A Tiered Approach for the Evaluation of the Safety of Botanicals Used as Dietary Supplements: An Industry Strategy

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Exposure to botanicals in dietary supplements is increasing across many geographies; with increased expectations from consumers, regulators, and industry stewards centered on quality and safety of these products. We present a tiered approach to assess the safety of botanicals, and an *in silico* decision tree to address toxicity data gaps. Tier 1 describes a Threshold of Toxicologic Concern (TTC) approach that can be used to assess the safety of conceptual levels of botanicals. Tier 2 is an approach to document a history of safe human use for botanical exposures higher than the TTC. An assessment of botanical-drug interaction (BDI) may also be necessary at this stage. Tier 3 involves botanical chemical constituent identification and safety assessment and the *in silico* approach as needed. Our novel approaches to identify potential hazards and establish safe human use levels for botanicals is cost and time efficient and minimizes reliance on animal testing.

Exposure to natural ingredients of botanical nature, particularly through the use of dietary supplements and herbal medicines, continues to increase globally. In the United States for example, 2016 marked the 13th consecutive year of sales growth for herbal-based supplements.[1] In a 2017 survey conducted by the Council for Responsible Nutrition, growth of dietary supplement use over the last decade shows that 76% of Americans are taking dietary supplements, up from 64% in 2008.[2] The Council for Responsible Nutrition survey also shows that 39% of the total dietary supplement use consists of herbals and botanicals, including green tea, cranberry, turmeric, garlic, ginseng, ginkgo biloba, milk thistle, and echinacea as the most popular. Furthermore, increases in supplement use is increasing across all age groups surveyed (18–55+ years of age), with the greatest increase in the 55 + age group (80% up from 76% in 2016). More recently, Agbabiaka *et al.*[3] using a systematic review of the literature, found that herbal medicinal product use was common in older adults (≥65 years of age). Exposure to dietary supplements, including botanicals, is also increasing in children. According to the National Health and Nutrition Examination Survey data from 2007 to 2010, 1.7% of children used supplements containing botanicals, primarily to boost immunity and prevent colds.[4] Most supplement use in children does not occur under the recommendation of a healthcare provider.[5] Kantor *et al.*[6] analyzed the National Health and Nutrition Examination Survey data from 1999–2012 and reported that only 23% of all supplement products were used at the recommendation of a health care provider.

The increased consumer exposure to botanical supplements has led to heightened scrutiny and compliance expectations by regulatory authorities, industry stewards, and consumers. Unfortunately, there is no global consensus on how to define dietary supplements, or regulatory expectations for quality, safety, and labeling across geographies. Even more challenging are the emotional and polarizing opinions on how to regulate this category; ranging from an approach that is similar to conventional drugs and foods, to a more tailored approach that relies on traditional or historical usages.[7] As evidenced above, the growing popularity and lucrative nature of the category has led to an increase in industry participants marketing novel and innovative botanical products.

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Although regulatory authorities and industry organizations are improving the safety expectations related to dietary supplements, there remain significant data gaps or conflicting data for critical toxicity end points. In many cases, safety data gaps may be attributed to an over-reliance on limited historical information rather than empirical testing of these complex botanical mixtures. Botanicals may include the use of whole plants or a specific plant part (e.g., flower, stem, leaves, bark, root, and fruit), in a dietary supplement. Typically, plant material is subjected to some form of extraction by a solvent or solvent mixture (e.g., water and ethanol) or high-pressure extraction (supercritical CO_2) to create the botanical ingredients that may be used in products. These botanical ingredients are typically complex mixtures consisting of numerous individual phytochemical constituents and potential contaminants; as shown in **Figure 1** with a representative chromatogram using high-performance liquid chromatography photodiode array detection for a botanical ingredient. Thus, comparing published literature reports for botanicals is challenging due to highly variable material in the marketplace and poorly described and characterized test materials. Likewise, testing botanicals is equally challenging due to the lack of characterized material, natural variations in botanical composition, presence of contaminants, and a lack of knowledge on toxicologically active phytochemical constituent(s). Testing these complex mixtures in *in vitro* systems pose particularly unique challenges, such as determining appropriate testing concentrations, solubility, and inherent antibacterial or cytotoxicity properties of plant-based constituents. Extrapolating findings from *in vitro* studies to typical human doses is also difficult without well characterized material and knowledge of dose form parameters (e.g., dissolution and disintegration) and bioavailability of phytochemical constituents.

Herein, we describe development of a tiered approach to support safety of botanical ingredients and a strategic way to fill certain toxicity end point gaps that allows for higher throughput, is more cost and time efficient, and may avoid the use of animal testing. The approach presented below provides guidance for the evaluation of safety of complex botanical mixtures for use in products marketed as herbal medicines, foods, or dietary supplements. Thus, it applies to botanical ingredients intended to deliver a product benefit to the consumer (e.g., health or function claim). In most geographies, a dietary supplement by definition is administered orally; however, our approach is applicable with other routes of administration as well as to consumer products (e.g., cosmetics).

Although identity quality of botanical raw materials is also a concern within the industry, and a significant effort is underway to make improvements in this arena, this topic will not be covered here. The approach outlined in this article assumes that the quality of the botanical ingredient(s) has been assured.

TIERED APPROACH FOR ASSESSING SAFETY OF BOTANICAL DIETARY SUPPLEMENTS

Tier 1—Conceptual levels of botanicals

Establishing safe botanical exposure levels in the absence of carcinogenicity and genotoxicity data, which address the most sensitive end point, becomes a critical and often rate-limiting step in botanical risk assessment. For chemical ingredients (including botanicals) that are used in products at low levels, a threshold of toxicological concern (TTC) approach can be used to rapidly assess safety when toxicological data are lacking.[8] The TTC allows for a level of exposure for any chemical, even without chemical-specific toxicity data, below which the assumption is that there would be no appreciable risk to human health.[9] This approach uses conservative assumptions for systemic exposure (i.e., assumes 100% bioavailability). The TTC decision tree approach starts with the identification and evaluation of possible structural alerts for genotoxicity and high potency carcinogenicity.[10] This step applies an exposure threshold of 0.15 µg/person/day.[11,12] Other authoritative bodies, including the European Food Safety Authority, have proposed the use of TTC to assess the safety of individual substances in botanical ingredients.[13]

Our laboratory has proposed to extend the TTC approach to botanicals, relying on this initial TTC exposure limit of 0.15 µg/day (0.0025 µg/kg bw/day) and adjusting it based on the concentration of phytochemical constituents of concern found in plants for genotoxicity and carcinogenicity end points.[14] We evaluated over 50 genotoxic/DNA reactive and carcinogenic phytochemical constituents found in plants and compiled concentration data from several hundred plant species (over 2,300 observations). Phytochemical constituent concentration values ranged from 0.00015 to 136,000 ppm; with the vast majority of the concentrations residing in lower ppm levels, which were best fitted with a Weibull distribution model. The distribution of the data took into account single chemical occurrences; co-occurrences remain to be done. The concentration probability at the 95th percentile for the concentration of phytochemical constituents of concern in plants can be used to adjust the TTC at the most conservative level for phytochemical constituents with genotoxic potential:

$$\text{Adjusted TTC exposure level} = \frac{\text{genotoxic TTC exposure level}}{\%\,\text{genotoxic concentration}\,(95\text{th percentile})}.$$

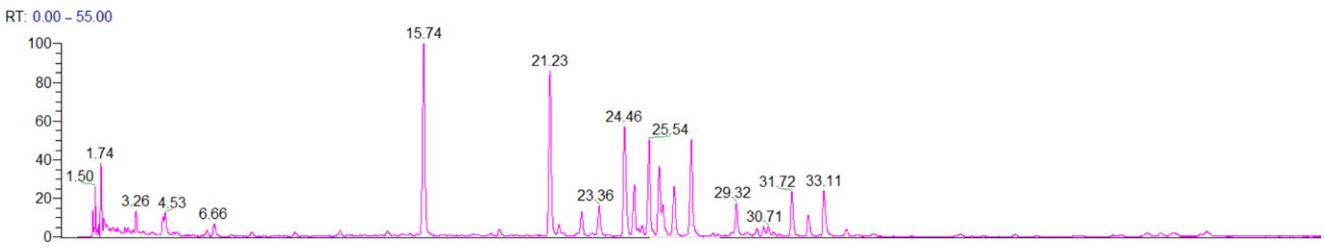

Figure 1 A representative chromatogram using high-performance liquid chromatography photodiode array detection for a botanical extract.

Table 1 Comparison between significant history of use and traditional use

Significant history of use	Traditional use
A concept used to describe the qualified presumption of safety, where there is evidence for safety from compositional data and from experience as an ongoing part of the diet (and possibly from other relevant routes of exposure) for a number of generations in a large, genetically diverse population.	Is based upon knowledge and experience in a population/culture but may have limited scientific documentation.
Includes a scientific evaluation of the information, which should include conclusions about safe use.	Traditional use in this regard may provide information on acute toxicity but it is unlikely to provide information on chronic toxicity and those effects that are delayed and, thus, less likely to be detected, such as cancer, developmental toxicity (including teratogenicity) and reproductive toxicity.
A description of history of use covers the use in different defined geographic areas with information on intake levels, intake patterns, years of use, preparation, handling methods, and impact on human health as well as addressing any potential adverse effect issues.	Information from traditional use will be influenced by the general health of the particular population and the available health care and health monitoring facilities.

Table 2 Sources to support significant history of use

Source	Weblink or Reference
WHO Monographs on Selected Medicinal Plants[14]	http://apps.who.int/medicinedocs/en/d/Js2200e/
German Commission E Monographs[15]	Translated from German and available online by the American Botanical Council, http://cms.herbalgram.org/commissione/intro/comm_e_int.html
European Medicines Agency Committee on Herbal Medicines[16]	http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/herbal_search.jsp&mid=WC0b01ac058001fa1d
European Scientific Cooperative on Phytotherapy[17]	http://escop.com/
Natural Standard Monographs[18]	https://naturalmedicines.therapeuticresearch.com/ J Med Libr Assoc 93, 4, (2005)

Thus, when classical toxicological data are lacking, we suggest using a health protective TTC exposure limit for the botanical mixtures and their simple extracts.[14]

Tier 2—Exposure > TTC with documented history of significant human use

When the botanical ingredient used in a dietary supplement exceeds the TTC, a logical starting place in the safety assessment is determining whether a documented significant human use (SHU) exists. An SHU may be considered as a surrogate measure for safety in the absence of relevant toxicological data. However, one must consider the context of SHU relative to traditional uses. A comparison of the two is presented in **Table 1**. As outlined in **Table 1**, an SHU considers relevant information about historical use, including route of administration (e.g., oral ingestion) and a comparison of intake levels and patterns (frequency of use). A variety of resources can help confirm food uses or components of food as well as food exposure data, including Research Institute for Fragrance Materials and Flavor Extract Manufacturers Association databases and Generally Recognized as Safe and Everything Added to Food in the United States for the US Food and Drug Administration approved food uses.[15–18] Additionally, a careful examination of the population that has traditionally used the botanical ingredient is also critical, including demographics, geographical location, method and purpose of use, and any associated side effects reported.[19]

A classic example of the importance of understanding traditional use of a botanical ingredient can be seen with Ephedra (ma-huang). This Chinese shrub has been known in Traditional Chinese Medicine for at least 5,000 years.[20] The historical use of Ephedra was as a tea, taken for the treatment of respiratory symptoms (e.g., cough and congestion), with minimal reported side effects. Beginning in the 1990s in the United States, Ephedra was frequently used in weight-loss and energy-enhancement products taken chronically. During this same time period, serious adverse effects associated with Ephedra-containing dietary supplements, including heart attack, stroke, seizure, high blood pressure, and heart rhythm problems were increasingly reported. Due to the serious nature of the adverse events, including several deaths in otherwise healthy individuals, the US Food and Drug Administration banned the use of Ephedra in dietary supplements in 2004. This example highlights the potential change in side effect profile that can occur when a botanical ingredient is used in a different form, indication, pattern of use, and population than its traditional use. In other words, no SHU was established for the new usage paradigm for Ephedra.

There are a number of reputable sources and databases available to confirm documented use (of same species, plant part, method of preparation, and similar exposure levels) to support SHU (**Table 2**). When utilizing traditional medicine sources, it is important to confirm widespread availability of use, and not just via a learned intermediary and/or preparation of tailor-made complex

mixtures. To further confirm widespread availability, a review of pharmacovigilance data and adverse event databases should also be conducted to ensure adequate reporting systems are in place and that no serious health problems exist with the botanicals under investigation. It should be noted that similar adverse event data are typically unavailable for traditional uses because formal reporting systems are not used.

Published literature, monographs, and assessment reports, examples of which are outlined in **Table 2**, may also provide data on safety end points, including nonclinical toxicity, clinical, and case report data to inform on an overall weight of evidence (WoE) conclusion for whether a safety concern exists regarding developmental and reproductive toxicity (DART), genotoxicity, and safety pharmacology parameters (cardiovascular, central nervous, and respiratory systems). These end points are particularly highlighted because, in the case of the latter, they are central to the function of primary organ systems or, in the case of genotoxicity/carcinogenicity and DART, these are end points that may be difficult to detect through human use alone. When applying a WoE approach, the relative robustness of published literature, including supporting references and scientific quality of reported studies, should be evaluated, as indicated in **Table 3**.

Epidemiology studies with safety-related end points can be very useful when the botanical composition and exposure being studied are very similar to the proposed product use. Prospective epidemiology studies can be expensive and retrospective studies are subject to recall bias. Clinical studies typically have smaller populations compared to epidemiology studies but can be carefully controlled as to the subject population, product use, and duration and safety end points measured. The regimented use in clinical studies may not mimic real world use and there is limited ability to detect rare events. With a robust monitoring system in place, human use experience outside of epidemiology or clinical studies can provide useful data, although may times these reports do not come from medical professionals and causality is difficult to assess. Nonclinical animal studies have been the traditional approach to safety testing and can be used to address many toxicity end points over critical developmental/reproductive periods or up to lifetime exposure. However, no one animal model can completely mimic a human response and animal testing bans exist for ingredients used in some product types in certain countries.[21] *In vitro* studies can be a fast, inexpensive way to obtain data on very specific end points but do not model the entire *in vivo* response. Structure-activity relationships (SARs) can be a fast and inexpensive tool for the chemical constituents of botanicals to provide an estimate of toxicity.

One assumes (but should verify) objective reporting when studies are published in peer-reviewed scientific journals; however, traditional uses may be reported in the form of anecdotal reports and, thus, potentially subjective reporting. An SHU should be based on fully vetted literature sources and/or well-established databases. As the WoE approach to safety evaluation of botanicals includes a comprehensive review of a diverse compilation of information and data (as shown in **Table 3** and discussed above), assistance from a number of experts may be required. Individuals with expertise in pharmacognosy and toxicology of botanicals, and healthcare practitioners with clinical experience in the use of herbal medicines, and natural products chemists can contribute to an overall WoE assessment.

Additionally, a consideration of interaction potential between the botanical ingredient and conventional medicines (botanical-drug interaction (BDI)) should be considered as part of Tier 2. This assessment is also made with a consideration of SHU but with recognition that as an aging population there is the potential for concomitant use of a number of prescription medications.[3,22] The scientific literature is replete with nonclinical reports of botanical ingredients and/or phytochemical constituents as potent inhibitors of drug-metabolizing enzymes.[23,24] However, without the use of robust analytical characterization and quantitation of phytochemical constituents, dose performance data, use of physiologically relevant *in vitro* metabolic systems, and follow-up human clinical studies when necessary; extrapolating these preliminary reports to determine clinical relevance in humans is likely impossible. This lack of clear determination of risk hinders healthcare professionals and consumers from making informed decisions about the safety of taking dietary supplements with prescription medications. Various strategies for assessing BDI potential have been proposed, including more systematic approaches similar to studying drug-drug interactions. For example, the integration of *in vitro* data with the pharmacokinetics of individual botanical constituents into physiologically based pharmacokinetic (PBPK) models can help prioritize and design follow-up clinical studies.[25–28] Other researchers have suggested quite extensive *in vitro* workflows to study BDI that include an initial assessment of CYP450 interactions by individual botanical constituents. Botanical constituents are then tested individually or as an extract for absorption, efflux, and transporter interactions using a Caco-2 permeability assay. Primary hepatocytes or cell lines, such as HepaRG cells, are proposed to study induction of CYP450 enzyme activity and mRNA expression.[26]

Important considerations that should be included in performing an overall BDI assessment are similar to those for other toxicity end points, including establishing an SHU, utilization of published literature data, and phytochemical constituent information based on careful analytical characterization (**Table 4**).[27] However, there are some nuances associated with some of these considerations that are unique to assessing BDI. For example, in assessing SHU in the context of BDI, a general consideration of prescription medication use (polypharmacy) in the population for which the botanical supplement is targeted may be helpful. Understanding the profile of the consumer who might be attracted to a particular botanical supplement can provide perspective on potential underlying diseases/conditions, and associated comedications used by that consumer population. For example, one might consider that a botanical-based dietary supplement aimed for inflammation and joint pain relief might be attractive as adjunctive therapy to individuals using nonsteroidal anti-inflammatory drugs. If there are literature data indicating the potential for the botanical under consideration to inhibit the enzymes involved in the metabolism of many nonsteroidal anti-inflammatory drugs, then perhaps follow-up BDI studies should be considered to further delineate risk associated with their co-administration.

A robust analytical characterization of phytochemical constituents in a given botanical can allow for further mining of the

Table 3 Considerations on relative robustness of available information on botanical of interest

Data type	Key considerations	General strengths	General limitations
Epidemiological studies involving safety-related endpoints	• The population studied in the epidemiology study vs. the population for which the product is intended (e.g., age, gender, race, cultural factors) • The composition of the material (i.e., key constituents, particularly any with anticipated toxicity) evaluated in the epidemiology study vs. the composition of the proposed product • How the material was used by the population in the epidemiology study vs. the proposed product uses (e.g., food vs. dietary supplement, duration of use)	• Ability to study rare events • Ability to evaluate risk under "real world" conditions of use (however, note the first three key considerations when applying available epidemiology data to a specific product) • Ability to measure exposure-specific incidence and prevalence of an outcome	• Requires additional information to address causality • Retrospective measurement of exposure is imprecise • Inability to totally control confounding factors • High cost of prospective studies
Clinical studies	• Rigour of safety evaluation incorporated into study design; High-active monitoring of safety endpoints (these might include vital signs, blood chemistry, hematology, urinalysis, or relevant end points based on the known or anticipated biological effects of the constituents) and recording of adverse events. Moderate—Recording of adverse events, Low—No information reported on adverse events • Duration of the clinical study(ies) compared to anticipated duration of use by consumers. • Dosing regimen employed in the clinical study(ies) compared to the dosing instructions in product labelling • Demographics of patients studied in the clinical study(ies) vs. intended consumer demographics (e.g., age race, gender, any medical exclusions in the clinical study(ies)) • Number of patients studied in the clinical study(ies). • Comparison of the composition of the test material used in the clinical study(ies) to the composition of product. Focus on constituents with known or anticipated biological effects particularly any constituents known to have the potential to cause adverse effects. • Nature and incidence of the adverse events. Have serious adverse events been reported? • Placebo controlled vs. uncontrolled. Is there a difference in the incidence of adverse events relative to placebo?	• Conducted in the species of interest for safety assessment (i.e., humans) • Often conducted under carefully controlled conditions • Provide a quantitative estimate of the frequency of adverse events • Ability to obtain subjective data pertinent to safety assessment from study participants	• Low capability to detect rare events • Low capability to obtain data under actual use condition • Inability to evaluate events requiring a long time to manifest • Measurements limited to non-invasive/minimally invasive procedures • Establishment of causality of adverse events can be difficult

(Continues)

Table 3 Continued

Data type	Key considerations	General strengths	General limitations
Human use experience (outside the context of epidemiology studies and clinical trials)	• Is the total daily exposure associated with the history of use comparable to anticipated total daily exposure via product use? • Comparison of the temporal pattern of historical use of the herbal preparation relative to intended use of the product (i.e., daily use, occasional use, etc.) • Comparison of the composition of the material used historically to the composition of our product. Focus on constituents with known or anticipated biological effects particularly any constituents known to have the potential to cause adverse effects. • Consideration of the known or anticipated biological effects of the constituents and the nature of any potential adverse event concerns that arise as a consequence of those effects. This is necessary to assess the degree of confidence that potential adverse events would be identified from the human use experience. For example, carcinogenic potential can be difficult to detect via spontaneous adverse event reports whereas acute effects are more readily identifiable. • Nature of monitoring systems in place to evaluate outcomes of exposure. • Are the adverse event data available for review? • Factors influencing reporting rate of adverse events. Is reporting mandatory for product manufacturers in the country(ies) where there is a history of use? • Nature of the adverse events. Have serious adverse events been reported? • Are the known biological properties of the constituents consistent with the nature of the adverse events reported?	• Capable of detecting rare events when a robust monitoring system is in place • Ability to collect information under "real world" conditions of use (however, note the first three key considerations above when applying adverse event reports from historical human use to a specific product)	• Accuracy and completeness of the information in spontaneous adverse event reports is sometimes poor, particularly in cases where the report is not made by a health care professional • Quantitation of adverse events is limited to reporting rate and it is not possible to draw firm conclusions regarding the incidence of certain events • Difficult or impossible to evaluate causality (however, note the last point under key considerations above)
Nonclinical animal studies	• Types of toxicological end points evaluated. Studies addressing endpoints relevant to serious adverse events that are not easily identifiable in humans in either clinical studies or as a result of human use experience (i.e., cancer, congenital anomaly) are particularly useful. This is especially true in cases where there are no epidemiological data addressing these endpoints. However, under certain circumstances, and even in the absence of data in humans, animal data on carcinogenicity or developmental toxicity may not be necessary. For carcinogenicity this would include situations where there are data to indicate lack of genotoxicity and no known pharmacological effects relevant to human carcinogenic potential (e.g., hormonal activity, immune suppression). Developmental toxicity data would not be necessary when the product would not be used in women of child-bearing potential. Other types of animal studies (e.g., subchronic toxicity, chronic toxicity, pharmacology) may be helpful in identifying what types of effects to look for in evaluating the human data and in adding to the weight of the evidence supporting the safety of long term use. • Access to sufficient detail concerning the study design, methods, and results of the animal studies to allow a judgment to be made regarding the study quality and reliability of the results. • Comparison of the composition of the test material used in the animal study(ies) to the composition of our product. Focus on constituents with known biological effects and any constituents expected to have the potential to cause adverse effects.	• Conducted under carefully controlled conditions • Incorporate the full spectrum of an *in vivo* response (in contrast to *in vitro* studies) • Ability to conduct invasive procedures and to euthanize animals for complete histopathological evaluation • Ability to evaluate the effects of life time exposure • Ability to define dose-response and time-response relationships • Can provide information on the mechanisms of toxicity	• No animal species mimics humans in all respects • Limited capability to detect rare events • Study design and methods may limit the ability to extrapolate the findings to humans (e.g., testing at high doses may produce results due to saturation of detoxification and elimination pathways)

(Continues)

Table 3 Continued

Data type	Key considerations	General strengths	General limitations
In vitro studies	• Types of toxicological endpoints evaluated. Genotoxicity studies are particularly useful because this type of data is helpful in making a weight of the evidence assessment regarding carcinogenic potential. *In vitro* assays for other biological activities may serve to guide the evaluation of other types of data. For example, *in vitro* evidence of estrogen receptor binding activity leads to the need to consider the potential for reproductive and developmental effects in animals and humans. • Access to sufficient detail concerning the study design, methods, and results of the *in vitro* studies to allow a judgment to be made regarding the study quality and reliability of the results. • Comparison of the composition of the test material used in the *in vitro* study(ies) to the composition of the ingredient to be used in our product. Focus on constituents with structural alerts that suggest the potential for certain biological properties.	• Low cost, speed, reduction in animal usage • May provide the opportunity to use human tissues/cells. • Simplified systems allow measurement of key biological events/responses (e.g., estrogen receptor binding) • Can provide information on the mechanisms of toxicity	• Do not model all elements of the *in vivo* response • May not replace the need for animal studies
Structure-activity relationships	This capability is available for individual constituents of botanicals	• Low cost, speed, no animal usage • May be able to provide both qualitative and quantitative estimates of toxicity	• Provides only an estimate of toxicity • Quality of the estimate is dependent on the quality and quantity of available data used to create the database. Requires an extensive data set for reliable estimates • Still an evolving technology with variable acceptance among the scientific/regulatory community • May not replace the need for animal studies

scientific literature for available absorption, distribution, metabolism, and excretion (ADME)-related data, including BDI potential. In addition, identification of phytochemical constituents may allow for molecular modeling (docking studies) to determine potential binding to enzyme active sites and *in silico* prediction of enzyme-inhibitor interactions.[29] Last, dose performance data that include dissolution of the dose formulation and solubility of the botanical extract/phytochemicals in gastric and intestinal fluids is also critical for predicting absorption of key constituents.

A number of well-established *in vitro* models exist for studying drug-drug interactions; some of which may be useful for reapplication to studying BDI.[30,31] Findings from screening-level studies in simplistic metabolic systems (e.g., liver microsomes and membrane vesicles) can be followed up in more physiologically relevant whole cell models, such as primary hepatocytes. Whole cell systems, particularly sandwich-cultured hepatocytes, have an advantage in that they integrate multiple processes (transport, metabolism, and associated cellular regulatory pathways), provide more *in vivo*-relevant intracellular concentrations, and allow for studying inhibition and induction simultaneously.[32] Recently, sandwich-cultured human hepatocytes have shown considerable promise in predicting clinically relevant BDI.[33] Other *in vitro* models (e.g., gut-liver co-cultures) need to be investigated in order to improve predictions of BDI occurring at the level of the gut. Data from an overall assessment of BDI potential, including *in vitro* assays, PBPK modeling, and/or human clinical studies can be used to inform on product label, formulation-adjusted or dose-adjusted, and postmarket surveillance strategies (**Figure 2**).

Tier 3—Decision tree approach to address botanical hazard assessment data gaps

When safety data are insufficient (specific gaps identified) from the Tier 2 assessment, an additional level of assessment may be necessary to confirm the safe use of a botanical ingredient (Tier 3). A novel decision tree approach has been developed that utilizes both SHU information and phytochemical constituent-based safety evaluations to resolve toxicity end points of concern (**Figure 3**).[34] This approach requires state-of-the-art analytical techniques to identify and quantify botanical constituents. Individual phytochemical constituents are then assessed according to both food intake levels and established *in silico* toxicology assessment tools to identify hazards. Combining this analysis with the appropriate dosing and phytochemical constituent co-exposure considerations can be used to establish a risk characterization for the various constituents of the botanical preparation.

Once a botanical ingredient of interest is fully characterized for individual phytochemical constituents and an estimate of human exposure identified, one can determine if the constituents are known structures (e.g., commonly consumed in the diet), and whether the proposed dose associated with the botanical supplement is comparable to dietary exposure levels. For many botanical constituents, this approach alone may be sufficient to support their safe use.

The decision tree approach can also be used to bridge safety between botanicals that are prepared by different methods. It has been well-established that different methods of botanical preparation and extraction (e.g., water, solvent, and CO_2) have potential to influence the phytochemical constituent profile. Situations can exist in which SHU, toxicology data, and clinical reports may exist for some preparations, whereas alternate methods of preparation of the same botanical may lack similar safety data. By making a comparison of both the similarities and unique chemical differences among two or more preparations, any qualitative or quantitative differences in the phytochemical constituents can then be addressed individually by using existing data or applying established *in silico* toxicology tools (e.g., TTC).

Last, this approach can be used to address toxicity end points for the identified phytochemical constituents, or as an early screen for toxicity alerts; particularly for less commonly known botanicals. As previously mentioned under Tier 2, toxicity data must address a number of different end points. When insufficient data

Table 4 Important considerations that warrant inclusion when assessing potential botanical-drug interactions (BDIs)

History of safe use:
- How do geography and culture of historical use compare to proposed product market?
- Is historical use the same as proposed product use?
- Same form (whole plant vs. plant part vs. single ingredient)?
- What is known about the consumer population that product targets (acute vs. chronic use, underlying disease/conditions, co-medications, and age group)?

Literature data:
- PK studies on constituents provide understanding of which constituents are readily absorbed and what relevant concentrations to use in *in vitro* assays.
- Which drug metabolizing enzymes/transporters are affected may guide the need to do additional studies (e.g., potent inhibition of CYP3A4 would likely be more concerning than moderate inhibition of CYP1A2).
- Are there clues in the clinical chemistry and/or histopathology from animal toxicity studies that may indicate potential effects on drug metabolizing enzymes or transporters (e.g., increases in bilirubin, cholestasis, increased liver weight, etc.)?

Incorporation of analytical characterization:
- Useful for assessing toxicity potential, but can also be applied to assessing HDI potential.
- Enables further data mining of literature for HDI information.
- Are there any structure-activity relationship (SAR) alerts for individual constituents of the herbal extract/constituent?
- Quantitation of individual constituents can be useful in predicting potential exposure levels, designing *in vitro* studies, or whether additional testing is necessary (cost effective).

Dose performance:
- Disintegration of dose form
- Dissolution of constituents
- Physical-chemical data on constituents
- Solubility information on extract/constituents

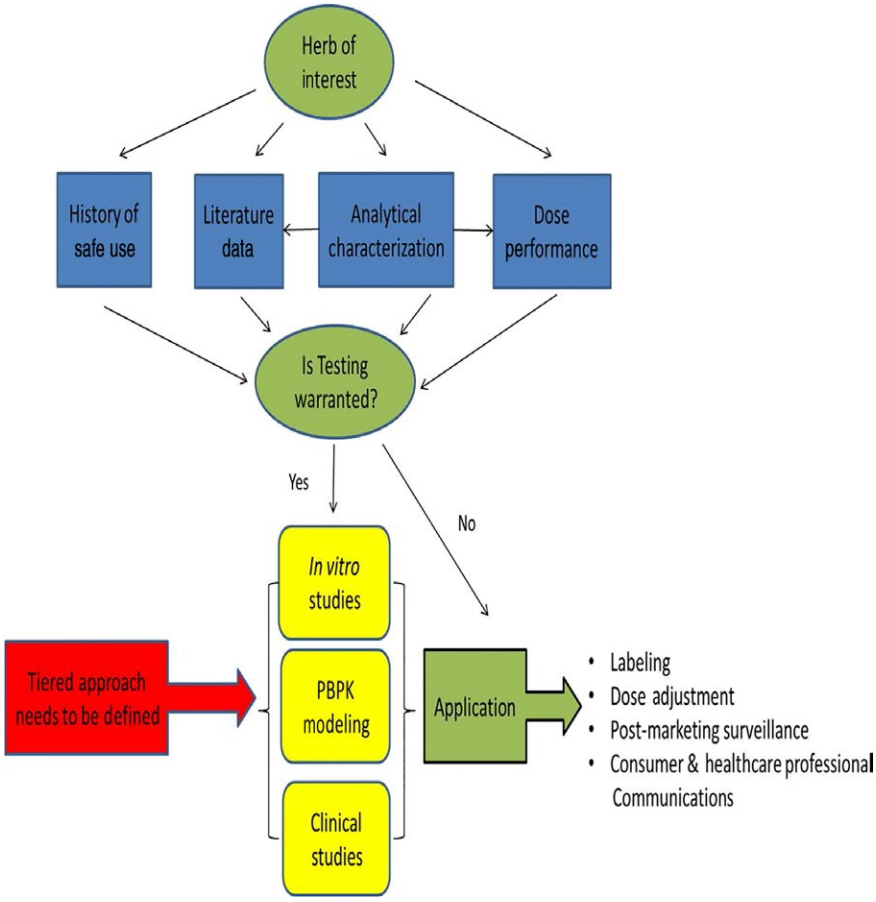

Figure 2 Key components of a framework for assessing botanical-drug interactions; ©2015 American Botanical Council. Reprinted with permission.

are available for one or more of these end points, the typical approach is to conduct traditional *in vitro* and *in vivo* animal toxicity studies to generate safety data on the botanical preparation. When botanical safety has been sufficiently characterized at the level of its phytochemical constituents, these toxicity studies become more meaningful. Using the *in silico* approach, a safety assessor can assess hazards and establish exposure levels of low safety concern for each phytochemical constituent by conducting a risk assessment using data on a constituent itself, or by using well-established toxicology assessment tools, such as TTC or SAR, for those constituents with insufficient toxicity data.[35,36] An SAR approach utilizes available toxicity data on structurally similar analogs of the phytochemical constituent in question. Additionally, phytochemical constituent structures with potential data gaps can be processed through rule-based software programs, such as DEREK, to evaluate them for possible structural alerts for various toxicity end points or ontologies where available (e.g., DART).[37] Alerts should be considered cautionary evidence and are not themselves definitive.

SPECIAL POPULATIONS—PREGNANT/BREASTFEEDING WOMEN AND CHILDREN

It is generally recognized that medicines and supplements should not be taken during pregnancy/breastfeeding unless the benefit to the mother outweighs any possible risk to the fetus or nursing infant. One of the major problems in drawing conclusions on the risk:benefit associated with an ingredient used during such periods is a lack of safety information (i.e., limited information during pregnancy or breastfeeding). Similarly, such data are often lacking in children. Furthermore, there is a subsection of products that are specifically designed to support pregnancy/nursing health and well-being, as well as child growth and development.[38] To ensure minimal risk to these special populations, ingredients used in products intended for these populations should be supported by appropriate toxicity data and/or a rationale for low toxicity risk based on SHU during pregnancy/period of nursing or infant/child development. Alternatively, if safety (or preclinical and/or clinical data) data are indicated for these populations, the ingredient may be used at sufficiently low levels in the formula to assure adequate margins of safety. Otherwise, in the absence of data to conclude safe use at a defined dose for the consumer, the mother or unborn/nursing infant or developing child, the ingredient should not be targeted toward pregnant/nursing women unless data gaps are satisfied through testing.

SUMMARY, CHALLENGES, AND FUTURE DIRECTIONS

Continued interest in botanical ingredients, particularly as botanical dietary supplements, coupled with challenges of testing these complex mixtures of phytochemical constituents, and cost, time,

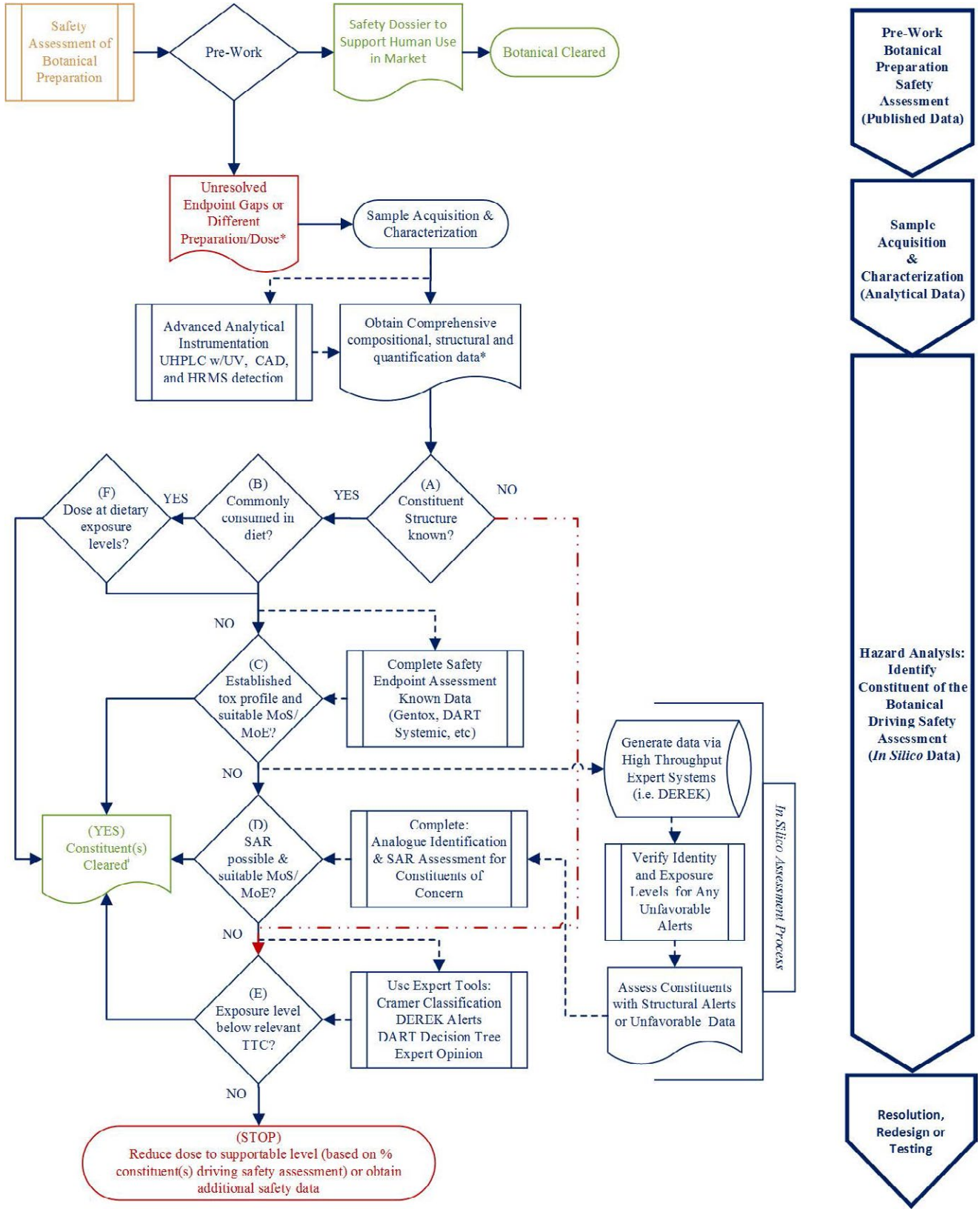

Figure 3 Decision tree for botanical constituent(s). CAD, Charged Aerosol Detector; HRMS, High Resolution Mass Spectrometry; MoE, Margin of Exposure; MoS, Margin of Safety; SAR, structure-activity relationship; TTC, threshold of toxicological concern; UHPLC, ultra-high-performance liquid chromatography. Reprinted from Food and Chemical Toxicology, Vol 107, Little, J., Marsman, D., Baker, T. & Mahony, C., In silico approach to safety of botanical dietary supplement ingredients utilizing constituent-level characterization. 418–429, (C) 2017, with permission from Elsevier.

and animal usage constraints, presents some unique challenges for this category of ingredients. For example, critical data end points that are often lacking may include raw material characterization, DART, genotoxicity/carcinogenicity testing, and/or ADME considerations (including botanical-drug and botanical-botanical interactions). Across the dietary supplement industry, there are various approaches for safety assessment of botanicals intended for use in food/dietary supplements, from heavy reliance on capturing adverse events via post-marketing surveillance, to tiered approaches building an assessment based on existing data and new data generated specifically to meet safety assessment needs as presented here.

We present a tiered-approach and proposed *in silico* decision tree methodology for botanical safety evaluation that provides a useful and pragmatic approach based on dietary intake levels and sufficient safe margins of exposure from existing safety information. We have also borrowed from traditional chemical-based safety assessment methodologies to include TTC and SAR approaches for establishing safe exposure thresholds. Although more work remains to be done, our botanical safety assessment approach begins to evaluate the critical data end points mentioned previously using more recent and advanced toxicology methodologies that are intended to eliminate or reduce the need for animal testing.

We believe that emphasis should be put against *in vitro* testing approaches to address critical end points where safety data are lacking. For example, high throughput approaches are assisting in characterizing the toxicological mode of action of botanical mixtures by way of gene expression studies, which allows for identification of functional analogues through the Connectivity Mapping Approach,[39] and receptor binding and enzyme activity data, which allows for identification of molecular targets.[40] These data streams (and extensions thereof) seem promising for follow-up on safety questions identified by the *in silico* decision tree methodology, either by informing on a lack of interaction with DART relevant targets or by enabling potency comparisons of *in vitro* targets, which can be linked to DART data for functional analogue(s) and used to judge whether human exposures would likely require follow-up (unpublished data Vandermolen, K., Naciff, J., Daston, G., & Mahony, C.). In the area of assessing BDI, utility of more physiologically relevant *in vitro* models, including human hepatocytes that are fully functional for uptake and efflux transporters, metabolism, and requisite regulatory pathways are now being used.[33] These advanced models, coupled with PBPK modeling techniques may be more useful for predicting clinical relevance of BDI, and/or aid in the design of follow-on clinical studies.

Central to our approach is the use of an advanced analytical method, using multiple simultaneous detectors, to adequately characterize botanical constituents sufficiently to enable the application of these modeling tools to botanical ingredients. In fact, the need to further develop and validate analytical methods to enable complete chemical characterization of complex botanical mixtures has been identified as a critical need by other experts in the field of botanical safety assessment.[41]

Future focus should include the investigation of relative source contributions or exposure source allocation factors in the assessment, as well as a dedicated effort toward building a database of chemical constituents of food substance mixtures (important for both botanical dietary supplements and botanical-containing consumer products). An emerging need is guidance on how to generate and interpret ADME-related data on complex botanical mixtures in order to extrapolate from *in vitro* assays to *in vivo* animal toxicity studies and ultimately to human exposures. The question of which marker of the botanical constituent(s) to extrapolate with across these studies is challenging. A "Best Practices" for characterizing ADME of botanicals should address which and how many constituents should be followed, and the best method(s) of analysis to use (unpublished data Ryan, K. *et al.*). Addressing these additional challenges will further support positive assurances of safety in the use of botanical preparations across various consumer product categories; and may ultimately lead to more innovative botanical-based products, including dietary supplements.

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CONFLICT OF INTEREST

Authors work for a company that manufactures and distributes dietary supplements.

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