Inferring Toxicological Similarity with Multidimensional Relational Analysis

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Ginkgo biloba extract (GBE; CASRN 90045-36-6) is a commonly used herbal supplement. A comprehensive toxicological assessment by the NTP identified GBE as hepatotoxic. The constituents of GBE can vary across lots, hence the question arises as to how representative the lot tested by NTP is of other lots of GBE and by extension to what degree the results of the NTP hazard characterization are more broadly applicable to the various GBEs on the market. In order to get a detailed fingerprint of the biological activity of five chemically distinct lots of GBE, extracts were administered at multiple dose levels to rats for five days after which global liver gene expression was examined. Genomic bench mark dose modeling was performed to provide detailed quantitative and qualitative description of the biological effects of the test articles. The GBE tested by NTP in its initial toxicological characterization had the overall largest and most potent effect on gene expression in the liver. A variety of multidimensional relational metrics found that two lots of GBE were similar in their biological effects to the lot used by NTP for their toxicological assessment. The two lots showing the greatest degree of multidimensional biological similarity also showed the highest degree of chemical fingerprint similarity, suggesting that in this case study chemical fingerprint similarity provides good approximation of biological similarity and can likely be used to identify lots of GBE that would be forecasted to have similar toxicological properties to the lot that has gone through comprehensive toxicological testing by the NTP.

The Complexity of Botanical Supplements

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Natural product research encompasses a spectrum of investigations ranging from new compound discovery to safety and efficacy studies. Common to such investigations is the need to demonstrate integrity and reproducibility of test articles. This effort includes assuring that biomass is properly identified and herbarium specimens cataloged and determining that quantitative measurements of phytochemicals are accurate and precise. Modern research on botanicals may include discovery of bioactive phytochemicals, including investigations of synergistic effects of complex mixtures in the botanical matrix. In the phytomedicine field, botanicals and their contained mixtures are considered the active pharmaceutical ingredient (API). Unlike single-chemical APIs, botanical products are variable because their composition depends on genotypic and phenotypic variation, geographical origin, weather exposure, harvesting practices, and processing methods. Complicating matters is the wide variety of product types in the marketplace ranging from relatively unprocessed dried pieces, powders, and teas made from a single plant species to highly processed concentrates, metabolites, constituents, and extracts of single or multiple plant species. As opposed to relatively unprocessed biomass, some highly processed ingredients such as native extracts and dry extracts are essentially manufactured materials - the nature and composition of which are defined by the processes used in their manufacture. Different proprietary processes used on biomass that is nominally the same will predictably result in raw materials that are chemically and biologically different from each other. These sometimes accidental and sometimes intentional variabilities in raw material composition can result in inconsistent research materials and commercial products that fundamentally different from one another even when the starting materials (biomass) are nominally the same for each. These differences can have implications for the bioactivity profile of the ingredients and should be addressed prior to the start of any investigation.

Integrating Biological and Chemical Datasets to Identify Active Constituents of Natural Products

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The critical barrier to effective clinical application of botanical medicines is the challenge of dealing with their complexity and variability. Complexity limits effective quality control and stymies efforts to design clinical trials. As a means of addressing this problem, there is a need to identify specific "marker compounds" of botanical extracts that can be used for quality control and standardization. The ideal marker compound is a compound responsible for the desired biological activity, and may vary depending on the desired therapeutic application. For effective marker compound identification, it is necessary to integrate biological data with chemical data. Even as –omics technologies have exploded, enabling the generation of very large and complex chemical datasets, the meaningful integration of these chemical datasets with biological data has remained a difficult bottleneck. This research presentation will illustrate the application of biochemometrics to address this bottleneck. Specific examples involving identification of antimicrobial metabolites from fungi and the selection of green tea products for clinical drug-interaction studies will be discussed.

Achieving Enhanced Benefit from Herbal Products for Personalized Cancer Chemotherapy – Efficacy and Safety Considerations

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Cancer is currently the number one killer of all diseases worldwide. One important factor for this high mortality is the development of chemotherapy resistance due to genetic heterogeneity and tumor physiology (environment). Recently, certain herbal product/compounds have been found to exert two important cytotoxic effects in resistant cancer cells at relatively low concentrations: chemosensitizing effect (reversing anticancer drug resistance when combined with a particular anticancer drug) and collateral sensitivity (selectively inhibit the growth or kill the resistant cells more than the non-resistant parent cells). Such products thus may be capable of achieving new therapeutic benefit in resistant cancer. One potential approach is applying a cycling or sequential treatment strategy based on these effects to individual patients whose cancer cell is sensitive to the herb. Since these herbs are usually already used in many patients with other diseases, their development cost and subsequent cost to patient can be considerably less than that of a new medicinal entity. Nevertheless, before application to treat individual patients, studies using appropriate culture and drug sensitivity testing as well as dose finding of an effective and safe regimen will be required. This presentation will further discuss potential techniques for pre-clinical studies to translate to future clinical trial of herbs for resistant cancer.

Poisonous Plant Active Constituents: Challenges of Natural Diversity

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We all understand the term “Poisonous” is a relative term and mostly determined by “Dose” and in cases of poisoning it is a direct result of an “overdose”. In our attempts to understand and ultimately control the dose of certain “poisonous” plants in the natural world, many of our challenges are a result of nature’s own diversity. We have the best analytical capabilities but many of the challenges in identification of the active toxins are posed by the diversity that is present in both the plants we study and the animals that may be exposed to those plants. Examples of plant diversity in toxin concentration, presence and absence, and geographical locations will be presented from case studies of *Delphinium barbeyi* (Larkspurs), *Oxytropis* and *Astragalus* (locoweeds) and *Gutierrezia* (broom snakeweed) plants. Diversity in animal response to various toxins can be demonstrated in species selectivity towards certain reproductive toxins, with examples taken from the teratogenic alkaloid anagyrine and the abortifacient compound isocupressic acid. Within a species, diversity will be demonstrated by recent data showing breed susceptibility to toxic larkspur alkaloids. Such natural diversity poses similar challenges in addressing the safety and use of botanical dietary supplements.

Statistical Strategy for Determining Sufficient Similarity of Related Botanicals: A Case Study of Ginkgo biloba Extract

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Equivalence testing methods have recently been applied to test for sufficient similarity comparing benchmark dose (BMD) estimates between a reference mixture (with available experimental *in vivo* data) and observed mixtures (eg., from exposure data) in data rich and data poor cases. The similarity region is defined by the radius around the BMD of the reference mixture (e.g., associated with a benchmark response of 1 SD above the control mean), where the radius is the distance between the BMD and an *a priori* selected effective dose (e.g., the dose associated with an additional increase in response of a specified level; or, based on the distance ordering among positive and negative controls). The database available to apply this approach to the analysis of herbals is developing. This talk will describe the method through an illustration and then address the data/decisions (e.g., study design, biological judgment about similarity bounds) required to use the approach. The methods are illustrated using mixtures of *ginkgo biloba* extract (GBE; Rider et al 2013) from different suppliers and lots. A reference mixture was experimentally evaluated in a 5-day toxicity dose-response study in rats measuring hepatotoxicity. The method identified at least 91% of the 22 mixtures as being sufficiently similar to the reference mixture.

Practical Considerations when Designing Clinical Herb-drug Interaction Studies

Bill Gurley, Ph.D., University of Arkansas for Medical Sciences, College of Pharmacy, Little Rock, AR

The medical literature is awash in contradictory findings from clinical herb-drug interaction studies. Such discrepancies stem from the fact that botanical dietary supplements are much unlike conventional drug dosage forms. In turn, clinicians unfamiliar with the eccentricities of these products often fail to account for them when designing herb-drug interaction studies. Botanical dietary supplements are not created equally; therefore, extra safeguards must be considered when designing and implementing clinical herb-drug interaction studies. Numerous influences affect phytochemical content and quality. Thus, it is imperative to utilize products manufactured according to current good manufacturing practices and to have their phytochemical content independently characterized and verified. (Product adulteration and contamination still plague the industry.) Failure to characterize basic pharmaceutical parameters like disintegration time and phytochemical dissolution profiles for botanical dosage forms can profoundly impact study outcomes. Unlike conventional oral drug dosage forms, dissolution testing is not required for botanical dietary supplements. Poor dosage performance can lead to misinterpretation of clinical findings. Utilization of proper probe substrates is essential when assessing the effect of botanical-mediated changes on specific drug metabolizing enzymes and/or transporters. In addition, failure to screen subjects for enzyme or transporter polymorphisms can lead to misinterpretation of phytochemical-mediated changes, or lack thereof. Finally, statistical significance does not always equate to clinical significance. Inclusion of positive controls for enzyme/transporter inhibition (e.g., clarithromycin, quinidine) or induction (e.g., rifampin) allow for the clinical relevance of any observed botanical-mediated changes in probe substrate pharmacokinetics to be assessed. Examples of these concerns will be presented.

Characterization of Botanical Materials Using Chemometric Methods

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Metabolite fingerprinting coupled with chemometric methods has proven to be a powerful tool for identifying compositional differences between plant materials. Chromatographic or spectral fingerprints from the powders or extracts of whole plant materials provide complex data patterns that have been used to identify genus, species, cultivar, growing site, growing year, harvest time, cultivation mode, and post-harvest processing. The methodology has also been used for authentication of botanical materials based on the comparison of the test sample to a collection of vouchered or authenticated samples. In this presentation, the application of chemometric methods to authentication of botanical materials will be illustrated by examining 26 commercial supplements of *Ginkgo biloba* collected by NIEHS and 2 Standard Reference Materials (SRM 3246 spray dried ginkgo extract and SRM 3248 ginkgo extract tablets). The chromatographic fingerprints were examined before and after hydrolysis. In addition, chromatograms for 12 pure standards were used to identify and quantify specific components. In general, 70% of the supplements fail to have the expected fingerprints, i.e. components were missing, unexpected components were present, or the ratios of the peaks were unexpected.

The Quest for Rigor and Reproducibility in Botanical Research

Craig Hopp, Ph.D., National Center for Complementary and Integrative Health (NCCIH), National Institutes of Health (NIH), Bethesda, MD

In response to reports highlighting the lack of reproducibility of published scientific research, NIH recently has placed an increased emphasis on the rigor and reproducibility of research supported through NIH grants. Due to the inherent variability in botanical products, the challenge of reproducibility in scientific research is something NCCIH has been actively managing for over 10 years. The primary way in which NCCIH endeavors to maximize the rigor and reproducibility of our funded botanical research is through our Product Integrity Policy. This policy places a strong emphasis on careful and thorough product characterization before initiating the research, including a requirement for independent verification of supplier specifications. The policy also emphasizes the need for ongoing product monitoring to confirm the stability of the product throughout the study. This presentation will provide details about this policy as well as examples demonstrating how it has helped improve the rigor and reproducibility of NIH supported research on botanical products.

Why Do We Care About Active Constituents?

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Man has always lived in a very dynamic environment. In recent history, many consumers have turned to dietary supplements as a source of dietary augmentation. As a result, individuals are consuming products that may vary in composition based on non-standardized harvesting and manufacturing practices. The food that we ingest is likewise dynamic, with the quality varying in response to weather, soil conditions, pests and harvest conditions, storage conditions, and preparation including cooking. The quality of meats and other animal products is likewise subject to variations in the animal environment, including the diets of the animals. Throughout the history of man, his/her health status is highly subject to the quality of the food, including herbs and dietary supplements, animal products and water that is ingested. In today's complex diets, food and other materials are being imported from many sources which may be from a different continent. It is entirely possible that an individual may purchase and consume a fruit or commodity today, and the next week or month, the source is from a different originator with a similar or different environment. This presentation will focus on the importance of delineating the causative agent that is responsible for the adverse event by focusing on two exposure scenarios: 1, the production of fumonisin B_1 by *Fusarium* species of fungi that contaminate food crops; 2, the putative active ingredient (aloin) in aloe vera and recent studies that detected cancer induction in rats. Understanding the active ingredient, and toxic ingredients, is especially necessary during this age where foods arise from non-local sources, and where differences in the environment can drive plants and fungi to produce widely different toxic products. Additionally, it is important to have quantitative procedures with which to measure the potential toxicity of a food to the consuming public. In light of this, it is critical to understand the active constituents in consumed materials, and develop quantitative assays for these active constituents.

The contents of this abstract should not be interpreted as current or future opinion or policy of the U.S. Food & Drug Administration.

Targeted Analysis of Herbs: Markers, Actives, Natural Toxins, and More

Kerri L. LeVanseler, Ph.D., Director, Chemistry Laboratory, NSF International, Ann Arbor, MI

In the study of herbal medicines and natural products, there is interest in understanding the chemical constituents that contribute to the properties of the material. Attempts are made to utilize analysis of individual chemicals or classes of chemicals to correlate to nutritional, health and efficacy benefits, toxicity, quality assessments and as an identification tool. Natural products present an analytical challenge due to the complexity and biodiversity of natural product samples. The presentation will focus on targeted analysis approaches employed for Ginkgo, Echinacea, and Black Cohosh evaluations. Advantages and disadvantages of the methods and techniques will be discussed. Specific attention will be given to the method's ability to detect adulteration in Ginkgo extract ingredients and products.

Challenges to Identifying Active Constituents

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Two challenges will be addressed. Firstly, there are two key methodological issues concerning the selection of appropriate test materials for studying bioactives in botanical dietary supplements. The polarity of solvent used for extracting phytochemicals will greatly affect the distribution of bioactives and the related bioactivity. A case with ginseng will be presented as an example. Another question is whether total extract or its sub-fractions should be used to identify key bioactive constituents. The concept of polypharmacology and the principles of Chinese decoction formulation will be introduced to discuss challenges in using the fractionation approach. Secondly, challenges in dealing with polysaccharides that are present in many botanical health products will be discussed. These are high molecular weight components derived mostly from cell walls and are not plant secondary metabolites. Recent studies have shown that they possess a wide range of significant pharmacological activity. They usually exist as heterogeneous mixture, and we have limited methodology to identify and quantify specific bioactive polysaccharides and their metabolites in biological system. Study on ginseng polysaccharides and polysaccharide-peptides derived from a medicinal mushroom (Coriolus versicolor) will be the focus for discussion.

Ensuring Safety of Botanical Dietary Supplements – The Industry's Role
Douglas MacKay, N.D., Council for Responsible Nutrition, Washington, DC

Humans have safely used herbs and botanicals as food and medicine for thousands of years. Traditional medicine systems, such as Ayurveda and Traditional Chinese Medicine (TCM), still include herbal therapy as a central modality. The WHO estimates that 80% of people worldwide rely on herbal medicines for some part of their health care. However, in societies that practice modern Western medicine, use of pharmaceutical drugs has virtually replaced use of herbs and herbal medicines.

With the growing public health emphasis on prevention, rather than treatment of disease, herbal and botanical dietary supplements have re-emerged as a popular choice for Americans to promote and maintain health. This spurred renewed interest in the safety of botanical dietary supplements. In the U.S., pharmaceutical drugs are the accepted treatment for most medical conditions, and subsequently the scientific paradigm used to evaluate the efficacy and safety of drugs has become the "gold standard" of safety and efficacy assessment. Using a drug paradigm for the scientific evaluation of botanical dietary supplements poses several unique challenges because of their inherent complexity and variability. Douglas MacKay, ND will present information on the roles and responsibilities of dietary supplement manufacturers in ensuring botanical dietary supplement safety. Dr. MacKay will also share an industry perspective on the National Toxicology Program's (NTP) Botanical Dietary Supplement Program.

Quantitative Prediction and Clinical Evaluation of Herb-Drug Interactions
Mary Paine, Ph.D., Washington State University, Spokane, Washington

Quantitative prediction of pharmacokinetic (PK) interactions between conventional medications and herbal products is an ongoing challenge. A systematic, translational approach was applied to a potential herb-drug interaction to evaluate a mechanism not previously explored in humans, specifically, inhibition of intestinal glucuronidation mediated by UDP-glucuronosyltransferase 1A (UGT1A). The semi-purified milk thistle product, silibinin, was selected as an exemplar herbal product precipitant, and raloxifene was selected as the intestinal UGT1A object drug. Requisite kinetic parameters were recovered using human intestinal microsomes and intestinal UGT1A-overexpressing HEK cell lysates. A simple static model predicted a 4-and 3-fold increase in raloxifene AUC in the presence of silibinin and a single constituent (silybin A or silybin B), respectively, indicating high interaction risk. A physiologically-based PK model was developed and used to determine the dynamic nature of this interaction and to inform clinical study design of a proof-of-concept healthy volunteer study. Clinical PK endpoints (geometric mean AUC and C_{max} ratio) were consistent with model predictions, providing credence to this systematic approach, as well a quantitative framework that could be applied to other herb-drug combinations. Successful quantitative predictions of herb-drug interactions would aid in establishing guidelines to identify clinically meaningful interactions prospectively, providing evidence-based information to both health care providers and patients about the risk or safety of adding a given herbal product to a conventional drug regimen.

Evaluating Sufficient Similarity among Whole Mixtures of Environmental Chemicals

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Many complex mixtures in the environment contain hundreds of component chemicals. The composition of such mixtures can vary over time, place or different conditions under which the mixture is produced, released, or encountered in the environment. When assessing human health risks associated with exposures to complex environmental mixtures, risk assessors prefer whole mixtures risk assessment approaches over component-based approaches that rely on single chemical data, because they require fewer assumptions and account for interactions among chemical constituents. Due to limitations in available dose-response data on whole mixtures of concern in the environment, it could be necessary to utilize dose-response information from a surrogate mixture that has been tested toxicologically and is judged to be sufficiently similar. However, methods for evaluating the similarity of a surrogate whole mixture to mixture(s) of concern have not been widely applied to date. This presentation will describe general concepts of sufficient similarity and highlight a biostatistical approach for evaluating sufficient similarity that uses principal component analysis of chemical and biological data from multiple whole mixtures. This method, among others, offers promise for increased application of whole mixtures risk assessment procedures. (This abstract does not reflect US EPA policy.)

How Similar Is Similar Enough? Case Studies Exploring Phytoequivalence of Botanicals

Cynthia Rider, Ph.D., Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Botanical dietary supplements are complex mixtures that can exhibit widely varying composition in products on the marketplace. Typically, a single test article is selected for evaluation of safety and there is an assumption that the test article is representative of other available products with similar labels. However, it is not clear how chemical differences among botanicals that have the same or similar plant source or label relate to the biological activity of those samples. In effect, what degree of chemical similarity is required to ensure that a representative sample is really representative? In order to develop methods for determining phytoequivalence (or sufficient similarity) of botanicals, the National Toxicology Program has developed case studies with *Ginkgo biloba* extract, black cohosh extract, and Echinacea. These botanicals were selected because they offer a range of chemical and biological profiles. *Ginkgo biloba* extract has a well-characterized chemical profile with known biologically-active marker constituents, while black cohosh and Echinacea are less well-characterized and exhibit different levels of biological activity. In each case, multiple lots of the botanical have been procured, undergone chemical analysis, and been evaluated for biological activity in *in vitro* assays. These case studies will provide a starting place for discussion of chemical similarity, biological similarity, and statistical methods for comparing across samples.

Assessing Herb-Drug Interactions: Screening Approaches

Amy Roe, Ph.D., DABT, The Procter & Gamble Company, Cincinnati, OH

The use of dietary supplements, including herbal medicines, by North Americans continues to increase across all age groups. This population has access to conventional medications, with significant polypharmacy observed in older adults. Thus, the safety of the interactions between multi-ingredient dietary supplements and drugs is a topic of paramount importance. Considerations such as history of safe use, literature data from animal toxicity and human clinical studies, and herbal constituent characterization would provide guidance on whether to assess herb-drug interactions experimentally. The literature is replete with reports of various herbal extracts and constituents as potent inhibitors of drug metabolizing enzymes and transporters. However, without standard methods for herbal characterization or *in vitro* testing, extrapolating these reports to clinically-relevant herb-drug interactions is difficult. This lack of a clear definition of risk precludes clinicians and consumers from making informed decisions about the safety of taking many dietary supplements and herbal medicines with conventional medications. A framework is needed that describes an integrated robust approach for assessing herb-drug interactions. This presentation will focus on *in silico* and *in vitro* approaches that may be used to screen for herb-drug interaction potential in the context of a defined framework approach.

Genotoxicity of Cohoshes Assessed Using the *In Vitro* Micronucleus Assay

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Products containing black cohosh extract (BCE) are marketed world-wide for relief of gynecological ailments. Although the efficacy of BCE has been evaluated, its toxicity has not been well characterized. The NTP tested a sample of an ethanolic black cohosh extract that showed a chromatographic profile that was consistent with a black cohosh standard reference material and with Remifemin®, a commercial formulation of black cohosh. This sample induced significant, dose-related increases in micronucleated reticulocytes (MN-RET) in the peripheral blood of female Wistar Han rats and B6C3F1 mice administered 15-1000 mg BCE/kg/day by gavage for 90 days. Significant, dose-dependent increases in mature erythrocytes (MN-E) were also induced in mice. In the two-year rodent cancer bioassay, significant, dose-dependent increases in MN-RET and MN-E were observed in female B6C3F1 mice at interim time points of 90 days and one year. Due to the heterogeneity of botanicals, we investigated whether cohosh samples in general exhibit genotoxic effects using the *in vitro* micronucleus assay. After confirming that the initial cohosh sample was genotoxic in human lymphoblastoid TK6 cells, an additional 11 cohosh extract samples were tested. Several samples met the criteria for a positive call for genotoxicity within the tested dose range. A targeted analysis of available marker terpene glycoside and polyphenol data did not reveal a chemical profile that correlated with cohosh genotoxicity. An untargeted chemical analysis with additional biological endpoints may provide more effective differentiation of cohosh samples.

Identification of Active Compounds in Botanical Dietary Supplements

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Many drugs in use today are natural products or derivatives of natural products. Despite the complexity of botanical dietary supplements, active compounds in these products can clearly still be identified. First, mechanisms of action need to be determined and then targets identified and validated. Based on this information, bioassays can be developed and used to guide the fractionation of botanical extracts with the goal of isolating the active constituents. Affinity assays based on liquid chromatography-mass spectrometry, many originally developed for high-throughput screening of combinatorial libraries, are being optimized for extract screening and can expedite bioassay-guided fractionation. High resolution MS/MS is ideal for dereplication (the rapid identification of previously characterized natural products) of active natural products. For structure determination, new natural products require additional spectroscopic analysis and interpretation using a combination of NMR, IR, UV, and/or X-ray crystallography.

Tracking Toxic Constituents in Botanicals: The Right Sample and the Right Question

Larry Walker, Ph.D., Amar Chittiboyina, Ph.D., Bharathi Avula, Ph.D., Christina Avonto, Ph.D., Yan-Hong Wang, Ph.D., Mohammad K. Ashfaq, Ph.D., Vamshi Manda, Ph.D., Shabana Khan, Ph.D., Mahmoud ElSohly, Ph.D., Ikhlas Khan, Ph.D., National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS

Although the safety record of botanical supplements, on the whole, is quite clean, there are some clear and striking examples of severe adverse events. Because of the lack of rigorous pre-market safety studies, often these first appear as isolated case reports which may or may not progress into case series or more direct evidence. Major obstacles to our understanding of these adverse effects derive from: 1) the questionable identity and authenticity of some botanical products; 2) the inherent complexity of botanical products and uncertainty about responsible constituents; 3) the lack of mechanistic understanding of the cellular targets for the adverse effects; 4) limited ability to replicate the observations in animal studies; and 5) the involvement of reactive metabolites, so that many standard *in vitro* approaches have limited relevance. To answer these questions and resolve the potential public health risks, involve considerable and wide-ranging expertise in botany, chemistry, toxicology, epidemiology, as well as knowledge of the market practices and legal/regulatory bases for action. Examples will be discussed of chemical adulteration, species misidentification, and sample processing with black cohosh, chamomile and other species, which highlight the challenges of tracking toxic constituents.

Understanding ADME of Botanicals: Challenges, Current Status, and Future Needs

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Botanical dietary supplements are complex mixtures containing one or more botanical product(s), each potentially containing multiple constituents responsible for efficacy and/or toxicity. ADME data and information on potential herb-herb and herb-drug interactions is critical to understanding the safety of botanicals. However, there is a paucity of ADME data on most botanicals, which may partly be due to: presence of large number of constituents and limited knowledge of active constituents; small percent of identified constituent-fraction; challenges in developing analytical methods to measure multiple and low levels of active constituents in systemic circulation; lack of consistency in the quality of products on the marketplace. The limited data in the literature utilize the traditional approach where ADME of either a single or multiple constituents is determined as representative of the complex mixture although the constituents(s) may or may not represent the active(s) of the botanical dietary supplement in question. Therefore, there is a critical need to develop approaches to generate relevant ADME data for complex botanical mixtures. Information from chemical analyses, in vitro and/or in vivo methods identifying active constituents, coupled with novel analytical chemistry and statistical approaches could be utilized to further our understanding of ADME of these complex mixtures.