Application of *in silico & in vitro* approaches for evaluating developmental & reproductive toxicity in botanical products

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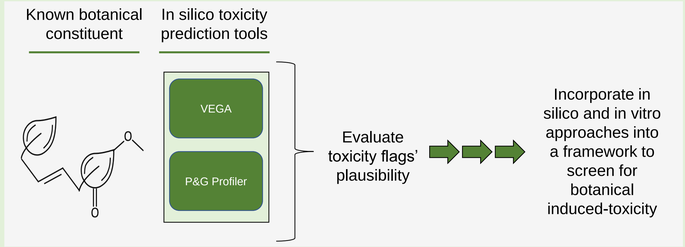
Background

- While exposure to botanicals and their constituents can occur during important life stages (reproductive years, pregnancy, childhood, menopause), developmental and reproductive toxicity (DART) has been understudied for many botanicals.
- 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 by the US FDA, NIEHS, and HESI. The BSC works to improve botanical safety by evaluating the suitability of new approach methodologies (NAMs) for botanicals as complex mixtures with DART being a key focus area.

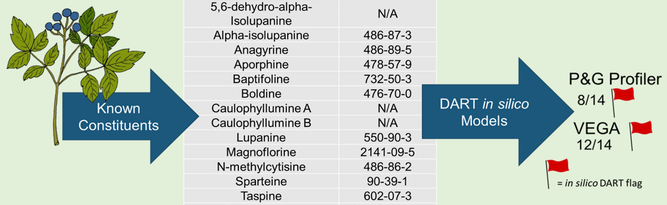
Methods

- Two in *silico tools* were utilized to predict potential DART liabilities of botanical constituents:
  1. P&G Profiler (identifies potential DART-active chemicals by integrating chemical structure with known DART modes of action)
  2. VEGA – provides numerous QSAR models to predict toxicity
- Compounds & constituents for evaluations were chosen from:
  - COlleCtion of Open Natural ProdUcTs COCONUT database (n=100,000)
  - Known constituents from well-studied botanicals (n=250)



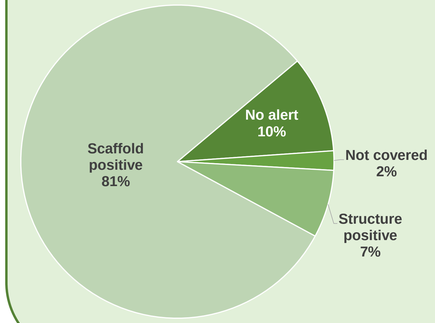
Blue cohosh: *in silico* case study

- Blue cohosh has reported teratogenic effects
- 14 of the known constituents were run through the P&G Profiler and VEGA tools
- The models predicted different numbers of flags
- For the P&G profiler, the 6 constituents not identified/not flagged were mapped to a scaffold for further scrutiny
- Overall, most constituents resulted in a flag or need to be further investigated.



Name	CASRN
5,6-dehydro-alpha-Isolupanine	N/A
Alpha-isolupanine	486-87-3
Anagyrine	486-89-5
Aporphine	478-57-9
Baptifoline	732-50-3
Boldine	476-70-0
Caulophyllumine A	N/A
Caulophyllumine B	N/A
Lupanine	550-90-3
Magnoflorine	2141-09-5
N-methylcytisine	486-86-2
Sparteine	90-39-1
Taspine	602-07-3
Thalictroidine	248259-06-5

in silico evaluation of COCONUT constituents



- We screened 100K of the +400K compounds in the COCONUT database for DART alerts.
- Using the P&G Profiler, only 10% of the phytochemicals screened did not yield an alert.

Botanical Case Studies

Botanical	Reported use(s)	Rationale for inclusion	Expected DART *in vitro*?
Ashwagandha root *Withania somnifera* (L.) Dunal	Brain function, lower blood sugar, anxiety and depression	Negative in DART animal studies	Negative
Blue cohosh root *Caulophyllum thalictroides* (L.) Michx.	Labor induction, menstruation	Teratogenic in animal studies	Positive
Asian ginseng root *Panax ginseng* C.A. Mey.	Increase energy, reduce stress	Large body of evidence pointing to safety	Negative
Usnea lichen *Usnea* spp.	Weight loss, pain, fever, wound healing	Reprotoxic in NTP study	Positive
Milk Thistle Seed *Silybum marianum* (L.) Gaertn.	Support liver function	Large body of evidence pointing to safety	Negative

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Future Directions

- Identify context-of-use for *in silico* assays
- Use ADME models to understand the large volume of toxicity flags
- Use multiple evidence streams (*in silico* and *in vitro*) in a Weight of Evidence analysis to provide information for safety evaluation
- Evaluate initial screening assays for their suitability for botanicals as complex mixtures
  - Transcriptomics in human cell lines
  - Zebrafish embryos
  - *C. elegans*
  - devTOX quickPredict assay