Research on Botanical Safety at the National Toxicology Program

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May 29, 2020
• 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*
• 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

Data mining

Knowledge integration

Chronic *in vivo*

Translation and communication

Science to inform decisions that impact human health

QSAR profiling

Hypothesis-driven research

Short-term *in vivo*

Bioactivity screening

In vitro studies
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
<table>
<thead>
<tr>
<th>Bisphenol A &amp; Analogues</th>
<th>Botanical Dietary Supplements</th>
<th>Cell Phones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate &amp; Formulations</td>
<td>Medicines &amp; Therapeutics</td>
<td>Mold</td>
</tr>
<tr>
<td>Nanomaterials</td>
<td>Polycyclic Aromatic Compounds</td>
<td>Synthetic Turf/ Crumb Rubber</td>
</tr>
</tbody>
</table>
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)
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

• 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

<table>
<thead>
<tr>
<th>Botanical</th>
<th>Male Rats</th>
<th>Female Rats</th>
<th>Male Mice</th>
<th>Female Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aloe vera</em></td>
<td>Clear</td>
<td>Clear</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>Ginkgo biloba</em></td>
<td>Some</td>
<td>Some</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Ginseng</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Goldenseal</td>
<td>Clear</td>
<td>Clear</td>
<td>Some</td>
<td>No</td>
</tr>
<tr>
<td>Green tea</td>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Kava Kava</td>
<td>Equivocal</td>
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<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Milk thistle</td>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Senna</td>
<td>Not tested</td>
<td>Not tested</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bitter orange</td>
<td></td>
<td></td>
<td></td>
<td>Increased heart rate and blood pressure</td>
</tr>
<tr>
<td>Ephedra</td>
<td></td>
<td></td>
<td></td>
<td>Cardiotoxicity</td>
</tr>
</tbody>
</table>

*Camellia sinensis*
April 26-27, 2016, NIH Campus, Bethesda, MD

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

- Identifying active constituents
  - Hazard characterization
  - Product development
  - Regulation
- Comparing across botanicals
- Understanding ADME of botanicals
Developing methods for comparing complex botanicals

How similar is similar enough? A sufficient similarity case study with *Ginkgo biloba* extract

Natasha R. Catlin, Bradley J. Collins, Scott S. Auerbach, Stephen S. Ferguson, James M. Harnly, Chris Gennings, Suramya Waidyanatha, Glenn E. Rice, Stephanie L. Smith-Roe, Kristine L. Witt, Cynthia V. Rider

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

Kristen R. Ryan, Madelyn C. Huang, 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
Determining sufficient similarity

Chemical data

**Figure 2.** Non-Targeted Fingerprint Chromatograms of First Set of GBE Samples (Not Hydrolyzed), BPLC-ELSD

Bioactivity data

CYP2B6 (CAR)

- Log$_{10}$ Relative Fold mRNA Content
- Log$_{10}$ Concentration (% v/v)

**Similar**

1F 1Z 1U 1T 1L 1I 1O 1Q

**Different**

P A B F G H

**Note:** System stopped after this injection. System was reinitiated the following day and a slight shift in retention times was noted.
Identifying active constituents

Licorice root
*Glycyrrhiza glabra*

Roberts et al., 2019. Food and Chemical Toxicology. 124: 431-438.
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., $C_{\text{max}}$, AUC, PBPK modeling)

*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

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