NTP
National Toxicology Program

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

Cynthia V. Rider, Ph.D.
Toxicology Branch
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

Search the NTP Website | search

Home | Testing Information | Study Results & Research Projects | Public Health | About NTP

Home » About NTP » Events, Meetings & Workshops » Workshop: Addressing Challenges in the Assessment of Botanical Dietary Supplement Safety

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

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?

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

Wellness Resources Thyroid

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

Herbal remedies pose 'global' health hazards, study claims

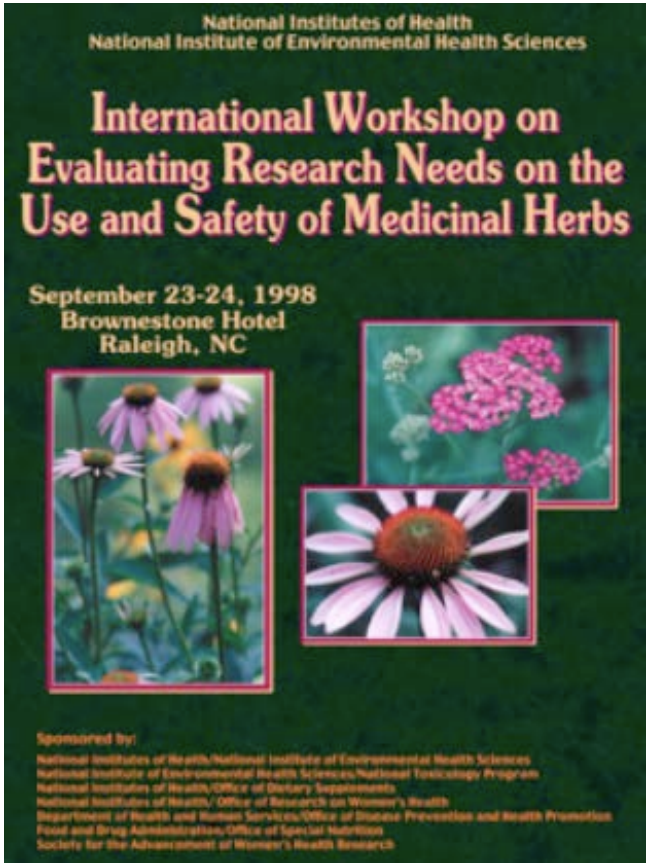
Robert Ferris | @RobertoFerris
Friday, 20 May 2016 | 3:35 PM ET

CNBC

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



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

History of NTP botanical research

Completed

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			

http://ntp.niehs.nih.gov/results/areas/botanicaldietarysupp/index.html

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 com

"The used tea infer rem

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

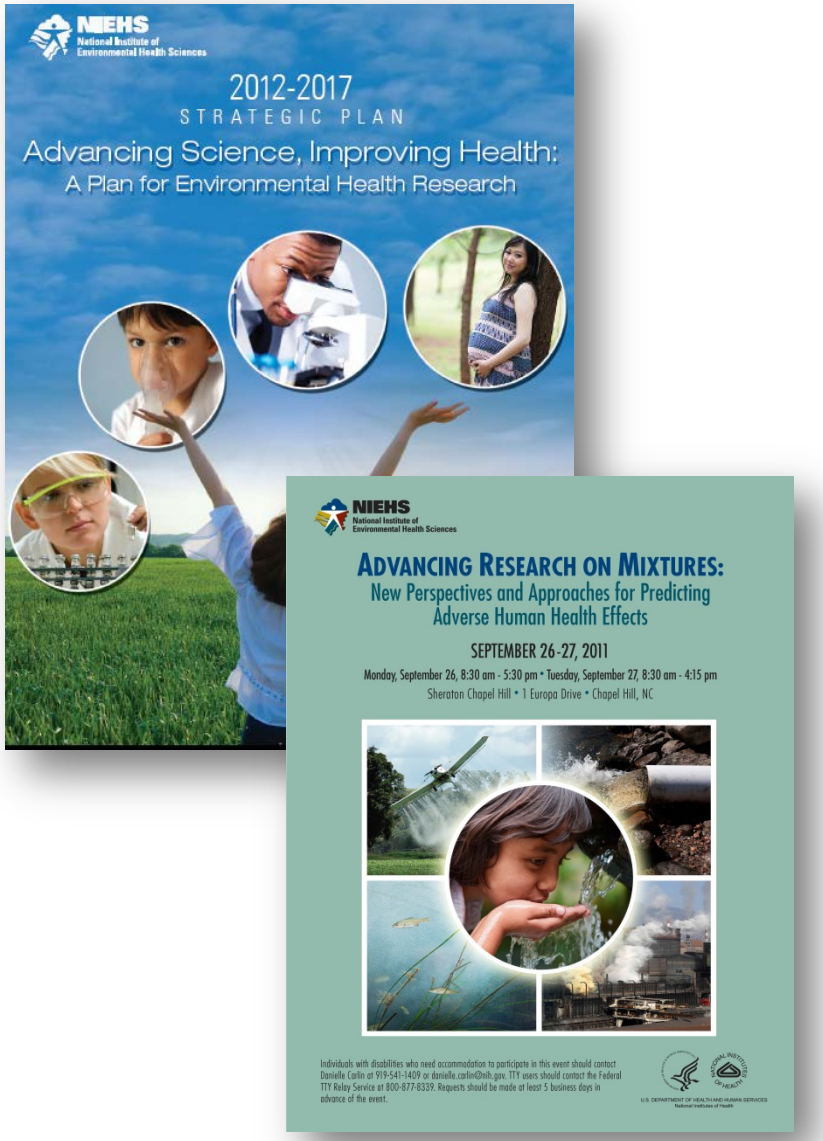
"In the context of implied human relevance, there are also concerns with the selection of doses utilized in the study. In this murine toxicity study, doses of the Shanghai Chinese GBE test doses given to both mice and rats were 5- to 55-fold larger than the highest level of consumption in hun
leve
test
sign
Am
7, 2

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

Mixtures context

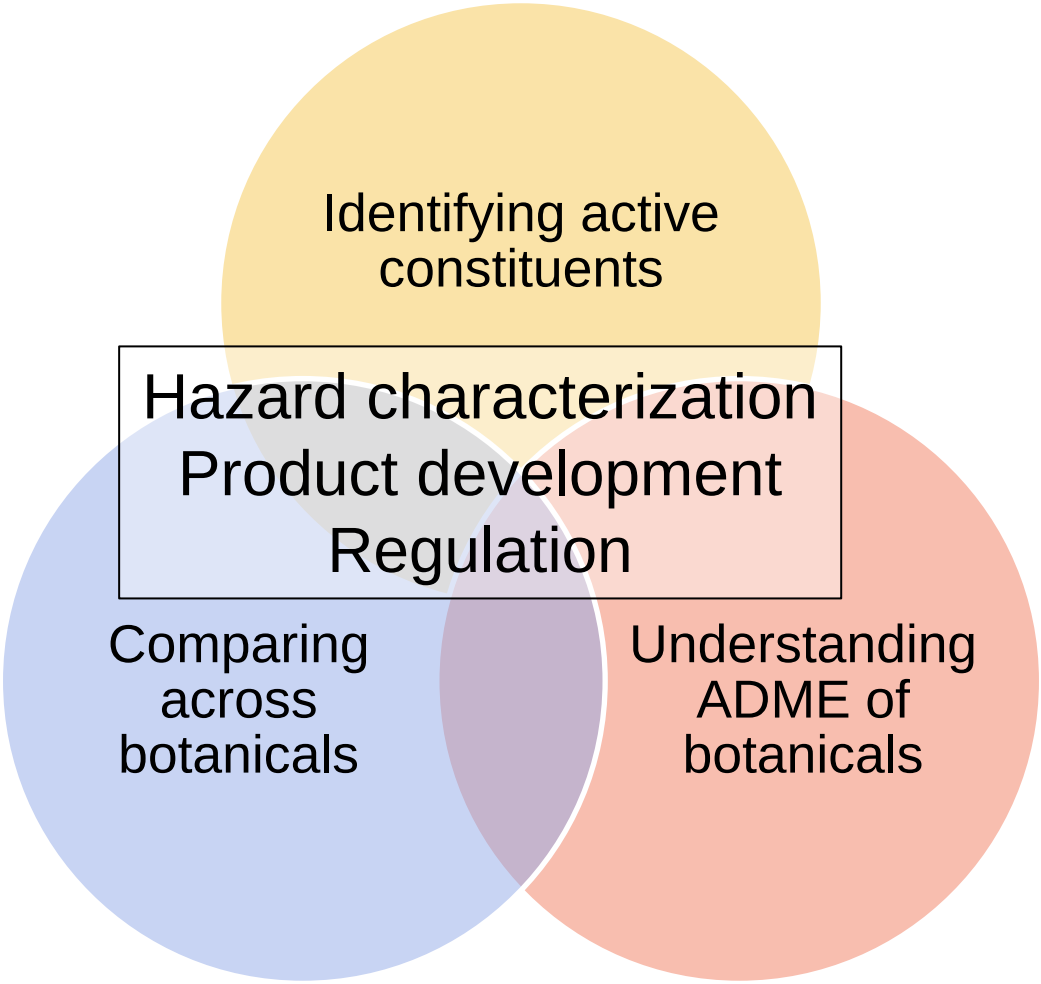
- Mixtures research is a priority for NIEHS and NTP
- Botanicals offer an opportunity to address key issues in understanding complex mixtures
- Knowledge gained will help us tackle other challenging problems (e.g., commercial formulations, environmental contaminant mixtures)



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



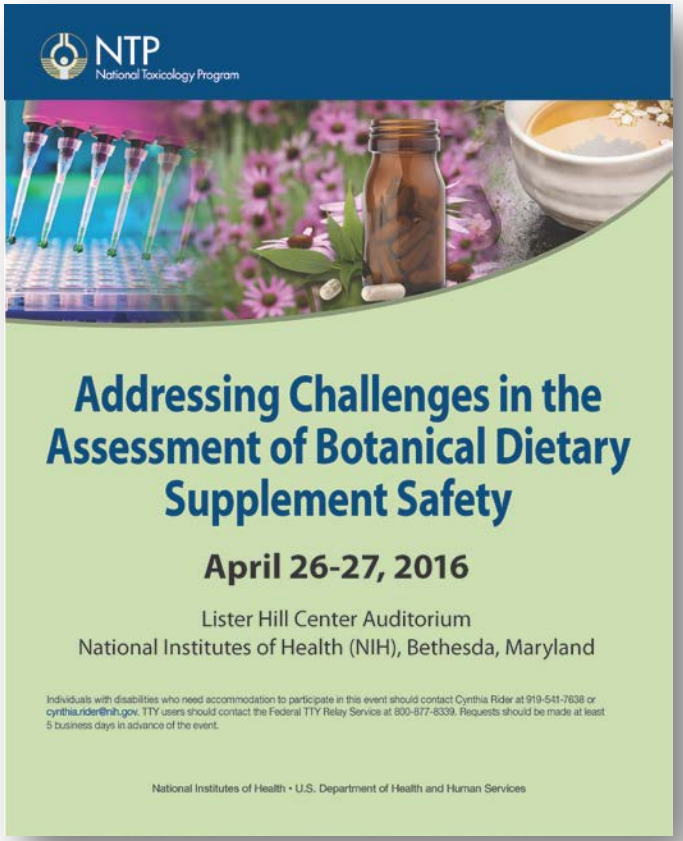
Workshop goal

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

Test article selection

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

Selection of botanicals

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

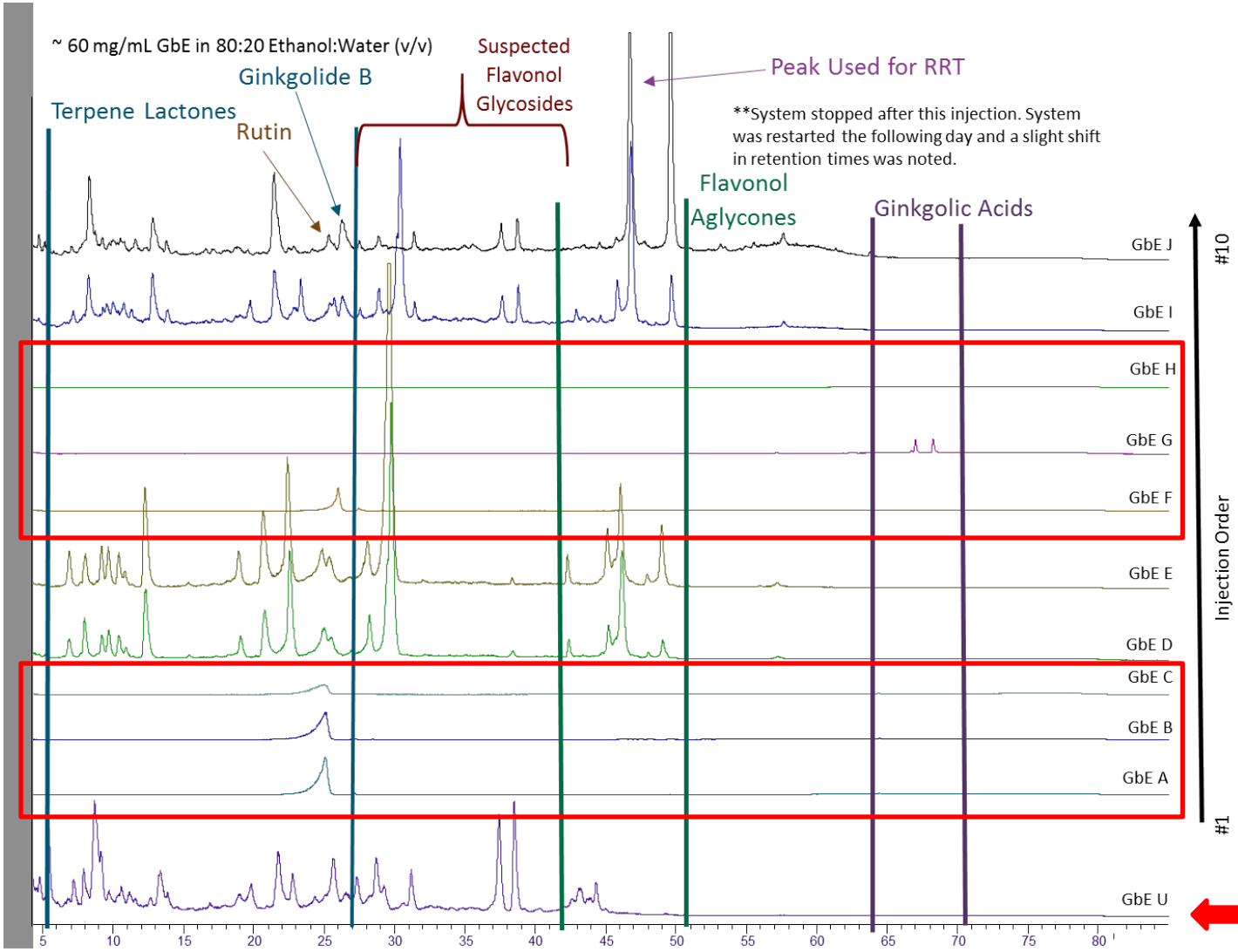
What we have…

Ginkgo biloba	**Black cohosh**	***Echinacea purpurea***
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
In vitro hepatocyte • Cytotoxicity • Pathways	*In vitro* hepatocytes • Cytotoxicity • Pathways	*In vitro* hepatocytes • Cytotoxicity • Pathways
	In vitro micronucleus	
In vivo rat • Liver weight • Gene expression		

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

Ginkgo biloba extract (GBE)

Untargeted chemistry: HPLC-ELSD

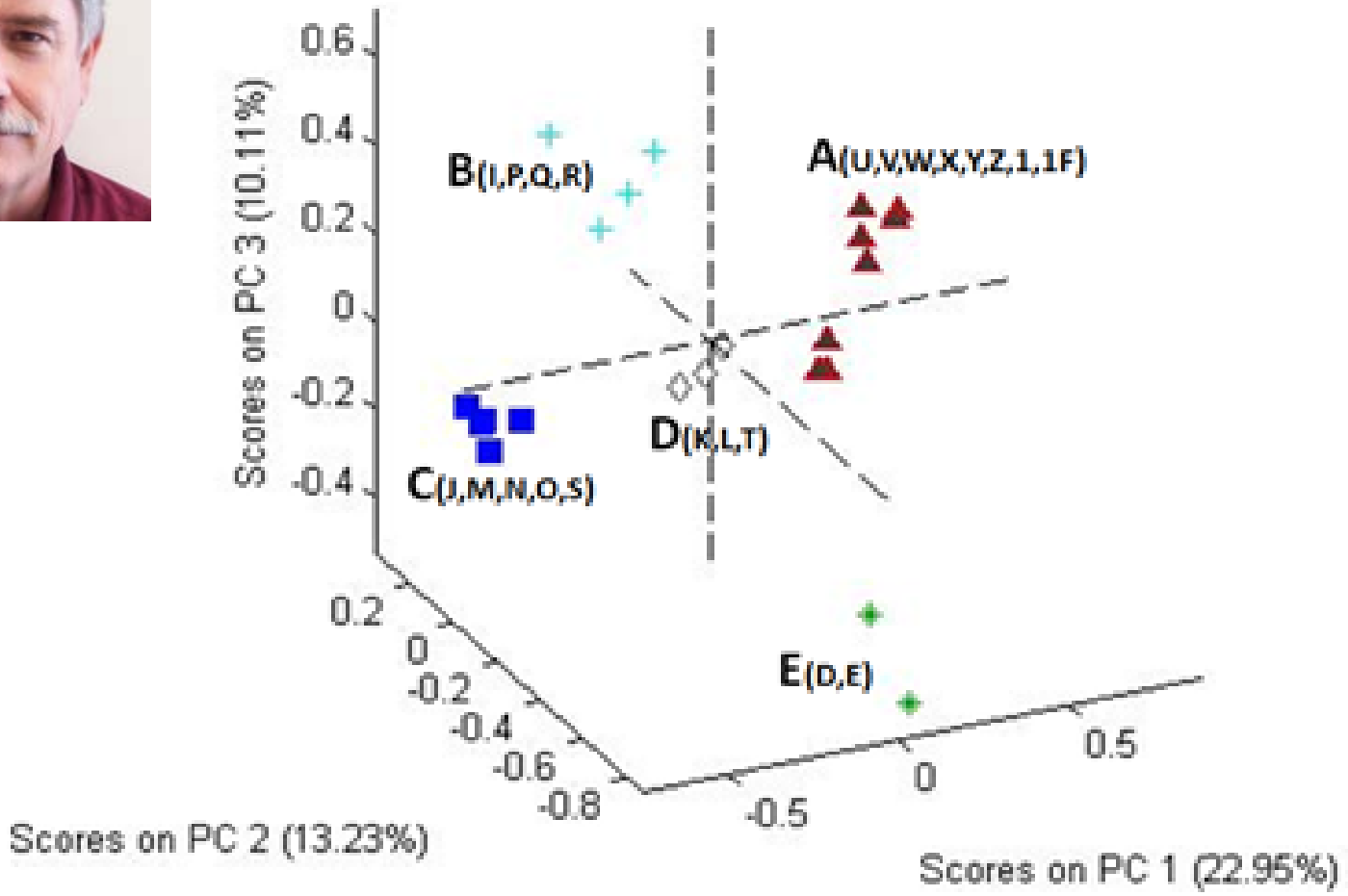


NIST SRM

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

Chemometric analysis of samples

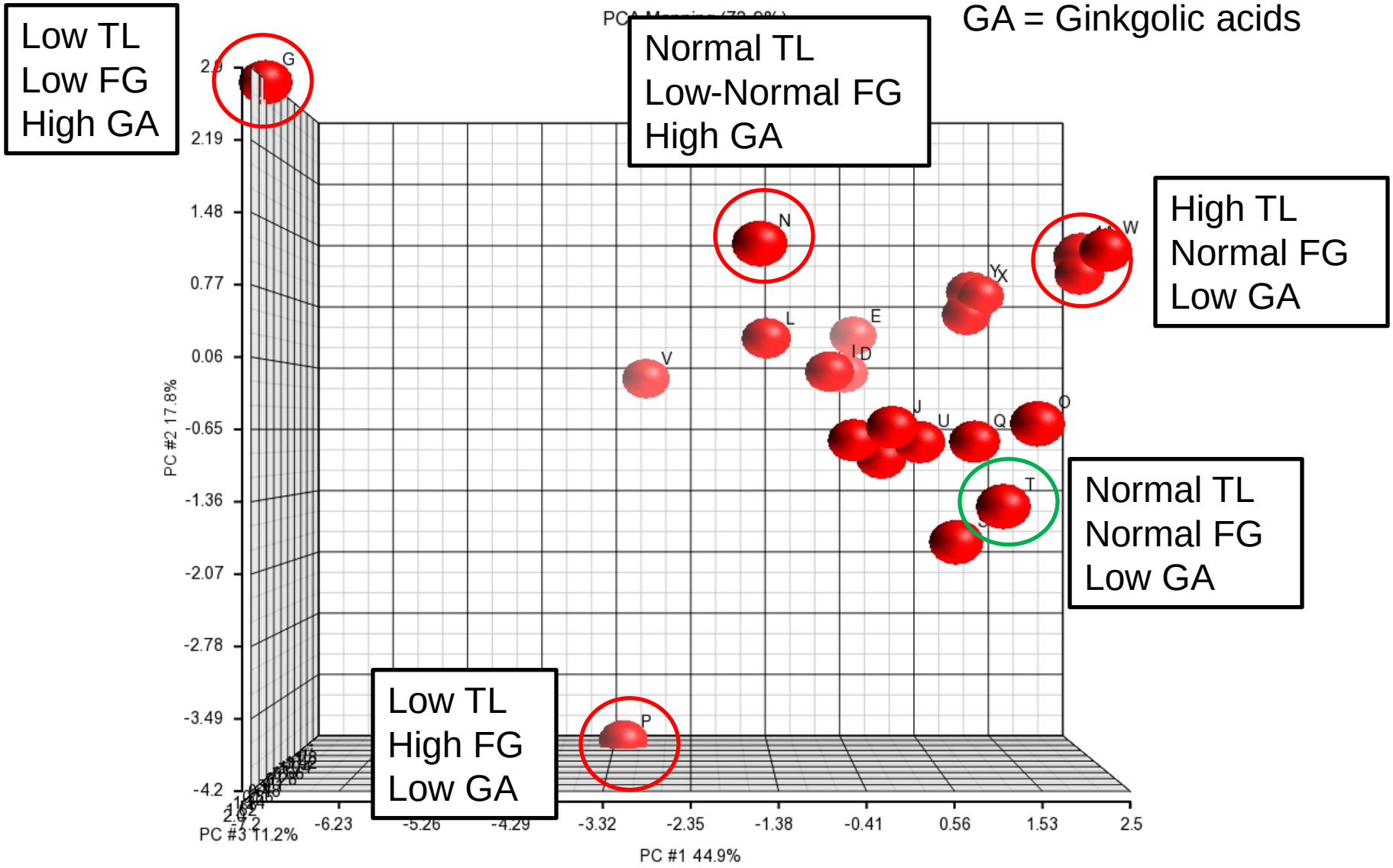
Jim Harnly (USDA)



Ginkgo biloba extract

Targeted chemistry

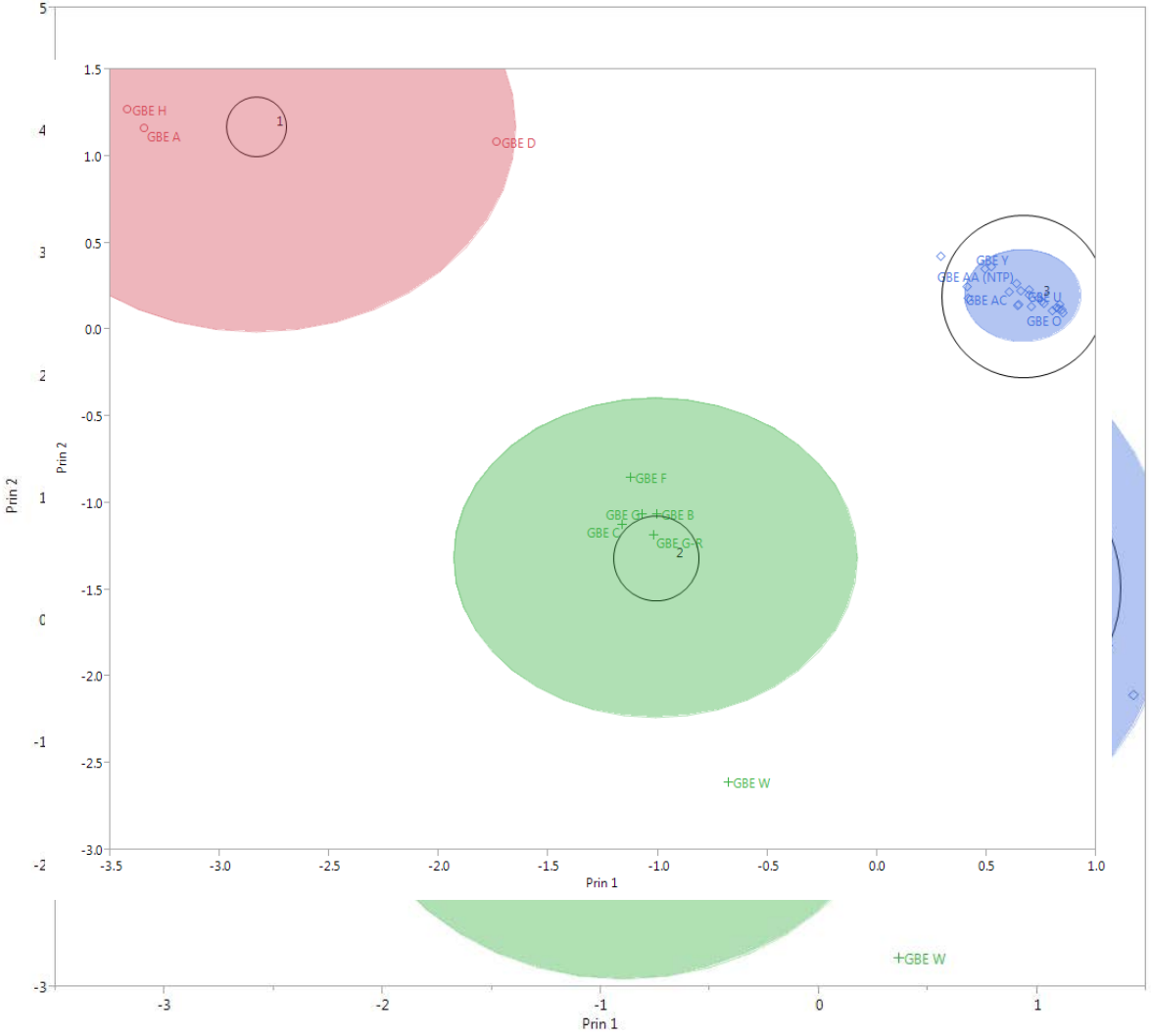
TL = Terpene lactones
FG = Flavonol glycosides
GA = Ginkgolic acids



Primary human hepatocyte data

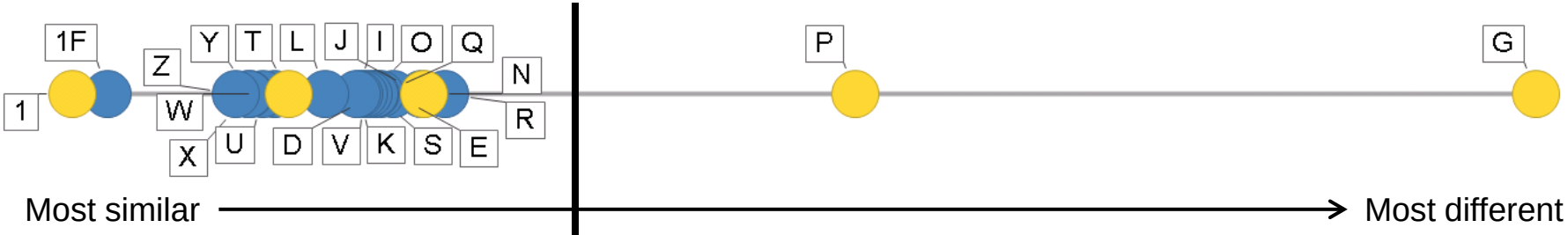
Liver enzyme induction

- AhR (CYP1A2)
- CAR (CYP2B6)
- PXR (CYP3A4)
- FXR (ABCB11)
- PPARα (HMGCS2)

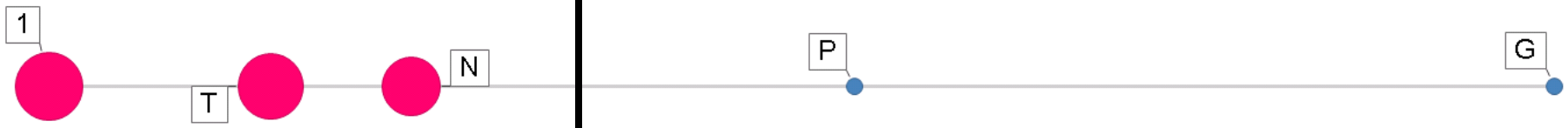


Determining sufficient similarity

Chemical fingerprint similarity



Liver weight (% increase)



Hepatocyte Lipid Accumulation Signature Score

Similarity cut-off

Identifying active constituents

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

Extraction

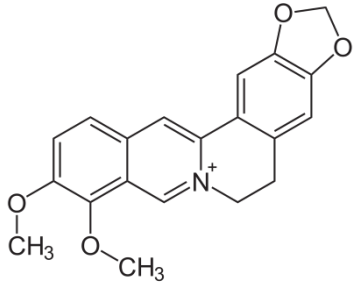
Bioassay

Active extract

Separation

Bioassay

Isolation/
Identification



Active fraction

Significance

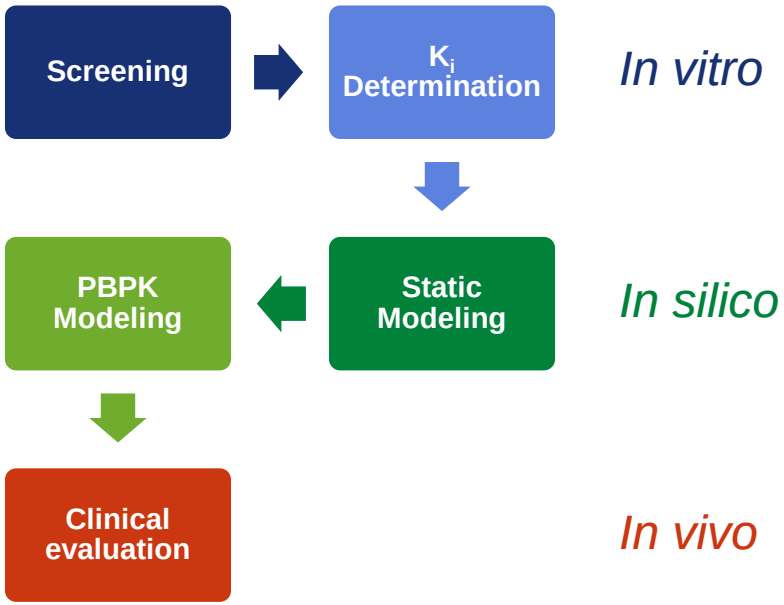
- Aid in the design of toxicology studies
  - Select doses, dosing paradigm, and route of exposure
- Provide information to link external exposure to internal or target site dose
  - Biological effects are best correlated with internal or target site dose rather than the administered dose
- Provide information 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



Products from workshop

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

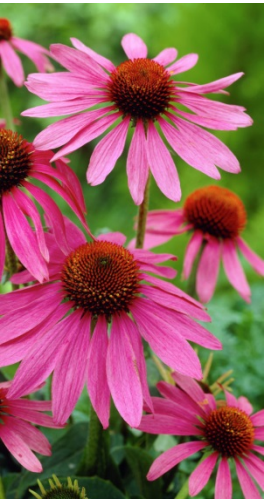
- Botanical dietary supplements are an important public health concern and an area of active research
  - Over 300 people registered to attend or view the webcast of the meeting
- Botanicals are complex entities that offer unique challenges for research, regulation, and manufacturing
  - Botanical quality 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

NIEHS: Scott Auerbach, Windy Boyd, Danielle Carlin, Michael DeVito, Paul Foster, Michelle Hooth, Scott Masten, Rick Paules, Diane Spencer, Suramya Waidyanatha, Nigel Walker, Kristine Witt, Mary Wolfe

- Case Study Development: Scott Auerbach, Brad Collins, Chris Gennings (Mt Sinai), James Harnly (USDA), Steve Ferguson, Stephanie Smith-Roe, Suramya Waidyanatha
- NIH Office of Dietary Supplements: funding for case study development
- NTP post doctoral trainees: Natasha Catlin, Georgia Roberts, Kristen Ryan, and Kelly Shipkowski
- NIEHS support staff: Denise Lasko and Anna Lee Mosley
- NLM Lister Hill staff: Melissa Hush, AV staff