

# **Report on Workshop: Addressing Challenges in the Assessment of Botanical Dietary Supplement Safety Workshop**

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National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting  
June 15 – 16, 2016



# NTP botanical workshop

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



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

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



[http://ntp.niehs.nih.gov/go/workshop\\_botanicals](http://ntp.niehs.nih.gov/go/workshop_botanicals)

**April 26-27, 2016**

**9 a.m. - 5 p.m. EDT**

**Location: Lister Hill Auditorium**

**National Institutes of Health (NIH), Bethesda, Maryland**

The safety of botanical dietary supplements, hereafter referred to as botanicals, is an important public health issue. According to the 2012 National Health Interview Survey, 17.7 percent of Americans reported having used nonvitamin, nonmineral dietary supplements (including botanicals) in the past 12 months (Clarke et al., 2015). Botanicals pose several unique challenges to efficacy and safety evaluation because of their inherent complexity and potential for wide variability in nominally related products. The interrelated challenges associated with the evaluation of botanicals include: (1) developing methods and criteria for assessing phytoequivalence (i.e., similarity in chemical composition and biological activity) of botanicals, (2) identifying the active constituent(s) or patterns of biological response of botanicals, and (3) assessing absorption, distribution, metabolism, and elimination (ADME) of botanicals. This workshop will engage experts from multiple disciplines to focus on practical approaches for addressing these challenges.

Multiple factors contribute to the variability in botanicals including complex and inconsistent source material, manufacturing processes, formulation, and storage. Botanicals in commerce often display a wide range in the concentration of known constituents. Robust procedures for comparing constituent profiles across multiple botanicals are needed to determine how broadly safety or efficacy evaluations with a specific product can be applied to related products. Topics for discussion at the workshop include definition of important chemical and biological activity features, statistical methods for comparing across complex mixtures, and how to define "similarity" across botanicals (i.e., how similar do botanicals have to be in order to apply safety data from a reference botanical to nominally-related botanicals).



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



# Workshop participants

Scott Auerbach (NIEHS/DNTP)  
Joseph Betz (NIH/ODS)  
Linda Birnbaum (NIEHS/NTP)  
John, Bucher (NIEHS/DNTP)  
Nadja Cech (University of North Carolina)  
Moses Chow (Western University)  
Paul Coates (NIH/ODS)  
Michael DeVito (NIEHS/DNTP)  
Stephen Ferguson (NIEHS/DNTP)  
Paul Foster (NIEHS/DNTP)  
Dale Gardner (USDA)  
Bill Gurley (University of Arkansas)  
James Harnly (USDA)  
Craig Hopp (NCCIH)  
Paul Howard (FDA/NCTR)  
Wei Jia (University of Hawaii)  
Ikhlas Khan (University of Mississippi)  
Kerri LeVanseler (NSF International)  
Edmund Lui (Western University)

James MacGregor (Toxicology Consulting Services)  
Duffy MacKay (CRN)  
Kenneth McMartin (LSU, BSC liaison)  
Hellen Oketch (USP)  
Mary Paine (Washington State University)  
Glenn Rice (US EPA)  
Cynthia Rider (NIEHS/DNTP)  
Amy Roe (P&G)  
Stephanie Smith-Roe (NIEHS/DNTP)  
Richard van Breemen (University of Illinois)  
Suramya Waidyanatha (NIEHS/DNTP)  
Larry Walker (University of Mississippi)  
Nigel Walker (NIEHS/DNTP)  
Cara Welch (FDA/CFSAN)  
Kevin Welch (USDA)  
Kristine Witt (NIEHS/DNTP)





# Why did NTP have the workshop?

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## Contributing factors

- Recent public concern over botanical dietary supplement quality and safety
- History of botanical research at NTP has revealed important data gaps
- Botanicals provide an excellent test case to develop methods for addressing complex mixtures



# Concerns over botanical quality and safety

FEB 24, 2016 @ 05:00 AM 4,611 VIEWS

## Poorly Regulated 'Herbal Supplements' Could Be Your Worst Nightmare

WHICH CRIMINAL CHARGES DID JUSTICE DEPARTMENT GIVE AGAINST  
DIETARY SUPPLEMENT FIRMS? — MESA DAILY SCIENCE

BLOG

## Sen. McCaskill Brings Herb-Drug Interactions into Regulatory Spotlight

by Steve Myers - © May 23, 2016

Wellness Resources Thvroid

## Herbal remedies pose 'global' health hazards, study claims

Robert Ferris | @RobertoFerris

Friday, 20 May 2016 | 3:35 PM ET



U.S. says supplements  
billed as natural can be  
toxic

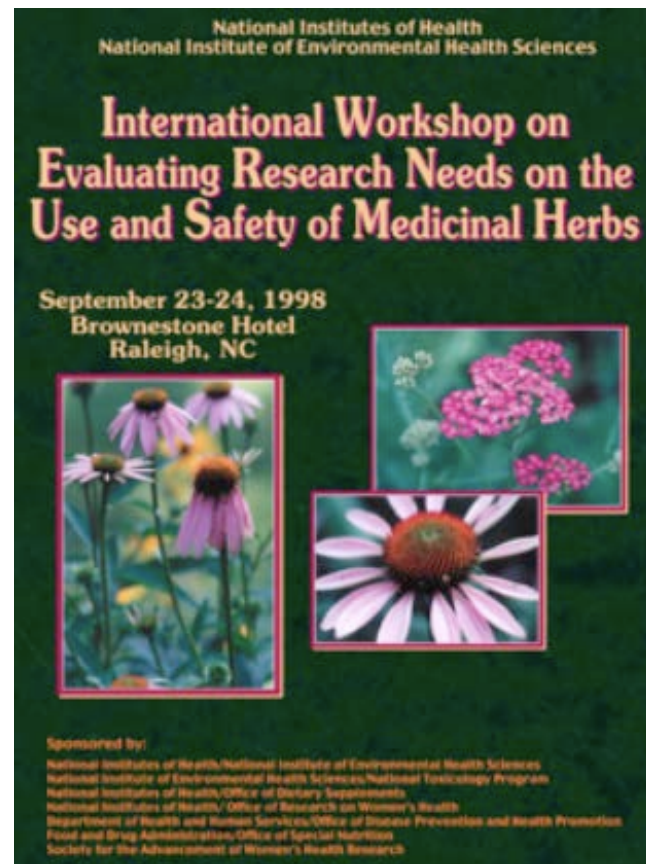




# History of botanical research at NTP

## 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 botanical-drug and botanical-botanical interactions
  - Research on risk to sensitive subpopulations





# History of NTP botanical research

## Completed

Botanical	Male Rats	Female Rats	Male Mice	Female Mice
<i>Aloe vera</i>	Clear	Clear	No	No
<i>Ginkgo biloba</i>	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			



# Feedback from botanical technical reports

## Test article selection

“The unique *Ginkgo biloba* leaf extract discussed in TR-578 is not representative of other *Ginkgo biloba* leaf extracts marketed in the United States, and is almost certainly not sold in the United States. It is incorrect to represent it as similar to other *Ginkgo biloba* leaf extracts based on the dissimilarity of its chemical composition to that of other commercially available *Ginkgo biloba* leaf extracts.” American Herbal Products Association (AHPA) public comment

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**NTP selected an inappropriate test article that is not representative of anything else in the marketplace.**

“...we are concerned that NTP researchers may be erroneously basing its oral consumption toxicity analysis on an Aloe Vera product sample that is not reflective of the products currently marketed in the US and exported in large quantities.” Congressional Inquiry, June 18, 2010

“The Committee urges NTP to be highly precise when describing the results of its studies on particular extracts of an herbal species to avoid any possible confusion about the relevance of such studies to other extracts of the species.” The United States Senate Appropriations Committee in report accompanying the fiscal year 2014 Labor, Health and Human Services and Education Appropriations spending bill





# Feedback from botanical technical reports

## Relevance to humans

“In the context of implied human relevance, there are also concerns with the selection of doses utilized in the study. In this murine toxicity study, doses of the Shanghai Chinese GBE test doses given to both mice and rats were 5- to 55-fold larger than the highest level of consumption in

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**NTP used doses that were too high  
and these studies have little  
relevance to humans.**

“There is an obvious issue of the applicability of findings in rodents to the safety of green tea extract in humans; there are questions about the appropriateness of the dosage levels used in the study and any suggestion that they have applicability with respect to the safety of the green tea at doses typically used as an extract or within a beverage during normal human intake” American Botanical Council written comments on TR 585, May 8, 2014




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- NIEHS**  
National Institute of  
Environmental Health Sciences
- 2012-2017  
STRATEGIC PLAN
- Advancing Science, Improving Health:  
A Plan for Environmental Health Research
- NIEHS**  
National Institute of  
Environmental Health Sciences
- ADVANCING RESEARCH**  
New Perspectives and Approaches to  
Adverse Human Health
- SEPTEMBER 26-27, 2012  
Monday, September 26, 8:30 am - 5:30 pm • Tuesday, September 27, 8:30 am - 5:30 pm  
Sheraton Chapel Hill • 1 Europa Lane

**NIHES**  
National Institute of  
Environmental Health Sciences



**ADVANCING RESEARCH ON MIXTURES:**  
New Perspectives and Approaches for Predicting  
Adverse Human Health Effects

SEPTEMBER 26-27, 2011

Monday, September 26, 8:30 am - 5:30 pm • Tuesday, September 27, 8:30 am - 4:15 pm  
Sheraton Chapel Hill • 1 Europa Drive • Chapel Hill, NC



Individuals with disabilities who need accommodation to participate in this event should contact  
Daniela Carlin at 919-541-1401 or [daniela.carlin@nih.gov](mailto:daniela.carlin@nih.gov). TTY users should contact the Federal  
TTY Relay Service at 800-877-8339. Requests should be made at least 5 business days in  
advance of the event.



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
National Institutes of Health



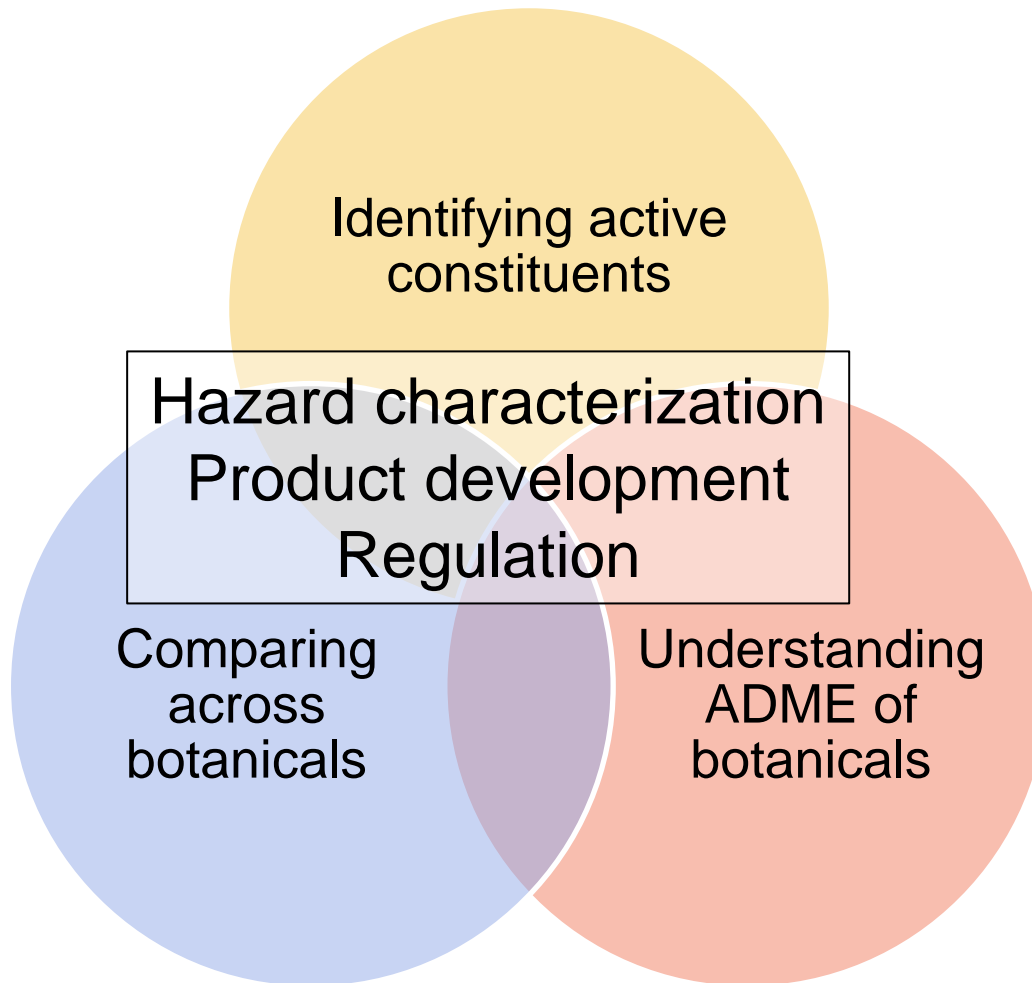
# Challenges with botanicals

- Complexity
  - Many constituents
  - Multiple “active” constituents
    - Pharmacological versus toxicological activity
    - Potential interactions among constituents
  - Large unidentified fraction
- Variability across marketplace
  - Differences in raw material due to source, season, plant part
  - Processing/manufacturing
  - Adulteration or combination





# Key topics for workshop





## Inform research on botanical safety

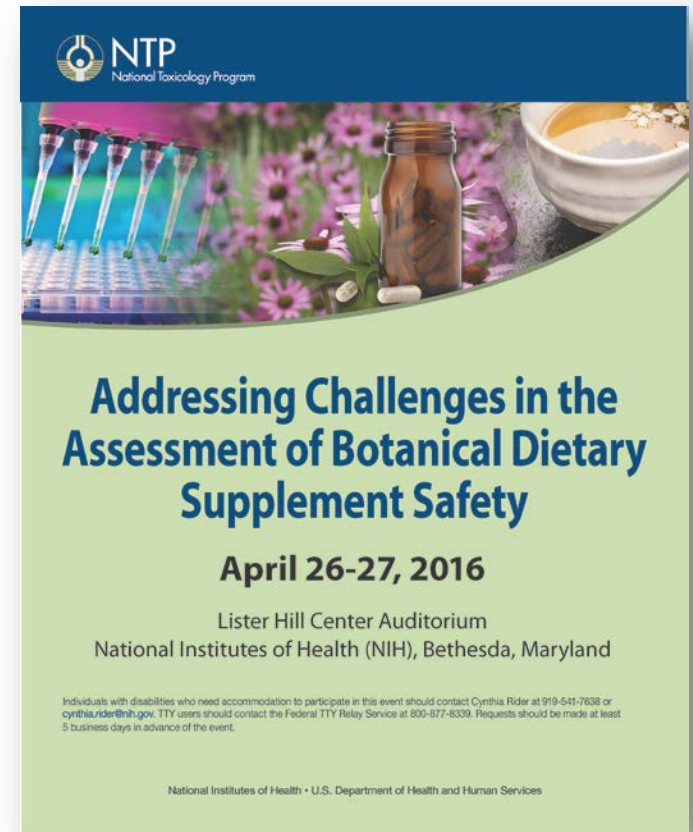
- Communicate current science in key topic areas
- Obtain feedback from stakeholders on presented approaches
- Identify data gaps and research needs





# Workshop outline

- Perspectives on the challenges associated with botanicals
  - Research, regulatory, industry
- Determining phytoequivalence of botanicals
  - Case studies
- Identifying active constituents in botanical dietary supplements
  - Approaches
- Best practices for assessing ADME of botanical dietary supplements
  - Information gathering







## Determining phytoequivalence

- What do we mean by “phytoequivalence” or “sufficient similarity”?
  - The tested lot is similar enough to an untested lot, so that data from the tested lot can be used as a surrogate for the untested lot
- Why do we care?
  - Provides a more transparent and defensible test article selection process for other botanicals (and beyond)
  - Allows for determination of how NTP test article relates to other products



## Current approach

- Evaluate multiple lots from various suppliers to find a single “representative” test article
- Considerations
  - Greatest exposure potential (e.g., most common, greatest marketshare)
  - Most like the reference standard
  - Highest level of active ingredients (most “potent”)
- Methods
  - Untargeted chemistry – compare chromatograms
  - Targeted chemistry – evaluate concentrations of marker compounds



## Goals

- Work through determining phytoequivalence (sufficient similarity) with multiple examples
- Compare different approaches for determining sufficient similarity
  - Chemical similarity
  - Biological similarity
  - Supervised approaches (require scientific judgement)
  - Unsupervised approaches (data-driven)
- Identify knowledge gaps



## Case studies

- *Ginkgo biloba* extract
  - Chemistry: Relatively large identified fraction; known marker constituents
  - Biology (NTP): Noted in vivo effects – hepatotoxicity, pathways identified
- Black cohosh extract
  - Chemistry: Large unidentified fraction; low confidence that marker constituents are associated with toxicity
  - Biology (NTP): Genotoxicity
- *Echinacea purpurea* extract
  - Chemistry: Large unidentified fraction
  - Biology (NTP): Weak activity – Enhanced immune response





## What we have...

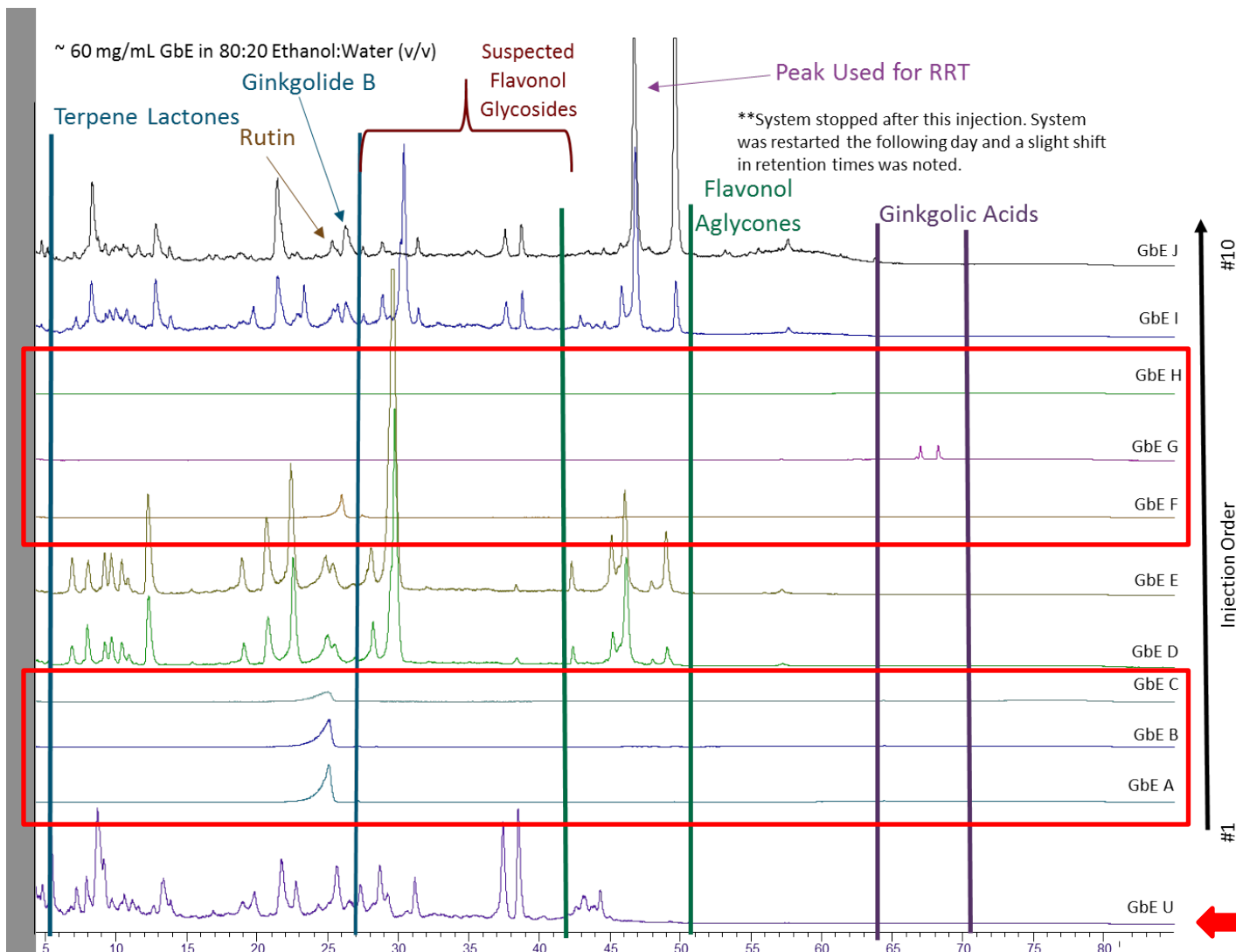
<b><i>Ginkgo biloba</i></b>	<b>Black cohosh</b>	<b><i>Echinacea purpurea</i></b>
3 NTP TA (reference) 20 Procured lots 2 SRM 4 Formulations (EGb761®) 12 Marker constituents	1 NTP TA (reference) 10 Procured lots 4 SRMs** 3 Formulations (Remifemin®) 9 Marker constituents	1 NTP TA (reference) 12 Procured lots 5 SRMs 9 Marker constituents
Untargeted chemistry	Untargeted chemistry	Untargeted chemistry
Marker concentrations	Marker concentrations	Marker concentrations
<i>In vitro</i> hepatocyte <ul style="list-style-type: none"><li>• Cytotoxicity</li><li>• Pathways</li></ul>	<i>In vitro</i> hepatocytes <ul style="list-style-type: none"><li>• Cytotoxicity</li><li>• Pathways</li></ul>	<i>In vitro</i> hepatocytes <ul style="list-style-type: none"><li>• Cytotoxicity</li><li>• Pathways</li></ul>
	<i>In vitro</i> micronucleus	
<i>In vivo</i> rat <ul style="list-style-type: none"><li>• Liver weight</li><li>• Gene expression</li></ul>		

\*Black cohosh, red cohosh, chinese cohosh, yellow cohosh



# *Ginkgo biloba* extract (GBE)

## Untargeted chemistry: HPLC-ELSD



← NIST SRM

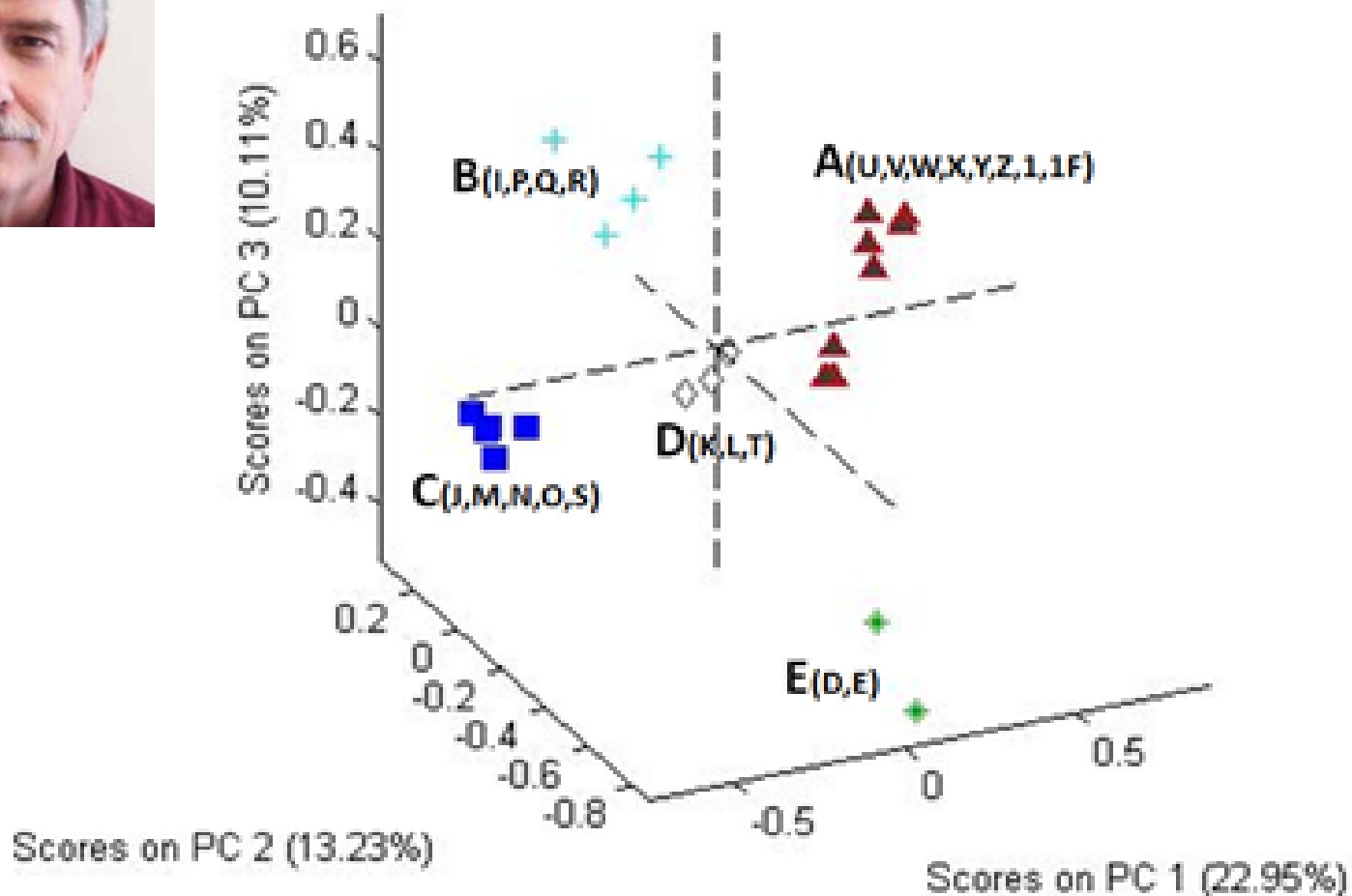
Figure 2. Non-Targeted Fingerprint Chromatograms of First Set of GbE Samples (Not Hydrolyzed), HPLC-ELSD





# Chemometric analysis of samples

Jim Harnly (USDA)

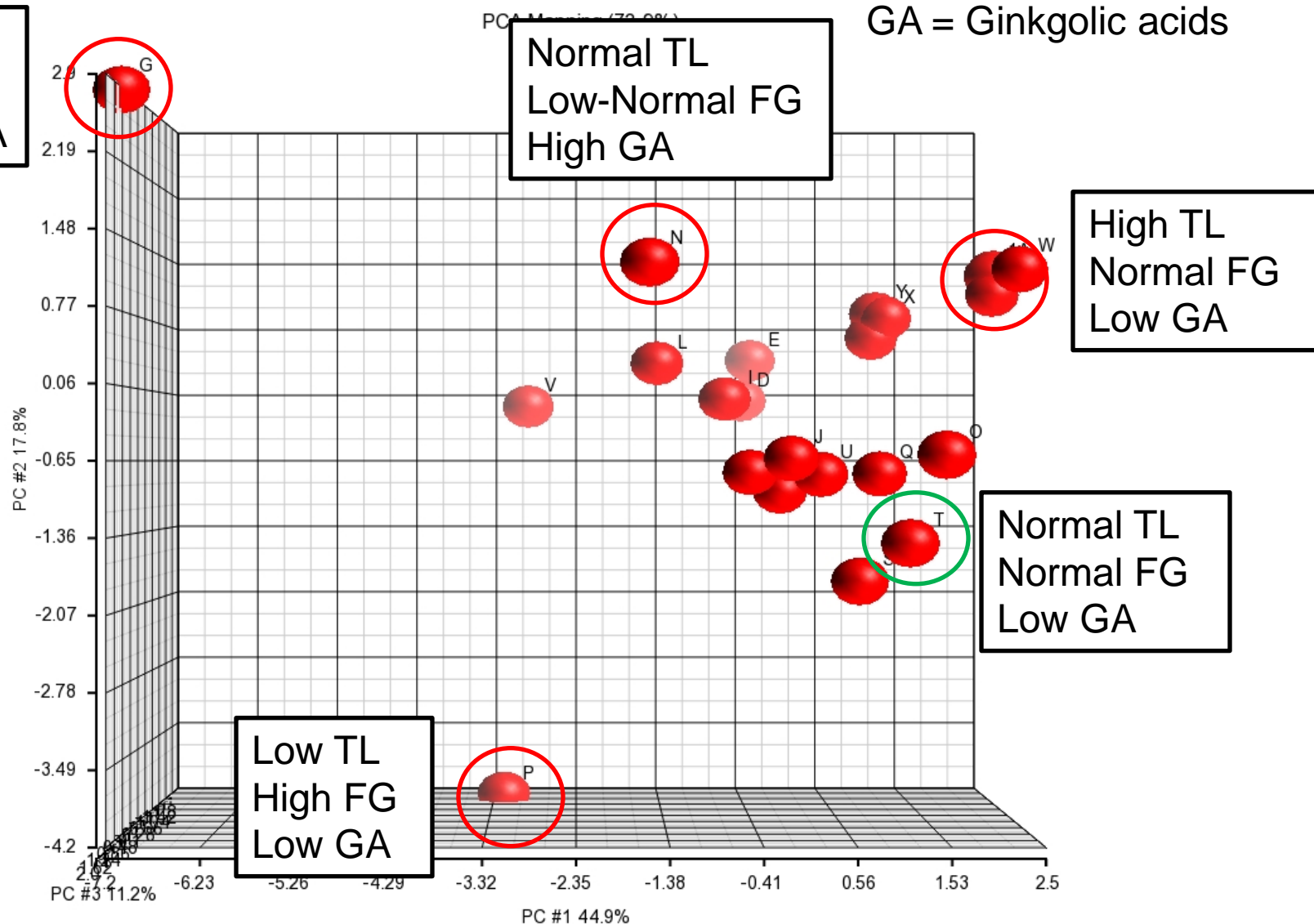




# *Ginkgo biloba* extract

## Targeted chemistry

TL = Terpene lactones  
FG = Flavonol glycosides  
GA = Ginkgolic acids

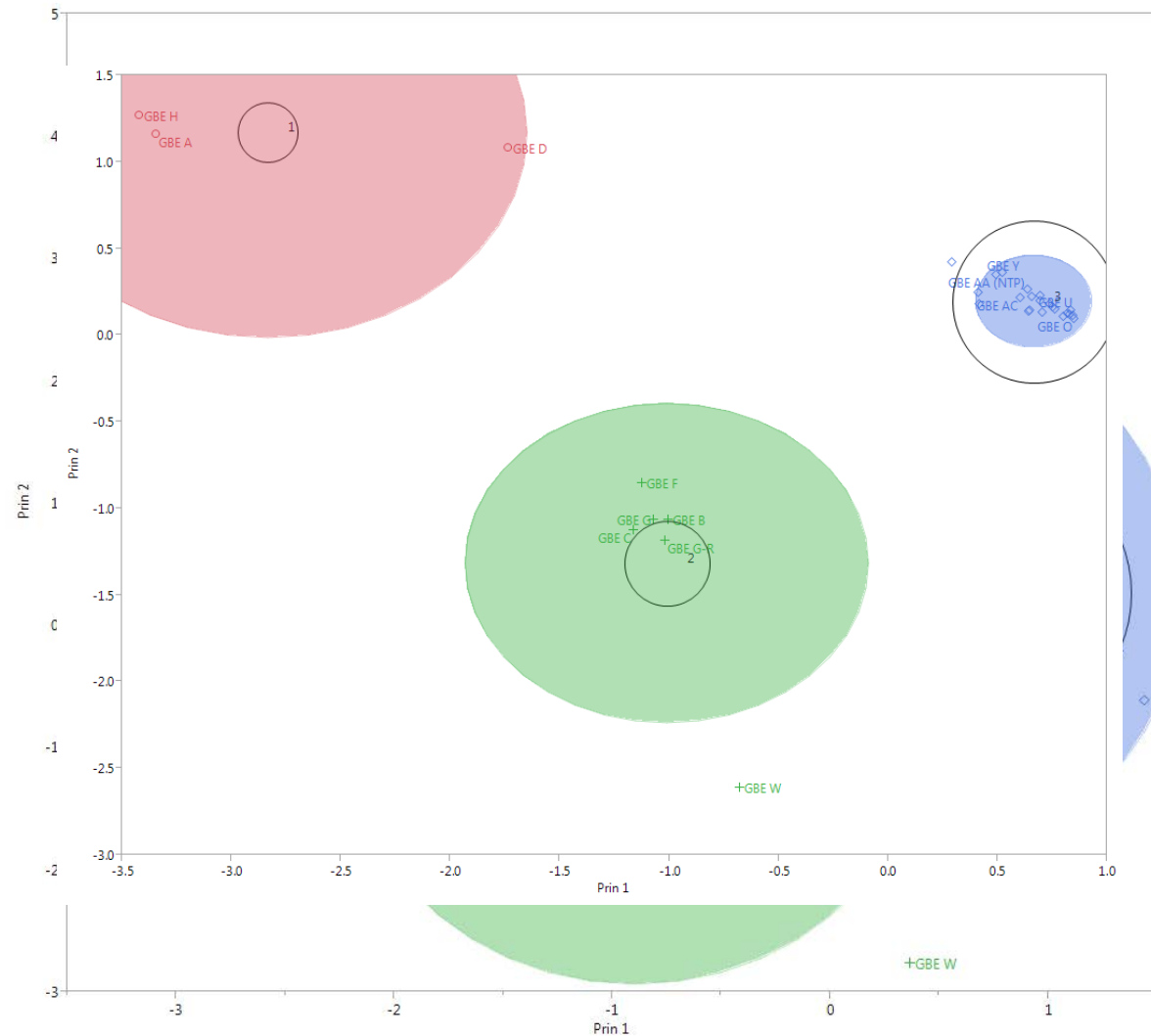




# Primary human hepatocyte data

## Liver enzyme induction

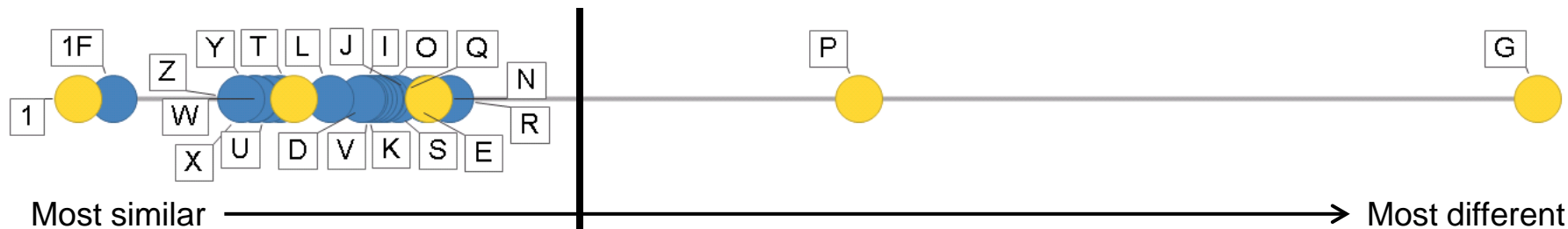
- AhR (CYP1A2)
- CAR (CYP2B6)
- PXR (CYP3A4)
- FXR (ABCB11)
- PPAR $\alpha$  (HMGCS2)





# Determining sufficient similarity

## Chemical fingerprint similarity



## Liver weight (% increase)



## Hepatocyte Lipid Accumulation Signature Score



Similarity cut-off





## Significance

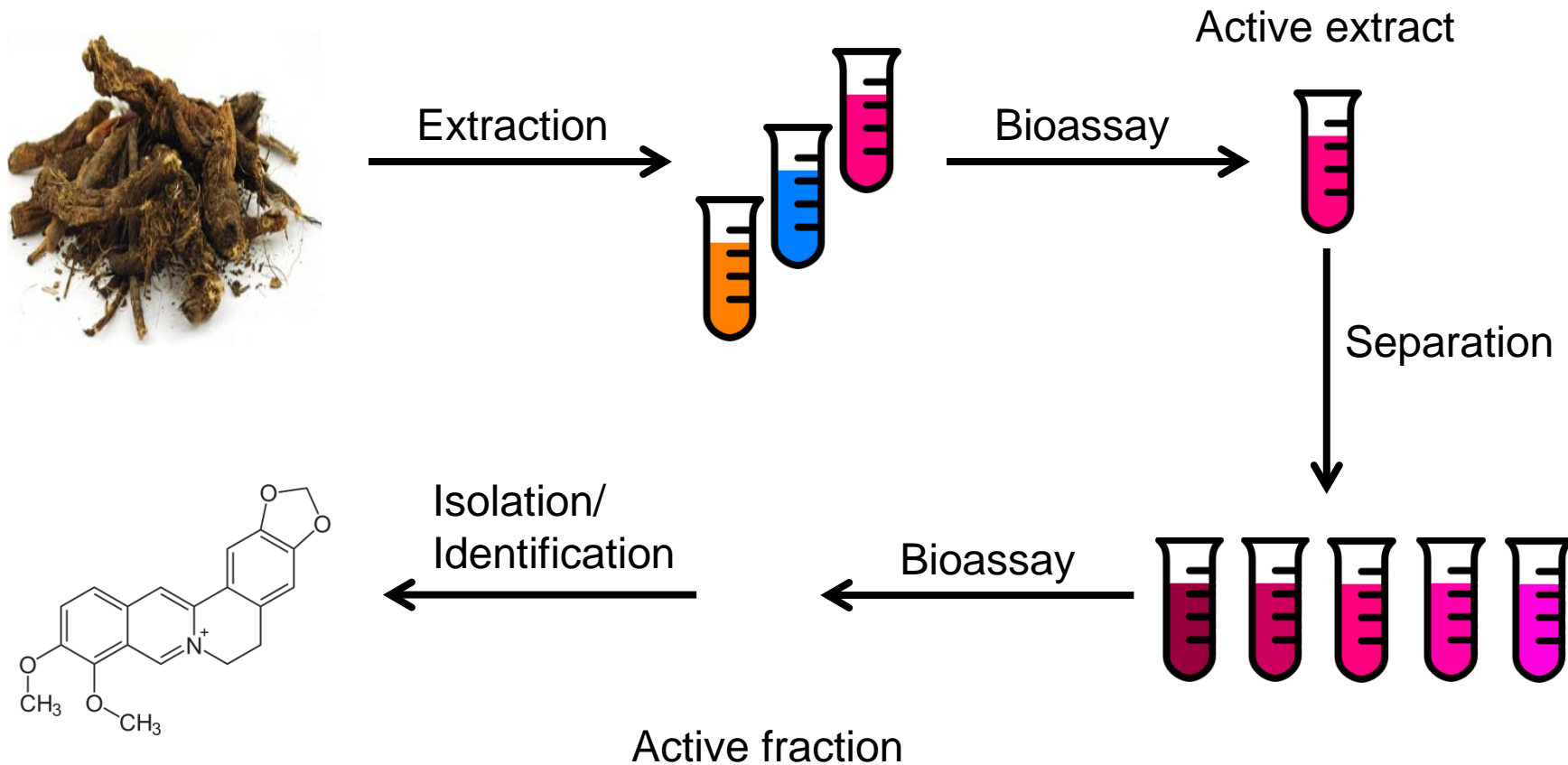
- Identification of the active constituent allows for:
  - Understanding mechanism of action and translation to humans
  - Develop tests for presence and activity
    - Biomarkers of exposure
    - Surveillance in commercial products
    - Ability to set action levels





# Identifying active constituents

## Basic steps







## Significance

- Aid in the design of toxicology studies
  - Select doses, dosing paradigm, and route of exposure
- Provide information to link external exposure to internal or target site dose
  - Biological effects are best correlated with internal or target site dose rather than the administered dose
- Provide information to extrapolate animal data to human safety assessment
- Improve our understanding of potential botanical-drug and botanical-botanical interactions

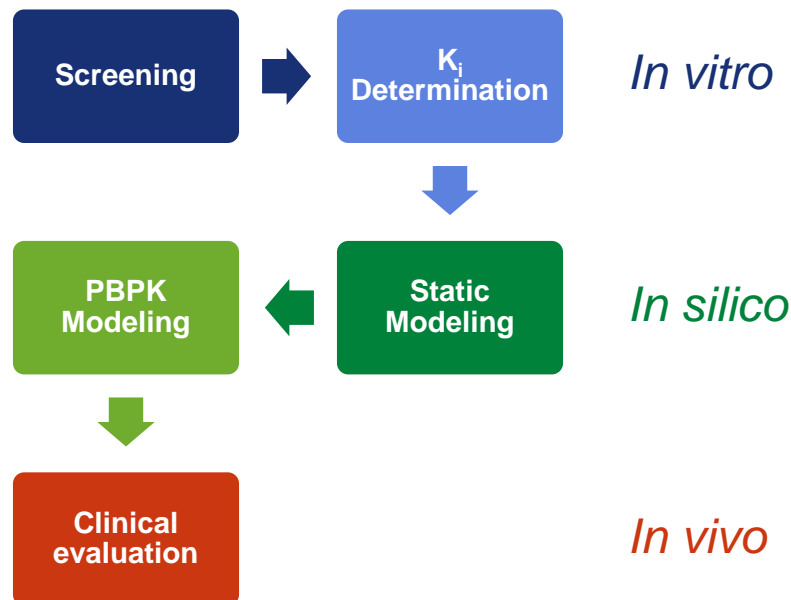


# Understanding ADME of botanicals

## Major challenges and proposed solutions

- Which constituent to track if active is unknown?
  - Polypharmacokinetics – Metabolomics and multivariate statistics to analyze small molecules in biofluids
- How can we identify and characterize botanical-botanical and drug-botanical interactions

Systematic approach





## Next steps

- Video of the workshop is available on the website <http://ntp.niehs.nih.gov/about/presscenter/events/2016/index.html>
- Publish summary and synthesis of workshop topics and discussion
  - Target journal: Food and Chemical Toxicology
- Complete case study research and publish results in the peer reviewed literature
  - *Ginkgo biloba* extract – 5 manuscripts in progress
  - Black cohosh extract – 1 manuscript in progress
  - *Echinacea purpurea* extract
- Make case study data available to others for methods development



# Take home messages

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- Botanical dietary supplements are an important public health concern and an area of active research
  - Over 300 people registered to attend or view the webcast of the meeting
- Botanicals are complex entities that offer unique challenges for research, regulation, and manufacturing
  - Botanical quality is a major concern
- Methods to determine sufficient similarity can be applied to botanicals to help with test article selection and relate findings from NTP studies to untested samples
  - Case studies were helpful in developing and applying approaches to determine sufficient similarity



# Take home messages

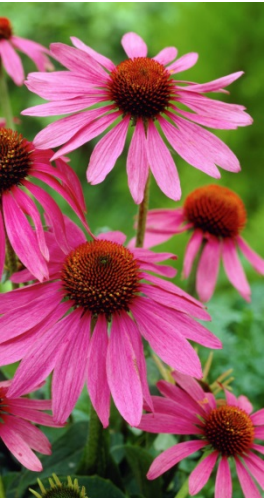
- Determining active constituents of botanicals remains a high priority and is typically accomplished using bioassay guided fractionation
  - Challenges include bioassay selection and possibility of whole mixture effects not captured in reductionist approach
- Both whole mixture and active constituent work are needed
- Developing best practices for assessing ADME of botanicals is a key area of research
  - Polypharmacokinetics is a promising method that requires further development
  - Framework for determining botanical-botanical and botanical-drug interactions involves *in vitro*, modeling, and clinical considerations





# Acknowledgements

- **Botanical Workshop Planning Committee:**



NIH/ODS: Joseph Betz

FDA/NCTR: Paul Howard

FDA/CFSAN: Susan Carlson, Suzanne Fitzpatrick, Leah Rosenfeld

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- Case Study Development: Scott Auerbach, Brad Collins, Chris Gennings (Mt Sinai), James Harnly (USDA), Steve Ferguson, Stephanie Smith-Roe, Suramya Waidyanatha
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- NIEHS support staff: Denise Lasko and Anna Lee Mosley
- NLM Lister Hill staff: Melissa Hush, AV staff