A Strategy for Developing a Framework of Genotoxicity Assays for Safety Assessment of Botanicals

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Introduction

Natural products, such as botanical dietary supplements, are used globally and are growing in popularity. Traditional *in vivo* animal toxicity testing on these complex and variable substances is not always practical and is resource intensive. The Botanical Safety Consortium (BSC) is a public-private partnership formed to improve botanical safety by evaluating the suitability of new approach methodologies (NAMs) for botanicals as complex mixtures. The BSC was formed via a Memorandum of Understanding between the US FDA, NIEHS, and HESI.

This toxicological assessment must include evaluation of genotoxicity, as genotoxicity is associated with a number of adverse human health effects not reliably predicted by adverse event reporting. Established in silico and in vitro methods are available for use in evaluating the genotoxicity of botanicals.

The BSC's Genotoxicity Technical Working Group (GTWG) is developing a pragmatic fit-for-purpose testing strategy for currently marketed botanicals.

Goals and Structure of the BSC

Current Charge to the BSC: To evaluate the suitability of selected assays for use in evaluating the toxicity of botanicals as complex mixtures.

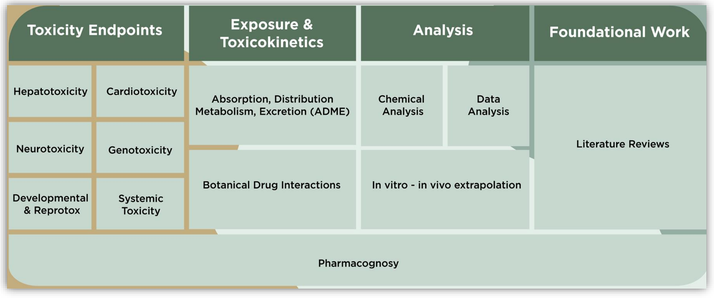

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Table 1. Initial Botanical Case Studies

Botanical*	Plant part
Ashwagandha	Root
Aristolochia fangchi	Root
Blue cohosh	Root
Comfrey	Root or leaf
Ephedra	Aerial parts
Ginseng, Asian	Root
Goldenseal	Root
Green Tea Extract	Leaf
Kava kava	Root
Kratom	Leaf
Milk thistle	Seed
Usnea lichen	Whole
Yohimbe	Bark

*Selected across working groups, based on known toxicity or safety and availability; botanicals in **green** font were nominated by the GTWG

Current Phase of Program Development

Tiered Testing Workflow Strategy for Genotoxicity

Tier 0 — ***In silico* predictive modeling** ← **Completed**
Leadscope: Ames, MN in vivo, CA in vivo/in vitro, MOLY

Tier 1a — **Initial in vitro screening assays** → Both (-), deprioritize
Ames (OECD 471) | *In vitro* MN (OECD 487)

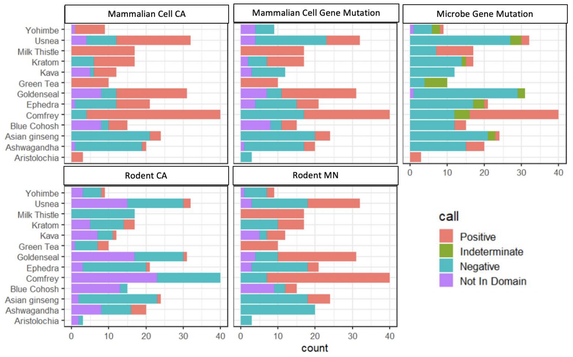
Tier 1b
- Ames + → HPRT (Mammalian cells)
- *In vitro* MN+ TK6 cells → MultiFlow® (Clastogenicity, Aneugenicity)

Follow-up options, as needed:
- **DNA-Damage Response**: p53 CALUX®, BlueScreen™ HC, GreenScreen® HC, ToxTracker®, Comet
- **MoA**: ToxTracker®, TGx, Ox stress, Topo inhibition
- **Higher tier *in vitro* assays**: Liver spheroids, 3D skin models, Hen's egg MN/ comet

What *in vitro* assays can be used to evaluate botanical safety?
- Focus on screening-level assays (*in vitro* + *in silico* approaches)
- Known botanicals with existing data/information **(13 currently prioritized)**
- Basic chemical analysis that allows authentication and basic constituent ID
- Lot-to-lot chemical analysis for multiple lots of **Ashwagandha**
- Single lots for all other botanicals
- Development of a toolkit / framework based on data collected

In Silico Predictive Modeling Output

OSAR Predictions by Model Category and Botanical*



- 246 constituents from 13 botanicals run in 5 genotoxicity prediction models* from Leadscope
- Aristolochia fangchi has the least (3) and comfrey the most (40) identified constituents
- 99% of compounds (244/246) were in domain for the microbe gene mutation model
- 73% of the constituents were predicted to be genotoxic in ≥ 1 model.

*CA, chromosome aberrations
MN, micronucleus

Information on the 7 Botanicals Nominated by the GTWG

Botanical (common name)	Existing Experimental Results	*In Silico* Prediction
Aristolochia fangchi	Contains Aristolochic acids, known human genotoxic carcinogen	Bacterial mutagen
Comfrey	Contains PAs, known genotoxicant	Mutagenic, clastogenic
Asian Ginseng	Negative in Ames, negative in vitro MN	Nongenotoxic
Goldenseal	Negative in Ames, negative in vitro MN, positive for cancer in rats	Clastogenic
Green Tea Extract	Positive in Ames , negative in vivo MN	Clastogenic
Kava kava	Negative in Ames, negative in vitro MN, positive for cancer in mice	Nongenotoxic
Milk thistle	Negative in vitro MN, mixed results in Ames, negative for cancer in rodents	Genotoxic

Next Steps

- Full chemical analysis of botanical case study materials
- Conduct Tier 1a tests – Ames OECD 471, *in vitro* MN OECD 487
- Evaluate data against extended *in silico* predictions (Tier 0)
- Initiate Tier 1b testing as needed for positive results (e.g., HPRT, MultiFlow)
- Determine need for follow-up MoA studies and higher tier *in vitro* assays
- Evaluate performance of the test battery for the 7 botanical substances nominated by the GTWG