



The Safety and Regulation of Natural Products Used as Foods and Food Ingredients

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The use of botanicals and dietary supplements derived from natural substances as an adjunct to an improved quality of life or for their purported medical benefits has become increasingly common in the United States. This review addresses the safety assessment and regulation of food products containing these substances by the U.S. Food and Drug Administration (FDA). The issue of safety is particularly critical given how little information is available on the toxicity of some of these products. The first section uses case studies for stevia and green tea extracts as examples of how FDA evaluates the safety of botanical and herbal products submitted for consideration as Generally Recognized as Safe under the Federal Food, Drug, and Cosmetics Act. The 1994 Dietary Supplement Health Education Act (DSHEA) created a regulatory framework for dietary supplements. The article also discusses the regulation of this class of dietary supplements under DSHEA and addresses the FDA experience in analyzing the safety of natural ingredients described in pre-market safety submissions. Lastly, we discuss an ongoing interagency collaboration to conduct safety testing of nominated dietary supplements.

Key Words: botanicals; dietary supplements; food; food ingredients; safety; regulation.

The art of healing comes from nature, not from the physician. Therefore the physician must start from nature, with an open mind. Attributed to Phillip von Hohenheim (aka Paracelsus), 1493–1541.

Poison is in everything, and nothing is without poison. The dosage makes it either a poison or a remedy. Attributed to Phillip von Hohenheim (aka Paracelsus), 1493–1541.

A large percentage of the U.S. population uses a botanical or nutritional or dietary supplement on a daily basis as an adjunct to an improved quality of life or for their alleged medical benefits. The regulation of botanicals by the U.S. Food and Drug Administration (FDA) is governed by the provisions of the Federal Food Drug and Cosmetic Act (FD&C Act). How FDA regulates the use of a substance is determined by its intended conditions of use. Products intended to diagnose, mitigate, treat, cure, or prevent disease are regulated by FDA as drugs. Drugs derived from botanical sources are outside the scope of this article. FDA's Center for Food Safety and Applied Nutrition (CFSAN) is responsible for regulating food ingredients and ensuring that those ingredients derived from botanical and other sources are safe and lawful. In 1994, the Dietary Supplement Health and Education Act (DSHEA) created a regulatory framework for dietary supplements that included provisions establishing current good manufacturing procedures, mechanisms for pre-market safety notifications for new ingredients, and a mechanism for establishing claims used in product labeling.

This article discusses botanical or other naturally derived product regulatory and/or safety assessment with emphasis on: (1) the regulation and safety assessment of botanicals and herbals submitted for consideration as food ingredients under the Generally Recognized as Safe (GRAS) provisions of the FD&C Act. This section uses case studies to illustrate the safety assessment process for products submitted to FDA under this program, (2) the regulation of these products as used under dietary supplement provisions of the FD&C Act. This section also presents a general framework for assessing the safety of these

products when they are new dietary ingredients (NDIs), and (3) an ongoing interagency collaborative effort to conduct safety assessment studies of nominated natural products used as dietary supplements.

FOOD INGREDIENTS DERIVED FROM BOTANICAL/ HERBAL SOURCES (Rebecca P. Danam and Gladys Erives)

Currently, there is an increased global interest in the use of botanicals or botanical-derived products as food ingredients because of the belief that they may be beneficial to health. Also, such products are commonly referred to as “natural” because their source is found in nature. An ingredient derived from a botanical/herbal source might require extensive purification for use in food or the botanical/herbal source itself might be the product added to food.

Through the FD&C Act, FDA is responsible for overseeing the safety of food ingredients (Under the FD&C Act, the legal status of a substance is dependent on whether it is offered for use in a conventional food or its use is as a dietary supplement or as a dietary ingredient in a dietary supplement product. This section of the article discusses only the use of a substance as an ingredient in conventional foods). The FD&C Act defines the term “food additive” to refer to any substance the intended use of which results in it becoming a component of food. The use of a food additive requires pre-market review and approval by FDA that results in a regulation prescribing safe conditions of use for the food additive. An exemption to the food additive definition, and as such, to the pre-market review and approval by FDA is for those substances that are generally recognized, among qualified experts, as having been adequately shown to be safe under their intended conditions of use (GRAS). The safety standard, “reasonable certainty of no harm” under the conditions of its intended use, is the same for all substances added to food as described under section 170.3(i) in Title 21 of the Code of Federal Regulations (CFR) (21 CFR 170.3(i)). Therefore, a GRAS substance is distinguished from a food additive based on the common knowledge about the safety of the substance for its intended use. GRAS status for a food ingredient can be achieved either based on the history of common use in foods before the year 1958 (21 CFR 170.30(c)(1) and 170.3(f)) or through scientific procedures (21 CFR 170.30(b) and 170.3(h)).

The basis for GRAS determination through experience based on common use in foods is rarely relied upon because it requires documentation that, prior to 1958, there was a substantial history of consumption for food use by a significant number of consumers using the same substance under similar conditions of intended use. For a substance to be GRAS through scientific procedures, the scientific data about the safety must be widely available and there must be consensus among qualified experts that the scientific data establish the substance to be safe under the conditions of its intended use. GRAS status does not require FDA’s determination that the intended use of a substance is

GRAS. Persons making an independent GRAS determination for a food ingredient can choose to inform the FDA of their determination that the use of a substance is GRAS and seek the agency’s review of the company’s basis for this determination by submitting a GRAS notice to FDA under the GRAS Notification Program (USFDA 1997).

Since the start of the GRAS Notification Program in 1997, more than 350 GRAS notices have been submitted. Of these, approximately 120 GRAS notices were for food ingredients that either are botanicals or derived from botanicals (CFSAN, FDA, 2011). Food ingredients from botanicals or botanical extracts present unique challenges for safety evaluation as they are complex mixtures of numerous chemical substances that exhibit compositional complexity and variation depending on several factors.

For the safety review of botanical substances, FDA considers the taxonomic identity of the source, the chemical identity, purity, and stability of the substance in question, its relationship to the article of commerce, description of the intended use, and consequent exposure. It is very important that the identity of the starting plant material is authenticated and standardized to ensure consistent quality of the desired final product. Safety studies should be conducted with the end product/substance that is substantially similar, if not identical to the article of commerce. Variations with respect to harvesting, storing, and processing methods can lead to compositional differences or contaminations and give rise to conflicting data rendering some test results irrelevant. Hence, validated methods are critical for the evaluation of pharmacological, toxicological, and clinical studies of botanical ingredients. Safety studies may need to be conducted on a single component of the mixture if toxicity is either known or suspected about a specific component. FDA considers on a case-by-case basis, different types of toxicological studies, including short- and long-term toxicity studies, metabolism and pharmacokinetic studies, reproductive and developmental toxicity studies, mutagenicity/carcinogenicity studies, immunotoxicity, and neurotoxicity studies to assess safety. Under the FD&C Act, only safety is considered when evaluating a substance added to food. The purported benefits of botanical/herbal ingredients are not considered in the safety assessment. FDA may consider such benefits or health claims in a separate notification process.

Herein, we discuss the different factors that affect the safety assessment of substances derived from botanicals either as highly purified single components or as “extracts” containing several components. As specific examples, we discuss two cases involving botanicals. The two cases, *Stevia rebaudiana* Bertoni (hereafter referred to as *S. rebaudiana* or stevia) and *Camellia sinensis* (hereafter referred to as *C. sinensis* or green tea extract), illustrate the various and complicated aspects involved in the process of the safety evaluation.

Stevia

Stevia rebaudiana leaves contain sweetening diterpene glycosides, known as steviol glycosides (SGs), which constitute

4–20% of the dry leaf weight. The leaf extracts are complex mixtures comprised of SGs (stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, dulcoside A, rubusoside, steviolbioside), labdane-type diterpenes, triterpenoids, steroids, volatile oils, and flavonoids (Kinghorn, 2002). FDA does not permit the use of crude whole-leaf extracts of stevia or uncharacterized stevia-derived compounds in conventional foods due to insufficient information to support its safety for such use and due to reports in the literature that raised safety concerns.

Initial toxicological studies in rats administered aqueous, crude, or partially purified stevia leaf extracts reported adverse reproductive (Planas and Kuc, 1968), renal, and cardiovascular (Melis, 1995, 1996) effects. For example, Planas and Kuc (1968) reported that female rats fed an aqueous leaf extract had reduced fertility. This finding was also confirmed in studies with female mice (Nuñez and Pereira, 1988). In addition, Melis (1999) reported that the crude leaf extract decreased fertility in the male rat. Traditionally, Guaraní Indians in Paraguay consumed the decoction of leaf extracts as an oral contraceptive (Kinghorn, 2002). These reports and other studies on its toxicity raised safety concerns about the use of stevia or stevia-derived substances. Recently, studies have been conducted with purified preparations of SGs, stevioside, rebaudioside A, or their degradation product steviol to address these safety issues.

Rebaudioside A and stevioside, the principal sweetening SGs of *S. rebaudiana*, were isolated and purified from the leaves, with 95% minimum purity (with respect to the total SGs content). Using these high-purity SGs, extensive toxicological *in vivo* studies were conducted in animal models as well as in humans. In particular, two subchronic toxicity studies of purified rebaudioside A in rats (Curry and Roberts, 2008; Nikiforov and Eapen, 2008), pharmacokinetic and metabolism studies in both rats (Roberts and Renwick, 2008) and humans (Wheeler *et al.*, 2008), and a two-generation reproductive/developmental toxicity study conducted with rebaudioside A in rats (Curry *et al.*, 2008) demonstrated lack of pharmacological activity and reproductive toxicity. Chronic toxicity studies on purified stevioside or rebaudioside A showed no adverse effects (Curry and Roberts, 2008; Toyoda *et al.*, 1997). In addition, clinical studies carried out in human volunteers to evaluate the potential pharmacological effects of rebaudioside A and stevioside on blood pressure and blood glucose levels indicated no adverse effects (Barriocanal *et al.*, 2008; Hsieh *et al.*, 2003; Maki *et al.*, 2008) in contrast to the previous studies conducted with crude leaf extracts or less pure SG preparations. The results of these recent toxicological studies, together with the clinical studies in humans, support the safety of high-purity ($\geq 95\%$) rebaudioside A, stevioside, or SG mixtures. The Joint FAO/WHO Expert Committee on Food Additives in 2008 reviewed the recent scientific data on SGs and established an acceptable daily intake for SGs as 0–4 mg/kg body weight (expressed as steviol equivalents).

Given these recent developments, FDA has received several GRAS notices since 2008 for high-purity rebaudioside A,

stevioside, or SG mixtures containing primarily rebaudioside A and stevioside. Based on the substantive toxicological data on high-purity SGs that is now publicly available, FDA had no questions about the notifier's determinations that these substances are GRAS for use as a sweetener in food. For the reasons described above, *S. rebaudiana* whole-leaf extracts or partially purified extracts containing low-purity SGs are not considered safe for use as food ingredients and FDA has an import alert in effect to exclude them from such use in U.S. commerce.

Green Tea Extract

Tea is the most commonly consumed beverage in the world, second only to water. Green, black, and oolong tea are all derived from the leaves of the *C. sinensis* plant. Tea has a long history of consumption as a beverage (hot water infusion) since ancient times. More recently, green tea extracts enriched with certain components are being consumed. They are also available as dietary supplements in the form of tablets and capsules.

Tea is a complex mixture comprised of several constituents. It is an abundant source of polyphenols, especially the complex group of compounds called flavonoids. Catechins (flavan-3-ols) are the major flavonoid compounds and contribute up to 30% of the dry weight of the tea leaf. The four major catechins in green tea are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). Their epimers include catechin (C), catechin-3-gallate (CG), gallic catechin (GC), and gallic catechin-3-gallate (GCG). Green tea also contains gallic acid, chlorogenic acid, alkaloids, namely theophylline, theobromine, and caffeine, volatile compounds, minerals, and a unique amino acid, L-theanine. The most abundant catechin, EGCG, is considered the most bioactive and the most studied.

The composition of green tea varies with growing conditions, horticultural practices, harvesting, and processing conditions. It is also influenced by environmental factors such as climate, season, the variety and age of the plant, as well as how the leaves are processed (Cabrera *et al.*, 2006).

Recently, green tea catechins (GTC) have garnered considerable attention in the popular press for their claimed health benefits such as “weight loss, reduction in body fat, maintenance of normal blood glucose levels and antioxidative properties.” Based on these purported beneficial effects, the marketing of concentrated green tea extracts and/or its components, particularly EGCG has been on the rise. Numerous studies in animal models using green tea extracts that contain catechins at various concentrations have attributed antioxidative, thermogenic, anti-carcinogenic, and anti-inflammatory properties to these GTC (Chacko *et al.*, 2010). In spite of these reported favorable effects, conflicting results have been reported from epidemiological studies and cancer intervention trials (Boehm *et al.*, 2009). Moreover, hepatic and intestinal toxicities associated with the

consumption of high doses of GTC preparations were reported in animal studies (Galati *et al.*, 2006; Isbrucker *et al.*, 2005). EGCG appears to act as an antioxidant as well as a pro-oxidant agent. The cytotoxic effects of EGCG and tea extracts to cancer cells have been attributed to their pro-oxidant effects (Weisburg *et al.*, 2004). EGCG-mediated mitochondrial toxicity and reactive oxygen species formation were implicated as the possible mechanism for the cytotoxicity to isolated rat hepatocytes and hepatotoxicity in mice (Galati *et al.*, 2006). Further, recent reviews of published case reports suggests a possible causal relationship between concentrated green tea extracts, particularly EGCG, and liver damage. A causative role has been suggested because the symptoms resolved upon cessation of consumption and reinjury were observed following rechallenge with the same tea-derived preparations. It is intriguing that the liver damage occurred mostly in women, suggesting gender differences in susceptibility (Mazzanti *et al.*, 2009; Sarma *et al.*, 2008). It is also reported that consumption of high doses of green tea could alter thyroid function adversely (Chandra and De, 2010).

Bioavailability is a factor positively associated with the severity of the toxicity of these catechins. For example, fasting increases the bioavailability of EGCG (Chow *et al.*, 2005; Isbrucker *et al.*, 2005) leading to increased severity of adverse effects when consumed on an empty stomach. Also, the risk of adverse effects is likely increased by factors that increase the bioavailability of these flavanols such as the genetic polymorphisms in metabolizing enzymes (Miller *et al.*, 2010) and herb-drug interactions. In light of the accumulating scientific evidence, it is becoming increasingly apparent that these catechins may have deleterious effects at pharmacological concentrations and in certain sensitive populations. Further research is necessary to better understand the nature and mechanism of action of the bioactive components, the differences in their bioavailability in humans and the safe range of consumption.

Conclusion

The two botanicals discussed above exemplify the complexity of the toxicological analysis of botanicals and the contrasting nature of toxicity scenarios presented by food ingredients derived from botanicals. In the case of *S. rebaudiana*, the whole-leaf extract was shown to exhibit toxic effects, whereas its purified constituents (SGs) were found to be safe for use as a sweetener. The reverse scenario is observed with *C. sinensis*. Green tea consumption has a long history of safe use whereas the isolated, purified, and concentrated catechin components of green tea appear to have adverse effects and currently are not considered safe for food ingredient use.

The safety assessment of botanical substances is complicated by various factors. Compositional diversity is a key factor because botanicals are complex mixtures, for which the identity of all the individual components is not known and the proportion of individual components varies with the source.

There are various other factors, among which are the lack of standardization of the botanical (in terms of both materials and analytical methods), lack of identity of the active ingredients, and the use of different formulations of the botanical in the article of commerce when compared with the test substance. The paucity of data on the toxicology of whole extracts or the individual components of botanicals makes it very challenging to determine the safety of botanical substances for use in conventional food. As such, the review of botanical substances for safe use in conventional food must be approached with some skepticism, an open mind and utilization of the full arsenal of scientific tools available to assess the safety of such substances. There is no set formula for dealing with the safety evaluation of such materials or combination of materials. Each new submission must be dealt with on a case-by-case basis.

NATURAL PRODUCTS USED AS DIETARY SUPPLEMENTS

(Corey J. Hilmas, Ali Abdel-Rahman, Njwen Anyangwe, Louis Carlucci, Steve Casper, Evaristus Enongene, Ramadevi Gudi, Fred Hines, Dan Levy, Ying Lin, Robert J. Moore, Erika A. Pfeiler, and Saleh Turujman, Daniel Fabricant)

The botanical and animal food sources we consume on a daily basis as part of the human diet contain a complex array of naturally occurring compounds with a wide range of chemical structures and physiological effects. The study and understanding of diet, its structural complexity, and its ability to both enhance and impair health presents a great scientific challenge to toxicologists, chemists, botanists, and microbiologists. The dietary supplement industry is an ever-increasing global market. In this section, the U.S. regulatory requirements for dietary supplements will be reviewed, followed by a discussion of the general framework for assessing the safety of botanical and herbal products as NDIs. Finally, examples from FDA response letters and landmark cases will highlight identity and safety issues regarding dietary supplements.

Overview of Regulation of Dietary Supplements in the United States

Dietary Supplement Use in the United States

Use of dietary supplements has increased over the past 20 years in the United States. Over one-half of the U.S. population report regularly consuming dietary supplements during 2003–2006 (Gahche *et al.*, 2011). Data from the National Health and Nutrition Examination Survey (NHANES) has been used to monitor use of dietary supplements since the 1970s. NHANES III data suggested that supplement use was highest among three population groups: children between 1 and 5 years of age, middle aged, and the elderly (Ervin *et al.*, 1999). The most recent analysis of NHANES (2003–2006) suggests that dietary supplement use is widespread among U.S. adults aged 20 and over (Gahche *et al.*, 2011). The percentage of the U.S.

population who used at least one dietary supplement increased from 42% in 1988–1994 to 53% in 2003–2006 (Gahche *et al.*, 2011). In particular, botanical products, more commonly used in older than younger age groups, are reportedly used by approximately 20% of all adults (Bailey *et al.*, 2011).

Between 1994 and 2000, dietary supplement sales increased 80% to near \$16 billion annually (Blendon *et al.*, 2001). Dietary supplement sales exceeded \$25 billion in 2008, and a recent report indicates that American consumers spent an estimated \$27 billion on dietary supplements in 2009 (Nutrition Business Journal, 2010). In 2009, 17 million U.S. adults were regular or heavy users of herbs or botanicals (Nutrition Business Journal, 2010). The upward trend in use of dietary supplements has been predicted to continue growing due to the aging baby boom generation, increased interest in self-sufficiency, and the rise in popularity of alternative botanical and herbal products over their pharmaceutical counterparts. In 2009, FDA estimated 55,600 dietary supplement products on the market (see Docket ID: FDA-2007-D-0209 [Document ID: FDA-2007-D-0209-0023]). In FY 2010, the CFSAN devoted 34 full-time equivalents and \$4.511 million for salaries and operating costs to dietary supplement work.

Regulatory Requirements for Dietary Supplements in the United States

The term “dietary supplement” is defined in section §201(ff) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act). Under the statutory definition, dietary supplements are defined as a food and must be one of several enumerated “dietary ingredients” such as a vitamin, mineral, or herb or other botanical. In addition, the product must be intended for ingestion and “not represented for use as a sole item of a meal or of the diet.”

For regulatory purposes in the United States, dietary supplements are regulated as foods under the FD&C Act, Title 21 Sections 301–399 of the United States Code (U.S.C.). Similar products may be defined and regulated in different ways in other countries. For example, in Canada, “natural health products” are a regulatory category separate from foods. Prior to 1994, the FD&C Act did not specifically define the term “dietary supplements” or the scope of products that could be dietary supplements. Through DSHEA, Congress amended the FD&C Act to define the term “dietary supplements” and establish a regulatory framework for safety and claims for dietary supplement products. DSHEA was intended to strike a balance between increased consumer access to dietary supplements and consumer protection against the toxicological health risks associated with consumption. See the following sections of the FD&C Act for the regulatory authority governing dietary supplements: §301 [21 U.S.C. §331] (Prohibited Acts), §402 [21 U.S.C. §342] (Adulterated Food), §411 [21 U.S.C. §350] (Vitamins and Minerals), and §413 [21 U.S.C. §350b] (New Dietary Ingredients).

The following example illustrates how the definition of a term like “ingestion,” when used in a statute, is ultimately defined in a legal context. Ener-B Nasal Gel was “a Vitamin B-12 supplement in gel form designed to be applied to the inside of the nose and absorbed into the blood stream through the nasal mucosal membranes” (72 F3d 285, 1995). In *United States versus Ten Cartons*, the U.S. district court “determined that Ener-B is not a ‘dietary supplement’ within the meaning of 21 U.S.C. Sec. 321(ff)” because “ingestion” means to take into the stomach and gastrointestinal tract by means of enteral administration through the mouth. “The district court concluded that Ener-B’s method of intake precludes its classification as a dietary supplement” (see 888 F Supp at 392–95).

Additional Federal Legislation on Dietary Supplements

Passage of DSHEA was followed by the Dietary Supplement and Nonprescription Drug Consumer Protection Act (Public Law 109-462) signed into law on 22 December 2006. This law amends the FD&C Act with respect to reporting of serious adverse events related to dietary supplements and nonprescription drugs. The law has four major provisions, (1) requires the collection of all adverse event reports by manufacturers, distributors, and retailers of dietary supplements; (2) requires the reporting of serious adverse event reports to the FDA; (3) requires firms to maintain records of reports of all adverse events and requires that FDA be allowed to inspect those records; and (4) requires that dietary supplement labels bear information to facilitate the reporting of serious adverse events associated with the use of dietary supplements by consumers. The requirements of this law became effective on 22 December 2007.

Although DSHEA amended the FD&C Act by adding dietary supplement-specific requirements, the statutory definition contained express language categorizing dietary supplements under the broader heading of foods in general. Accordingly, the legal requirements that apply to the manufacturing and marketing of dietary supplements are not limited solely to those found in DSHEA. For example, dietary supplement firms must register their physical facilities with the FDA under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (Pub. L. 107-188) and provide prior notice when importing dietary supplements or dietary supplement ingredients. Dietary supplements must also conform with labeling requirements imposed by DSHEA as well as other broader labeling amendments such as the Nutrition Labeling and Education Act of 1990 (Publ. L. 101-535) and Food Allergen Labeling and Consumer Protection Act of 2004 (Pub. L. 108-282).

Under the authority of the FD&C Act and the Public Health Service Act (42 U.S.C § 201 et seq.), the FDA has imposed a number of regulatory requirements that address quality and safety of dietary supplements. Regulations prescribing Current Good Manufacturing Practices require that all manufacturers and distributors of dietary supplements have in place

procedures to ensure quality, potency, and identity of dietary supplements (21 CFR 111). Manufacturers may also have to comply with other manufacturing regulations if their products fit within the scope of products subject to manufacturing regulations for seafood HACCP (21 CFR 123), juice HACCP (21 CFR 120), acidified foods (21 CFR 114), or low acid foods (21 CFR 113).

Dietary Supplement Safety

The safety of dietary supplements is addressed in §402 (Adulterated Food), which has provisions that apply to all foods and provisions specific to dietary supplements. The FD&C Act has adulteration standards specific to supplements including “significant and unreasonable risk of illness or injury” although the FD&C Act makes clear that the government bears the burden of proof in showing that a supplement is adulterated using this standard. Supplements are also adulterated if they are prepared, packed, or held under conditions that do not meet current good manufacturing practice regulations.

In addition, the DSHEA amended the FD&C Act to require a pre-market safety notification for some ingredients. Products containing NDIs are adulterated in the absence of a required notification or if there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. The statute specifies that the notification, when required, should include the “history of use or other evidence of safety that the dietary ingredient, when used under the conditions of use suggested or recommended in the labeling of the dietary supplement will reasonably be expected to be safe . . .” (see §413 of the FD&C Act).

General Framework for Assessing the Safety of Natural Products as New Dietary Ingredients

FDA has received over 410 NDI notifications containing some form of botanical ingredient as of February 2011. Botanical ingredients represent 61% of the entire NDI notification portfolio, which contains over 700 NDIs since

1994. Figure 1 illustrates the various ingredient types found in NDI notifications received by FDA to date and their representation as a percent among the entire NDI notification portfolio. This figure takes into account that NDIs often include more than one category due to the presence of multiple ingredients. The statute created a notification system whereby FDA is required to respond to the notification, acknowledging receipt, and after 90 days, posting the notification on the public docket. FDA does review the notifications and frequently comments on the adequacy of the basis for the safety of the product. Figure 2 represents the types of letters, issued by FDA, for NDIs derived from botanical products. Approximately 18% of letters are acknowledgements without objection or comment. The majority of FDA response letters provide comments on various aspects of identity and safety. The category of ‘Other’ represents letters where the filing date has been reset due to receipt of a substantial amendment from the notifier because of extra time required to evaluate the submission, as authorized by the regulation that implements the statute.

Identity

Establishing NDI identity is the first step toward evaluating safety of a dietary supplement product containing an NDI. If the identity of the ingredient is described only in a general way, it may become unclear how the NDI qualitatively and quantitatively relates to the substances described in safety evidence. Therefore, the relevance of the safety evidence as a basis for a reasonable expectation of safety of the NDI under the intended conditions of use becomes unclear. As a new ingredient, the NDI is typically different from previously consumed material, and relating the composition of the various versions of an ingredient to one another is often the subject of discussion in the notifications. The approaches to evaluating botanical and live microbial product NDIs will be addressed in the following section.

General Safety Considerations for Botanical Products

The statute refers to “history of use or other evidence of safety” as the basis for the safety of dietary supplements described in NDI

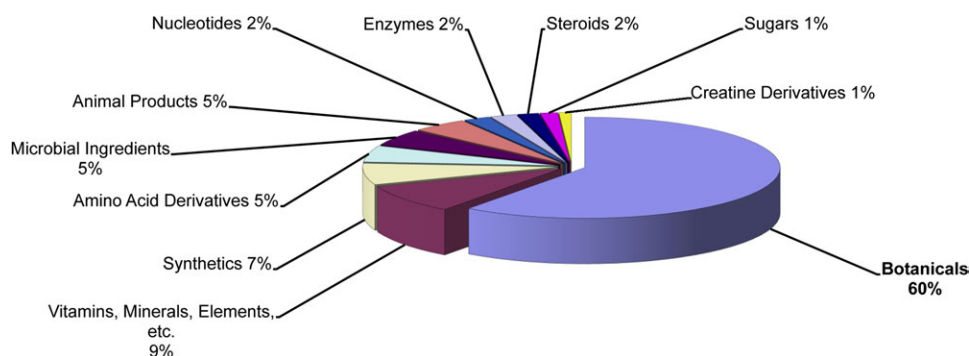


FIG. 1. Category types of NDIs received by FDA.

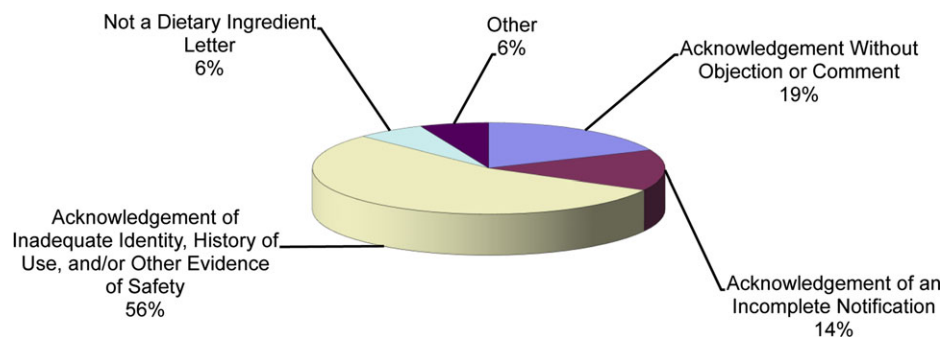


FIG. 2. FDA response letter types for NDIs involving botanical product ingredients.

notifications. Notifications typically include published literature and unpublished toxicology or clinical studies, in addition to descriptions of historically used materials that are the same as or in some ways similar to the new ingredient. One general method for ranking botanical ingredients according to possible safety concerns is to examine the history of use. Botanicals that are traditionally consumed as food rely on that history of food use as evidence of safety. A key consideration is the assumption that the conditions of use of the botanical are the same or very similar to the form, quantity, and frequency of consumption that is documented in the history of use. Foods are often consumed either intermittently over the course of time or seasonally. This pattern is different from many traditional medical uses such as when a botanical is used for a short period once or only intermittently to treat an acute medical condition. Safety problems that might be obvious once a large number of individuals begin regular daily use might not be evident when the botanical is used only intermittently.

Safety Considerations for Live Microbial Dietary Ingredients

The concept of natural products as dietary supplements includes live bacteria and yeast. Live microbial dietary ingredients (LMDIs), commonly promoted for probiotic properties, have gained popularity among consumers. Although fermented foods which retain live microorganisms have been consumed by humans for centuries, factors such as the use of newly isolated strains, changes in methods of industrial cultivation, or dosage at levels higher than those typically found in foods might alter the safety profile in the context of novel uses of the organisms. A recent evidence-based review of clinical literature on probiotics found significant gaps in knowledge about the safety of probiotics particularly with respect to chronic use and evaluation of frequency of infections (AHRQ, 2011). To date, FDA has received notifications for new LMDIs that include species of *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Saccharomyces*, *Streptococcus*, and *Clostridium*. Because of their uniqueness among natural products, safety reviews examine parameters based on the potential for particular risks of consuming LMDIs. A number of published assessments focus on the safety of these ingredients

(Bernardeau *et al.*, 2006; FAO/WHO, 2002; Meile *et al.*, 2008; Sanders *et al.*, 2010; Wassenaar and Klein, 2008; Wright, 2005).

Participants at a workshop on nomenclature for organisms used as probiotic food ingredients concluded that scientifically valid names for the genus and species should be used on the label and that the organisms should be identified below the species level to the strain (Sanders and Levy, 2011). Other factors that might be relevant to the safety of the ingredient include: (1) the number of viable microorganisms per serving, (2) properties of the organism that are dependent on the fermentation medium and growth conditions, (3) any phylogenetically related microbes that are pathogens that produce mammalian toxins, (4) the ability of the LMDI to persist in the gastrointestinal tract, and (5) the resistance of the LMDI to clinically important antibiotics with special attention given to any transferable genetic elements encoding antibiotic resistance genes (Sanders *et al.*, 2010).

History of Use

Although evaluation through toxicology or clinical studies are the most common ways notifiers have used to establish a reasonable expectation of safety (Section 413(a)(2) of the FD&C Act), a documented history of safe food use without any additional animal toxicity or human clinical data have also been used. Factors described in notifications relying on this strategy include (1) the dose (amount per serving), (2) duration of use, (3) frequency of intake, and (4) a comparison of the historically consumed material to the NDI. Exposure assessments for food frequently rely on estimations of a distribution of intake within the population (e.g., the amount consumed by the mean or the highest 90% of the population). Both the European Union (EU) and Canada expect at least 25 years of historical use data before the historical information can be used as a basis for a safety determination. The EU has spelled out their history of use criteria in the Council of the EU Regulation (EC) No. 509/2006 March on agricultural product and foodstuffs as traditional specialities guaranteed. FDA has not published guidance with respect to history of use for NDIs.

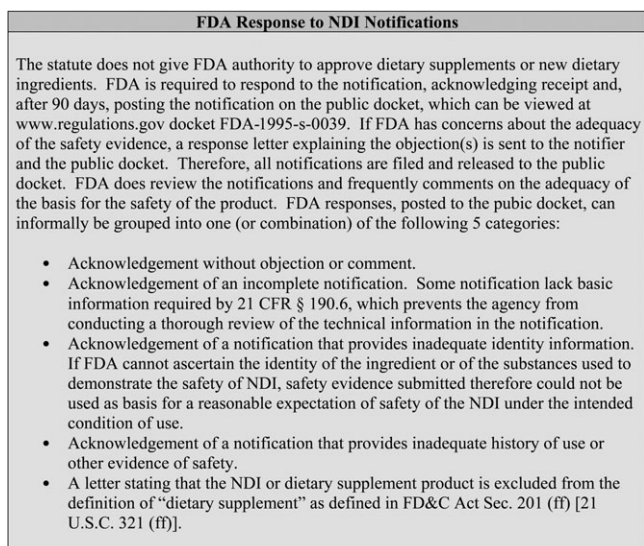


FIG. 3. FDA response letter to NDI notifications.

Evaluation of Other Evidence of Safety

FDA has not yet published guidance concerning the safety evaluation of NDIs, although a guidance document is under development. However, the agency receives approximately 50 NDI notifications a year, and the comments in FDA response letters, which raise concerns about the adequacy of the safety information in the notifications, illustrate some of the issues raised by the safety of natural products used in dietary supplements.

Evaluation of NDI Notifications and Dietary Supplements

The Division of Dietary Supplement Programs NDI Review Team in the Office of Nutrition, Labeling and Dietary Supplements at the Center for Food Safety and Applied Nutrition reviews NDI notifications. An informal characterization of the types of FDA response letters to those notifications is shown in Figure 3. The following sections describe issues raised in letters sent to notifiers.

NDIs Failing to Meet Administrative Requirements

The administrative requirements for an NDI submission in 21 CFR §190.6 are critical to evaluate an NDI submission. Notifications which do not comply with the basic information required in 21 CFR §190.6 generally cannot be reviewed for safety. The FDA letter concerning an ingredient made from *Toona sinensis*, dated 1 September 2005 in [Docket Report #339 (Docket ID: 95s-0316-rpt0294)], illustrates a response related to compliance with §190.6. "Federal regulations found at 21 CFR 190.6 specify the requirements for a pre-market notification for a new dietary ingredient. Your notification concerning '*Toona*

sinensis' does not comply with the requirements of 21 CFR 190.6 and is incomplete. The following items were not included with your submission: (1) an original and two copies of the notification, (2) a description of the dietary supplement or dietary supplements that contains your new dietary ingredient, (3) the level of the dietary ingredient in the dietary supplement, and (4) the conditions of use recommended or suggested in the labeling of the dietary supplement. Your notification describes the physical characteristics and edible uses for the tree, fruit, and leaves, but no information for the product that you intend to market. Your notification provided three reference articles; the remainder consisted of citations and abstracts of the references that you relied on as evidence of safety. Any references to published information offered in support of the notification shall be accompanied by reprints or photostatic copies of such references. In addition, your notification did not include documented history of use of your new dietary ingredient as an article present in the food supply."

NDIs Excluded from the Definition of "Dietary Supplement"

Sec. 201(ff) [21 U.S.C. 321(ff)] defines a "dietary supplement." If the NDI does not fall within the meaning of 201(ff), it is not a dietary supplement. One notification involved "obestilin" (3'-hydroxystilbene), which was determined not to be a dietary ingredient. In this case, the FDA letter, dated 12 March 2010 in [Docket Report #625], responded with the following: "Based on the information in your notification, your synthetically derived 3'-hydroxystilbene is not a dietary ingredient within the meaning of Section 321(ff)(1) as explained below. Although you state in your notification that 3'-hydroxystilbene has been reported to be a constituent of plants such as *Pterocarpus marsupium* and *Sphaerophysa salsula*, your synthetically derived 3'-hydroxystilbene is not prepared by extracting and purifying 3'-hydroxystilbene from a plant source. Therefore, your synthetic 3'-hydroxystilbene is not a dietary ingredient under 21 U.S.C. 321(ff)(1)(F) because it is not a constituent or an extract of a botanical, nor does your notification present information establishing that synthetic 3'-hydroxystilbene qualifies as any other type of dietary ingredient listed in Section 321(ff)(1). For these reasons, synthetic 3'-hydroxystilbene cannot be marketed as a dietary ingredient in a dietary supplement." This response highlights that FDA has stated that synthetic copies of botanical constituents are not equivalent to the natural counterpart in the regulatory determination of the status of the ingredient because they do not fit under 201(ff)(1)(F). The Federal Register announcement of the 21 CFR 119 Ephedra Final Rule explains "... synthetic sources of ephedrine cannot be dietary ingredients because they are not constituents or extracts of a botanical, nor do they qualify as any other type of dietary ingredient. For these reasons, products containing synthetic ephedrine cannot be legally marketed as dietary supplements" (USFDA, 2004).

Another reason for a product to be excluded from the definition of a dietary supplement is that it was authorized for investigation as a new drug. The FDA response letter for Cotinine, dated 20 April 2004 in [Docket Report #277], demonstrates this point. “Your notification identified the substance ‘Cotinine’ [derived from] (*Duboisia hopwoodii* (F. Muell.)) as the substance that you intend to market as a new dietary ingredient. . . . Cotinine may be excluded from the definition of ‘dietary supplement’ under 21 U.S.C. 321(ff)(3)(B). ‘Cotinine’ is an article authorized for investigation as a drug for which substantial clinical investigations have been instituted in the United States, and the investigations have been made public, and which was not before such authorization marketed as a dietary supplement or as a food. Because the information in your submission does not specify the form for your new dietary ingredient, we are unable to determine if your ingredient has been the subject of previous investigational new drug applications.”

Finally, an NDI can be excluded from the meaning of 201(ff) based upon its route of administration or intended effect. The notification describing a ‘*Hoodia gordonii* extract’ in ‘mint’ form was excluded from the definition in the FDA response letter dated 15 January 2004 [Docket Report #262]. *Hoodia gordonii* in the Asclepiadaceae family contains steroidal glycosides with cardiac activity. “An article that is delivered orally, but that exerts its effect prior to being swallowed (for example, a gum, lozenge, or mint that stimulates salivation) or that is a delivery system for a substance that is absorbed buccolingually is not ‘intended for ingestion’. A gum, lozenge or mint preparation which is not intended for ingestion can function as a delivery system for a substance that is absorbed buccolingually. As stated above, the definition of dietary supplement in 21 U.S.C. 321(ff) states that a dietary supplement is a product ‘intended for ingestion.’ The term ‘ingestion’ has been addressed by the court in *United States v. Ten Cartons, Ener-B Nasal Gel*, 888 F. Supp. 381, 393–94 (E.D.N.Y.), *aff’d*, 72 F.3d 285 (2d Cir. 1995), which states:

The ordinary and plain meaning of the term ‘ingestion’ means to take into the stomach and gastrointestinal tract by means of enteral administration. See *Stedman’s Medical Dictionary* (4th Lawyer’s Ed. 1976) (defining ingestion as the ‘introduction of food and drink into the stomach.’); *Webster’s Third New International Dictionary* (1976) (defining ingestion as ‘the taking of material (as food) into the digestive system’). . .

The interpretation of the term ‘ingestion’ to mean enteral administration into the stomach and gastrointestinal tract is also supported by the language of the statutory sections immediately preceding and following Section 350(c)(1)(B)(ii).” See the §411 [21 U.S.C. §350] (Vitamins and Minerals) of the FD&C Act for the statutory language.

Identity Issues with Old and New Dietary Ingredients

Chomper. In 1997, FDA demonstrated that Chomper, an early dietary ingredient with laxative properties and multiple botanical ingredients, and the plant material identified as the

ingredient “plantain” was adulterated and misbranded (Slifman *et al.*, 1998). In its 6 October 1997 warning letter to Neutraceutical, FDA “received a complaint regarding injuries sustained by a young woman who experienced an abnormal heart rate with complete heart block, a potentially life-threatening condition. The consumer’s symptoms were consistent with an overdose of digitalis-like cardiac glycosides. The young woman experienced this condition after ingesting a regimen of dietary supplements. FDA’s investigation determined that the problem was due to the ingredient plantain found in your dietary supplement ‘Chomper’. FDA collected multiple samples of plantain.” “FDA analyses of these samples showed that the plant material identified as ‘plantain’ contained lanatosides (cardiac glycosides). The presence of lanatosides support that the plant material contains *Digitalis* glycosides. *Digitalis lanata* has been reported to contain these lanatosides. Plantain has not been reported to contain any cardiac glycosides. FDA also conducted an analysis of a sample of plantain to determine whether the material identified as plantain actually contained plantain. The analysis found that the characteristic trichomes for plantain were low in concentration in the sample when compared with reference specimens. These analyses indicate that the plantain was contaminated with *Digitalis*.” FDA determined the involved parties responsible for the adulteration and misbranding product under Sections 402(a)(1), 402(f)(1)(A) and 403(a)(1) of the FD&C Act.

Milkweed seed oil. Milkweed seed oil presented another ingredient that raised concerns about cardiotoxic glycosides, this time in the context of an NDI notification. The FDA response letter for Milkweed Seed Oil, dated 17 September 2009 (Report 597—Natural Fibers Corporation (Milkweed Seed Oil), describes safety concerns raised by this notification. “Your notification concerns the new dietary ingredient ‘Milkweed Seed Oil (MSO)’ derived from the pod seeds of milkweed plants that you call ‘*Asclepias Syriaca*’ [*sic*] and ‘*Asclepias Speciosa*’ [*sic*] that you intend to market as a dietary supplement. FDA was unable to establish the identity of your new dietary ingredient ‘Milkweed Seed Oil (MSO).’ For example, plants in the genus *Asclepias* are widely regarded as toxic to humans and grazing animals due not only to the cardiotoxic glycosides (e.g. cardenolides) mentioned in your notification but also due to toxic resinoids (e.g. galitoxin) and alkaloids (e.g. phenanthroindolizidines) found in the seeds and other parts of the plants. In addition, your notification states that you will use petroleum ether or other, unspecified organic solvents to produce your ingredient. Your notification does not provide adequate information about the processing or manufacturing of your ingredient, specifications for your ingredient or specifications for the materials that you will use to make your ingredient that address these poisonous and deleterious substances which may be present in a dietary supplement made by petroleum ether extraction of milkweed seed pods. . . . Therefore it is unclear how your ‘Milkweed

Seed Oil (MSO)' is qualitatively or quantitatively related to the materials used to establish the composition of your product or how the composition of those materials can be used to provide a basis for the safety of a product containing 'Milkweed Seed Oil (MSO).' Because your notification asserts the safety of 'Milkweed Seed Oil (MSO)' based on the safety of each and all of its constituents, FDA cannot determine the safety of 'Milkweed Seed Oil (MSO)' until the identity of the ingredient you intend to manufacture can be established. For the reasons discussed above, the information in your submission and the other scientific literature the agency has reviewed FDA disagrees with your conclusion that 'Milkweed Seed Oil', when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v)."

Lessons learned. Consequently, the two cases presented above indicate that composition, adequate species identification, and setting proper limits in a specification table for known toxic constituents in a botanical are critical factors in assessing the identity and safety of dietary supplements containing dietary ingredients derived from herbs or botanicals. Botanical identification is critical and should be a major factor for dietary supplement manufacturers to consider. Simply inspecting the leaves of an herbal to verify herbal/botanical identification may not be adequate to describe the identity of the plant. Indeed, distinguishing between species at such a superficial level can be problematic even for expert taxonomists. Many genera contain species that are nearly indistinguishable at a macroscopic level. Knowledge of morphological characteristics is crucial to proper identification and may require microscopic or chemical analysis to properly determine identity.

Aristolochic acid. Aristolochic acids are a family of nitrophenanthrene compounds found in plants of the family Aristolochiaceae, particularly those in two genera, *Aristolochia* and *Assarum*. They came to worldwide attention following an outbreak of about 100 cases of renal failure, many with urinary tract carcinoma, in Belgium in the 1990s (Debelle *et al.*, 2008). In 1992, this cluster of patients in Belgium, who consumed a "slimming" regimen containing a mixture of several Chinese herbs combined with western pharmaceutical products, was found to have a severe and progressive kidney failure often progressing to end-stage renal disease (Cosyns *et al.*, 1994; Depierreux *et al.*, 1994; Vanherweghem, 1998; Vanherweghem *et al.*, 1993). At least 70 patients required renal transplant or dialysis. The diagnosis was made based on an unusual and distinctive pattern of nephropathy that included a characteristic degeneration in the proximal renal tubules and interstitial

fibroses in surrounding tissue. Subsequent investigations detected aristolochic acid-DNA adducts in epithelial tissue in the urinary tract (Schmeiser *et al.*, 2009). The herbal product was labeled to have contained *Stephania tetrandia*—a botanical not known to contain aristolochic acid—but was later found to be inadvertently substituted with the botanical *Aristolochia fangchi*, which contains aristolochic acid. The substitution has been attributed to the similarity of the Chinese names of the two species (*Guang fang ji* vs. *Fen fang ji*). Subsequent reports have shown the toxicity to be related to exposure to aristolochic acids (Muniz-Martinez *et al.*, 2002; Nortier *et al.*, 2000; Schmeiser *et al.*, 1996; Vanhaelen *et al.*, 1994). Nephropathy and end-stage renal disease were all found to be associated with *Aristolochia* consumption, and in some cases, dialysis or transplant was necessary. Many of the patients also had transitional cell carcinomas in the upper urinary tract (ureters, bladder, renal pelvis) most of which were discovered in organs removed during renal transplant.

Since then similar diagnoses were reported elsewhere in Europe, Asia, and the United States, and aristolochic acids have been implicated as the etiologic agent in "Balken endemic nephropathy," possibly due to contamination of wheat by *Aristolochia clematis* growing as weeds in or near crops (Grollman and Jelakovic, 2007). Botanicals containing aristolochic acid have been proposed for inclusion in the 12th Report on Carcinogens (<http://ntp.niehs.nih.gov/?objectid=BD1A20B5-F1F6-975E-7CF8CBFACF0FC7EF>) and FDA continues to maintain an import alert excluding certain botanicals from importation for use in dietary supplements (http://www.accessdata.fda.gov/cms_ia/importalert_141.html). A list of plants with similar names that might be found in commerce was also published by FDA (<http://www.fda.gov/Food/DietarySupplements/Alerts/ucm095283.htm>).

Lessons learned. In spite of a long history of traditional medical use, it was not until nephrologists in Belgium noticed an unusual pattern of renal disease that the association between chronic consumption of botanical products and either kidney failure or urinary tract cancers (Debelle *et al.*, 2008). The severity of the disease, distinctive pathology, and relatively rapid onset of disease were all key factors in the discovery of the association. Prior to the initial discovery, there were no published chronic or subchronic toxicity studies of these botanicals that might have been used to signal a risk of chronic renal failure, an endpoint unlikely to be detected using adverse event reporting due the gradual nature of its onset.

History of Use

Although the history of use for botanicals like *Aristolochia* spp. in traditional medicines may be considerable, this usage may not equate to a history of safe use as a food. Although a well-documented history of safe use can be used as the basis for establishing reasonable expectation of safety for an NDI or

dietary supplement product, an inadequate history of use is often cited in FDA response letters. A botanical used to make an NDI may have a history of use in traditional medicine, but the traditional use has to be described relative to the form, quantity, and frequency of consumption of the new dietary ingredient. An example is the notification for “Yuen Foong Yu Herba Dendrobii,” derived from the plant *Dendrobium huoshanense*. The 17 February 2009 FDA response letter [Docket Report #566] to this notification explains that “[t]he notification does not provide adequate information to determine how your new dietary ingredient is related to materials derived from the *Dendrobium huoshanense* that are described in the history of use information you provide as evidence of safety. For example, your notification informs FDA that Yuen Foong Yu Biotech Co., Ltd. did select, breed and propagate superior strains ... [to develop] a uniquely efficacious ophthalmological herbal drug. According to information cited in your notification, your ingredient Yuen Foong Yu Herba Dendrobii is actually a hybrid of *Dendrobium tosaense* Makino and *Dendrobium huoshanense*. C.Z. Tang and S.J. Cheng specifically selected and propagated for fast growth rate and superior bioactivity. The use of the name *D. huoshanense* C.Z. Tang and S.J. Cheng to describe your new ingredient may be misleading because it is unclear how the properties relevant to the safety of consumption of cultivar YFY-HS1 are related to the properties of historically consumed cultivars of *D. huoshanense* or to *D. tosaense*. In addition, it is unclear how *D. huoshanense* was used to make tea or was used in traditional medicines. For example, the articles that you provide to illustrate the use of *D. huoshanense* to make tea do not describe the frequency (daily or occasional) of consumption or the serving levels of *D. huoshanense* in the tea. Therefore, it is unclear how Yuen Foong Yu Herba Dendrobii is qualitatively or quantitatively related to the material described in the history of use or how that history of use provides a basis for the safety of your product.”

The FDA response to an ingredient derived from *Polypodium leucotomos*, dated 28 September 2009 [Docket Report #602], illustrates another set of concerns regarding history of use. “FDA has carefully considered the information in your submission and the agency has significant concerns about the evidence you rely on to support your conclusion that a dietary supplement containing your new dietary ingredient, ‘*Polypodium leucotomos* leaf extract’ will reasonably be expected to be safe under the conditions of use described in your notification. FDA was unable to establish the safety of your new dietary ingredient ‘*Polypodium leucotomos* leaf extract’ based on the history of use of your product in more than 26 countries because you have not provided information on how many people have eaten products containing your ‘*Polypodium leucotomos* leaf extract’ or a description of those products. For example, you have not provided information about serving levels, duration and frequency of consumption, as well as other conditions of use or any information about adverse event monitoring.”

Lessons learned. Providing an adequate history of use is important because an articulated, logical, detailed history of use for the NDI in question can supersede the need to perform expensive safety tests. Indeed, the regulation implies that the history of use alone may be sufficient to demonstrate the safety of the NDI. A description of the form, quantity, and frequency of consumption of a botanical ingredient can be used to demonstrate the safety of the ingredient. However, many new botanical dietary supplement ingredients are in a form, quantity, or frequency of consumption that differs from the traditional use. The notification for *D. huoshanense* failed to describe the details of how much is consumed and how the dried, ground leaves and stems in a capsule relate to the amount and frequency consumed as a beverage.

Other Evidence of Safety

When the history of use information is insufficient alone to support that the dietary supplement or product containing the NDI will reasonably be expected to be safe, then toxicological studies and clinical data in humans are sometimes used to support the safety of the product containing the ingredient. Examples of inadequate demonstration of safety in toxicology and clinical studies are discussed below. An example is a highly concentrated extract from a plant widely used to flavor food. The FDA response letter for “Tarragon extract,” dated 4 June 2010 [Docket Report #645] identified significant safety concerns about the evidence presented in the notification to conclude reasonable expectation of safety. “Your notification concerned a new dietary ingredient you call ‘Tarragon extract’ which you derive from the leaves, stems, shoots, and flowers of *Artemisia dracuncululus* L. that you intend to market in a dietary supplement product in a capsule form. FDA was unable to establish the safety of ‘Tarragon extract’ based on the toxicology studies you provided. It is unclear how these data provide basis for the determination that a dietary supplement containing your proposed new dietary ingredient is reasonably likely to be safe. For example, it is unclear that the maximum administered dose in the subchronic rodent study was the maximum tolerated dose for your ingredient, nor does it provide an adequate margin of safety. In addition, tarragon extracts are known to contain allyl benzene compounds such as methyleugenol and estragole. These compounds have been shown to cause cancer in rats and mice but do not induce mutations in bacterial mutagenesis assays such as the one you used as a short-term indicator of carcinogenicity. Your notification does not provide history of use or other evidence of safety that addresses the risk of cancer from constituents that may be present at unsafe levels in ‘Tarragon extract’.”

When history of use is inadequate and the margin of safety, derived from rodent toxicology studies, is clearly insufficient, clinical toxicology data in humans can contribute to a safety argument. However, it is important for the clinical study to be designed in such a way as to address safety endpoints that are

appropriate to the characteristics of the ingredient. The FDA response letter for an ingredient called “Sesame Peptide KM-20,” dated 13 October 2009 [Docket Report #605] illustrates this point. “Your notification concerned a powder derived from *Sesamum indicum* L. which you identify as a new dietary ingredient that you intend to market in a dietary supplement product under the trade name ‘Sesame Peptide KM-20’. . . . For example, you state that ‘Sesame Peptide KM-20’ is intended to be used to reduce ‘both the systolic and diastolic blood pressure in individuals with high-normal blood pressure and mildly hypertensive individuals’. You also state that ‘Sesame Peptide KM-20’ is intended to lower blood pressure by inhibiting angiotensin conversion enzyme (ACE). In addition, FDA was unable to establish the safety of your ‘Sesame Peptide KM-20’ based on history of use provided in your notification. For example, your notification does not mention the history of use of people eating sesame seeds for lowering blood pressure or that sesame seeds or products derived from sesame seeds are known to inhibit ACE or lower blood pressure. It is unclear how the history of use in your notification of sesame seed as a food source provides a basis for the safety of your ingredient. In addition, FDA was unable to establish the safety of your ‘Sesame Peptide KM-20’ from the toxicology and clinical studies provided in your notification. For example, the toxicology studies you provide do not address the safety of the use of your ingredient in normal and hypotensive individuals; nor were the endpoints of blood pressure, heart rhythm, and other cardiac endpoints collected which would have been appropriate for a test article known to affect the cardiovascular system. Based on the above information, it is unclear how these studies provide a basis for the safety of ingestion of dietary supplements containing ‘Sesame Peptide KM-20’.”

The FDA response letter for *Cissus quadrangularis*, dated 30 November 2009 [Docket Report #612], demonstrates how a variety of elements (identity, history of use, rodent toxicology, genetic toxicology, and clinical toxicology) come together in assessing the safe use of a botanical ingredient. In the case of *C. quadrangularis*, multiple threads of evidence related to a single endpoint, hepatotoxicity, led to “significant concerns about the evidence on which . . . to support [a] conclusion that the dietary supplement product containing ‘Cissus CQR-300’ will reasonably be expected to be safe under the conditions of use described in [the] notification.”

Lessons learned. The examples provided above are all common problems in NDI notifications that do not support the basis for reasonable expectation of safety. Providing proper chemical, botanical, and microbiological identity is essential for the safety review. Identity provides evidence to support that the dietary supplement product or NDI is qualitatively and quantitatively similar to the substances described in the information relied upon in the notification as evidence of safety. Safety, in terms of history of use and toxicology data, is then evaluated to determine

how that information forms the basis for a reasonable expectation of safety under the intended conditions of use.

TOXICITY ASSESSMENT OF DIETARY SUPPLEMENTS BY THE NATIONAL TOXICOLOGY PROGRAM (Nigel J. Walker and Paul Howard)

The National Toxicology Program (NTP; <http://www.ntp.niehs.nih.gov>) is a federal interagency program established in 1978, as a cooperative effort to coordinate toxicology testing programs within the federal government, strengthen the science base in toxicology, develop and validate improved testing methods, and provide information about potentially toxic chemicals to health, regulatory, and research agencies, scientific and medical communities, and the public. The three agencies that form the core of the NTP are the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention, and the National Center for Toxicological Research (NCTR) of the FDA.

The NTP actively identifies and selects for study chemicals and other substances for which sufficient information is not available to adequately evaluate potential human health hazards. The NTP accomplishes this goal through a formal open nomination and selection process. Substances considered appropriate for study generally fall into two broad yet overlapping categories: (1) substances judged to have high concern as a possible public health hazard based on the extent of human exposure and/or suspicion of toxicity and (2) substances for which toxicological data gaps exist and additional studies would aid in assessing potential human health risks.

The NTP held a workshop in 1998 on herbal medicines in response to public concerns regarding the use and efficacy of medicinal herbs and due to continued increase in nominations of herbal products for toxicology studies. Recommendations from the workshop included a call for: (1) more research on herbals, (2) the identification and standardization of product ingredients by industry, (3) increased consumer education through package inserts, (4) identification of herb-drug and herb-herb interactions, and (5) research on risk to sensitive subpopulations (Mathews *et al.*, 1999).

Subsequent to this workshop, NTP initiated a program of studies to evaluate the toxicity of a series of botanical-derived dietary supplements and their constituents. Currently, staffs at both NIEHS and NCTR are actively working with the NIH Office of Dietary Supplements, the Center for Food Safety and Nutrition of the FDA, the academic community, and others to conduct research that will address deficiencies in our knowledge about dietary supplements and their potential toxicities. This has led to the recent formation of an Interagency Working Group to discuss among federal agencies a prioritization of herbals that should be studied for potential toxicity. The formation of this Interagency Working Group meets the mission of the NTP, where it is to

TABLE 1
Dietary Supplements/Botanicals Evaluated and/or Under Evaluation by NTP

Aloe vera	Plant widely used as a treatment for minor burns and is increasingly being used in products for internal consumption.
Bitter orange (<i>Citrus aurantium</i>)	Bitter orange peel and its constituent synephrine have adrenergic activity and may result in cardiovascular or other adverse effects similar to those induced by ephedra alkaloids.
Black cohosh	Used to treat symptoms of premenstrual syndrome, dysmenorrhea, and menopause.
Dong quai (<i>Angelica sinensis</i> root) and extract	Use as an antispasmodic or blood purifier and for reduction of pain, dilation of blood vessels, and stimulation, as well as relaxation of uterine muscles.
<i>Garcinia cambogia</i>	<i>Garcinia cambogia</i> is marketed as an ephedra-free diet aid and there is consumer exposure through increasing dietary supplement use.
<i>Ginkgo biloba</i> extract	Ginkgo fruit and seeds have been used medicinally for thousands of years to promote improved blood flow and short-term memory and to treat headache and depression.
Ginseng	Ginsenosides are thought to be the active ingredients in ginseng. Ginseng has been used as a laxative, tonic, and diuretic.
Glucosamine/chondroitin sulfate	Widely used alone and in combination with glucosamine to alleviate pain and inflammation from osteoarthritis.
Goldenseal root	Traditionally used to treat wounds, digestive problems, and infections. Current uses include as a laxative, tonic, and diuretic.
Green tea extract	Used for its antioxidative properties.
Gum Guggul extract	Used as a dietary supplement and has demonstrated biological effects on lipid metabolism, thyroid hormone homeostasis, female reproductive tissues, and endogenous nuclear hormone receptors.
Indole-3-carbinol	Found in Brassica vegetables and marketed as a dietary supplement for cancer chemoprevention.
Kava kava extract	A widely used medicinal herb with psychoactive properties sold as a calmate and antidepressant. A recent report of severe liver toxicity has led to restrictions of its sale in Europe.
Milk thistle extract	Used to treat depression and several liver conditions including cirrhosis and hepatitis and to increase breast milk production.
Pulegone	A major terpenoid constituent of the herb pennyroyal. Has been used as a carminative, insect repellent, nervous system.
Resveratrol	<i>Trans</i> -Resveratrol is found in grapes and wine and is currently marketed in pure or extract form as a dietary supplement.
Senna	Laxative with increased use due to the removal of a widely used chemical-stimulant type laxative from the market.
Thujone	Terpenoid is found in a variety of herbs including sage and tansy and in high concentrations in wormwood. Suspected as the causative toxic agent associated with drinking absinthe, a liqueur flavored with wormwood extract.
<i>Usnea Lichen</i>	<i>Usnea lichen</i> extracts (e.g., <i>Usnea barbata</i>) are traditionally used as antimicrobials. Recently, usnic acid extracted and purified from <i>U. lichen</i> has been utilized as a weight loss agent; with subsequent reports of extract-associated hepatotoxicity.

participate in the coordination of toxicology studies among federal agencies. An additional advantage is that the respective strengths of the participating federal agencies can be combined to ensure that the limited resources of the NTP (and other agencies) are used to address the toxicity of the appropriate test material thus maximizing the regulatory impact of any data that is generated.

NTP's evaluations of these dietary supplements includes extensive physicochemical characterizations of the materials and their constituents, *in vitro* studies that test specific mechanisms of action, mechanism-based investigative studies, evaluation of pharmacokinetics, and *in vivo* toxicology studies under the Good Laboratory Practice guidelines.

Dietary supplements, containing biologically active constituents found in some herbs and herbal extracts, continue to be nominated to the NTP and selected for study, including some of the most common dietary supplements used by consumers in the United States (Table 1). Studies have been designed for many dietary supplements and herbal products that focus on

the characterization of potential adverse health effects, including general toxicity associated with short-term high-dose exposure and/or long-term exposure to lower doses, as well as system-specific toxicities including reproductive toxicity, neurotoxicity, cardiovascular toxicity, and immunotoxicity.

The rationale and prioritization of selection of a given botanical or botanical extract for study by NTP is often determined by an assessment of potential for exposure and/or suspicion of potential toxicity based on known or inferred potential pharmacological activity, based on use pattern or chemical constituent profile. First, exposure information is critical to determining prioritization; however, precise information is often lacking for specific type of materials, and as a result, sales figures are often used as a surrogate for assessing relative differences in use of specific products, although these types of data tend to be not fully comprehensive for all retail outlets. Second, the probable duration of exposure is critical for prioritization and can be inferred from the intended or marketed

use of the product. For example, an herbal product that is intended for long-term daily use will receive more attention than a product that is only intended for episodic or brief use. Third, the possibility of intended or unintended use of the product by a sensitive target population (e.g., children, women of child bearing age, elderly) can raise the priority, especially in light of differences in metabolism between children and adults, and the likelihood of polypharmacy in the elderly.

The evaluation of potential toxicity of herbal supplements requires special considerations compared with other agents evaluated by NTP. In general, the NTP does not study specific commercial products but rather focuses on materials or constituents that are in some way representative of many different specific products. This can be a challenge especially for herbals because preparations can have very different composition and chemical constituent profiles due to variations in which part of the plant was included (or excluded), growing conditions including location and local weather, harvesting conditions, and processing conditions. As a result, physico-chemical characterization is a key aspect in the development of a testing program by NTP. Because analytical techniques are selective, a characterization program must be developed to provide the essential information for the selection of the test article, test article stability (e.g., diet, dosed-water, dosing vehicle), and test article dosimetry during the course of the study. A general approach that NTP uses includes initially evaluating the presence of known biologically active components. Use of chemical fingerprinting approaches allows for the assessment of complex pattern for comparison across different formulations. Whenever possible NTP attempts to detect as many components as possible, so that "marker" constituents can be assessed during the study for dose certification and to evaluate the presence of potential actives discovered after bioassay is complete.

Another reason for full characterization of the test article is to be able to assess what the impact of exposure will have on nutritional status including things such as vitamins, metals, amino acids, and fatty acids. Efforts for such total composition include evaluation of presence of nutritional components including protein, moisture, fat, ash, fiber vitamins A, E, B12, thiamin, biotin, folic acid, riboflavin, niacin, pantothenic acid, choline, pyridoxine hydrochloride, fatty acids, and amino acids. Additionally, possible contaminants such as metals, pesticides, and mold toxins are also quantified to ensure that any toxicities are not due to these contaminants.

Dietary supplements currently under evaluation by NTP include components in several overlapping categories including: (1) those intended for multipurpose use such as aloe vera, Echinacea, gum guggul, kava, milk thistle, pulegone/pennyroyal, and senna (laxative); (2) those intended for use in relation to women's health issues such as black cohosh, gum guggul, and dong quai; (3) those marketed as cancer chemopreventatives such as green tea extract, indole carbinol, milk thistle extract, resveratrol, and melatonin; (4) those marketed

for anti-aging, antioxidants, or wellness such as ginseng, glucosamine/chondroitin sulfate, *Ginkgo biloba*, and vincamine; and (5) those marketed for either weight loss or as sports aids including *usnea lichen*/usnic acid, chitosan, *Garcinia cambogia*, bitter orange extract, and androstenedione.

Starting in 2009, the results of the first round of chronic 2-year studies on herbals were reported by the NTP. These included long-term studies of Goldenseal Root Powder (NTP Technical Report [TR] 562), Ginseng (TR 567), Milk Thistle (TR 565), and Pulegone (TR 563). More recently, NTP technical reports were released for chronic studies of Aloe vera (TR 577), Alpha/beta Thujone (TR 570), and Kava kava (TR 571). A full description of the results of these is beyond the scope of this short review so readers should refer to the NTP Web site (<http://www.ntp.niehs.nih.gov>) for free, downloadable NTP technical reports on these herbals. Long-term studies have also been conducted on Green tea extract, *G. biloba*, and indole-3 carbinol and will be reported later.

Given the limitations on pre-market safety assessment of herbals and other agents covered by DSHEA, the NTP program on herbals remains one of the only independent safety assessment programs available for assessing potential toxicity of these compounds. For that reason, the herbals research at NTP has been and continues to be a key part of the overall mission of NTP, that is, to "provide information about potentially toxic chemicals to health, regulatory, and research agencies, scientific and medical communities, and the public." Combining the resources and expertise of multiple government agencies into the Interagency Working Group to prioritize the toxicology studies being conducted on botanicals will ensure that the limited resources will be used to study the botanicals with the highest public risk.

SUMMARY

There are challenges in establishing the safety of naturally occurring products proposed to be used as food ingredients under FDA's GRAS notification program or dietary ingredients in dietary supplements under FDA's NDI notification program. The 1958 Food Additives Amendment established criteria for the use of a substance in food to be GRAS and, in 1994, DSHEA established criteria for NDIs.

For a food ingredient evaluated under the GRAS notification program, two criteria must be met: (1) publicly available data to support safe use in conventional food and (2) general recognition of safety. In terms of the first criterion, the safety assessment of a naturally-occurring product is complicated by a number of factors including the compositional complexity of extracts of whole products, the need for complete biochemical characterization of a product's components, and the need for toxicity data on the whole product, as well as the components of the extract. In terms of the second criterion, there must be consensus among experts regarding the published safety data.

History of safe use in food prior to 1958 is another way to establish general recognition of safety, but there needs to be sufficient documentation regarding the nature of the substance and its use in food before 1958 to establish that it is the same substance intended to be added to the current food supply.

The toxicological evaluation of the safety of dietary supplements under the NDI notification program is often complicated by the compositional complexity of a product and a lack of information regarding its chemical, botanical, and microbiological identity. Identity provides evidence to establish that the product is qualitatively and quantitatively sufficiently similar to the substances described in the information relied upon as evidence of safety in the notification. The history of use of NDI products is necessary to establish that the information confirms a reasonable expectation of safety under the intended conditions of use.

Over the past decade, the NTP has been working with the FDA to address gaps in our knowledge of the safety of naturally derived substances regulated under GRAS and NDI notification programs. This effort has utilized *in vitro* (e.g., P450 interactions and genetic toxicity assays) and *in vivo* (e.g., short-term toxicity studies such as 90-day oral toxicity studies in animals and long-term bioassays evaluating the carcinogenic potential of different compounds) studies. These studies provide additional information to help fill the safety data gaps for these food ingredients and/or their components and facilitate the FDA's mission of ensuring the safety of our food supply.

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