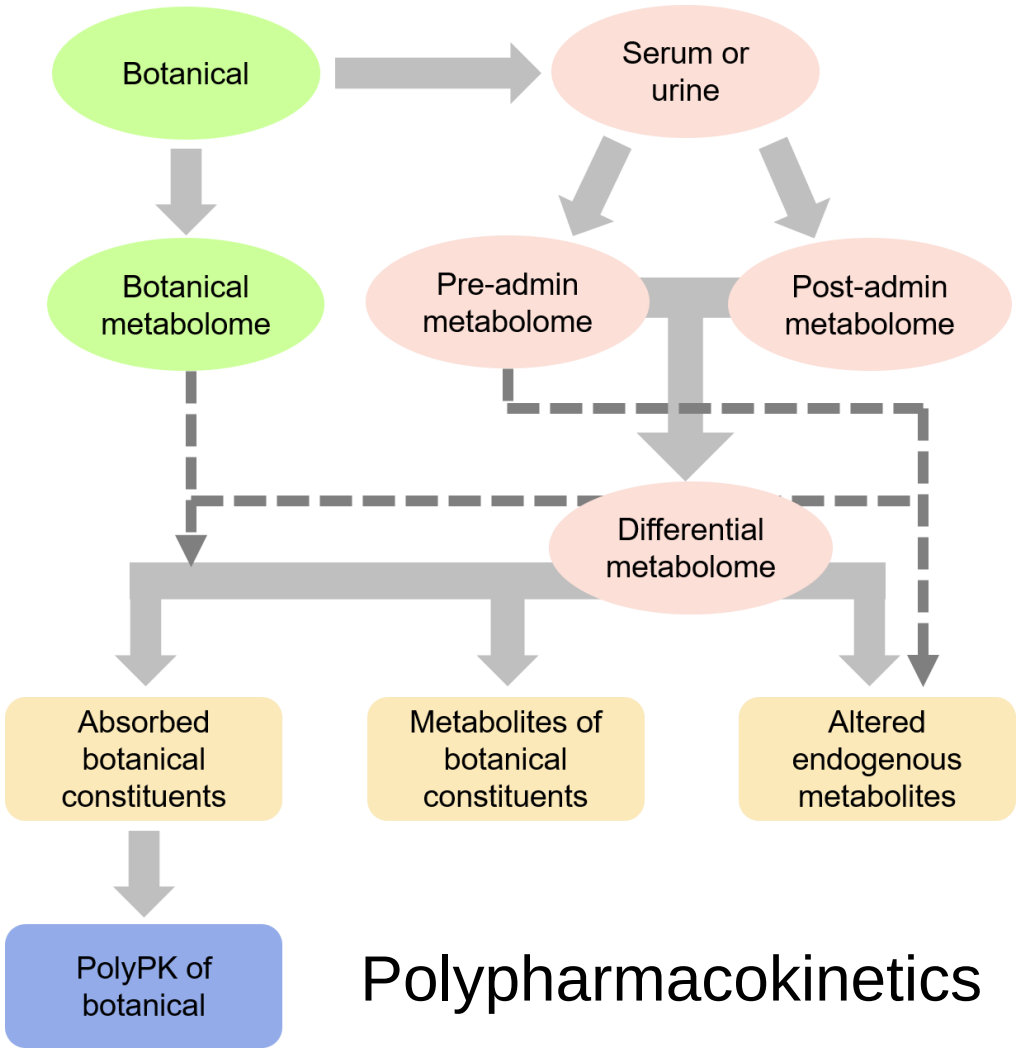
Advancing ADME of botanicals

Standard practice

- Rarely assess ADME in animal studies
- Follow ‘marker’ constituents
- Drug-botanical interactions rarely evaluated with emphasis on clinical assessment
- Animal to human dose comparisons rely on administered dose

Recommendations

- Regularly assess ADME in animal studies
- Follow toxicologically important constituents (identify active constituents) or employ polypharmacokinetics
- Leverage *in silico* and *in vitro* approaches to identify potential drug-botanical interactions
- Animal to human dose comparisons based on systemic exposure (e.g., C_{max}, AUC, PBPK modeling)

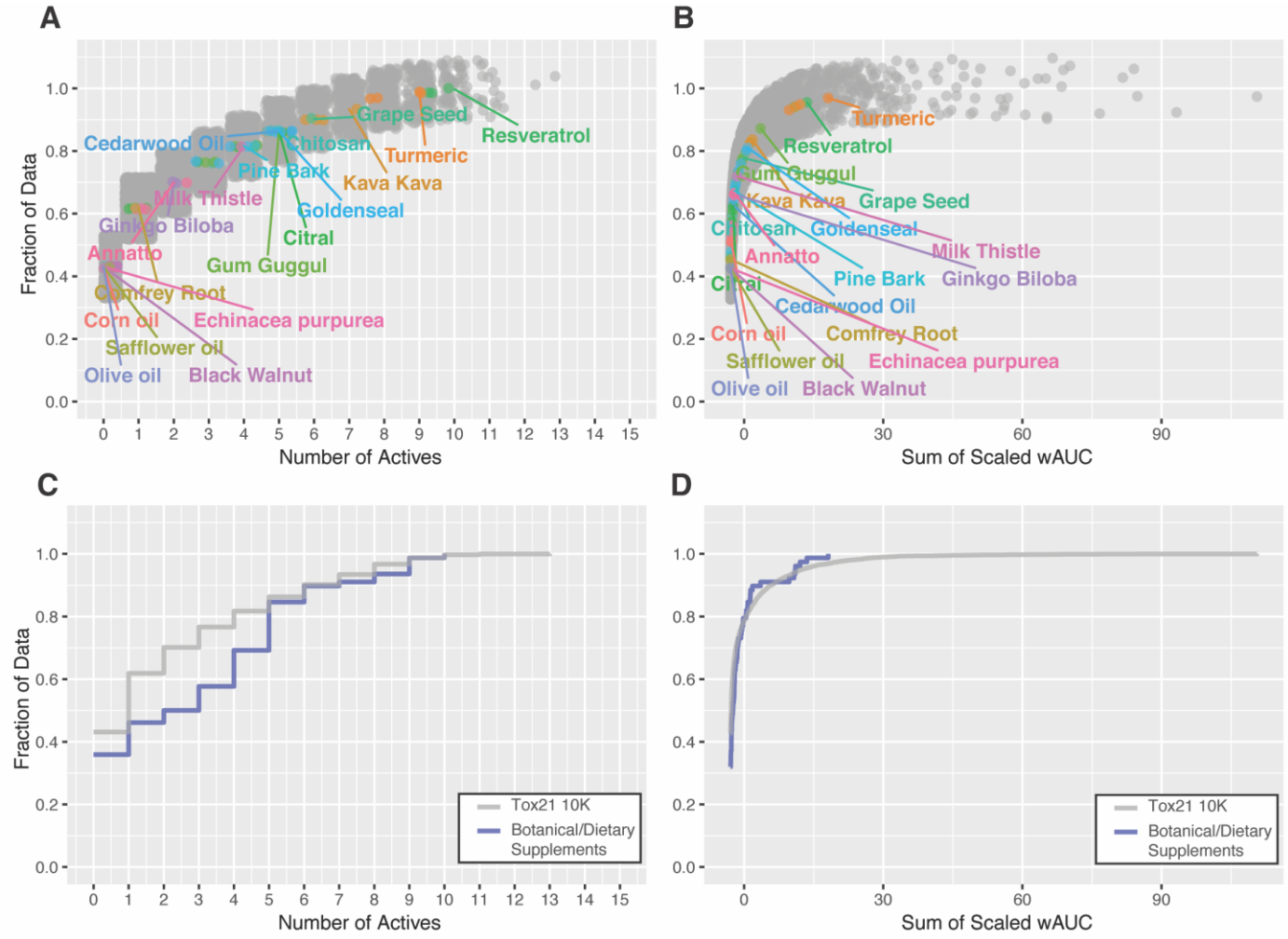


Polypharmacokinetics

Waidyanatha et al., 2018. Food and Chemical Toxicology. 121: 194-202.

Botanicals in High Throughput Screening

- Toxicology in the 21st Century (Tox21)
  - Government partnership with FDA, EPA, NIEHS, and NCATS
  - Evaluation of 10,000 chemicals in high throughput screening assays that measure biological activity in human cells
  - Pilot study to assess feasibility of including botanicals in the HTS paradigm



Hubbard et al., 2019. Appl In Vitro Toxicol. 2019 Mar 1; 5(1): 10–25.

Acknowledgements

- Chemistry
  - Suramya Waidyanatha
  - Brad Collins
  - Esra Mutlu
  - MRI
  - Battelle
- Black cohosh
  - Stephanie Smith-Roe
  - ILS
- *Echinacea*
  - Kristen Ryan
  - Mimi Huang
- *Ginkgo biloba* extract case study
  - Stephen Ferguson
  - Scott Auerbach
  - Sreenivasa Ramaiahgari
  - Julie Rice
  - Paul Dunlap
  - Arun Pandiri
- Botanical Safety Consortium
  - Michelle Embry
  - Connie Mitchell
- High throughput screening of botanicals
  - Troy Hubbard
  - Jui-Hua Hsieh
  - NCATS

Garcinia cambogia
Garcinia gummi-gutta