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FEMA GRAS assessment of natural flavor complexes: Clove, cinnamon leaf and West Indian bay leaf-derived flavoring ingredients

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ABSTRACT

In 2015, the Expert Panel of the Flavor and Extract Manufacturers Association initiated the safety re-evaluation of over 250 natural flavor complexes (NFCs) used as flavor ingredients. This publication, 4th in a series focusing on the safety evaluation of NFCs, presents an evaluation of NFCs rich in hydroxyallylbenzene and hydroxypropenylbenzene constituents using a procedure initially published in 2005 and updated in 2018 that evaluates the safety of naturally occurring mixtures for their intended use as flavoring ingredients. The procedure requires the characterization of the chemical composition for each NFC and subsequent organization of the constituents into defined congeneric groups. The safety of each NFC is evaluated using the conservative threshold of toxicological concern (TTC) approach together with studies on absorption, metabolism and toxicology of the NFC and its constituent congeneric groups. By the application of this procedure, seven NFCs, derived from clove, cinnamon leaf and West Indian bay leaf were affirmed as "generally recognized as safe (GRAS)" under their conditions of intended use as flavor ingredients. An eighth NFC, an oleoresin of West Indian bay leaf, was affirmed based on its estimated intake, which is below the TTC of 0.15 μg/person per day for compounds with structural alerts for genotoxicity.

1. Introduction

For almost six decades, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) has been the principal, independent body evaluating flavoring ingredient safety in the United States. Flavor ingredients are evaluated based on their usage and toxicological properties to determine their 'generally recognized as safe' (GRAS) status for their intended flavoring uses consistent with the 1958 Food Additive Amendment to the Federal Food, Drug and Cosmetic Act (Hallagan and Hall, 1995, 2009; Hallagan et al., 2020). To date, the FEMA Expert Panel has determined that over 2,700 flavoring ingredients have met GRAS criteria for their intended uses.

An essential part of FEMA's GRAS program is the periodic re-evaluation of the GRAS status of flavoring ingredients. Flavoring ingredients are divided into two general categories: chemically defined flavoring materials and natural flavor complexes (NFCs). Chemically defined flavoring materials are typically single chemical substances

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Abbreviations

ASTA	American Spice Trade Association
BMDL10	Lower confidence limit of the benchmark dose resulting in a 10% extra cancer incidence
CF	Correction factor
CFR	Code of Federal Regulations
CG	Congeneric group
DTC	Decision tree class
EFFA	European Flavour Association
EFSA	European Food Safety Authority
ERS/USDA	Economic Research Service/United States Department of Agriculture
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FID	Flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GLP	Good laboratory practice
GMP	Good manufacturing practice
GRAS	Generally recognized as safe
IFEAT	International Federation of Essential Oils and Aroma Trades
IOFI	International Organization of the Flavor Industry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JFFMA	Japan Fragrance and Flavor Materials Association
LC-MS	Liquid chromatography-mass spectrometry
MOE	Margin of exposure
MoS	Margin of safety
ND	No data
NFC	Natural flavoring complex
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organization for Economic Co-Operation and Development
PBBK	Physiologically based biokinetic (model)
PCI	Per capita intake
TD50	Dose giving a 50% tumor incidence
TDI	Tolerable daily intake
TTC	Threshold of toxicological concern
WHO	World Health Organization

while NFCs are complex mixtures usually derived from botanical or other natural sources; both are used to flavor food. The FEMA Expert Panel previously completed two re-evaluations of chemically defined flavor ingredients and in 2015, expanded the re-evaluation program to include NFCs. The Panel's safety evaluation procedure, published in 2005 (Smith et al., 2005), applies a stepwise analysis of an NFC based on its chemical composition that was re-evaluated and updated in 2018 (Cohen et al., 2018a). NFC constituents, which are often the products of well-established biochemical pathways, can be organized into a finite number of well-defined congeneric groups. The estimated intakes for the constituent congeneric groups of each NFC are evaluated using the threshold of toxicological concern (TTC) concept (Kroes et al., 2000; Munro et al., 1996). Data on the absorption, metabolism and toxicology of members of each congeneric group and the NFC are also considered. This procedure has previously been applied for the re-evaluation of FEMA GRAS status for *Citrus*-derived NFCs (Cohen et al., 2019), NFCs derived from mint, buchu, dill and caraway plants (Cohen et al., 2020) and *Cassia*, *Cinnamomum* and *Myroxylon*-derived NFCs (Rietjens et al., 2020). This manuscript is focused on NFCs whose constituent profile is dominated by eugenol and other constituents of congeneric Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives).

In 2015, the FEMA Expert Panel issued a call for data requesting detailed chemical analysis for the *Eugenia*, *Cinnamomum* and *Pimenta*-derived NFCs listed in Table 1. Members from various organizations, including the International Organization of the Flavor Industry (IOFI), FEMA, the Japan Fragrance and Flavor Materials Association (JFFMA), the European Flavour Association (EFFA), and the International Federation of Essential Oils and Aroma Trades (IFEAT) provided data for the safety evaluation of these NFCs that are used for flavoring of food and beverage products.

2. History of food use

Cloves and other eugenol producing botanicals have historically been used by diverse cultures for flavoring, food preservation and traditional medicine. Dried clove buds, more familiarly known as whole cloves, remain a popular culinary spice globally. Cinnamon leaves and West Indian bay leaves are also used to flavor foods but their popularity is more localized to their native growing regions.

The cinnamon leaf and clove NFCs in this group originate from Southeast Asia and have a long history of use. Cinnamon is a well-recognized culinary spice that is harvested from the inner bark of the plant. Several *Cinnamomum*-derived flavoring ingredients derived from the inner bark of the plant were recently evaluated by the FEMA Expert Panel (Rietjens et al., 2020). While cinnamon bark oil is characterized by its high cinnamaldehyde content, the essential oils derived from distillation of the leaves *C. zeylanicum* Nees, *C. loureirii* Blume and *C. cassia* Blume are rich in eugenol. Clove is also a common culinary spice with a long history of use as flavoring and in traditional medicine. Third century B.C. Chinese court officials chewed on cloves to sweeten their breath when addressing the emperor (ASTA, 2002; Purseglove et al., 1981). Trade of these spices from the East to the West remained under strict control during much of the Middle Ages, making them expensive luxuries in the West (Coppen, 1995; Purseglove et al., 1981; Schivelbusch, 1992). With the beginning of the Age of Exploration (1500s–1700s), sea trade routes, dominated by European naval powers, became the prominent means for transport and trade of spices to Europe (Osborne; Purseglove et al., 1981; Ravindran et al., 2003). In contrast, the West Indian bay tree, of the genus *Pimenta,* originates in the West Indian islands and is different than bay or "sweet bay" of the genus *Laurus,* that originated in Asia and whose leaves are a commonly known culinary spice in the USA. Cinnamon, West Indian bay and sweet bay leaves have historically been used to flavor foods and teas. Over time, cultivation of these spices has been transplanted into new regions and their essential oils and oleoresins have become widely available for use in flavoring and fragrance applications.

In the twentieth century, the use of spice oleoresins became increasingly prevalent in processed foods. Spice oleoresins, prepared by the extraction of a spice such as cloves or West Indian bay leaves, contain both the essential oil and resinous fractions of the spice and are highly concentrated flavor ingredients compared to the spices from which they are derived. Spice oleoresins used as flavoring ingredients are often standardized to contain a specific percentage of essential oil by dilution with food grade ingredients. Because spice oleoresins can be concentrated, standardized and more easily stored and handled, they have found use in some processed foods in place of whole or ground spices.

3. Current usage

The most recent annual usage (Harman and Murray, 2018) and exposure calculations for each NFC are listed in Table 1. The clove oils,

Table 1
NFCs evaluated by the FEMA Expert Panel.

Name	FEMA No.	Estimated Intake (μg/person/day)[a]	Most recent annual volume (kg)[b]
Bay Leaves West Indian Oil (*Pimenta acris* Kostel; *P. racemosa*)[c]	2122	60	560
Bay Leaves West Indian Oleoresin (*Pimenta acris* Kostel; *P. racemosa*)[c]	2123	0.01	0.1
Clove Bud Extract (*Eugenia caryophyllata* Thunb. [*Eugenia aromatica* (L.) Baill.or *Syzygium aromaticum* (L.) Merr. et Perry])[d]	2322	60	530
Clove Bud Oil (*Eugenia caryophyllata* Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])[d]	2323	2,350	22,000
Clove Bud Oleoresin (*Eugenia caryophyllata* Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])[d]	2324	390	3,640
Clove Leaf Oil (*Eugenia caryophyllata* Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])[d]	2325	430	40,300
Clove Stem Oil (*Eugenia caryophyllata* Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])[d]	2328	190	1,790
Cinnamon Leaf Oil (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)[c]	2292	560	5,260

[a] For high volume materials (greater than 22,700 kg/year), the PCI per capita is shown. For materials with a lower surveyed volume (less than 22,700 kg/year, PCI × 10 (“eaters only’) calculation is shown.
[b] Harman, C.L. and Murray, I.J. 2018. Flavor and Extract Manufacturers Association of the United States (FEMA) 2015 Poundage and Technical Effects Survey, Washington DC, USA.
[c] Federal Code of Regulation 21 CFR § 182.20 (Essential oils, solvent-free oleoresins, and natural extractives, including distillates).
[d] Federal Code of Regulation 21 CFR § 184.1257 (Direct food substances affirmed as generally recognized as safe – Clove and its derivatives).

Clove Bud Oil (FEMA 2323), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328) have estimated per capita intakes ranging from 190 to 2350 μg/person/day. Cinnamon Leaf Oil (FEMA 2292) is within this range as well while the estimated intakes of Clove Bud Extract (FEMA 2322) and Bay Leaves West Indian Oil (FEMA 2122) are lower, 60 μg/person/day. For the oleoresins, the estimated per capita intake for Clove Bud Oleoresin (FEMA 2324) is 390 μg/person/day and 0.01 μg/person/day for Bay Leaves West Indian Oleoresin (FEMA 2123).

Cloves, cinnamon leaves and West Indian bay leaves are culinary spices. The Economic Research Service of the United States Department of Agriculture (ERS/USDA) routinely collects import poundage data on major spices and herbs and their composites. The estimated annual volume of cloves imported into the USA in 2015 was 1.7 million kg (ERS/USDA, 2019). The concentration of volatile oil in cloves ranges from 11 to 17% volatile oil (Al-Hilphy, 2015; Guan et al., 2007; Safrudin et al., 2015). Using these figures, the estimated intake of clove oil from the consumption of cloves as food is estimated to be between 1600 and 2500 μg/person/day. A similar estimation of intake for the consumption of the volatile oils from cinnamon leaves and West Indian bay leaves is not possible due to the lack of quantitative data on their consumption as spices.

4. Manufacturing methodology

Clove plants are indigenous to the Maluku islands, also known as the Spice Islands and are currently cultivated in Indonesia as well as several African and South Asian countries. Clove plants produce viable cloves after 4–5 years, but normally the plant does not reach full bearing capacity until after 20 years (Purseglove et al., 1981). Clove clusters are manually picked when buds have reached full size and begin developing a pink flush prior to opening (ASTA, 2002). Clusters are taken for processing where stems, buds, and leaves are separated and allowed to dry. Dried clove buds are steam distilled, either whole or pulverized, yielding on average 11–17% volatile oil (Al-Hilphy, 2015; Guan et al., 2007; Safrudin et al., 2015). Clove stems are also steam distilled, yielding on average 5–7% oil. Clove leaves yield 1.5–1.8% volatile oil by steam distillation (Milind and Deepa, 2011; Purseglove et al., 1981). Clove extracts are also produced by extraction with organic solvents of the clove bud, followed by solvent evaporation.

During the harvesting of cinnamon bark, the plant leaves are trimmed, collected, and stored separately from the bark. The leaves are allowed to dry for a limited time before proceeding to processing. Steam distillation of a batch of leaves normally results in a 1% volatile oil yield on a dry weight basis (Ravindran et al., 2003).

Spice oleoresins such as clove bud oleoresin and oleoresin from West Indian bay leaves are prepared by the extraction of the spice with a volatile solvent such as acetone, isopropanol, methanol, hexane or a chlorinated hydrocarbon followed by removal of the solvent from the extract by distillation. Alternatively, following the collection of the volatile oil of the spice by distillation, the non-volatile spice fraction is extracted with an approved solvent, concentrated by solvent removal then combined with the volatile portion collected earlier in the process. Acceptable solvents for the manufacture of spice oleoresins and allowable levels of residual solvents in the finished oleoresin vary across different countries. In the USA, permissible solvents and allowable levels of residual solvents are listed in 21 C.F.R. § Sec. 173 subpart C and in the FCC monograph on spice oleoresins (FCC, 2019). In addition, the FCC standard on spice oleoresins requires that the essential oil of an oleoresin be similar in its physical and chemical properties, including its infrared spectrum, to that distilled from the spice of the same origin.

5. Chemical Composition

Constituent data for the essential oil and extract NFCs listed in Table 1 were collected using gas-chromatography (GC) coupled to a flame ionization detector (FID) for quantitation. Peaks were identified by mass spectrometry (MS) or retention time using standard reference compounds. Both identified and unidentified GC peaks were reported as the percent area of the chromatogram. Constituent data for the NFC were compiled and the constituents present at greater than 1% are listed in Appendix A. The Cramer decision tree class (DTC) and congeneric group were determined for each constituent, as outlined in the safety evaluation procedure (Cohen et al., 2018a). The DTC assigned to each congeneric group was determined by assigning the most conservative class for the constituents within each group. The constituent profile for each NFC is presented in Appendix A, organized by congeneric group.

The constituent profiles for these NFCs are characterized by a high percentage of eugenol and other Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) constituents, Group 19 (Aliphatic and aromatic hydrocarbons) constituents and other terpenoid constituents such as β-caryophyllene and β-myrcene (structures shown in Fig. 1). Pie chart representations of the constituent congeneric group profiles for the essential oil and extract NFCs are shown in Fig. 2.

Because of the variable nature of the constituent profile of spice oleoresins, they are characterized separately from the essential oil and extract NFCs. Raw spice oleoresins are highly concentrated and

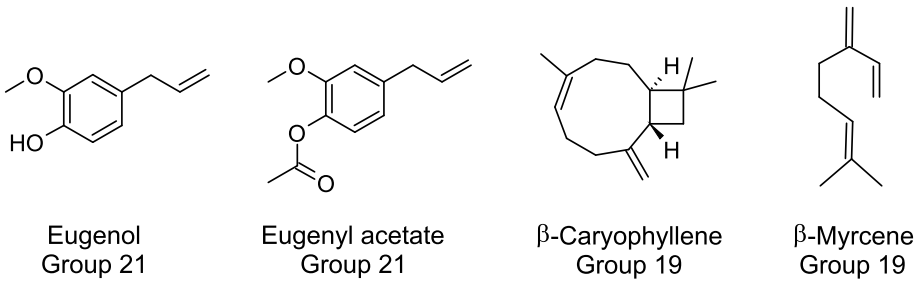


Fig. 1. Some commonly reported constituents of clove, cinnamon leaf and West Indian bay leaf-derived NFCs and their respective congeneric groups.

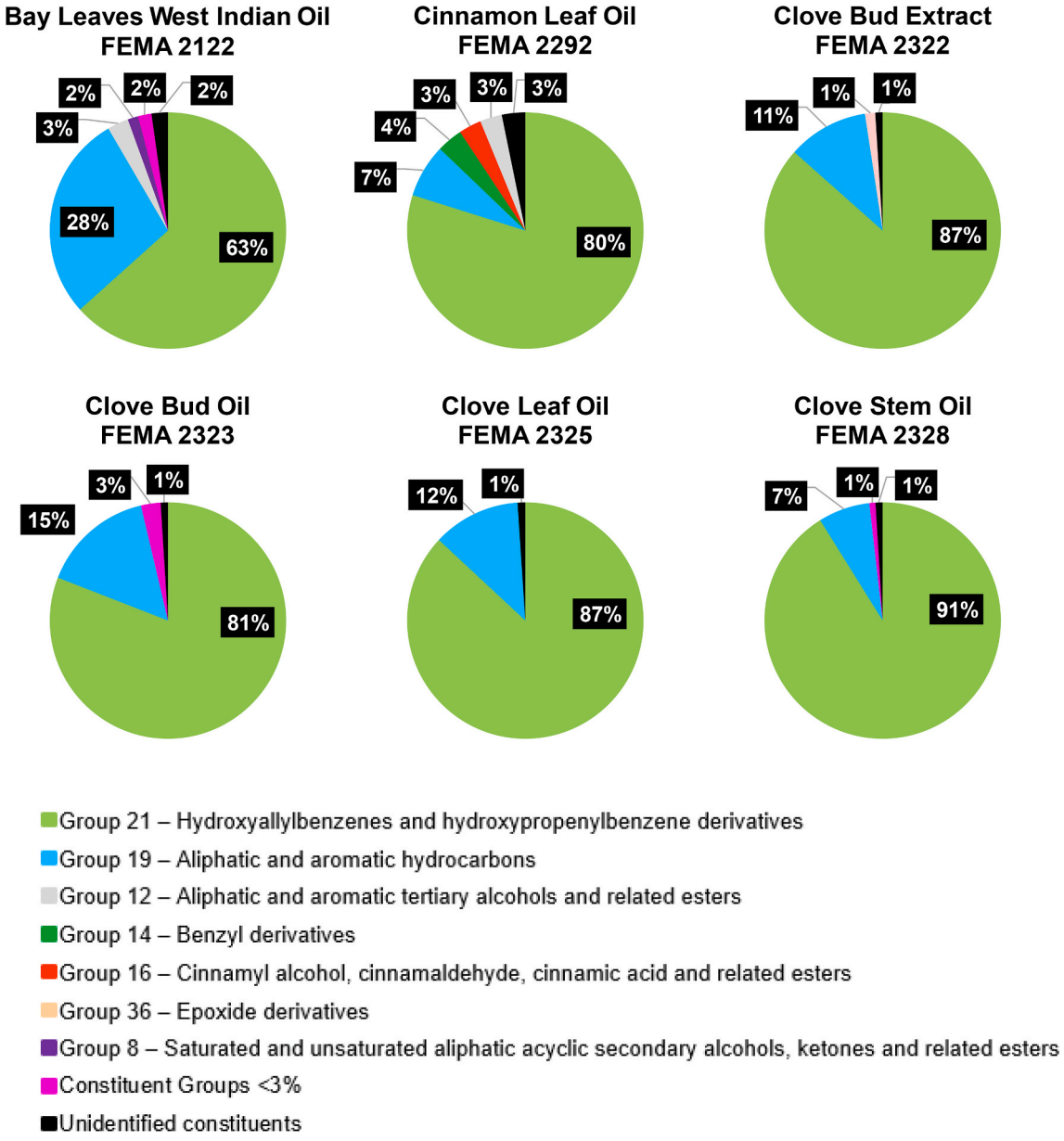


Fig. 2. Constituent congeneric group profiles for essential oil and extract NFCs.
*The composition of Bay Leaves West Indian Oleoresin (FEMA 2123) and Clove Bud Oleoresin (FEMA 2324) are not included in this figure due to the variable nature of the spice oleoresins.

consequently they are often standardized by dilution with a food grade ingredient that also often provides an associated solubility profile for the standardized oleoresin. For oil-based applications, an oleoresin may be standardized with an edible vegetable oil. A raw oleoresin may be standardized with a polysorbate ester that results in a water-soluble standardized oleoresin. Oleoresins may be spray-dried with a modified starch or dispersed on a food grade carrier such as salt or dextrose (Reineccius, 1994). For example, raw clove bud oleoresin may contain approximately 90% essential oil with 10% non-volatile resinous material but be standardized to contain a much lower percentage of essential oil using a food grade diluent, as shown in Fig. 3 (Nurdjannah and Bermawie, 2001). In this case, a standardization of raw clove bud oleoresin estimated to consist of 90% essential oil, 10% non-volatile resin with a food grade diluent resulting in an oleoresin characterized as consisting of 25% essential oil is depicted. Clove oleoresin standardized to contain 25% essential oil is representative of Clove Bud Oleoresin (FEMA 2324) used as a flavoring ingredient, although Clove Bud Oleoresins (FEMA 2324) standardized to contain essential oil ranging from 82% to less than 25% may be used, depending on the application. The customization of spice oleoresins for specific applications does not allow for the determination of a single chemical composition, although ranges for volatile oil contents for some standardized spice oleoresins are listed in the Food and Chemical Codex (FCC, 2019). Although the composition of a spice oleoresin, such as Clove Bud Oleoresin (FEMA 2324) or Bay Leaves West Indian Oleoresin (FEMA 2123) is variable, the safety evaluation can be based on the ranges expected for essential oil, resin and standardization agent content.

6. Safety evaluation

The procedure for the safety evaluation for NFCs, summarized in Fig. 4, is guided by a set of criteria initially outlined in two publications (Smith et al., 2004, 2005) and subsequently updated in 2018 (Cohen et al., 2018a). Briefly, the NFC passes through a 14-step process; Step 1 requires the gathering of data and assesses the consumption of the NFC as a flavor relative to intake from the natural source when consumed as food; Steps 2 through 6 evaluate the exposure and potential toxicity of the identified constituents (organized by congeneric group) based on scientific data on metabolism and toxicity and on the application of the TTC approach (Kroes et al., 2000)[1]; Steps 7-12 address the potential toxicity, including genotoxicity of the unidentified constituents; in Step 13 the overall safety is evaluated along with considerations of safety for use by children, given their lower body weights; Step 14 makes a determination of GRAS status. Below, the safety evaluation is presented in which each step in the procedure (Cohen et al., 2018a) (provided in italics) is answered for the NFCs under consideration.

Step 1

To conduct a safety evaluation of an NFC, the Panel requires that comprehensive analytical data are provided. The analytical methodologies employed should reflect the expected composition of the NFC and provide data that identify, to the greatest extent possible, the constituents of the NFC and the levels (%) at which they are present. It is anticipated that GC-MS and LC-MS would be used for characterization of most NFCs, and that the chromatographic peaks based on peak area of total ion current will be almost completely identified. The percentage of unknowns should be low enough to not raise a safety concern. Other appropriate methods (e.g., Karl Fischer titration, amino acid analysis, etc.) should be employed as necessary. The analytical parameters should be submitted for each type of analysis, including the method of quantitation for both identified and unidentified constituents and libraries as well as databases and methodology employed for the identification of analytes. The Panel requires data from multiple batches to understand the inherent variability of the NFC.

a. Consumption of foods from which the NFCs are derived

Calculate the per capita daily intake (PCI) of the NFC based on the annual volume added to food.

For NFCs with a reported volume of use greater than 22,700 kg (50,000 lbs), the intake may be calculated by assuming that consumption of the NFC is spread among the entire population, on a case-by-case basis. In these cases, the PCI is calculated as follows:

$$PCI\ \ (\mu g\ /\ person\ /\ day) = \frac{annual\ volume\ in\ kg\ \times 10^9}{population\ \times\ CF\ \times\ 365\ days}$$

where:

The annual volume of use of NFCs currently used as flavorings for food is reported in flavor industry surveys (Gavin et al., 2008; Harman et al., 2013; Harman and Murray, 2018; Lucas et al., 1999). A correction factor (CF) is used in the calculation to correct for possible incompleteness of the annual volume survey. For flavorings, including NFCs, that are undergoing GRAS re-evaluation, the CF, currently 0.8, is established based on the response rate from the most recently reported flavor industry volume-of-use surveys.

For new flavorings undergoing an initial GRAS evaluation, the anticipated volume is used and a correction factor of 0.6 is applied which is a conservative assumption that only 60% of the total anticipated volume is reported.

For NFCs with a reported volume of use less than 22,700 kg (50,000 lbs), the eaters' population intake assumes that consumption of the NFC is distributed among only 10% of the entire population. In these cases, the per capita intake for assuming a 10% "eaters only" population (PCI × 10) is calculated as follows:

$$PCI\ \times\ 10\ (\mu g\ /\ person\ /\ day) = \frac{annual\ volume\ in\ kg\ \times 10^9}{population\ \times\ CF\ \times\ 365\ days}\ \times\ 10$$

If applicable, estimate the intake resulting from consumption of the commonly consumed food from which the NFC is derived. The aspect of food use is particularly important. It determines whether intake of the NFC occurs predominantly from the food of which it is derived, or from the NFC itself when it is added as a flavoring ingredient (Stofberg and Grundschober, 1987).[2] At this step, if the conditions of use[3] for the NFC result in levels that differ from intake of the same constituents in the food source, it should be reported.

The NFCs under consideration here are derived from the leaves, buds and stems of botanicals from the *Eugenia, Cinnamomum and Pimenta* genera that have been traditionally used in the preparation of food. For 2015, the ERS/USDA reported that 1,700,000 kg of cloves (includes buds and stems) were imported into the USA (ERS/USDA, 2019). The intake of clove oil from use of cloves in food is conservatively estimated to be 1600 μg/person/day, assuming an 11% volatile oil content. As discussed earlier, the volatile oil concentration in cloves ranges from 11 to 17% (Al-Hilphy, 2015; Guan et al., 2007; Safrudin et al., 2015). The estimated intake of clove oil consumed from the consumption of cloves as a spice is reported in Table 2 in addition to the estimated intakes of Clove Bud Oil (FEMA 2323) and similar eugenol-rich NFCs used as flavoring ingredients. The estimated intake of clove oil from the

[1] In Step 5, the estimated intake for each congeneric group of the NFC is compared to the TTC threshold for the structural class of the group. TTC thresholds were determined for structural classes I, II and III based on the 5th percentiles of the NOAEL of each class with an additional 100-fold uncertainty factor, providing a highly conservative threshold for each class (Cramer et al., 1978; Munro et al., 1996; Kroes et al., 2000).

[2] See Stofberg and Grundschober, 1987 for data on the consumption of NFCs from commonly consumed foods.

[3] The focus throughout this evaluation sequence is on the intake of the constituents of the NFC. To the extent that processing conditions, for example, alter the intake of constituents, those conditions of use need to be noted, and their consequences evaluated in arriving at the safety judgments that are the purpose of this procedure.

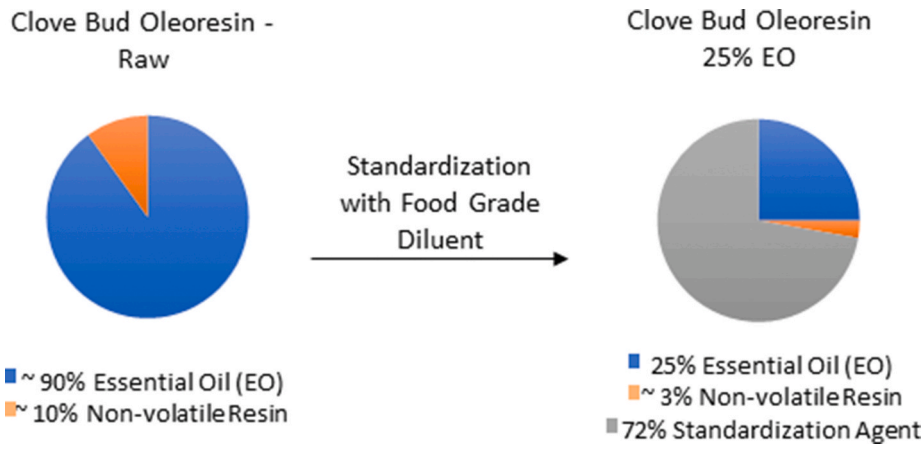
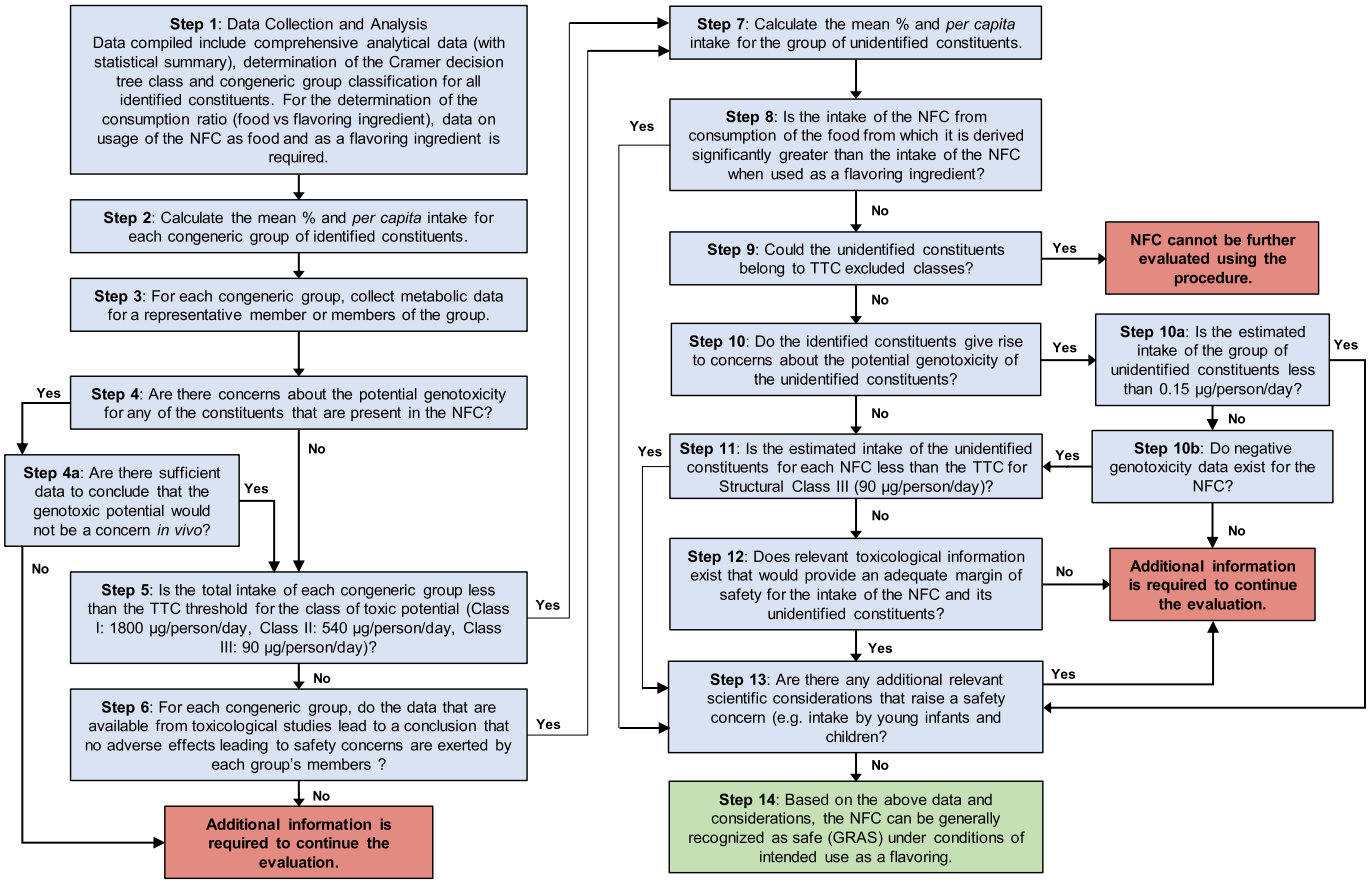


Fig. 3. Standardization of raw spice oleoresins, using clove bud oleoresin as an example. Here, the raw clove bud oleoresin is standardized by dilution with a food grade standardization agent, such as vegetable oil or salt, resulting in a Clove Bud Oleoresin (FEMA 2324) composed of 25% essential oil approximately 72% standardization agent and 3% non-volatile resins. Clove bud oleoresin standardized to contain 25% essential oil is representative of a clove bud oleoresin used as a flavoring ingredient. However, clove bud oleoresin standardized to contain up to 82% essential oil may also be used as a flavoring ingredient.



This scheme presents a summary of the revised procedure for the evaluation of NFCs to give an overall structural view. When applying the procedure, the full procedure described in the manuscript should be followed.

Fig. 4. Procedure for the safety evaluation of NFCs (Cohen et al., 2018a).

consumption of clove spice is significantly higher than the estimated intake for Clove Bud Extract (FEMA 2322), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf Oil (FEMA 2292) but significantly lower than the estimated intake of Clove Bud Oil (FEMA 2323). The consumption ratio comparing the consumption of clove oil from food sources versus as an added flavoring ingredient is considered again in Step 8.

Clove Bud Oleoresin (FEMA 2324) is extracted from clove buds with typical yields ranging from 22 to 31% when ethyl alcohol is used as the solvent (Nurdjannah and Bermawie, 2001). Based on the USA import data for cloves in 2015 reported above, the consumption of clove bud oleoresin from the consumption of cloves as a spice is estimated to be 374,000 kg, assuming a conservative 22% oleoresin content. Based on this annual usage, an estimated intake of 3000 µg/person/day was calculated, assuming consumption by the entire population. This is many times higher than the most recent volume for Clove Bud Oleoresin (FEMA 2324) of 390 µg/person/day.

Finally, cinnamon leaves and West Indian bay leaves are used in local cuisine for the flavoring of food. However, data on annual volumes of consumption or per capita estimated intakes are not available for these spices for the calculation of the ratio of consumption from food sources versus consumption as a flavoring ingredient.

Table 2
Estimated Intake of Clove oil from food (in bold) and estimated intakes of NFCs used as flavoring in food.

	Estimated Intake (μg/person/day)
Clove Oil from use as spice (ERS/USDA)	1,600
FEMA 2322 Clove Bud Extract	60
FEMA 2323 Clove Bud Oil	2,350
FEMA 2325 Clove Leaf Oil	430[a]
FEMA 2328 Clove Stem Oil	190
FEMA 2292 Cinnamon Leaf Oil	560

[a] For high volume materials (greater than 22,700 kg/year), the PCI per capita is shown. For all other NFCs listed here, the estimated intake was calculated using the PCI × 10 method.

Table 3
Estimated intake of methyl eugenol, estragole and safrole in NFCs.

Name (FEMA No.)	Constituent of Concern	Mean %	Estimated Intake (μg/person/day)
Bay Leaves West Indian Oil (2122)	Methyl eugenol	1	0.7
	Estragole	2	1
Clove Bud Oil (2323)	Methyl eugenol	0.04	0.9
Clove Bud Oleoresin (2324)	Methyl eugenol	0.01–0.03	0.04–0.13
Clove Leaf Oil (2325)	Methyl eugenol	0.01	0.04
Clove Stem Oil (2328)	Methyl eugenol	0.05	0.1
Cinnamon Leaf Oil (2292)	Safrole	1	6

b. Identification of all known constituents and assignment of DTC

In this step, the results of the complete chemical analyses for each NFC are examined, and the DTC is determined for each constituent (Cramer et al., 1978).

In Appendix A, the congeneric groups with constituents with a mean % greater or equal to 1% of the NFC are listed in order of highest to lowest mean%. For each congeneric group listed, the constituents with a mean % equal or greater than 1% are also shown and the minor constituents (<1%) are summed and reported.

c. Assignment of the constituents to Congeneric groups; assignment of congeneric group DTC

$$\text{Intake of congeneric group } (\mu g / person / day) = \frac{\text{Mean \% congeneric group} \times \text{Intake of NFC } (\mu g/person/day)}{100}$$

In this step, the identified constituents are sorted by their structural features into congeneric groups. Each congeneric group should be expected, based on established data, to consistently exhibit similar rates and pathways of absorption, distribution, metabolism and excretion, and common toxicological endpoints (e.g. benzyl acetate, benzaldehyde, and benzoic acid are expected to have similar toxicological properties).

Assign a decision tree structural class to each congeneric group. Within a congeneric group, when there are multiple decision tree structural classes for individual constituents, the class of highest toxicological concern is assigned to the group. In cases where constituents do not belong to a congeneric group, potential safety concerns would be addressed in Step 13.

Proceed to Step 2.

For the essential oil and extract NFCs, all reported constituents were organized by congeneric group and constituent tables for each NFC, organized by congeneric group are presented in Appendix A. Congeneric groups with constituents with a mean percent greater than or equal to 1% of the NFC are listed in order of highest to lowest and the minor constituents (<1%) are summed and reported. The total mean % for each congeneric group is subtotaled and reported with the DTC for the group.

Because detailed analyses were not available for the spice oleoresins, their constituent profile has been derived from the information available on the volatile oil content and standardization procedures used for each oleoresin. For Clove Bud Oleoresin (FEMA 2324), a constituent table and summary report were prepared from data collected on the volatile oil and resinoid composition of the raw oleoresins and the standardized oleoresins in commerce. Constituents are listed and a range of the values for the mean % and estimated intake are provided, reflecting the range of products in commerce. Due to a lack of data on volatile oil and resinoid content and standardization levels for Bay Leaves West Indian Oleoresin (FEMA 2123) a detailed constituent table for this NFC could not be prepared and is not evaluated using this procedure. All NFCs listed in Table 1, with the exception of Bay Leaves West Indian Oleoresin (FEMA 2123) which is addressed in Step 14, proceed to Step 2.

Step 2

Determine (a) the mean percentage (%) of each congeneric group in NFCs, and (b) the daily per capita intake[4] of each congeneric group. (a) is calculated by summing the mean percentage of each of the constituents within a congeneric group, and (b) is calculated from consumption of the NFC and the mean percentage.

Calculation of PCI for each constituent congeneric group of the NFC:

where:

The mean % is the mean percentage % of the congeneric group.

The intake of NFC (μg/person/day) is calculated using the PCI × 10 or PCI equation as appropriate.

Proceed to Step 3.

In the summary reports provided in Appendix A, the total mean percent for each congeneric group is subtotaled and reported with the DTC and estimated intake (PCI × 10 or PCI, as appropriate).

Step 3

For each congeneric group, collect metabolic data for a representative member or members of the group. Step 3 is critical in assessing whether the metabolism of the members of each congeneric group would require additional considerations at Step 13 of the procedure.

Proceed to Step 4.

Appendix A lists the constituent congeneric groups for each NFC. For each congeneric group present in these NFCs, sufficient data on the metabolism of their constituents or related compounds exist to conclude that members of the respective groups are metabolized to innocuous products. The use of metabolic data in the safety evaluation of flavoring compounds and a summary of the expected metabolism of flavoring compounds by congeneric group is described in a recent FEMA Expert Panel publication (Smith et al., 2018). The relationship of structure to the toxicity of Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) flavoring compounds has been reviewed by the Panel (Rietjens et al., 2014). In addition, the Panel has also published evaluations of metabolic data for Group 19 (Aliphatic and

[4] See Smith et al., 2005 for a discussion on the use of PCI × 10 for exposure calculations in the procedure.

aromatic hydrocarbons), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters constituents), Group 14 (Benzyl derivatives) and Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) flavoring compounds (Adams et al., 2004, 2005a, 2011; Marnett et al., 2014) and assessments of other groups or individual constituents (Adams et al., 2005b, c; Adams et al., 2002; Adams et al., 1997; Adams et al., 2008; Adams et al., 1998; Adams et al., 1996; Adams et al., 2007).

Step 4

Are there concerns about potential genotoxicity for any of the constituents that are present in the NFC?

If Yes, proceed to Step 4a.

If No, proceed to Step 5.

The FEMA Expert Panel has previously reviewed *in vitro* and *in vivo* genotoxicity studies for Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) flavoring ingredients that are major constituents for the NFCs under consideration and, in general, the structural features of the congeneric groups present in the *Eugenia*-derived NFCs, Cinnamon Leaf Oil (FEMA 2292) and Bay Leaves West Indian Oil (FEMA 2122) do not raise concerns for genotoxic potential (Rietjens et al., 2014). In addition, genotoxicity studies on the NFCs, described later under "Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation" and a review of the minor constituent profile of these NFCs indicate no genotoxic concern.

However, for a subset of constituents of Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) with an allyl alkoxybenzene structural motif, a concern for genotoxic potential is raised (Rietjens et al., 2014). Three constituents of this subgroup are: methyl eugenol, which is naturally occurring in low concentrations, 0.01–1% in Clove Bud Oil (FEMA 2323), Clove Leaf Oil (FEMA 2325) Clove Stem Oil (FEMA 2328) and Bay Leaves West Indian Oil (FEMA 2122); estragole which is naturally occurring at low concentrations in Bay Leaves West Indian Oil (FEMA 2122); and safrole which is naturally occurring in low concentrations in Cinnamon Leaf Oil (FEMA 2292). Since the essential oil profile of the spice oleoresins must be similar to their corresponding essential oil, naturally occurring methyl eugenol is also expected to be present in Clove Bud Oleoresin (FEMA 2324). The occurrence and estimated intakes for these constituents are shown for each NFC in Table 3. These NFCs proceed to Step 4a. Clove Bud Extract (FEMA 2322), which does not contain any of these alkoxybenzenes, proceeds to Step 5.

Step 4a

Are there sufficient data to conclude that the genotoxic potential would not be a concern in vivo?

If Yes, proceed to Step 5.

If No, additional information is required to continue the evaluation.

The structures of estragole, methyl eugenol and safrole (see Fig. 5) share a motif of a benzene ring substituted with an alkoxy group located *para* to a 2-propenyl substituent. These allylalkoxybenzene compounds have been shown to be capable of forming DNA adducts upon bioactivation in which cytochrome P450s catalyze the formation of a 1′-hydroxy metabolite followed by sulfation at this site by a sulfotransferase. The elimination of sulfate creates a DNA reactive species (Herrmann et al., 2012, 2014; Jeurissen et al., 2004, 2006, 2007; Punt et al., 2008; Rietjens et al., 2014; Ueng et al., 2004; Wislocki et al., 1976). Rodent studies have indicated that safrole, methyl eugenol and estragole are hepatocarcinogens at high dose levels (Abbott et al., 1961; Drinkwater et al., 1976; Homburger et al., 1965; Homburger et al., 1962; Long et al., 1963; Miller et al., 1983; NTP, 2000).

The direct addition of safrole to food is prohibited in the USA (21 CFR §189.180) and the addition of estragole, methyl eugenol and safrole as such to food is prohibited in the European Union (Regulation EC No 1334/2008). In 2018, the FEMA Expert Panel removed methyl eugenol from the FEMA GRAS list, citing the need for additional data to clarify the relevance of DNA adducts formed by methyl eugenol in humans (Cohen et al., 2018b). Later, in October 2018, FDA's food additive regulations were amended to no longer authorize the use of methyl eugenol as synthetic flavoring substances and adjuvants for use in food (83 Fed. Reg. 50490. October 9, 2018) in response to a food additive petition. The FDA explained that it had based its decision "as a matter of law" on the "extraordinarily rigid" Delaney Clause of the Federal Food, Drug, and Cosmetic Act and further noted that based on the data evaluated, that "it is unlikely that consumption of methyl eugenol presents a risk to the public health from use as a flavoring substance" (83 Fed. Reg. 50490. October 9, 2018).

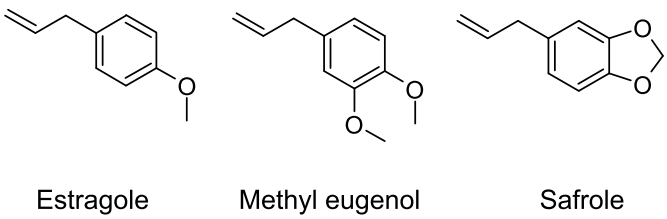


Fig. 5. Structures of estragole, methyl eugenol and safrole.

Estragole, methyl eugenol and safrole, however, are naturally occurring constituents in common culinary herbs and spices such as basil, tarragon, allspice, cinnamon, anise, nutmeg and mace. Regarding the natural occurrence of methyl eugenol in herbs, spices and their essential oils and extracts, the FEMA Expert Panel stated, "that these flavorings continue to meet the criteria for FEMA GRAS under their conditions of intended use as flavorings" (Cohen et al., 2018b). In its decision to amend the food additive regulations permitting the addition of synthetic methyl eugenol to food, the FDA states "… there is nothing in the data FDA has reviewed in responding to the pending food additive petition that causes FDA concern about the safety of foods that contain natural counterparts or extracts from such foods" (83 Fed. Reg. 50490. October 9, 2018). Similarly, the European Union established maximum levels for estragole, methyl eugenol and safrole in finished foods that have been flavored with flavorings and food ingredients in which these constituents occur naturally (European Commission, 2008).

For the essential oil NFCs listed in Table 3, the estimated intakes of methyl eugenol, estragole and safrole from the consumption of these NFCs are low, ranging from 0.04 to 6 μg/person/day. For Clove Bud Oleoresin (FEMA 2324) standardized to contain 25–82% essential oil, the range for the estimated intake of methyl eugenol from the use as flavoring is 0.04–0.13 μg/person/day. As indicated in Table 3, the estimated intakes from the natural occurrence of methyl eugenol in Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328) are below the TTC of 0.15 μg/person/day for compounds with structural alerts for genotoxicity, as originally stated by Kroes et al. in 2004 (Kroes et al., 2004). This value was determined based on an analysis of the dose-response data for carcinogenic compounds, provided by the Gold database of carcinogens presenting the dose giving a 50% tumor incidence (TD50) (Gold et al., 1984; Kroes et al., 2004). By linear extrapolation of these TD50 data to a 1 in 10^6 tumor incidence, an exposure level or TTC at which the lifetime risk of cancer was less than 1 in 10^6 was determined to be 0.15 μg/person/day (Kroes et al., 2004). In a recent EFSA/WHO review of the TTC approach, a 0.15 μg/person/day threshold was proposed and considered sufficiently protective for compounds with structural alerts for genotoxicity with the exclusion of high potency carcinogens (the Cohort of Concern) specified by Kroes and co-workers (EFSA, 2016; Kroes et al., 2004; Nohmi, 2018). Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328) proceed to Step 5.

In cases where the intake of a naturally occurring carcinogen from

Table 4
MOE Analyses for naturally occurring estragole, methyl eugenol and safrole in NFCs.

Name (FEMA No.)	Constituent of Concern	Estimated Intake (mg/kg bw/day)	$BMDL_{10}$ for carcinogenicity (mg/kg bw/day)	MOE
Bay Leaves West Indian Oil (FEMA 2122)	Estragole	2×10^{-5}	3.3	>180,000
Bay Leaves West Indian Oil (FEMA 2122)	Methyl eugenol	1×10^{-5}	22.2	>1,900,000
Cinnamon Leaf Oil (FEMA 2292)	Safrole	9.7×10^{-5}	1.9	>19,000
Clove Bud Oil (FEMA 2323)	Methyl eugenol	1.6×10^{-5}	22.2	>1,400,000

Table 5
Consideration of congeneric groups for NFCs where the estimated intake exceeds the TTC for the congeneric group.

Name (FEMA No.)	DTC	Estimated Intake of CG (μg/person/day)	Estimated Intake of CG (mg/kg bw/day)	NOAEL (mg/kg bw/day)	MoS
Congeneric Group 21 - Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives					
Cinnamon Leaf Oil (FEMA 2292)	III	450	7.5×10^{-3}	300	>40,000
Clove Bud Oil (FEMA 2323)	III	1900	3.2×10^{-2}	300	>9,000
Clove Bud Oleoresin (FEMA 2324)	III	78–258	$1.3 \times 10^{-3} - 4.3 \times 10^{-3}$	300	>60,000
Clove Leaf Oil (FEMA 2325)	III	370	6.2×10^{-3}	300	>48,000
Clove Stem Oil (FEMA 2328)	III	180	2.8×10^{-3}	300	>100,000

food exceeds the TTC for genotoxic substances, a Margin of Exposure (MOE) approach can be applied (EFSA, 2009). The MOE is calculated based on the lower confidence limit of the benchmark dose resulting in a 10% extra cancer incidence ($BMDL_{10}$) determined from the mathematical modeling of *in vivo* study data on tumor formation in experimental animals. For safrole, a $BMDL_{10}$ of 1.9 mg/kg bw/day was calculated based on a carcinogenicity study in female mice (Miller et al., 1983; van den Berg et al., 2011). For methyl eugenol, a $BMDL_{10}$ of 22.2 mg/kg bw/day was calculated based on a carcinogenicity study in male rats (Suparmi et al., 2019; van den Berg et al., 2011). For estragole, a $BMDL_{10}$ of 3.3 mg/kg bw/day was calculated based on a carcinogenicity study in female mice (Miller et al., 1983; van den Berg et al., 2011). EFSA has stated, and the FEMA Expert Panel concurs with the opinion, that MOE values greater than 10,000 that are based on a $BMDL_{10}$ derived from an animal study would be of low concern from a public health point of view and of low priority for risk management (EFSA, 2005). Table 4 lists the MOE values for estragole and methyl eugenol from the estimated intake of Bay Leaves West Indian Oil (FEMA 2122), safrole from the estimated intake of Cinnamon Leaf Oil (FEMA 2292) and methyl eugenol from the estimated intakes of Clove Bud Oil (FEMA 2323). In each instance, the calculated MOE is much greater than 10,000 indicating low concern. Bay Leaves West Indian Oil (FEMA 2122), Cinnamon Leaf Oil (FEMA 2292) and Clove Bud Oil (FEMA 2323) proceed to Step 5.

Step 5

Is the total intake of the congeneric group less than the TTC for the class of toxic potential assigned to the group (i.e. Class I: 1800 μg/person/day, Class II: 540 μg/person/day, Class III: 90 μg/person/day) (Kroes et al., 2000; Munro et al., 1996)? For congeneric groups that contain members of different structural classes, the class of highest toxicological concern is selected.

If Yes, proceed to Step 7.

If No, proceed to Step 6.

For five NFCs under consideration, Cinnamon Leaf Oil (FEMA 2292), Clove Bud Oil (FEMA 2323), Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328), the estimated intake for Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) constituents (summarized in Table 5) is above the TTC for the structural class of the group, Class III, in these instances. The estimated intakes for the other constituent groups of these NFCs are below the TTC. These NFC flavoring ingredients proceed to Step 6 of the procedure. The constituent congeneric groups of Bay Leaves West Indian Oil (FEMA 2122) and Clove Bud Extract (FEMA 2322) have estimated intakes below the TTC for their respective structural classes. These NFC flavoring ingredients proceed to Step 7.

Table 6
Estimated intake of unidentified constituents.

Name	FEMA No.	Estimated Intake μg/person/day
Bay Leaves West Indian Oil	2122	1
Cinnamon Leaf Oil	2292	18
Clove Bud Extract	2322	0.5
Clove Bud Oil	2323	23
Clove Bud Oleoresin	2324	12–35
Clove Leaf Oil	2325	5
Clove Stem, Oil	2328	2

Step 6

For each congeneric group, do the data that are available from toxicological studies lead to a conclusion that no adverse effects leading to safety concerns are exerted by each group's members?

This question can commonly be answered by considering the database of relevant metabolic and toxicological data that exist for a representative member or members of the congeneric group, or the NFC itself. A comprehensive safety evaluation of the congeneric group and a sufficient margin of safety (MoS) based on the data available is to be determined on a case-by-case basis. Examples of factors that contribute to the determination of a safety margin include 1) species differences, 2) inter-individual variation, 3) the extent of natural occurrence of each of the constituents of the congeneric group throughout the food supply, 4) the nature and concentration of constituents in related botanical genera and species. 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 should be taken into consideration in the safety evaluation (Kroes et al., 2000).

If Yes, proceed to Step 7.

If No, additional information is required to continue the evaluation.

For the NFCs listed in Table 5, the TTC is exceeded for Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) constituents. The Margin of Safety (MoS) is calculated for Group 21 constituents based on a two-year NTP dietary study of eugenol in rats which determined a no observed adverse effect level (NOAEL) of 300 mg/kg bw/day for males and 625 mg/kg bw/day for females (NTP, 1983). The more conservative NOAEL, 300 mg/kg bw/day, was used to calculate the MoS. Eugenol is the dominant constituent of Group 21 constituents in Cinnamon Leaf Oil (FEMA 2292), Clove Bud Oil (FEMA 2323), Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328). This NOAEL does not apply to Group 21 constituents methyl eugenol, estragole and safrole, whose genotoxic potential and safety is analyzed above in Steps 4 and 4a. These NFC

flavoring substances proceed to Step 7 of the procedure.

Step 7

Calculate the mean percentage (%) for the group of unidentified constituents of unknown structure in each NFC (as noted in Step 1) and determine the daily per capita intake (PCI or PCI × 10) for this group.

Proceed to Step 8.

Appendix A reports the mean % for the group of unidentified constituents and the per capita intake for each NFC. These data are also summarized below in Table 6.

Step 8

Using the data from Step 1, is the intake of the NFC from consumption of the food[5] from which it is derived significantly greater than the intake of the NFC when used as a flavoring ingredient?

If Yes, proceed to Step 13.

If No, proceed to Step 9.

As discussed in Step 1, a conservative calculation of the intake of clove essential oil from the consumption of the spice/food is 1600 μg/person/day. The consumption ratios (food intake to intake as added flavoring) of Clove Bud Extract (FEMA 2322), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf (FEMA 2292) are 28, 4, 8 and 3, respectively. In addition, the consumption of the constituents of Clove Bud Oleoresin (FEMA 2324) from spice as food is estimated to be greater than that as added flavoring. These NFCs proceed to Step 13. The consumption ratio of Clove Bud Oil (FEMA 2323) is 0.7, indicating higher consumption as flavoring in food. A consumption ratio could not be determined for Bay Leaves West Indian Oil (FEMA 2122) due to lack of data on consumption as a spice in food. For these NFCs, the estimated intake is calculated or assumed to be predominantly from flavoring added to food and they proceed to Step 9.

Step 9

Could the unidentified constituents belong to TTC excluded classes?[6] The excluded classes are defined as high potency carcinogens, certain inorganic substances, metals and organometallics, certain proteins, steroids known or predicted bio-accumulators, nanomaterials, and radioactive materials (EFSA, 2016; Kroes et al., 2004).

If Yes, the NFC is not appropriate for consideration via this procedure.

If No, proceed to Step 10.

Clove Bud Oil (FEMA 2323) and Bay Leaves West Indian Oil (FEMA 2122) are harvested from the botanical material by steam distillation and further rectified by fractional distillation. The oil is primarily composed of low molecular weight alcohols, esters and hydrocarbons derived from the phenylpropanoid and isoprene pathways. Based on the identified constituents, production process and current literature, members of the TTC excluded classes are not present in these oils.

Step 10

Do the identified constituents give rise to concerns about the potential genotoxicity of the unidentified constituents?

If Yes, proceed to Step 10a.

If No, proceed to Step 11.

The identified constituents of the *Eugenia*, *Cinnamomum* and *Pimenta* derived NFCs include eugenol with smaller amounts of mono- and sesquiterpene hydrocarbons, compounds that are not genotoxic. The composition of the unidentified constituent fraction is expected to be similar to the identified constituent profile and consist of products of the shikimate pathway and isoprene pathways which are not genotoxic. In Step 4, the occurrence of genotoxins estragole, methyl eugenol and safrole were reported. Because of these natural occurrences, the possibility for the presence of additional allylalkoxybenzene compounds was evaluated. Allylalkoxybenzene compounds such as estragole, methyl eugenol, safrole, myristicin and elemicin are represented in current mass spectral libraries and are readily detected and identified by GC-MS instruments. Consequently, these compounds will only be part of the unidentified fraction when they occur at concentrations below the limit of detection. For this reason and a lack of other reports of the occurrence of allylalkoxybenzenes in Bay Leaves West Indian Oil (FEMA 2122) and Clove Bud Oil (FEMA 2323), the FEMA Expert Panel determined that these compounds are unlikely to be present in the unidentified constituent fraction and that there is not a genotoxic concern for the unidentified constituents. Proceed to Step 11.

Step 10a

Is the estimated intake of the group of unidentified constituents less than 0.15 μg/person/day? A TTC of 0.15 μg/person/day has been proposed for potentially genotoxic substances that are not from the TTC excluded classes (Kroes et al., 2004).

If Yes, proceed to Step 13.

If No, proceed to Step 10b.

Not Required.

Step 10b

Do negative genotoxicity data exist for the NFC?

If Yes, proceed to Step 11.

If No, retain for further evaluation, which would include the collecting of data from appropriate genotoxicity tests, obtaining further analytical data to reduce the fraction of unidentified constituents, and/or considering toxicity data for other NFCs having a similar composition. When additional data are available, the NFC could be reconsidered for further evaluation.

Not Required.

Step 11

Is the estimated intake of the unidentified constituents (calculated in Step 7) less than the TTC (Kroes et al., 2000; Munro et al., 1996) for Structural Class III (90 μg/person/day)?[7]

If Yes, proceed to Step 13.

If No, proceed to Step 12.

Yes, the estimated intakes for the group of unidentified constituents in Clove Bud Oil (FEMA 2323) and Bay Leaves West Indian Oil (FEMA 2122) listed above in Table 6 are lower than the TTC threshold for Structural Class III. Proceed to Step 13.

[5] 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.

[6] This can be based on arguments including: Expert judgement; Nature of the identified ingredients; Knowledge on the production/extraction process (see also Koster et al. (2011); EFSA, 2016).

[7] The human exposure threshold of 90 μg/person/day is determined from a database of NOAELs 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 NOAEL (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 90 μg/person/day. However, no flavoring substance or food additive in this structural class exhibited a NOAEL less than 25 mg/kg bw/d. Therefore the 90 μg/person/day 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

Does relevant toxicological information exist that would provide an adequate margin of safety for the intake of the NFC and its unidentified constituents?

This question may be addressed by considering data for the NFC or an NFC with similar composition. It may have to be considered further on a case-by-case basis, particularly for NFCs with primarily non-volatile constituents.

If Yes, proceed to Step 13.

If No, perform appropriate toxicity tests or obtain further analytical data to reduce the fraction of unidentified constituents. Resubmit for further evaluation.

Not required.

Step 13

Are there any additional relevant scientific considerations that raise a safety concern (e.g. intake by young infants and children)?

If Yes, acquire and evaluate additional data required to address the concern before proceeding to Step 14.

If No, proceed to Step 14.

A further evaluation to consider possible exposure of children and infants given their lower body weights and the potential for differences in toxicokinetics and toxicodynamics as compared to adults, was conducted for each NFC evaluated. Table 5 lists the congeneric groups that exceed TTC threshold and in each instance, the margin of safety remains >3000 using a body weight of 20 kg. A review of the estimated intakes for the constituent congeneric groups for each NFC shows none with estimated intakes close to the TTC threshold. Table 6 lists the estimated intake of the unknown constituent fraction, none of which is close to or exceeding the TTC threshold for Class III.

In consideration of the NFCs containing low concentrations of methyl eugenol, estragole or safrole, the estimated intakes from the natural occurrence of methyl eugenol in Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328) are below the TTC of 0.15 μg/person/day for compounds with structural alerts for genotoxicity and not a safety concern. For Bay Leaves West Indian Oil (FEMA 2122), Cinnamon Leaf Oil (FEMA 2292) and Clove Bud Oil (FEMA 2323), where the estimated intakes of these compounds exceeded the TTC of 0.15 μg/person/day, MOE analyses were presented in Table 4 in Step 4a showing, that in each case, the calculated MOE significantly exceeded the limit of concern, 10,000. Since the spice oleoresins are composed of the essential oil, a food grade agent (for standardization) and resinous material of the spice, there are no safety concerns for these oleoresins as flavoring ingredients under conditions of intended use.

Step 14

Based on the above data and considerations, the NFC can be GRAS under conditions of intended use as a flavoring ingredient.

Based on the application of the procedure, the FEMA Expert Panel concluded that the current FEMA GRAS NFCs listed in Table 7, Bay Leaves West Indian Oil (FEMA 2122), Clove Bud Extract (FEMA 2322), Clove Bud Oil (FEMA 2323), Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf Oil (FEMA 2292) are affirmed as GRAS under conditions of intended use as flavor substances. Bay Leaves West Indian Oleoresin (FEMA 2123) is also affirmed as GRAS under conditions of intended use as a flavor substance based on its estimated intake, 0.01 μg/person/day, which is below the TTC for compounds with structural alerts for genotoxicity.

7. Biochemical and Toxicological Supporting Information Relevant to the safety evaluation

The constituent profiles of Bay Leaves West Indian Oil (FEMA 2122), Clove Bud Extract (FEMA 2322), Clove Bud Oil (FEMA 2323), Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf Oil (FEMA 2292) are dominated by eugenol with smaller amounts of eugenyl acetate and other members of congeneric Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives). The toxicity of these constituents has been reviewed by the FEMA Expert Panel (Rietjens et al., 2014). The FEMA Expert Panel has also reviewed flavoring ingredients from the other congeneric groups present in minor amounts, including Group 19 (Aliphatic and aromatic hydrocarbons), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters constituents), Group 14 (Benzyl derivatives) and Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) flavoring ingredients (Adams et al., 2004, 2005a, 2011; Marnett et al., 2014). The additional information presented here includes studies on the NFCs themselves, studies on the principal constituents of these materials and newly available studies on relevant constituents.

7.1. Eugenol

Numerous genotoxicity and mutagenicity studies of eugenol have been reported (FDA. 1978; JECFA, 1982), including Ames, rec, L5178Y forward mutation, sister chromatid exchange, chromosomal aberration, and unscheduled DNA synthesis assays. *In vivo* micronucleus induction assays have also been reported for eugenol. In its review of these studies, the FEMA Expert Panel concluded that eugenol is genotoxic only at higher concentrations that result in significant cellular toxicity (Rietjens et al., 2014). The Panel also noted that in the National Toxicology Program's two-year bioassay of eugenol in F344/N rats described below, carcinogenicity was not observed and no significant dose-related increase in hepatocellular or other neoplasms was observed in B6C3F1 mice (NTP, 1983; Rietjens et al., 2014).

Two-year bioassays for carcinogenicity were conducted for eugenol in both rats and mice by the National Toxicology Program (NTP, 1983). These studies were preceded by 14- and 90-day toxicity studies. In a 2-year dietary study in B6C3F$_1$ mice, mice (50/sex/dose) were administered 0, 3000 or 6000 ppm of eugenol corresponding to estimated intakes of 0, 450 and 900 mg/kg bw/day (FDA, 1993). Histopathological analyses conducted at the end of the study found an increased incidence of hepatocellular adenomas and carcinomas at the low dose but not at the high dose in male mice. The p values for male mice for adenomas and carcinomas for the low dose were 0.016 and 0.024, respectively

Table 7
Clove, cinnamon leaf and West Indian bay leaf-derived NFCs affirmed FEMA GRAS.

FEMA No.	Name
2122	Bay Leaves West Indian Oil (*Pimenta acris* Kostel; *P. racemosa*)
2123	Bay Leaves West Indian Oleoresin (*Pimenta acris* Kostel; *P. racemosa*)
2292	Cinnamon Leaf Oil (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)
2322	Clove Bud Extract (*Eugenia* caryophyllata Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])
2323	Clove Bud Oil (*Eugenia* caryophyllata Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])
2324	Clove Bud Oleoresin (*Eugenia* caryophyllata Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])
2325	Clove Leaf Oil (*Eugenia* caryophyllata Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])
2328	Clove Stem Oil (*Eugenia* caryophyllata Thunb. [*Eugenia aromatica* (L.) Baill. or *Syzygium aromaticum* (L.) Merr. et Perry])

[adenomas control: 4/50 (8%), low dose: 13/50 (26%), high dose: 10/49 (20%), and carcinomas, control: 10/50 (20%), low dose: 20/50 (40%), high dose: 9/49 (18%)], and it was 0.004 for adenomas and carcinomas combined. At the $p < 0.01$ significance level, a statistical value considered more appropriate for common tumors such as mouse liver tumors (FDA, 2001; Haseman, 1983; OECD, 2014), only the increase in the combination of hepatocellular adenoma and carcinoma in low dose male mice was significant. The incidences of adenomas, carcinomas and combined tumors were not significantly increased in female mice and the positive dose-related trend observed ($p = 0.021$) for the incidence of combined tumors was also not significant [control: 2/50 (4%), low dose: 7/49 (14%), high dose: 9/49 (18%)]. Although the NTP concluded there was equivocal evidence of carcinogenicity in mice for eugenol based on the liver findings, the lack of a dose-response in male mice, the lack of statistical significance in female mice, combined with analysis of historical NTP data showing high levels of background hepatocellular neoplasms in $B6C3F_1$ mice, indicate that the hepatocellular adenomas and the hepatocellular carcinomas of the liver in this study were likely not related to administration of the test substance (Maronpot and Boorman, 1982; Maronpot et al., 1986).

In a 2-year dietary study in F344/N rats, groups of male F344/N rats (40 rats in control group, 50 rats in each treatment group) were fed a diet containing 0, 3000 or 6000 ppm eugenol, corresponding to a daily intake of 0, 150 or 300 mg/kg bw eugenol five days per week for 105 weeks (NTP, 1983). Female F344/N rats (40 rats in control group, 50 rats in each treatment group) were fed a diet containing 0, 6000 or 12500 ppm of eugenol, corresponding to a daily intake of 0, 300 or 625 mg/kg bw eugenol five days per week, for 105 weeks (FDA, 1993). Animals were observed twice daily for mortality and body weight changes, and clinical findings were recorded every four weeks for the duration of the study. Survival of all treated rat groups was similar to the control groups. Findings included a decrease in mean body weights and food consumption compared to controls for female rats in the highest dose group. Alveolar/bronchiolar adenomas or carcinomas of the lung (control: 0/40, low dose: 5/49 (10%), high dose: 2/50 (4%)) occurred in the low but not high dose male rats. An increased incidence of C-cell adenomas of the thyroid were observed in the low dose female rats but not in the high dose group (control: 3/40 (8%), low dose: 11/49 (22%), high dose: 2/50 (4%)). C-cell proliferative lesions are common in F344 rats, especially hyperplasia and adenoma (Chandra and Frith, 1992; Haseman et al., 1984). C-cell thyroid tumors were reported to range from 2 to 20% in male F344 rats and 0–18% in females (Haseman et al., 1984). Similar to the analysis of mouse liver tumors, a p value of <0.01 is the more appropriate statistical comparison for rat C-cell thyroid tumors (Haseman, 1953; FDA, 2001; OECD, 2014). When the incidences of female rats with either C-cell thyroid carcinomas or adenomas were combined, there were no significant results, even at $p < 0.05$. There was a statistically non-significant decrease ($p > 0.01$) in the incidence of C-cell adenomas and the combined incidence of adenomas and carcinomas of the thyroid in treated males compared to that of controls. In treated female rats, a dose-related increase in the incidence of endometrial stromal polyps was observed in the high dose group, but this effect was not statistically significant ($p > 0.01$) and was not considered associated with the administration of eugenol (NTP, 1983). The NTP considered the tumor findings in rats to not be treatment related and concluded that eugenol was not carcinogenic in rats. Based on the lack of significant findings of neoplastic and non-neoplastic lesions at all dose levels in both male and female rats, the no observed adverse effect level (NOAEL) is 300 mg/kg bw/day in male rats and 625 mg/kg bw/day in female rats. The more conservative NOAEL of 300 mg/kg bw/day observed for male rats was used to calculate the margin of safety for Cinnamon Leaf Oil (FEMA 2292, Clove Bud Oil (FEMA 2323), Clove Leaf Oil (FEMA 2325) and Clove Stem Oil (FEMA 2328) in Step 6, Table 5 of the safety evaluation.

7.2. Clove bud extract

Samples of ground clove bud and water extracts of clove bud (*Eugenia caryophyllus* Bullock et Harris) were not mutagenic when tested in a rec assay conducted in *B. subtilis* using both the cold and standard streak methods. The constituent profile of the test substances was not provided (Ungsurungsie et al., 1982) and it should be added that the rec assay does not have an OECD guideline; the OECD has noted that indicator tests such as the rec assay should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015). When tested in a bacterial reverse mutation assay, an ethanolic flower extract of *E. caryophyllus* Thunb. was positive in strains TA98 and TA100 of *S. typhimurium* at the only concentration tested, 10,000 μg/plate, without metabolic activation (Mahmoud et al., 1992). The test substance was prepared by extraction of the dried, powdered plant material with 95% cold ethanol, followed by concentration under vacuum until the sample was of syrupy consistency. The constituent profile of the test substance was not specified by the authors, nor was any verification provided of the identity of the botanical sample extracted for this study. In addition, this study did not evaluate the cytotoxicity of the test substance and only reports results at a concentration that exceeds the 5000 μg/plate limit recommended in the OECD guideline (OECD, 1997). Because of the uncertain identity of the test substances and non-standard assay conditions employed, this study is not considered relevant to the safety evaluation of Clove Bud Extract (FEMA 2322). The constituent analysis of Clove Bud Extract (FEMA 2322) reports a high eugenol content, approximately 86% (see Appendix A) with approximately 11% sesquiterpene hydrocarbons. Given this composition and the lack of mutagenic potential of eugenol, Clove Bud Extract (FEMA 2322) is expected to lack relevant genotoxic potential.

7.3. Clove and cinnamon leaf oils

Clove leaf oil was non-mutagenic in an Ames assay conducted under GLP standards in both the presence and absence of Aroclor 1254-induced rat liver S9 metabolic activation. Clove leaf oil was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations ranging from 10 to 5200 μg/plate and was determined to be negative for mutagenicity up to the limit of cytotoxicity. The onset of cytotoxicity by clove leaf oil was observed at 2340 μg/plate in strain TA100 (DeGraff, 1983; Heck et al., 1989). In a separate Ames assay in *S. typhimurium* strain TA100, a clove bud oil (*E. caryophyllata*) was tested in the presence and absence of S9 metabolic activation. The test sample was prepared by steam distillation of the botanical raw material to collect the volatile oil which was then partitioned into ether in three successive extractions following which the extracts were combined and concentrated. This sample was negative for mutagenicity except at the highest concentration tested, 500 μg/plate without S9 metabolic activation (Park, 2002). Equivocal Ames assay results were reported for mutagenicity of two commercial samples of clove oil. The "Clove I" sample (chemical composition and part(s) of botanical from which the sample was derived were not specified) was determined to be weakly positive in strain TA98 and strongly positive in strain TA1538 at a concentration of 5.2 ng/plate[8] but negative in all strains tested (TA98, TA1535, TA1537, and TA1538) at a concentration of 10.4 ng/plate. These experiments were performed without a metabolic activation system. A second sample, "Clove II" (chemical composition and part(s) of the botanical from which the sample was derived were not specified) was negative for mutagenic potential in strains TA98, TA1535, and TA1538 tested at concentrations of 5.2 and 10.4 ng/plate and positive in strain TA1538 at the higher concentration in the absence of metabolic

[8] Based on density of 1.038 g/mL (Source: Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD, USA).

activation (Sivaswamy et al., 1991). Under the same assay conditions, cinnamon leaf oil was weakly positive in strain TA98 and strongly positive in strain TA1538 at the lower concentration but negative at the higher concentration in all strains tested (TA98, TA1535, TA1537, and TA1538) (Sivaswamy et al., 1991). Because the Sivaswamy et al. studies did not report the chemical composition of the tested cinnamon leaf and clove oil samples, did not demonstrate a dose response and did not evaluate the cytotoxicity of the test substance and reported test concentrations that are remarkably low (OECD, 1997), the results of their study are not considered relevant to the safety evaluation of the NFCs under consideration. Similarly, because the test sample of clove bud oil used in the Park (2002) study was partitioned into ether, a practice not used in the preparation of the clove NFCs under consideration, that is likely to result in a different constituent profile, the results of this study are also not considered helpful to the safety assessment of the NFCs under consideration. The GLP-compliant Ames study on clove leaf oil is considered the most valid of the studies described here. The negative results reported for clove leaf oil, which contains typically more than 80% eugenol, are consistent with the negative Ames results reported for eugenol.

Two *B. subtilis* rec assay studies have been reported for clove oil. In the first, a sample of clove oil (whether bud, stem, leaf or combination thereof was not specified; chemical composition reported: 76% eugenol, 17% eugenyl acetate, 5% caryophyllene) was positive in the *B. subtilis* rec assay using strains H17 Rec^{+} and M45 Rec^{-} when tested at a dose of 0.2 mg/disk (Sekizawa and Shibamoto, 1982). The authors noted that there were significant differences in the growth of the H17 and M45 strains and the amount of diffusion of the test substance throughout the aqueous agar was unclear, which may have had an impact on the results (Sekizawa and Shibamoto, 1982). In the second study, also in *B. subtilis* strains H17 Rec^{+} and M45 Rec^{-}, an uncharacterized, unspecified type of clove oil, was positive in the presence and absence of S9 activation in a dose range of 0.63–10 μL (Kuroda et al., 1989). The relevance of these studies is limited because the rec assay has not been standardized in the OECD guideline for genotoxicity testing, which notes that it, as well as other indicator tests, should be correlated to other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015).

In an *in vitro* assay, clove oil (type and composition not specified) did not induce chromosomal aberrations in Chinese hamster fibroblast cells at concentrations up to 0.04 mg/mL. For this assay, the maximum dose did not exceed the dose required for 50% cell growth inhibition and was controlled for increases in osmolality (Ishidate et al., 1984).

In an *in vivo* micronucleus assay conducted in male ddY mice, clove oil (type and composition not specified) was negative for the induction of micronuclei in bone marrow cells. Six male mice were administered clove oil in olive oil by intraperitoneal injection at a single dose of 700 mg/kg bw followed by four doses of 175 mg/kg bw (Ishidate et al., 1988). Control mice were treated with olive oil and mice in the positive control group were administered mitomycin C. Animals were sacrificed 24 h post-dosing and bone marrow smears were prepared. Clove oil did not increase the induction of micronuclei based on the examination of 1000 polychromatic erythrocytes; however, a clear positive response was noted for the positive control (Ishidate et al., 1988).

7.4. Summary on genotoxicity for clove and cinnamon leaf oils

The review of the genotoxicity assay results reported for clove and cinnamon leaf oils is complicated by mixed results and limited information on the chemical composition of the test substance or the part of the plant (leaf, bud or stem) from which the samples were derived. The positive results reported for cinnamon leaf oil and clove oil are from Ames assays conducted under non-standard conditions or from the non-standardized rec assay. In contrast, negative results for mutagenicity were reported for clove leaf oil in a GLP-compliant Ames assay and negative results for clastogenicity and aneugenicity for clove oil were reported in an *in vitro* chromosomal aberration assay and the *in vivo* micronucleus assay, respectively (DeGraff, 1983; Ishidate et al., 1984, 1988). These negative results for mutagenicity, clastogenicity and aneugenicity are consistent with results from analogous studies for eugenol (FDA. 1978; JECFA, 1982; Rietjens et al., 2014). The constituent analyses of Clove Bud Oil (FEMA 2332), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf Oil (FEMA 2292) demonstrate a high eugenol/eugenyl acetate content of approximately 80% (see Appendix A) with smaller amounts of sesquiterpene hydrocarbons and other minor constituents. Given this composition in which eugenol is the primary constituent, Clove Bud Oil (FEMA 2332), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf Oil (FEMA 2292) are expected to lack genotoxic potential. The negative GLP-compliant Ames assay and negative results for clastogenicity and aneugenicity for clove oil in an *in vitro* chromosomal aberration assay and the *in vivo* micronucleus assay, support this conclusion (DeGraff, 1983; Ishidate et al., 1984, 1988).

7.5. West Indian Bay leaf oil

In an OECD compliant study, West Indian bay leaf oil was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvrA* using the plate incorporation method up to 1600 μg/plate in both the presence and absence of Aroclor 1254-induced rat liver S9 metabolic activation system. The composition of the test substance, determined by GC analysis, was 47% eugenol, 27% myrcene, 11% 4-allylphenol (chavicol), 3% α- and β-pinene, 2% methyl eugenol and approximately 10% unidentified constituents. Cytotoxicity, measured as the reduction of the incidence of spontaneous revertant colonies or as a reduction in the growth of the background lawns, was observed at 50 μg/plate in the absence of S9 metabolic activation and above 160 μg/plate in the presence of S9 metabolic activation for the *S. typhimurium* strains tested. In *E. coli* WP2*uvrA* cytotoxicity was observed at concentrations greater than 500 μg/plate in the presence of S9 metabolic activation (Mee, 2017). Dried and water extracts of West Indian bay leaf (*Pimenta racemosa* Moiller) were also found to be non-mutagenic when tested in a non-standard rec assay conducted in *B. subtilis* using both the cold and standard streak methods (Ungsurungsie et al., 1982). The rec assay has not been standardized with an OECD guideline for genotoxicity testing, which notes that indicator tests such as the rec assay should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015). In summary, West Indian bay leaf an oil was found not be mutagenic in an OECD-compliant Ames assay as well as in the rec assay.

8. Recognition of GRAS status

Based on the application of the safety evaluation procedure for NFCs, it is concluded that Bay Leaves West Indian Oil (FEMA 2122), Clove Bud Extract (FEMA 2322), Clove Bud Oil (FEMA 2323), Clove Bud Oleoresin (FEMA 2324), Clove Leaf Oil (FEMA 2325), Clove Stem Oil (FEMA 2328) and Cinnamon Leaf Oil (FEMA 2292) do not present safety concerns under conditions of intended use as flavoring ingredients. The safety of these NFCs is supported by their self-limiting properties as flavoring ingredients in food resulting in use levels that do not saturate pathways of metabolism and excretion. The estimated intakes of the majority of the constituent congeneric groups for each NFC were below the TTC, giving adequate margins of safety. In cases where the estimated intake of Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) constituents exceeded the TTC, adequate margins of safety were determined based on a long-term toxicity study. The exposure of low concentrations of naturally occurring allylalkoxybenzene constituents methyl eugenol, estragole and safrole in these NFCs was evaluated and found to not present a safety concern based on their low estimated intakes that were either less than the TTC of 0.15 μg/person/day for

compounds with structural alerts for genotoxicity or used to calculate a MOE greater than 10,000, indicating a low concern. Although Bay Leaves West Indian Oleoresin (FEMA 2123) could not be evaluated by the procedure, it also does not present a safety concern, since its estimated intake is below the TTC for compounds with structural alerts for genotoxicity.

The *Eugenia*, *Cinnamomum* and *Pimenta*-derived NFCs listed in Table 7 were initially determined to be GRAS in 1965 (Hall and Oser, 1965). Based on the application of the safety evaluation procedure, the FEMA Expert Panel has affirmed the GRAS status of these NFCs under conditions of intended use as flavor ingredients.

CRediT authorship contribution statement

Nigel J. Gooderham: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Samuel M. Cohen:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Gerhard Eisenbrand:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Shoji Fukushima:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **F. Peter Guengerich:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Stephen S. Hecht:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Ivonne M.C.M. Rietjens:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Thomas J. Rosol:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Jeanne M. Davidsen:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing, Supervision. **Christie L. Harman:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Ian J. Murray:** Formal analysis, Investigation, Data curation, Visualization, Writing - original draft. **Sean V. Taylor:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Drs. Cohen, Eisenbrand, Fukushima, Gooderham, Guengerich, Hecht, Rietjens and Rosol are members of the Expert Panel of the Flavor and Extract Manufacturers Association. Authors Davidsen, Harman, Murray and Taylor are employed by Verto Solutions which provides scientific and management support services to FEMA. A full description of the conflict of interest protections and procedures used to ensure that the FEMA Expert Panel decisions are fully objective and based solely on the merits of the available information are available on the FEMA website at https://www.femaflavor.org/gras#conflict.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111585.

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