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A Procedure for the Safety Evaluation of Natural Flavor Complexes Used as Ingredients in Food: Essential Oils

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Abstract

A scientifically-based guide has been developed to evaluate the safety of naturally occurring mixtures, particularly essential oils, for their intended use as flavor ingredients. The approach relies on the complete chemical characterization of the essential oil and the variability of the composition of the oil in the product intended for commerce. Being products of common plant biochemical pathways, the chemically-identified constituents are organized according to a limited number of well-established chemical groups called congeneric groups. The safety of the intake of the each congeneric group from consumption of the essential oil is evaluated in the context of data on absorption, metabolism, and toxicology of members of the congeneric group.

The intake of the group of unidentified constituents is evaluated in the context of the consumption of the essential oil as a food, a highly conservative toxicologic threshold, and toxicity data on the essential oil or an essential oil of similar chemotaxonomy. The flexibility of the guide is reflected in the fact that high intake of major congeneric groups of low toxicologic concern will be evaluated along with low intake of minor congeneric groups of significant toxicological concern (i.e., higher structural class). The guide also provides a comprehensive evaluation of all congeneric groups and constituents that account for the majority of the composition of the essential oil. The overall objective of the guide is to organize and prioritize the chemical constituents of an essential oil in order that no reasonably possible significant risk associated with the intake of essential oil goes unevaluated. The guide is, however, not intended to be a rigid checklist. The Panel will continue to evaluate each essential oil on a case by case basis applying their scientific judgment to insure that each NFC is exhaustively evaluated.

I. Introduction

Throughout the development of both Western and Eastern civilization, plants, plant parts, and derived oils and extracts have functioned as sources of food and medicine, symbolic articles in religious and social ceremonies, and remedies to modify behavior. In many cases, substances gaining widespread acceptance as

multifunctional agents were ones that strongly stimulate the human senses of taste (gustatory) and smell (olfactory). Cinnamon oil exhibits a pleasing warm spicy aftertaste, characteristic spicy aroma and preservative properties that made it attractive as a food flavoring and fragrance millennia ago. It was also the principal ingredient in the oil of holy ointment mentioned in Exodus 32:22-26. Because of its perceived preservative properties, cinnamon and cinnamon oil were sought by Egyptians for embalming. According to Discorides (Discorides, 50AD), cinnamon was a breath freshener, would aid in digestion, would counteract the bites of venomous beasts, reduce inflammation of the intestines and the kidneys, and act as a diuretic. Applied to the face, it was purported to remove undesirable spots. It is no wonder that, at one time, cinnamon was more expensive than gold.

Based on rich histories of use of selected plants and plant products that strongly impact the senses, it is not unexpected that society would bestow powers to heal, cure diseases, and spur desirable emotions, in the effort to improve the human condition. The perception that these products are “natural” and have a long history of use has, in part, mitigated the public’s need to know whether these products work or are safe under conditions of intended use. In the absence of information on the efficacy and safety of the natural product, recommendations of the quantity and quality of natural product to be consumed remain ambiguous. However, when the intended use is as a flavor or fragrance, effective and safe levels of use are defined by fundamental biological limits.

Flavors and fragrances act directly on the gustatory and olfactory receptors in the mouth and nose leading to taste and aroma responses, respectively. Saturation of these receptors occurs at very low levels in animals. Hence, with few exceptions the effects of flavors and fragrances are self-limiting. The evolution of the human diet is tightly tied to the function of these receptors. Taste and aroma not only determine what we eat but often allow us to evaluate the quality of food and, in some cases, identify unwanted contaminants. The principle of self-limitation taken together with the long history of use of natural flavor complexes in food argues that these substances are safe under intended conditions of use. The conclusion by the US Food and Drug Administration (21 CFR Sec. 182.10, 182.20, 482.40, and 182.50) that natural flavor complexes are “generally recognized as safe” (GRAS) for their intended use was based, in large part, on these two considerations.

For other intended uses of natural products (e.g., dietary supplements or direct food additives), a more traditional toxicological approach has been used to demonstrate safety. This approach relies on performing toxicity tests on laboratory animals, evaluating intake for the intended use, and determining adequate margins of safety between daily intake by humans and toxic levels resulting from animal studies. In light of the many new products in the marketplace, the resources necessary to test all natural products for each intended use is simply economically unfeasible. Additionally, in the context of natural products that are complex mixtures of chemicals, the traditional approach is effective only when specifications for the composition and purity are clearly defined and adequate quality controls are in place. In the absence of such specifications, the results of toxicity testing apply specifically to the article tested. Recent safety evaluation approaches (Schilter et al., 2003) suggest that a multifaceted decision-tree approach can be applied to prioritize natural products and the extent of data required to demonstrate safety under conditions of use. The latter approach offers many advantages, both economic as well as scientific, over more traditional approaches. However, various levels of toxicity testing of the natural product are required in this approach.

No attempt has yet been made to evaluate the safety of a natural product based on its chemical composition and the variability of that composition for the intended use. The chemical constitution of a natural product is fundamental to understanding the product's intended use and factors affecting its safety. Advances in analytical methodology have made intensive investigation of the chemical composition of a natural product feasible. High through-put instrumentation necessary to perform extensive qualitative and quantitative analysis of complex chemical mixtures and to evaluate the variation in the composition of the mixture is now a reality. In fact, analytical tools needed to chemically characterize these complex mixtures are becoming more cost effective while the cost of traditional toxicology is ever-increasing. If such a chemically-based evaluation of natural products could be developed and properly documented, the wealth of existing chemical and biological data on individual constituents, not on the natural product itself, could provide the basis to evaluate the safety of the natural product.

Independent of the above considerations, it is scientifically sound to evaluate the safety of a natural mixture based on its actual chemical composition. Fundamentally, it is the interaction of one or more molecules in the natural product with macromolecules (proteins, enzymes, etc.) that yield the

biological response whether it is a desired functional effect such as a pleasing taste or a potential toxic effect such as liver necrosis. It is the chemical constituents in the natural product that are the basis for the pharmacologic activity of a herbal product or the respective gustatory and olfactory response of a flavor or fragrance. Many of the advertised beneficial properties of ephedra are based on the presence of the CNS stimulant ephedrine. So too, the gustatory and olfactory properties of coriander oil are, in part, based on the binding of the linalool, benzyl benzoate, and other molecules to the appropriate receptors. It is these molecular interactions of chemical constituents that ultimately determine conditions of use of the natural product.

The principal objective of this article is to present a guide for the safety evaluation of naturally-occurring essential oils for their intended use as flavoring substances (natural flavor complexes, NFCs).

II. Scope of Natural Flavor Complexes Used as Flavoring Substances

A. Types of Natural Flavor Complexes

Natural flavor complexes are mixtures of mainly low molecular weight chemical substances separated from plants by physical means such as distillation, extraction, and cold pressing. Sources of natural flavor complexes include components (e.g. pulp, bark, peel, leaf, berry, blossom) of fruits, vegetables, spices, and other plants. The most common NFCs are essential oils. The essential oil is typically obtained by steam distillation of the plant or plant part whereas an oleoresin is produced by extraction of the same with an appropriate organic solvent. With few exceptions, plants are dependent on their essential oil content for their unique aroma and gustatory profile. In other words, the volatile constituents of the plant isolated in the essential oil are primarily responsible for aroma and taste of the plant. Hence, borneol, bornyl acetate, camphor and other volatile constituents in rosemary oil provide a flavor intensity as potent as the mass of dried rosemary used to produce the oil. The few exceptions (cayenne pepper, black pepper, ginger, paprika, sesame seeds) include those plants containing key non-volatile flavor constituents (e.g., gingerol and zingerone in ginger). These non-volatile constituents are often higher molecular weight, hydrophilic substances contained in the fixed oil of an oleoresin. For economic reasons, crude essential oils are often produced via distillation at the source of the plant raw material and subsequently further processed at modern flavor facilities. The methods of preparation of essential oils are reviewed in Appendix C.

Natural flavor complexes are prepared from foods and non-food sources. Many of the approximately 100 essential oils used as flavoring substances in food are derived directly from food (i.e. lemon oil, basil oil, and cardamom oil); far fewer are extracts from plants not normally consumed as food (e.g., cedar leaf oil or balsam fir oil).

B. Processing of Essential Oils

Because essential oils are a product of nature, environmental and genetic factors will impact the chemical composition of the plant. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The Preamble to the Guide for the Safety Evaluation of NFCs (see Appendix A) addresses these factors in detail. The variability of the composition of the NFC as isolated from nature has been the subject of much research and development since plant and oils yields are major economic factors in crop production of NFCs.

However, the crude essential oil that arrives at the flavor processing plant is not normally used as such. The crude may be subjected to a number of processes intended to purify the oil and produce a product with the intended flavor characteristics. Some essential oils may be distilled and cooled to remove natural waxes and improve clarity while others are distilled more than once (i.e., rectified) to remove undesirable fractions or increase essential oil content. Some oils are dry or vacuum distilled.

However, at some point during processing the essential oil is evaluated for its technical function as a flavor. This evaluation typically involves analysis (normally by GLC or liquid chromatography) of the composition of the essential oil for chemical constituents that are markers for the desired technical flavor effect. For an essential oil such as cardamom oil, levels of target constituents such as terpinyl acetate, 1,8-cineole, and limonene are markers for technical viability as a flavoring substance. Based on this initial assessment, the crude NFC may be blended with other sources of the same oil or chemical constituents isolated from the oil to reach target ranges for key constituent markers that reflect flavor function. The mixture may then be further rectified by distillation. Each step of the process is driven by flavor function. Therefore, the chemical composition of product to be marketed is, in almost all cases, significantly different from that of the crude oil. Also, the chemical composition of the processed essential oil is more

consistent than that of the crude batches of oil isolated from various plant harvests. The range of concentrations for individual constituents and groups of structurally related constituents in an essential oil are dictated, in large part, by the requirement that target levels of flavor-marker constituents (e.g., (-)-carvone and limonene in spearmint oil or terpinyl acetate, limonene, and 1,8-cineole in cardamom oil) must be maintained.

Many citations in the scientific literature record the effect of changes in subspecies, geography, harvest time, and method of extraction on chemical composition of an NFC as isolated. These analyses provide some information on the variation in composition. However, with regards to safety, the attempted complete analysis of batches of NFC intended for the marketplace represents the chemical composition of the product intended for consumption. Rigorous chemically-based safety evaluation should be performed on the product intended for human consumption.

C. Chemical Composition of Essential Oils

In addition to the key chemical markers for the technical flavor effect, the NFC marketed normally contains many other chemical constituents, some having little or no flavor function. However, the types of chemicals in NFCs are not infinite in structural variation. Being derived from higher plants, chemical constituents are formed by one of five or six major biosynthetic pathways: lipoxygenase oxidation of lipids, shikimic acid, isoprenoid (terpenoid), peptide, and photosynthetic pathways. In ripening vegetables, lipoxygenases oxidize polyunsaturated fatty acids eventually yielding low molecular weight aldehydes (2-hexenal), alcohols (2,6-nonadienol) and esters, many exhibiting flavoring properties. Plant amino acids phenylalanine and tyrosine formed via the shikimic acid pathway are deaminated, oxidized and reduced to yield important aromatic substances such as cinnamaldehyde and eugenol. Formed via the isoprene pathway, the vast majority of constituents detected in commercially viable essential oils are terpenes {e.g., hydrocarbons (limonene), alcohols (menthol), aldehydes (citral), ketones (carvone), acids, and esters (geranyl acetate)} (Roe and Field, 1965). Since all of these pathways operate to different extent in plants, many of the same chemical constituents are present in a wide variety of essential oils.

A consequence of having a limited number of plant biosynthetic pathways is that structural variation of chemical constituents in an essential oil is limited. Essential oils typically contain 5 to 10 distinct chemical classes or congeneric groups with some congeneric groups, such as aliphatic terpene hydrocarbons containing upwards of 100 chemically-identified constituents. In some essential oils, a single constituent (e.g., citral in lemongrass oil) or congeneric group of constituents (e.g. hydroxyallylbenzene derivatives; eugneol, eugenyl acetate, etc in clove bud oil) comprise the majority of the mass of the NFC. In others, no one congeneric group predominates. For instance, although ten congeneric groups comprise > 98 % of the composition of cardamom oil, greater than 95% of the oil is accounted for by three chemical groups:

1) terpene tertiary alcohols and related esters
2) terpene aliphatic ethers
3) terpene aliphatic and aromatic hydrocarbons

Typically, in each of these groups one or two constituents predominate (alpha-terpinyl acetate, eucalyptol, limonene and myrcene). The presence of a limited number of congeneric groups in an essential oil is key to the organization of constituents and subsequent safety evaluation of the oil itself. Members of each congeneric group exhibit common structural features and participate in common pathways of pharmacokinetics and metabolism and exhibit similar toxicologic potential. If the mass of the essential oil (>95%) can be adequately characterized chemically and constituents assigned to well-defined congeneric groups, the safety evaluation of the NFC reduces to 1) a safety evaluation of each of the congeneric groups comprising the essential oil and, 2) a “sum of the parts” evaluation of the all congeneric groups to account for any chemical or biological interactions between congeneric groups in the essential oil under conditions of intended use.

Potential interactions between congeneric groups can, to some extent, be analyzed by an in depth comparison of the biochemical and toxicologic properties of different congeneric groups in the essential oil. For some representative essential oils that have been the subject of toxicology studies, a comparison of data for the congeneric groups in the essential oil with data on the essential oil itself (congeneric groups together) is a basis for analyzing for the presence or absence of interactions. Therefore, the impact of

interaction between congeneric groups is minimal if the levels of and endpoints for toxicity of congeneric groups (e.g., tertiary terpene alcohols) are similar to those of the essential oil (e.g., coriander oil)

In some cases, essentially complete chemical characterization of the essential oil may be difficult or economically unfeasible based on the small volume of essential oil used as a flavor ingredient. If the structures of a large number of constituents cannot or are not identified, a significant mass of NFC will go uncharacterized. In these few cases, mainly for low volume essential oils, the unknown fraction may be appreciable. However, if the intake of the essential oil is low or significantly less than its intake from consumption of food (thyme) from which the essential oil is derived (thyme oil), there should be no significant concern for safety in use. For some cases in which chemical characterization of the NFC is limited and the volume of intake is not insignificant, it may be necessary to perform additional analytical work to decrease the number of unidentified constituent or, in other cases, perform selected toxicity studies on the essential oil itself. A principal goal of the safety evaluation is that no significant portion of the essential oil should go unevaluated.

D. Specifications and Chemical Description of Essential Oil

A necessary part of the safety evaluation of an essential oil involves specifying the biological origin, physical and chemical properties, and other identifying characteristics of the substance being evaluated. An essential oil produced under good manufacturing practices (GMP) should be of a purity (quality) and chemical composition sufficiently high to represent a reasonable certainty of safety under conditions of intended use. Because the evaluation is based primarily on the actual chemical composition of the essential oil, specifications must necessarily include chemical assay for the essential oil in commerce. In addition to information on the origin of the essential oil (commercial botanical sources, geographical sources, plant parts used, degree of maturity, and methods of isolation) and physical properties (specific gravity, refractive index, optical rotation, solubility, etc.), specifications on composition link the chemical identity of the essential oil to its safety evaluation. Therefore data on the % range or upper limit of concentration of congeneric groups in the essential oil, target constituents monitored in an ongoing quality control program, and the amount of trace unidentified constituents that

stipulate the composition of the essential oil become key specifications linking the product distributed in the marketplace to the chemically-based safety evaluation.

Limited specifications for the chemical composition of some essential oils to be used as food flavorings are currently listed in the Food Chemical Codex (FCC, 4th Edition, 1996). For instance, the chemical assay for cinnamon oil is "not less than 80%, by volume, as total aldehydes". Any specification developed related to this safety evaluation procedure should be consistent with already published specifications. However, based on complete chemical analyses for the commercially-available oil, the chemical specification or assay can and should be expanded to:

1) specify the upper limits of concentrations for congeneric groups that constitute the vast majority of the oil.

2) identify key constituents in these groups that can be used to efficiently monitor the quality of the oil placed into commerce over time.

3) provide information on trace constituents or levels of unidentified constituents that may be of a safety concern at sufficiently high concentrations and levels of intake.

It is anticipated that a chemical specification for lemongrass oil would include: 1) greater than 97% of the composition chemically identified; 2) not more than 92% aliphatic terpene primary alcohols, aldehydes, acids, and related esters, typically measured as citral; 2) not more than 15% aliphatic terpene hydrocarbons, typically measured as myrcene. The principal goal of a chemical specification is to provide sufficient chemical characterization to ensure safety of the essential oil from use as a food flavoring. From an industry standpoint the specification should be such as to require timely quality control monitoring for constituents that are responsible for the technical flavor function. These constituents should also be representative of the major congeneric group or groups in the essential oil. Also, monitored constituents should include those that may be of a safety concern at sufficiently high levels of intake of the essential oil. The scope of a specification should be sufficient to ensure safety in use, but not impose an unnecessary burden on industry to perform ongoing analyses for constituents unrelated to the safety or flavor of the essential oil.

Chemical Description of the Essential Oil Evaluated as GRAS

A prerequisite for GRAS consideration of a chemically-identified flavoring substance is that the chemical identity and purity of the GRAS candidate must be specified. Included in each GRAS application is a request for spectral data to support the assignment of chemical structure, and chromatographic data to quantify the purity of the substance and the presence of secondary components. For essential oils, there is a requirement to specify the chemical constituents and their range of concentrations for the oil to be evaluated for GRAS. However, the chemical description represents the chemical composition of material considered for GRAS. It is not a required specification, since different batches of the commercial oil will not contain all listed constituents in the reference concentration ranges. Instead different batches will be required to exhibit upper concentration limits for congeneric group that comprises the essential oil. Practically speaking, although the aliphatic terpene primary alcohols, aldehydes, acids, and esters derivatives geraniol, nerol, citronellol, citral, and geranyl acetate vary quite widely in different batches of lemongrass oil, the upper limit of exposure to the congeneric group provides a practical specification for the safe use of the product in commerce.

III. Safety of Essential Oils, Constituents and Congeneric Groups

A. Essential Oils

Safety of NFCs - Relationship to Food

The close relationship of flavor complexes to food itself has made it difficult to evaluate the safety and regulate the use of essential oils. The Federal Food Drug and Cosmetic Act (FFDCA) recognizes that a different, lower, standard of safety must apply to naturally-occurring substances in food than applies to an ingredient intentionally added to food. For naturally occurring substances, the Act applies a realistic standard that the substance must "... not ordinarily render it [the food] injurious to health." (21 CFR 172.30). For added substances, a much higher standard applies. The food is adulterated if the added substance "... may render it [the food] injurious to health (21 CFR 172.20)." Essential oils used as flavoring substances occupy an intermediate position in that they are composed of naturally occurring substances many of which are intentionally added to food as individual chemical substances. Because they are considered neither a direct food additive nor a food itself, no current standard can be easily applied to the safety evaluation of essential oils.

The evaluation of the safety of essential oils that have a documented history of use in foods starts with the presumption that they are safe based on their long history of use over a wide range of human exposures without known adverse effects. With a high degree of confidence one may presume that essential oils derived from food are likely to be safe. Annual surveys of the use of flavoring substances in the United States (Lucas et al., 1999; NAS, 1965, 1970, 1975, 1981, 1987; 21 CFR 172.510) in part, document the history of use of many essential oils. Conversely, confidence in the presumption of safety decreases for natural complexes that exhibit a significant change in the pattern of use or when novel natural complexes with unique flavor properties enter the food supply. The recent trends in the popular diet have changed the exposure levels to essential oils in a variety of ways. For example, changes in the use of cinnamon oil in low-fat cinnamon pastries may alter exposure in a specialized population of eaters. Secondly, increased international trade has led to the introduction of novel plants and plant extracts from previously remote geographical locations. Osmanthus absolute (FEMA No. 3750) and Jambu oleoresin (FEMA No. 3783) are examples of natural complexes recently added to the FEMA GRAS list which are derived from plants not indigenous to the USA. Furthermore, the consumption of some NFCs may not occur solely from intake as flavoring substances; rather, they may be regularly consumed as dietary supplements with advertised functional benefits. These impacts have brought renewed interest in the safety evaluation of natural flavor complexes including essential oils. Although the safety evaluation of natural complexes must still rely heavily on knowledge of the history of use, a flexible science-based approach would allow for rigorous safety evaluation of different uses for the same essential oil.

B. Safety of Constituents and Congeneric Groups in NFC's

Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose, or reasonably might be expected to pose a significant risk to human health at current low levels of intake when used as flavoring substances. When consumed in higher quantities, normally for other functions, some plants do indeed exhibit toxicity. Historically, humans have used plants as poisons (e.g., hemlock) and many of the intended medicinal uses of plants (pennyroyal oil as an abortifacient) have produced undesirable toxicity. High levels of exposure to selected constituents (pulegone in pennyroyal oil) of the plant have been associated with the observed toxicity. However, with

regard to flavor use, experience through long term use and the predominant self-limiting impact of flavorings on our senses have restricted the amount of a plant or plant part we use in or on food.

Extensive scientific data on major constituents in essential oils have not revealed any results giving rise to safety concerns. Chronic studies have been performed on over 30 major chemical constituents (menthol, carvone, limonene, citral, cinnamaldehyde, benzaldehyde, benzyl acetate, 2-ethyl-1-hexanol, methyl anthranilate, geranyl acetate, furfural, eugneol, etc.) found in many essential oils, the majority of which were sponsored by the National Toxicology Program (NTP). Given that the studies were hazard determinations, they were normally performed at dose levels orders of magnitude greater than the daily intakes of these constituents from consumption of the NFC. Even at these high intake levels, the majority of the constituents show no carcinogenic potential (Smith, 2004, in press). In addition to dose, the carcinogenic potential of some flavor ingredients are related to several factors including mode of administration, species and sex of the animal model, and target organ specificity. In the vast majority of studies, the carcinogenic effect occurs through a non-genotoxic mechanism in which tumors form secondary to pre-existing high-dose, chronic organ toxicity. Selected subgroups of structurally related substances (e.g., aldehydes, terpene hydrocarbons) are associated with a single target organ and tumor type in a specific species and sex of rodent (*i.e.*, male rat kidney tumors secondary to alpha-2u-globulin neoplasms with limonene in male rats) or using a single mode of administration (*i.e.*, forestomach tumors that arise due to high doses of benzaldehyde and hexadienal given by gavage).

There may well be constituents not yet studied which are weak nongenotoxic carcinogens at chronic high dose levels. However, because of the relatively low intake (Lucas et al., 1999) as constituents of essential oils, they are expected to be many orders of magnitude less potent than similar levels of aflatoxins, the polycyclic heterocyclic amines, or the polynuclear aromatic hydrocarbons. There is nothing to suggest that the major biosynthetic pathways available to higher plants are capable of producing substances that exhibit a high level of toxicity or carcinogenicity. Thus, while the minor constituents should be considered, particularly in those plant families and genera known to contain constituents of concern, there is less need for caution than when dealing with xenobiotics, or with substances from origins other than those considered here.

The toxic and carcinogenic potentials exhibited by constituent chemicals in essential oils can largely be equated with the toxic potential of the congeneric group to which that chemical belongs. A comparison of the oral toxicity data (JECFA, 2004) for limonene, myrcene, pinene and other members of the congeneric group of terpene hydrocarbons show similar low levels of toxicity with the same high-dose target organ endpoint (kidney). Likewise, dietary toxicity and carcinogenicity data (JECFA, 2001) for cinnamyl alcohol, cinnamaldehyde, cinnamyl acetate and other members of the congeneric group of 3-phenyl-1-propanol derivatives show similar toxic and carcinogenic endpoints. The safety data for the congeneric chemical groups that are found in vast majority of NFCs have been reviewed (Adams *et al*., 1996, 1997, 1998, 2004; Newberne et al., 1999; Smith et al., 2002a, 2002b; JECFA 1997-2004). Available data for different representative members in each of these congeneric groups support the conclusion that the toxic and carcinogenic potential of individual constituents adequately represent similar potentials for the corresponding congeneric group.

A second key factor in the determination of safety is level of intake of the congeneric group from consumption of the NFC. Intake of the congeneric group will, in turn, depend upon the variability of the chemical composition of the NFC in the marketplace and on the conditions of use. The complete chemical analysis of the different batches of NFC obtained from the same and different manufacturers will produce a range of concentrations for individual constituents in each congeneric group of the essential oil. The upper limit of those concentration ranges (%) times the estimated daily intake of the essential oil provides a conservative estimate of exposure to each congeneric group from consumption of the essential oil.

In some essential oils, the intake of one constituent, and therefore, one congeneric group, may account for essentially all of the oil (e.g., linalool in coriander oil, citral in lemongrass oil, benzaldehyde in bitter almond oil). In other oils, exposure to a variety of congeneric groups over a broad concentration range may occur. As noted earlier, cardamom oil is an example of such an NFC. Ultimately, it is the relative intake and the toxic potential of each congeneric group that is the basis of the chemically-based safety evaluation. The combination of relative intake and toxic potential will prioritize congeneric groups for the safety evaluation. Hypothetically, a congeneric group of increased toxic potential that accounts for only 5% of the essential oil may be prioritized higher than a congeneric group of lower toxic potential accounting for 95%.

The following guide is intended to be applied only to the safety evaluation of essential oils derived from higher plants for the intended use as flavoring substances in food. Fermentation products, process flavors, substances derived from fungi, microorganisms, or animals; and direct food additives are explicitly excluded. The guide is designed specifically for application to approximately 300 NFCs currently in use as flavoring substances and any new natural complexes anticipated to be marketed as flavoring substances. The guide is a tool to organize and prioritize the chemical constituents and congeneric groups in a NFC in such a way as to allow a detailed analysis of their chemical and biological properties. This analysis as well as consideration of other relevant scientific data provides the basis for a safety evaluation of the essential oil under conditions of intended use.

IV. The Guide for the Safety Evaluation of NFCs

A. Introduction

The FEMA Expert Panel has implemented new guidelines for the safety evaluation of natural flavoring complexes (NFCs) including essential oils. This novel procedure will be used in the GRAS reassessment of more than 300 NFCs including more than 100 essential oils being used as flavoring substances. The procedure will also be applied to the GRAS assessment of new NFCs. Many of the 300 natural complexes that have been used as flavoring substances for decades may still be assumed to be safe by virtue of their long history of use. However, the guide provides for a scientifically-based evaluation of NFCs based on their chemical composition. The procedure was initially developed by a subcommittee chaired by Dr. Nancy Higley and composed of Panel members, Dr. Ian Munro, Dr. Bernard Wagner, and Dr. John Doull, and members of the flavor industry, Dr. Richard Hall, Dr. Klaus Bauer; and Dr. Timothy Adams. The guidelines will almost certainly undergo many revisions and refinements, given the early stages of the NFC safety evaluation program and the fact that this endeavor is the first practical attempt to evaluate naturally-occurring mixtures.

The guide does not use the conventional criteria used for the safety evaluation of individual chemical substances. Instead, it is a procedure involving a comprehensive evaluation of the chemical and

biological properties of the constituents of the NFC. Constituents of known structure in the NFC are organized into congeneric groups that exhibit similar metabolic and toxicologic properties. The congeneric groups are further classified according to levels of toxicologic concern; Structural Classes I, II, and III (Cramer et al., 1978; Munro et al., 1996). Based on intake data for the NFC and constituent concentrations, the congeneric groups are prioritized according to intake and toxicity potential. The procedure ultimately focuses on those congeneric groups which due to their structural features and intake may pose some significant risk from the consumption of the NFC. Key elements used by the Expert Panel to evaluate congeneric groups include exposure, structural analogy, metabolism, and toxicology, including toxicity, carcinogenicity, and genotoxicity potential (Adams *et al.*, 1996, 1997, 1998, 2004; Woods and Doull, 1991; Oser and Ford, 1991; Oser and Hall, 1977; Newberne et all, 1999; Smith et al., 2002a and b). Upon completion of this analysis, the members of the Expert Panel apply professional judgment and s cientific expertise to complete the safety evaluation of the NFC.

B. Elements of the Guide for the Safety Evaluation of the NFC

B.1. Introduction

The guide (Appendix A) is structured to prioritize the essential oil according to sources of intake (Step 1). It then organizes the chemically-identified constituents into congeneric groups and prioritizes them according to relative intake and their toxic potential (Steps 2, 3, and 4). The intake of the total of unidentified constituents is also determined. A Preamble to the Guide specifies the data (e.g., botanical, physical, chemical) required to completely describe the product being evaluated and factors to be considered in the collection of data on the essential oil such as chemical composition, intake of the essential oil, and data on related essential oils. In order to effectively evaluate an essential oil, attempted complete analyses must be available for the product intended for the marketplace. Additional quality control data is useful since it demonstrates consistency in the chemical composition of the product being marketed. A Technical Information Paper drafted for the particular essential oil under consideration organizes and prioritizes these data for efficient sequential evaluation of the essential oil.

In Steps 5 thru 10, each congeneric group of known structure and the total of unidentified constituents are evaluated for safety under conditions of intended use. In Steps 11 and 12, the safety of the essential oil is evaluated in the context of all congeneric groups, the fraction of the essential oil accounted for by unidentified substances, and any other related data (e.g., data on the essential oil itself or on an essential oil of similar composition). The guide organizes the extensive database of information on NFC constituents in order to efficiently evaluate the safety of the essential oil under conditions of use. It is, however, not intended to be a rigid checklist. The Panel will continue to evaluate each essential oil on a case by case basis applying their scientific judgment to insure that each NFC is exhaustively evaluated. One of the principal objectives of the guide and subsequent evaluation is that no significant portion of the essential oil should go unevaluated.

B.2. Prioritization of NFC According to Presence in Food

In Step 1, essential oils are prioritized according to their presence or absence as components of commonly consumed foods (Step 1). This question evaluates the relative intake of the NFC as an intentionally added flavoring substance versus its intake as a component part of food. Many NFCs are isolated from plants that are commonly consumed as a food. Little or no safety concerns should exist for the intentional addition of the NFC to the diet, if intake of the NFC from consumption of traditional foods

(garlic) substantially exceeds intake as an intentionally added flavoring substance (garlic oil). The first step in many ways, applies the concept of "long history of safe use" to essential oils. That is, a conclusion of safety is straight forward, if exposure to the essential oil occurs predominantly from consumption of a normal diet. This step clearly prioritizes essential oils consumed as part of a traditional diet on a lower level of concern that oils derived from plants that are either not part of the traditional diet or whose intake is not predominantly from the diet. The first step also mitigates the need to perform comprehensive chemical analysis for essential oils in those cases where intake is low and occurs predominantly from consumption of food.

B.3. Organization of Chemical Data-Congeneric Groups and Classes of Toxicity

In Steps 2 and 3, each identified chemical constituent is broadly classified according to toxic potential (Cramer *et al*., 1978) and then assigned to a congeneric group of structurally related substances that exhibit similar pathways of metabolism and toxicologic potential.

In Step 2, constituents are assigned to one of three structural classes (I, II, or III) based on toxic potential (Cramer *et al*., 1978). Class I substances contain structural features which suggest a low order of oral toxicity. Class II substances are clearly less innocuous than Class I substances, but do not contain structural features that provide a positive indication of toxicity. Class III substances contain structural features (epoxide functional group or un-substituted heteroaromatic derivatives) that permit no strong presumption of safety or may even suggest significant toxicity. For instance, the simple aliphatic hydrocarbon, limonene, is assigned to structural class I while elemicin which is an allyl-substituted benzene derivative with a reactive benzylic/allylic position is assigned to Class III. Likewise, chemically unidentified constituents of the essential oil will routinely be placed in Structural Class III.

The toxic potential of each of the three structural classes has been quantified (Munro *et al*., 1996). An extensive toxicity database has been compiled for substances in each structural class. The database covers a wide range of chemical structures, including food additives, naturally-occurring substances, pesticides, drugs, antioxidants, industrial chemicals, flavors and fragrances. Conservative no observable effect levels (5^{th} percentile NOELs) have been determined for each class. These 5^{th} percentile NOELs in each structural class are converted to human exposure thresholds levels by applying a 100-fold safety

factor and correcting for mean bodyweight (60/100). The 5th percentile NOELs and human exposure thresholds are recorded for each structural class (see Table 1). With regards to flavoring substances, these thresholds are even more conservative, given that the vast majority of NOELs for flavoring substances are above the 90th percentile. These conservative exposure thresholds have since been adopted by the World Health Organizations Joint Expert Committee on Food Additives (JECFA, 1997) and Commission of the European Communities (EC, 1999) for use in the evaluation of chemically-identified flavoring agents.

Table 1. Structural Class Definitions and their Human Intake Thresholds

CLASS	DESCRIPTION	5th PERCENTILE NOEL (mg/kg/day)	HUMAN EXPOSURE THRESHOLD* µg/day
I	Structure and related data suggest a low order of toxicity. If combined with low human exposure, they should enjoy an extremely low priority for investigation. The criteria for adequate evidence of safety would also be minimal. Greater exposures would require proportionately higher priority for more exhaustive study.	3.0	1800
II	Intermediate substances. They are less clearly innocuous than those of Class I, but do not offer the basis either of the positive indication of toxicity or of the lack of knowledge characteristic of those in Class III	0.91	540
III	Permit no strong initial presumptions of safety, or that may even suggest significant toxicity. They thus deserve the highest priority for investigation. Particularly when per capita intake is high of a significant subsection of the population has a high intake, the implied hazard would then require the most extensive evidence for safety-in-use.	0.15	90

* The human exposure threshold was calculated by multiplying the fifth percentile NOEL by 60 (assuming an individual weighs 60 kg) and dividing by a safety factor of 100.

Step 3 is a key step in the guide. It organizes the chemical constituents into congeneric groups that exhibit common chemical and biological properties. Based on the well-recognized biochemical pathways operating in plants, essentially all of the volatile constituents found in essential oils, extracts, and oleoresins belong to well-recognized congeneric groups. Recent reports (Maarse et al., 1992, 1994, 2000; Njissen et al., 2003) of the identification of new naturally occurring constituents indicate that newly identified substances fall into existing congeneric groups. The Expert Panel, JECFA, and the EC have acknowledged that individual chemical substances can be evaluated in the context of their respective congeneric group. The congeneric group approach provides the basis for understanding the relationship between the biochemical fate of members of a chemical group and their

toxicologic potential. Within this framework, the objective is to continuously build a more complete understanding of the absorption, distribution, metabolism and excretion of members of the congeneric group and their potential to cause systemic toxicity. Within the guidelines, the structural class of each congeneric group is assigned based on the highest structural class of any member of the group. Therefore, if a group of furanone derivatives contained members in Classes II and III, the congeneric group would, in a conservative manner, be assigned to Class III..

The types and numbers of congeneric groups in a safety evaluation program are, by no means, static. As new scientific data and information become available, some congeneric groups are combined while others are subdivided. This has been the case for the group of alicyclic secondary alcohols and ketones that were the subject of a comprehensive scientific literature review (SLR) in 1975 (FEMA, 1975). Over the last two decades, experimental data has become available indicating that a few members of this group exhibit biochemical fate and toxicologic potential inconsistent with that for other members of the same group. These inconsistencies, almost without exception, arise at high dose levels that are irrelevant to the safety evaluation of low levels of exposure to flavor use of the substance. However, given the importance of the congeneric group approach in the safety assessment program, it is critical to resolve these inconsistencies. Additional metabolic and toxicologic studies may be required to distinguish the factors that determine these differences. Often the effect of dose and a unique structural feature results in utilization of a metabolic activation pathway not utilized by other members of a congeneric group. Currently, evaluating bodies including JECFA, the European Union, and the FEMA Expert Panel have classified flavoring substances into the same congeneric groups for the purpose of safety evaluation.

B.4. Determination of Intake

Step 4 deals with the intake of congeneric groups and the unknown fraction from consumption of the essential oil. A range of concentration of each congeneric group is determined from multiple attempted complete analyses of the essential oil intended for commerce. The intake of each congeneric group is determined from highest concentration (%) recorded for the group and the daily *per capita* intake of the essential oil derived from the annual volumes reported in industry surveys (NAS, 1965, 1970, 1975, 1982, 1987; Lucas et al., 1999). The daily *per capita* intake of each congeneric group due to consumption of the essential oil is key data for the subsequent safety evaluation both of the congeneric group and the oil *in toto*. In similar manner, intake of the

fraction of unidentified constituents in the essential oil is determined from the highest concentration (%) reported for the total of the constituents and the intake of the NFC.

Intake is calculated using a method known as the *per capita* intake (PCI) x 10 method (Rulis *et al*. 1984; Woods and Doull, 1991). The method assumes that only 10% of the population consumes the total annual reported volume of use of a flavor ingredient. This approximation provides a practical and cost effective approach to the estimation of intake for flavoring substances. The annual volumes of flavoring agents are relatively easy to obtain by industry-wide surveys, which can be performed on a regular basis to account for changes in food trends and flavor consumption. The 1995 poundage survey of US flavor producers was published by FEMA in 1999 (Lucas *et al.*, 1999).

Calculation of intake using the PCI X 10 method has been shown to result in conservative estimates of intake and this is appropriate for safety evaluation. Over the last three decades, two comprehensive studies of flavor intake have been undertaken. One involved a detailed dietary analysis (DDA) of a panel of 12,000 consumers (Hall, 1976; Hall and Ford, 1999). The other is based on a robust flavor stochastic model (FSM) (Lambe et al., 2002). The results of the data intensive DDA method and the model-based FSM support the use of per capita intake data as a conservative estimate of intake.

With regards to essential oils, the PCI x 10 method provides overestimates of intake for oils that are widely distributed in food. The large annual volume of use reported for essential oils such as orange oil and lemon oil indicate widespread use in a large variety of foods by a high percentage of the population. Citrus flavor is pervasive in a multitude of foods and beverages. Therefore, for selected high volume essential oils, a simple *per capita* intake rather than a *per capita* x 10 intake may be more appropriate. However, unless otherwise noted, the intake of the congeneric groups and the group of unidentified constituents will be determined by the PCI x 10 method.

C. Safety Evaluation

C.1 Congeneric Groups in the Essential oil

In steps 5, 6, and 7, each congeneric group in the essential oil is evaluated for safety in use. In step 5, an evaluation of the metabolism and disposition is performed to determine, if under current conditions of intake, whether the group of congeneric constituents is metabolized by well-established detoxication pathways to yield innocuous products. If the congeneric group is metabolized to innocuous products, the answer to Step 5 is yes. That is, such pathways exist for the congeneric group of constituents in an essential oil and safety concerns will arise only if intake of the congeneric group is sufficient to saturate these pathways potentially leading to toxicity. Therefore, step 6 of the guide considers the intake of the congeneric group relative to the respective human exposure thresholds for one of the three structural classes (1800 ug/day for Class I; 540 ug/day for Class II; 90 ug/day for Class III). If the intake of the congeneric group is less than the threshold for the respective structural class, the intake of the congeneric group presents no significant safety concerns. The group passes the first phase of the evaluation and is then referred to step 11, the step in which the safety of the congeneric group is evaluated in the context of all congeneric groups in the essential oil.

If, at step 5, no sufficient metabolic data exist to establish safe excretion of the product or, in fact, activation pathways have been identified for a particular congeneric group, then toxicity data (Step 7) are required to establish safe use of the congeneric group under current conditions of intake. We are aware of examples where low levels of xenobiotic substances can be metabolized to reactive substances. In the event that reactive metabolites are formed at low levels of intake of naturally occurring substances, a detailed analysis of dose-dependent toxicity data will be performed. Also, if the intake of the congeneric group is greater than the human exposure threshold (suggesting metabolic saturation may occur), then toxicity data are also required. If, at step 7, a database of relevant toxicological data for a representative member or members of the congeneric group indicates a sufficient margin of safety exists for the intake of the congeneric group, the members of that congeneric group are concluded to be safe under conditions of use of the essential oil. The congeneric group then moves to step 11.

In the event a congeneric group either does not have sufficient data to evaluate step 7 or exhibits data in which sufficient margins of safety are not established, the essential oil cannot be further evaluated by this guide and must be set aside for further considerations.

Use of the guide requires scientific judgment at each step of the sequence. The need to evaluate congeneric groups that accounts for less than 2% of a low volume essential oil is not necessary, if the same

congeneric group that accounted for 20% of a high volume NFC was previously evaluated and found to be safe under intended conditions of use.

C.2 Unidentified Constituents in the Essential Oil

In step 8, 9, and 10, the total of unidentified constituents are considered. In most essential oils of commercial importance (i.e., significant annual volume of use), the % of the essential oil not chemically identified will be relatively small, typically <5%. Depending on the analytical method, it is possible to detect and identify constituents of a volatile essential oil at concentrations of 0.01%. However, there will remain a number of volatile constituents detected but not identified and ones that go undetected. Given that newly identified volatile constituents of food (Maarse et al., 1992, 1994, 2000; Njissen et al., 2003) can be assigned to already existing congeneric groups, there is a high probability that a significant portion of unidentified constituents belong to these same group. However, as a practical matter, consideration for the safety of this fraction of unidentified constituents is required. Therefore, the group of unidentified constituents is placed in the structural class of highest concern (Class III).

Another factor to consider in prioritization of the unidentified constituents for safety evaluation is the method of isolation. Steam distillation used to isolate the essential oil and additional distillation during processing of the essential oil limits the types of chemicals in the consumed. Substances such as higher molecular weight polyaromatic hydrocarbons and halogenated polyaromatic hydrocarbons will not be present in any significant quantity in the distilled essential oil. Considering that essential oils are derived from plant sources, many of which are consumed as food, safety concern for the presence of the unidentified constituents is reduced.

If, however, a constituent or group of constituents is known to exhibit increased toxic potential, rigorous chemical analysis is performed. Lemon oil contains a group of photosensitive chemicals named psoralens that have been routinely quantified at ppm levels in the oil. Manufacturers regularly monitor psoralen levels and maintain low levels in the finished product. However, even in these cases, a practical perspective is needed, given that we safely consume most if not all of these constituents in traditional food products (e.g., lemons). Although little concern for safety exists for naturally occurring constituents

regardless of whether they are chemically characterized, the safety evaluation will evaluate the intake and sources of exposure to unidentified constituents contained in the essential oil.

In step 8, the principal source of exposure to the unidentified constituents is evaluated. If the source of the essential oil is also a food (e.g., basil oil), there are two sources of dietary exposure. If intake of the essential oil as a component of commonly consumed food far exceeds (5-10x) intake from its intentional addition to food, then no significant safety concerns exist for the presence of unidentified constituents in the essential oil. The evaluation of the unidentified constituents is then moved to step 11.

If the essential oil is not a component of a food or its intake from added flavor use is greater than its intake from food, the intake of the group of unidentified constituents in the essential oil must be considered relative to a conservative threshold for toxicity (Step 9). This would certainly apply to an essential oil with a significant fraction of unidentified constituents that isn't consumed as a food (e.g., myrrh oil) or one in which intake of the unidentified constituents from added flavor use exceeds that from consumption in food (*e.g.*, clove bud oil). As a highly conservative assumption, the total intake of all unidentified constituents are considered together and placed in the structural class of greatest toxic potential (i.e., Class III). If the total intake of unidentified constituents is less than the conservative human exposure threshold (90 ug/p/d), it is considered safe because of the very low level of exposure. If it exceeds the threshold, exposure to the unknown fraction has reached a level that demands additional action (Step 10).

Options are available to address safety concerns for significant exposure to unknowns that are not normally consumed in food (e.g., myrrh oil). Since the number of unidentified constituents depends upon the rigor of analytical methodology, further analysis may reduce the level of unknowns such that it no longer raises a safety concern. As second option, toxicity data could be collected or generated for the essential oil to provide an adequate margin of safety for intake of the unidentified constituents present in that essential oil. Third, toxicity data on an essential oil of similar composition (e.g., coriander oil) could also be used to provide margins of safety for the intake of the essential oil (e.g., Bois de Rose) containing the unidentified constituents. Finally, the safety factor typically used to ensure a margin of safety (100) may not be relevant, if the essential oil or botanically related essential oil is widely consumed in food. On a

case-by-case basis, practical considerations and scientific judgment should determine the most effective option to evaluate the safety due to the presence of an unknown fraction.

D. Evaluation of Total Chemical Composition of Essential Oil –GRAS Decision

Results of safety evaluations of all congeneric groups and the group of unidentified constituents that comprise the essential oil funnel into Step 11. Step 11 deals with relevant scientific considerations (e.g. synergistic interactions) that may raise safety concerns for the essential oil under conditions of intended use. This question provides the opportunity to consider the potential for chemical and biological interactions between constituents of different congeneric groups of the essential oil or the effect of intake of the essential oil by a specialized group of eaters.

Step 11 considers additivity or synergistic interactions of individual substances and the different congeneric groups in the essential oil. The level of exposure to congeneric groups is relevant to whether additive or synergistic effects present a significant health hazard. The vast majority of NFCs are used in food in extremely low concentrations; obviously, also resulting in very low intake levels of different congeneric groups. Moreover, major representative constituents of each congeneric group have been tested individually and pose no toxicological threat even at dose levels that are orders of magnitude greater than normal levels of intake of essential oils from use in traditional foods. Based on the results of toxicity studies both on major constituents of different congeneric groups in the NFC and on the NFC itself, it can be concluded that the toxic potential of these major constituents is representative of that of the NFC itself, indicating the likely absence of additivity and synergistic interaction. Therefore, as a rule the margin of safety is so wide and the possibility of additivity or synergistic interaction so remote that combined exposure to the different congeneric groups and the unknowns are considered of no health concern, even if expert judgement cannot fully rule out additivity or synergism. However, case-by-case considerations are appropriate. Where possible combined effects might be considered to have toxicological relevance, additional data may be needed for an adequate safety evaluation of the NFC.

Additivity of toxicologic effect or synergistic interaction is a conservative default assumption which may be applied whenever the available metabolic data do not clearly suggest otherwise. The extensive database of metabolic information on congeneric groups (JECFA 1997-2004) that occur in

essential oils suggest the potential for additive effects and synergistic interactions among congeneric groups in essential oils is extremely low. Although additivity of effect is the approach recommended by NAS/NRC committees (NRC, 1994; NRC, 1988) and regulatory agencies (EPA, 1988), the Presidential Commission of Risk Assessment and Risk Management has recently recommended (Presidential Commission, 1996) that: "For risk assessments involving multiple chemical exposures at low concentrations, without information on mechanisms, risks should be added. If the chemicals act through separate mechanisms, their attendant risks should not be added but should be considered separately." Thus, the risks of chemicals which act through different mechanisms, on different target systems, or are toxicologically dissimilar should be considered to be independent of each other. The congeneric groups in essential oils are considered separately.

Further, the majority of individual constituents that comprise essential oils are themselves used as flavoring substances that pose no toxicological threat at doses that are magnitudes greater than their level of intake from the essential oil. Rulis (1986) reported that "The overwhelming majority of additives present a high likelihood of having safety assurance margins in excess of 10^5". He points out that this is particularly true for additives used in the U.S. at less than 100,000 lbs./year. Because more than 90% of all flavoring ingredients are used at less than 10,000 lbs./year (Hall and Oser, 1968), this alone implies intakes commonly many orders of magnitude below the no-effect level. Non-additivity thus can often be assumed. As is customary in the evaluation of any substance, high-end data for exposure (consumption) are used, and multiple other conservatisms are employed to guard against underestimation of possible risk. All of these apply to complex mixtures as well as to individual substances.

E. Summary

The safety evaluation of an essential oil is performed in the context of the data for congeneric groups of identified constituents and the group of unidentified constituents, available data on the essential oil or a related essential oil, and any potential interactions that that may occur in the essential oil when consumed as a flavoring substance.

In summary, the guide provides a chemically-based approach to the safety evaluation of an essential oil. The approach depends on attempted complete quantitative analysis of chemical constituents

in the essential oil intended for commerce. The chemical constituents are then assigned to well-defined congeneric groups that are established based on extensive biochemical and toxicologic information. Metabolic and toxicological data for each congeneric group is, in turn, is evaluated in the context of intake of the congeneric group resulting from consumption of the essential oil. The intake of unidentified constituents is evaluated in the context of the consumption of the essential oil as a food, a highly conservative toxicologic threshold, and toxicity data on the essential oil or an essential oil of similar chemical composition. The flexibility of the guide is reflected in the fact that high intake of major congeneric groups of low toxicologic concern will be evaluated along with low intake of minor congeneric groups of significant toxicological concern (i.e., higher structural class). The guide also provides a comprehensive evaluation of all congeneric groups and constituents that account for the majority of the composition of the essential oil. The overall objective of the guide is to organize and prioritize the chemical constituents of an essential oil in order that no reasonably possible significant risk associated with the intake of essential oil goes unevaluated. Future publications on this subject will provide examples on the application of the guide to a wide variety of essential oils under different conditions of exposure.

Appendix A

Guide for the Safety Evaluation of Natural Flavoring Complexes (NFCs): Essential Oils

Preamble

This document provides ***guidance*** for the safety evaluation of essential oils; it is not to be viewed as a rigid check-list.

The preamble identifies the data that must be available to successfully employ this safety evaluation sequence. These data constitute a description of the commercial product(s) to be evaluated. These data are essential for assembly of the congeneric groups[1] on which the safety evaluation will be based.

Description of Product for GRAS Evaluation

The six (a-f) factors listed below can, and often do, have so extensive an influence on composition as to result in a wholly distinctive product. Therefore, in all cases, it is essential to define these factors in order to ensure that commercial products conform to the composition limits that describe the evaluated product.

Specify the range of concentrations of each known constituent of the final essential oil intended for commerce. These data may be obtained by using analytical data from currently or recently available commercial products derived only from the botanical source named plus carefully reviewed literature data and supplemented as necessary with new analytical data. It is essential to provide a thorough characterization of the chemical composition of the product to be used as a flavoring agent. If available, list existing specifications (*e.g.*, Food Chemicals Codex) for the composition of the product intended for use in food.

That range must take into account:

a. all recognized commercially practical botanical sources[2],
b. all relevant geographical sources,
c. all commercially used plant parts,
d. all commercially used degrees of maturity,
e. all commercially used methods of isolation, and
f. the variability inherent in each method of isolation.

Product Specification

A product specification shall include existing relevant specifications (for example, Food Chemical Codex specifications) and additional data that assure the identity, purity, and safety of the

[1] See Appendix B.
[2] The botanical source should be described phytogenically by family and by genera, species and sub-species within each family.

commercial product. The data on identity shall include all constituents that characterize the product and any of particular safety concern.

Exposure Data

Provide data on the total exposure to the essential oil through:

a. **history of use**
b. **intake of the parent botanical source of the essential oil when that source is itself consumed as a food, and**
c. **intake of the essential oil when it is used as an added flavoring ingredient.**
d. **any other relevant data on individual constituents**

Note: If there are other closely related essential oils, it will be necessary to submit compositional and consumption data, as defined above, on each such essential oil. In some cases, the composition of closely related essential oils may be so similar that multiple botanical and geographic sources, plant parts, and/or methods of isolation may be combined into one defined essential oil and exposure calculation.

Unidentified Constituents

Provide data on the range of concentration of the total of unidentified constituents in the essential oil intended for commerce, consistent with the factors outlined above and provide a discussion of how these data were calculated.

Guide for the Safety Evaluation of Essential Oils

STEP 1. If the essential oil is comprised of the volatile or extractable components from a commonly consumed food and the NFC is added to food in such manner and at levels comparable to those encountered by consumers of that food, determine the daily *per capita* intake of the essential oil resulting from consumption of 1) the food itself and 2) as an added flavoring agent. The aspect of food use is particularly important. It determines whether exposure to the essential oil occurs predominantly from intake of the parent botanical when it is used as food, or from the essential oil itself when it is added as a flavoring ingredient. These data are important in Steps 8 and 12. If the conditions of use of the essential oil differ from those of the same constituents in the food source, list these differences. Proceed to Step 2.

STEP 2. Classify each known constituent according to its decision tree structural class (Cramer *et al.*, 1978) and proceed to Step 3. All unidentified constituents will be assigned to the default classification of Structural Class III (highest level of concern) and proceed to Step 4b.

STEP 3. Classify the chemically identified constituents into one of the groups of structurally related substances (congeneric groups) in the list in Appendix B. Each group should be expected, on the basis of established data, to exhibit consistently similar rates and pathways of absorption, distribution, metabolism and excretion, and common toxicological endpoints (e.g. benzyl acetate, benzaldehyde, and benzoic acid). Assign a decision tree structural class to each congeneric group. If constituents of a congeneric group exhibit different decision tree structural classes, assign the highest structural class to the congeneric group. Proceed to Step 4a.

STEP 4a. Calculate a concentration range for each group of congeneric substances. The upper limit of the range is the highest concentration of the sum of the members of the congeneric group in the essential oil from any one attempted complete analysis of any one product (see note in the preamble). Based on the upper limit concentration (%) for each congeneric group in the essential oil and the reported annual volume of consumption, determine the daily *per capita* intake of each congeneric group from consumption of the essential oil. Proceed to Step 5.

STEP 4b. Choose the highest % concentration in the range given for the group of unidentified constituents from any credible effort at a complete analysis, and determine the daily *per capita* intake of the group of unidentified constituents. Proceed to Step 8.

Congeneric Groups of Chemically Identified Constituents

Note: Repeat Steps 5 through 7 for each congeneric group in the essential oil.

STEP 5. Within each congeneric group, do metabolic data exist for a representative member or members of the group, that indicate, in the context of current estimated levels of intake, that the group would be expected to be metabolized primarily by well established detoxication pathways to innocuous products?

If yes, go to Step 6.
If no, go to Step 7.

STEP 6. Is the total intake of the congeneric group less than the human exposure threshold (Munro *et al.*, 1996) for the respective structural class? Note: If the group contains members from different structural classes, select the structural class with the highest level of concern.

If yes, go to Step 11.
If no, proceed to Step 7.

STEP 7. Does a database of relevant toxicological data (NOAEL, genotoxicity, metabolism, etc.) exist for a representative member or members of the congeneric group that would allow for a comprehensive safety evaluation of the congeneric group and provide a sufficient margin of safety for intake of the congeneric group derived from the essential oil? "A sufficient margin of safety" must be determined on a case-by-case basis. Examples of factors that contribute to the determination of a safety margin include 1) the extent of natural occurrence of each of the constituents of the congeneric group throughout the food supply[3], 2) recognition that the maximum concentration for the group, based the highest concentration of each constituent, is necessary for prudence, but wholly unrealistic, 3) structural alerts, 4) the nature and concentration of constituents in related botanical genera and species and 5) metabolic detoxication thresholds for the congeneric group of substances.

If yes, proceed to Step 11.
If no, retain for further evaluation.

[3] Although natural occurrence is no guarantee of safety, if exposure to the intentionally added constituent is trivial compared to intake of the constituent from consumption of food, then this must be taken into consideration in the determination of a safety factor (Kroes *et al*, 2000).

Unidentified Constituents

STEP 8. Using data from Step 1, is the intake of the essential oil from consumption of the food itself significantly greater[4] than the intake of the added essential oil?

If yes, proceed to Step 11.
If no, the intake of the group of unidentified constituents from consumption of the essential oil is significant. Proceed to Step 9.

STEP 9. Is the estimated intake of the group of unidentified constituents less than the human exposure threshold (Munro, 1996; Kroes *et al,* 2000) for structural class III, the level of highest toxicological concern (90 μg/p/d)[5]?

If Yes, proceed to Step 11.
If No, proceed to Step 10.

STEP 10. Do toxicity data (NOAEL, etc.) exist for the essential oil, an essential oil of similar composition, or from the same botanical species, that would provide an adequate margin of safety for intake of the essential oil?

If Yes, proceed to Step 11.
If No, perform the appropriate toxicity test, obtain further analytical data to reduce the fraction of unknown components, consider instituting a lower safety factor [1] where appropriate, and/or consider toxicity data for other essential oils having a similar composition. Resubmit for further evaluation.

Conclusion on the Safety of the Essential Oil

STEP 11. Are there any other relevant scientific considerations (e.g. non-interaction, joint actions or interactions) that raise safety concerns?

If Yes, retain for further evaluation.
If No, proceed to Step 12.

[4] Provided the intake of the unidentified constituents is greater from consumption of the food itself, the intake of unidentified constituents from the added essential oil is considered trivial.

[5] The human exposure threshold of 90 ug/person/d is determined from a database of NOELs obtained from 448 subchronic and chronic studies of substances of the highest toxic potential (structural class III) mainly herbicides, pesticides and pharmacologically active substances (Munro *et al.*,1996). The 5th percentile NOEL (lowest 5%) was determined to be 0.15 mg/kg bw/day which upon incorporation of a 100-fold safety factor for a 60 kg person yielded a human exposure threshold of the 90 ug/person/d. However, no flavoring substance or food additive in this structural class exhibited a NOEL less than 25 mg/kg bw/d. Therefore the 90 ug/person/d threshold is an extremely conservative threshold for the types of substances expected in natural flavoring complexes. Additional data on other specific toxic endpoints (*e.g.*, neurotoxicity, reproductive and endocrine disruption) support the use of this threshold value (Kroes *et al,* 2000).

STEP 12. Based on the above considerations, can the essential oil safely be used as a flavoring agent under current conditions of intended use?

If Yes, the essential oil is concluded to be of no safety concern under current conditions of intended use.

If No, withhold for further testing and evaluation.

Appendix B

Table 1. Chemical Groups of Flavor Materials	
1	Saturated Aliphatic, Acyclic, Linear Primary Alcohols, Aldehydes, Carboxylic Acids and Related Esters
2	Saturated Aliphatic, Acyclic, Branched-chain Primary Alcohols, Aldehydes, Carboxylic Acids and Related Esters
3	Aliphatic Linear and Branched-chain alpha,beta-Unsaturated Aldehydes and Related Alcohols, Acids and Esters
4	Aliphatic Allyl Esters
5	Unsaturated Linear and Branched-chain Aliphatic, Non-conjugated Aldehydes, Related Primary Alcohols, Carboxylic Acids and Esters
6	Aliphatic Primary Alcohols, Aldehydes, Carboxylic Acids, Acetals and Esters Containing Additional Oxygenated Functional Groups
7	Saturated Alicyclic Primary Alcohols, Aldehydes, Acids, and Related Esters
8	Saturated and Unsaturated Aliphatic Acyclic Secondary Alcohols, Ketones and Related Esters
9	Aliphatic Acyclic and Alicyclic alpha-Diketones and Related alpha-Hydroxyketones
10	Alicyclic Ketones, Secondary Alcohols and Related Esters*
11	Pulegone and Structurally- and Metabollically-related Substances
12	Aliphatic and Aromatic Tertiary Alcohols and Related Esters
13	Aliphatic, Alicyclic, Alicyclic-Fused and Aromatic-Fused Ring Lactones *
13	Aliphatic and Aromatic Hydrocarbons
14	Benzyl Derivatives
15	Hydroxy- and Alkoxy-substituted Benzyl Derivatives
16	Cinnamyl Alcohol, Cinnamaldehyde, Cinnamic Acid, and Related Esters
17	Phenyl-substituted primary Alcohols, Aldehydes, Carboxylic Acids, and Related esters
18	Phenyl-substituted Secondary Alcohols, Ketones, and Related Esters
20	Phenol Derivatives
21	Hydroxyallylbenzene and Hydroxypropenylbenzenes Derivatives
22	Phenethyl Alcohol, Phenylacetaldehyde and Related Acetals and Esters
23	Aliphatic and Aromatic Ethers
24	Furfuryl Alcohol, Furfural, and Related Substances
25	Furan Derivatives
26	Aliphatic and Aromatic Sulfides and Thiols
27	Sulfur-substituted Furan Derivatives
28	Sulfur-containing Heterocyclic and Heteroaromatic Derivatives
30	Aliphatic and Aromatic Amines and Related Amides
31	Nitrogen-containing Heterocyclicand Heteroaromtic Substances
32	Pyrazine Derivatives
33	Anthranilate Derivatives
34	Amino Acids
35	Maltol Derivatives
36	Epoxide Derivatives

Appendix C. Natural Flavor Complexes:Essential Oils

1. Method of Preparation

Although flavors are often created exclusively from discrete chemical raw materials, certain flavor types cannot be satisfactorily reproduced without the use of natural oils or extracts. Natural complexes are the volatile, ethereal fraction obtained from botanicals by a physical separation method. The essential oils obtained by distillation, or expression represent the odorous part and are distinguished from the "fixed" or non-volatile oils which have virtually no odor or flavor value (Schay, 1971). Further distillation of the essential oil results in folded, rectified, terpeneless, or sesquiterpeneless oils. Over the years, various methods have evolved to obtain a concentrate of volatiles which are more representative of the original botanical. Most of these methods include some form of distillation of the plant part or treatment of the botanical with one or more organic solvents followed by the concentration of the extracted solute. Because solvent extraction often results in a product with properties more representative than that of a distilled oil, many natural complexes are available as extracts in addition to the essential oil.

Essential oils, with few exceptions, are liquids isolated from various plant parts. Essential oils are either distilled or expressed. Distillation can be water, steam, water-steam, or dry with steam distillation being the most common. High pressure steam distillation is applied when the botanical material and its essential oil are sufficiently heat-resistant and non-hydrolyzable. Some essential oils require the enzymatic release of the volatile components prior to steam distillation. A rectified oil refers to one from which certain fractions from a fractional distillation are blended or bulked. Rectification often results in a significant loss of material (Arctander, 1969) in an effort to improve a particular property or characteristic, such as flavor or aroma. Distillations and redistillations are applied to remove color, water, resinous matter, and unpleasant topnotes.

The physical process of expression is applied almost exclusively to citrus fruits. Concentrated or folded oils are processed to remove undesirable or nonflavor components. The processing methods include fractional distillation, topping, solvent extraction, countercurrent extraction, supercritical extraction, thin-film evaporation, and molecular distillation (Mookherjee, 1996). These processed oils are referred to as terpeneless or sesquiterpeneless. Although termed concentration, the process of concentration or folding results in a concentrate with flavor body weaker than the complete essential oil, suggesting that valuable constituents are lost in the process to remove terpenes. The "fold" terminology refers to the reduction of the volatile constituent; e.g.

when half of the volatile constituents are removed, their removal “doubles” the concentration and the oil, therefore, is called twofold. Concentrated (folded) oils are used extensively in flavors where a high amount of unstable and insoluble terpenes may be undesirable. The percentages of oxygenated compounds in terpeneless and sesquiterpeneless oils are higher than that of the complete essential oil.

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