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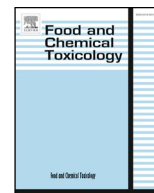
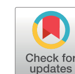


FEMA GRAS assessment of natural flavor complexes: *Cinnamomum* and *Myroxylon*-derived flavoring ingredients



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ABSTRACT

In 2015, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) initiated a program for the re-evaluation of the safety of over 250 natural flavor complexes (NFCs) used as flavor ingredients. This publication, third in the series, considers NFCs composed primarily of constituents with the 3-phenyl-2-propenyl or a cinnamyl functional group, using the procedure outlined in 2005 and updated in 2018 to evaluate the safety of naturally-occurring mixtures for their intended use as flavor ingredients. The procedure relies on a complete chemical characterization of the NFC intended for commerce and organization of each NFC's chemical constituents into well-defined congeneric groups. The safety of the NFC is evaluated using the well-established and conservative threshold of toxicological concern (TTC) concept in addition to data on absorption, metabolism and toxicology of members of the congeneric groups and the NFC under evaluation. Six NFCs from the *Myroxylon and Cinnamomum* genera, Balsam Oil, Peru (FEMA 2117), Tolu Balsam Extract (FEMA 3069), Cassia Bark Extract (FEMA 2257), Cassia Bark Oil (FEMA 2258), Cinnamon Bark Extract (FEMA 2290) and Cinnamon Bark Oil (FEMA 2291) were evaluated and affirmed as generally recognized as safe (GRAS) under their conditions of intended use as flavor ingredients.

1. Introduction

For over five decades, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) has been the primary, independent body evaluating the safety of flavoring ingredients for use in human foods in the United States. Flavor ingredients are evaluated for "generally recognized as safe" (GRAS) status for intended use consistent with the 1958 Food Additive Amendment to the Federal Food Drug and Cosmetic Act. To date, the FEMA Expert Panel has determined that over 2,700 flavoring ingredients have met the GRAS criteria for their intended uses.

A key part of the FEMA GRAS program is the cyclical re-evaluation of GRAS flavoring ingredients. Flavoring ingredients are generally divided into two categories: chemically defined flavoring materials and natural flavor complexes (NFCs). NFCs are mixtures, often derived from botanical sources that are used for flavoring food. The FEMA Expert Panel has completed two re-evaluations of chemically defined flavor ingredients and in 2015 expanded the re-evaluation program to encompass FEMA GRAS NFCs and other relevant NFCs. A scientifically-based procedure was developed to assist in this determination via a step-wise analysis of the chemical composition of the NFC (Smith et al., 2005). Recently, this procedure has been reviewed and updated (Cohen

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Abbreviations

BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
$BMDL_{10}$	Lower confidence limit of the benchmark dose resulting in a 10% extra cancer incidence
CA	Chromosomal aberration
CF	Correction factor
CFR	Code of federal regulations
CHO	Chinese hamster ovary (cells)
DTC	Decision tree class
EFFA	European Flavour Association
EFSA	European Food Safety Authority
ERS/USDA	Economic Research Service/United States Department of Agriculture
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FID	Flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally recognized as safe
HPBL	Human peripheral blood lymphocytes
IFEAT	International Federation of Essential Oils and Aroma Trades
IOFI	International Organization of the Flavor Industry
JFFMA	Japan Fragrance and Flavor Materials Association
MOE	Margin of exposure
MoS	Margin of safety
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
SCE	Sister chromatid exchange
TDI	Tolerable daily intake
TTC	Threshold of toxicological concern
WHO	World Health Organization

et al., 2018a). Because the constituents of NFCs are typically derived from common biochemical pathways, the constituents can be organized into a finite number of well-established chemical groupings called congeneric groups. For each NFC, information is gathered on estimated intake, absorption, metabolism and toxicology for each constituent congeneric group and evaluated as part of the safety evaluation. In addition, rigorous consideration is given to the unidentified constituents. NFCs have been grouped by similarity of their chemical composition or taxonomy to facilitate the re-evaluation of all the NFCs. This procedure has previously been applied in the safety evaluation of *Citrus*-derived NFCs (Cohen et al., 2019a) and mint, buchu, dill and caraway-derived NFCs (Cohen et al., 2019b). The chemical composition of the NFCs in the group evaluated in the present paper is dominated by the presence of 3-phenyl-2-propenyl or cinnamyl constituents.

In 2015, the FEMA Expert Panel issued a call for data requesting detailed chemical analysis for five cinnamyl NFCs. Members from the International Organization of the Flavor Industry (IOFI), including FEMA, the Japan Fragrance and Flavor Materials Association (JFFMA), the European Flavour Association (EFFA), in addition to the International Federation of Essential Oils and Aroma Trades (IFEAT), provided data on *Cinnamomum*-derived NFCs and *Myroxylon*-derived balsam oil of Peru which are currently used for flavoring food and beverage products. A sixth NFC, tolu balsam extract, was included on a later call for data and was classified into this cluster. These NFCs are listed in Table 1.

2. History of food use

The *Cinnamomum* genus consists of some of the world's economically and historically most important plants. These plants are valued for their inner bark, which when dried and ground into a fine powder, can give food both sweet and savory notes or be distilled to capture its essential oil. While the *Cinnamomum* family consists of over 300 species, historically the global trades have focused on only a few types of this plant. The four most prominent *Cinnamomum* species originate from Southeast Asia and China. References to cinnamon date back to ancient Egyptian and Chinese civilizations (ASTA, 2002; Parry, 1969). Cinnamon is mentioned in the Bible several times and it is noted that Nero burned a year's worth of Rome's cinnamon stockpile upon the death of his wife (Ravindran and Babu, 2004). Prior to the discovery of naval shipping routes, cinnamon was transported by land through the Middle East into Europe making the spice an expensive luxury in the West (Coppen, 1995; Schivelbusch, 1992). During the Age of Exploration (1500's – 1700's), Western European powers sought control of both naval cinnamon trading routes and their lands of production as a means of revenue (Ravindran and Babu, 2004). Over time, cinnamon and cassia became commodity items and are widely available worldwide.

There are two types of cinnamon available in food markets, “True Cinnamon” and “Cassia”. While both are commonly known as cinnamon, True Cinnamon (*C. verum* syn. *C. zeylanicum*) is native to Sri Lanka (formerly Ceylon) and lighter in color and milder in taste than Cassia cinnamons (ASTA, 2002, 2008). Sri Lanka remains the predominant producer of True Cinnamon (Ratwatte, 1991) and cinnamon bark essential oil. Cassia cinnamon normally refers to the product of three plants: *C. burmanii* (Indonesian Cinnamon), *C. loureirii* (Saigon Cinnamon), and *C. cassia* (Chinese Cinnamon). The volatile oil content of these cinnamons ranges from 1.5 to 7%, with Saigon cinnamon typically yielding the highest oil content (ASTA, 2002; Geng et al., 2011). The quality of these cinnamons is traditionally determined by their oil

Table 1
NFCs evaluated by the Expert Panel.

Name	FEMA No.	Estimated Intake (μg/person/day)[a]	Most recent annual volume (kg)[b]
Balsam Oil, Peru (*Myroxylon pereirae* Klotzsch)	2117	190	1770
Tolu Balsam Extract (*Myroxylon balsamum* L. Harms (*M. toluiferum* HBK))	3069	2	20
Cassia Bark Extract (*Cinnamomum cassia* Blume)	2257	250	2290
Cassia Bark Oil (*Cinnamomum cassia* Blume)	2258	280	26,200
Cinnamon Bark Extract (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)	2290	690	6480
Cinnamon Bark Oil (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)	2291	440	4150

[a] For high volume materials (greater than 22,500 kg/year), the PCI *per capita* is shown. For materials with a lower surveyed volume (less than 22,500 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.

content (ASTA, 2002). It is not uncommon for cinnamon purchasers to buy blends of these four spices to achieve their desired products.

The *Myroxylon*-derived NFCs considered here are prepared from tree balsams and are rich in cinnamic and benzyl acids and esters. Balsam oil Peru and tolu balsam extract are prepared from the tree resin, called balsam, collected from the outer bark. Balsam oil Peru is harvested from the balsam of *Myroxylon pereirae* by a solvent extraction or distillation. Tolu balsam extract is a hexane extract obtained from the ethanolic solution of balsam collected from *Myroxylon balsamum* trees (Custódio and Veiga-Junior, 2012). Initially discovered growing in Central America (not Peru) during early Spanish domination, the balsam of *Myroxylon pereirae* was exported to Europe from Peru, resulting in its name, Balsam of Peru (Guenther, 1949). *Myroxylon balsamum,* the tree from which tolu balsam extract is derived, is native to Columbia and Venezuela. Over the last several decades, these NFCs have been used in various flavoring and fragrance applications.

3. Current usage

Cinnamyl flavoring materials are used in a variety of foods including frozen dairy products, gelatins, candies, gums and various beverages. The most recent annual poundage and exposure calculations for these NFCs are summarized in Table 1. The estimated intake is calculated using the PCI × 10 approach which assumes that the annual usage is consumed by 10% of the population. For NFCs with annual usage of greater than 22,700 kg, the assumption that the NFC is only consumed by only part of the population is not used and the estimated intake is calculated based on consumption of the NFC by the entire population (Hall and Ford, 1999; Smith et al., 2005).

Cinnamon Bark Extract had the highest estimated *per capita* consumption of 690 μg/person/day. The remaining four Cinnamon and Cassia-derived NFCs exposure range from 2 to 440 μg/person/day. In Western diets, cinnamon bark is commonly consumed as a ground spice and is a common addition to various baked goods and coffee beverages. Cinnamon and Cassia contain 0.5–7% total volatile oil by dry weight (ASTA, 2002; Geng et al., 2011). The annual volume of cassia and cinnamon imported into the USA in 2014 was estimated by the Economic Research Service of the United States Department of Agriculture (ERS/USDA)[1] to be 29 million kg. The ERS/USDA groups cassia and cinnamon together when compiling market supply and disappearance estimations. Separate data on cassia and cinnamon are not available. Both cassia and cinnamon are used under the name "cinnamon" for use as a spice.[2] Assuming that the import total was consumed in food by use as a spice and contained 2% volatile oil, which is on the low range of the expected content of a middle quality cassia quill (CBI, 2018), an estimated 348,000 kg of cassia/cinnamon oil would be consumed in the USA per year by consuming cinnamon spice, resulting in an estimated intake of 5000 μg/person/day, if consumed by the entire population. This estimated intake from food is significantly higher than the *per capita* intake of the cinnamon and cassia derived NFCs listed in Table 1. This estimate will be used to calculate consumption ratios in the safety evaluation of these NFCs.

Balsam oil, Peru and tolu balsam extract are derived from balsam trees grown in Central and South America which are not known to be used as a food. The essential oil derived from these trees, however, has a warm, sweet, "balsamic" flavor and is used as a flavoring added to food.

[1] Data obtained from ERS/USDA based on data from various sources (see https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/). Data last updated Feb. 1, 2017. Information was downloaded on May 4, 2017. Annual volume naturally occurring in foods calculated from the *per capita* consumption of each in 2014 multiplied by the estimated population for the United States.

[2] US FDA CPG Sec. 525.750 Spices - Definitions.

4. Manufacturing methodology

The bark oils and extracts from *Cinnamomum* and *Cassia* plants are harvested using similar processes. Oil producing *Cassia* cinnamon plants (*C. loureirii* and *C. cassia*) take 3–20 years to grow to maturity. At maturity, the plant's shoots are trimmed of leaves and clipped slightly above the ground. The outer cork is removed and specialized cutting tools are used to strip the inner bark from the woody stem. The strips are then rolled and allowed to air dry out of direct sunlight. The drying process causes the strips to curl forming cinnamon's characteristic quills (ASTA, 2008; Dayananda et al., 2004). Ceylon (Sri Lankan) cinnamon (*C. verum* or *C. zeylanicum*) is collected twice a year in concurrence with the area's rainy seasons. After the leaves are removed and the bark is collected, the Ceylon cinnamon bark is taken through a curing process involving several wash and dry cycles, with the bark eventually curling into quills, similar to *Cassia* cinnamon. The highest quality quills—-based on color and size—are bundled and exported. The quillings, featherings, and chips are collected for steam distillation to make oils and extracts (Dayananda et al., 2004; Wijesekera and Chichester, 1978). Cinnamon and cassia extracts are prepared by solvent extraction of the final dried bark product and may be further processed to remove the solvents, yielding a more concentrated flavoring material. Cinnamon and cassia extracts may also be an aqueous ethanolic solution of the essential oil (Merory, 1960).

Other cinnamyl NFCs surveyed are Balsam Oil Peru and Tolu Balsam Extract. Both are produced from the resin excreted by the *Myroxylon* plant upon cutting or burning into the bark of the tree (Khan and Abourashed, 2011), collecting the balsam, and extracting the essential oil using solvent extraction or distillation techniques.

5. Chemical composition

The constituent profiles for the six NFCs listed in Table 1 were collected using gas-chromatography-mass spectrometry (GC-MS) techniques to identify constituents in comparison to a standard library and a flame ionization detection (FID) for quantitation of each chromatographic peak. Both identified and unidentified GC peaks were reported as the percent area of the chromatogram. Constituent data for each NFC were compiled and the mean % for constituents reported at greater or equal to 1.0% are listed in Appendix A, ordered by congeneric group. The constituents are classified into congeneric groups based on the chemical structure and the functional groups outlined in the NFC safety evaluation procedure (Cohen et al., 2018a). The Cramer decision tree class (Cramer et al., 1978) for each constituent in Appendix A is also reported. The Cramer decision tree class assigned to each congeneric group is determined by assigning the most conservative class for the constituents within each group.

Structures of the commonly found constituents reported in the NFCs under consideration are shown in Fig. 1. Pie charts depicting the constituent profiles for each NFC are shown in Fig. 2. The major constituent of three of the *Cinnamomum* NFCs - Cassia Bark Extract (FEMA 2257), Cassia Bark Oil (FEMA 2258), and Cinnamon Bark Oil (FEMA 2291)- is cinnamaldehyde. Overall, these NFCs show very high percentages (89, 93 and 76% respectively) of Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) constituents. Cinnamon Bark Extract (FEMA 2290), an ethanolic extract of cinnamon bark (spice), is composed of a high percentage of ethyl alcohol, a member of Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters) constituents and a smaller percentage of cinnamaldehyde. The primary congeneric groups of Balsam Oil, Peru (FEMA 2117) and Tolu Balsam Extract (FEMA 3069) are Groups 16 and 14 (Benzyl derivatives). The constituent groups comprising the remaining 10–20% of the NFCs under consideration are Group 19 (Aliphatic and aromatic hydrocarbons), Group 13 (Aliphatic, alicyclic-fused and aromatic-fused ring lactones) and Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives).

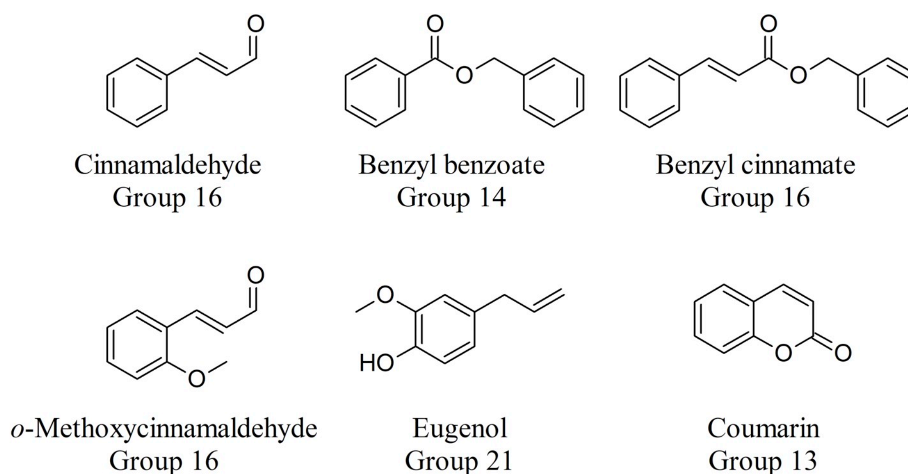

Fig. 1. Structures of commonly found constituents in cinnamyl natural flavor complexes and their congeneric group.

Unidentified constituents account for less than 3% of the profile for each NFC. The safety of the unidentified constituent fraction is considered in Steps 7 to 12 in the safety evaluation procedure.

6. Safety evaluation

The procedure for the safety evaluation for NFCs was guided by a set of criteria that were initially outlined in two publications (Smith et al., 2004, 2005) and updated recently (Cohen et al., 2018a). Briefly, as shown in Fig. 3, 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 data on metabolism and toxicity and on the application of the Threshold of Toxicologic Concern (TTC) approach (Kroes et al., 2000).[3] Steps 7-12 address the potential toxicity, including genotoxicity of the unidentified constituents; lastly, in Steps 13 and 14 the overall safety is evaluated along with considerations of potential biologically relevant synergistic or antagonistic interactions among constituents. The safety evaluation is presented below. Each step of the procedure (Cohen et al., 2018a) (provided in italics), is considered and 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, 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 (Adams 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).[4] *At this step, if the conditions of use*[5] *for the NFC result in levels that differ from intake of the same constituents in the food source, it should be reported.*

b. Identification of all known constituents and assignment of Cramer

[3] 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).

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

[5] 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.

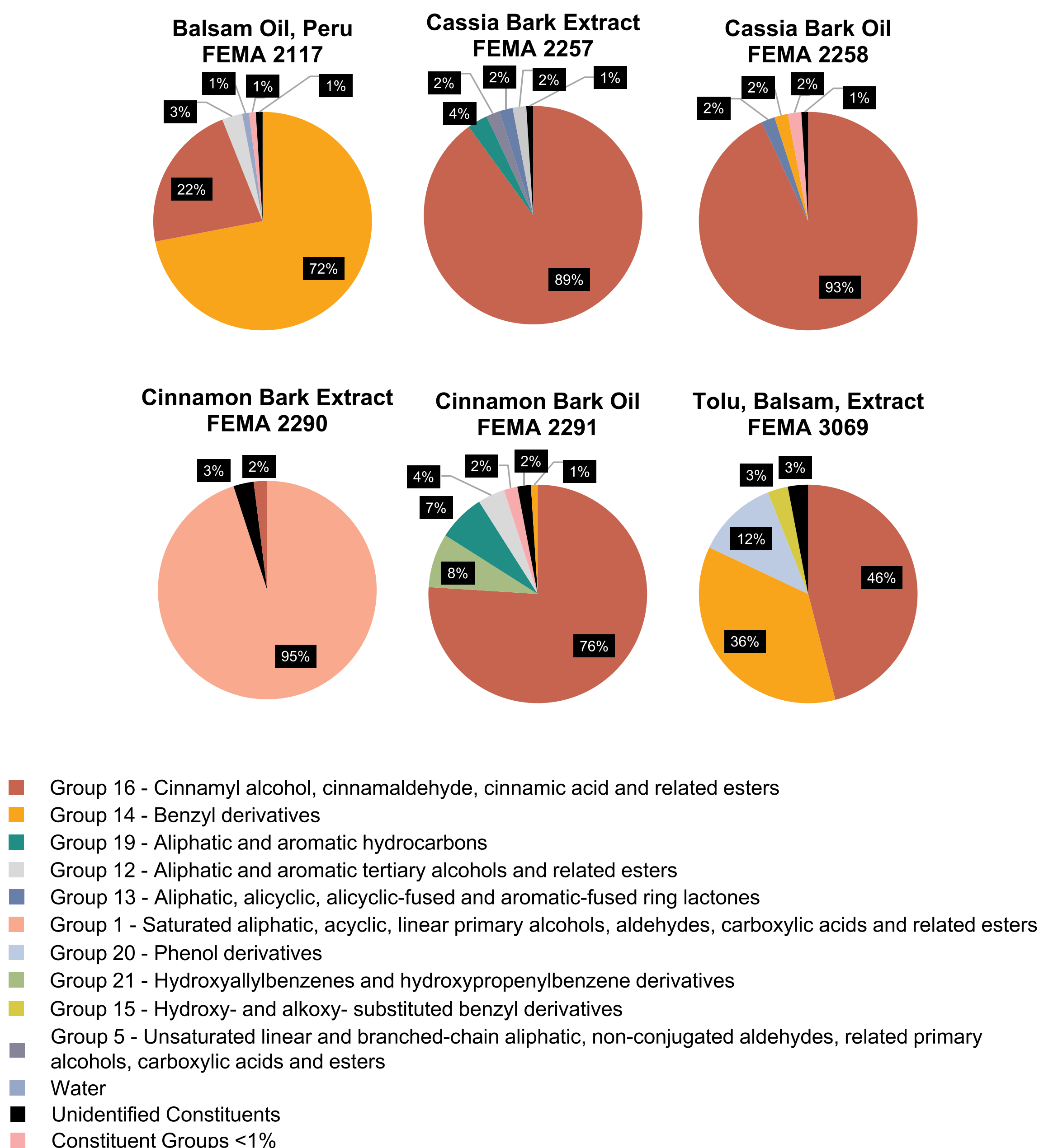

Fig. 2. Constituent congeneric group profiles for the NFCs under consideration.

Decision Tree Class

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

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

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 exhibit consistently 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.

Cassia Bark Oil (FEMA 2258) and Cinnamon Bark Oil (FEMA 2291) are derived from *Cinnamonum* species which are also the source of cinnamon commonly used as a spice for the flavoring of food. Based on data compiled by the ERS/USDA, approximately 29 million kg of cassia and cinnamon were imported in 2014. Unfortunately, separate data on cassia and cinnamon are not available, however, both cassia and cinnamon are used under the name "cinnamon" for use as a spice[4]. Assuming that the import total was consumed in food by use as a spice and contained 2% volatile oil, which is on the low range of the expected content of a middle quality cassia quill (CBI, 2018), an estimated 348,000 kg of cassia/cinnamon oil would be consumed in the USA per year by consuming cinnamon spice, resulting in an estimated intake of 5000 μg/person/day, if consumed by the entire population. This volume/intake is substantially larger than the most recent volume/intake reported for the essential oil NFCs Cinnamon Bark Oil (FEMA 2291) and Cassia Bark Oil (FEMA 2258) and the botanical extract NFCs Cassia Bark Extract (FEMA 2257) and Cinnamon Bark Extract (FEMA 2290) (summarized in Table 2) indicating that intake of the NFC occurs

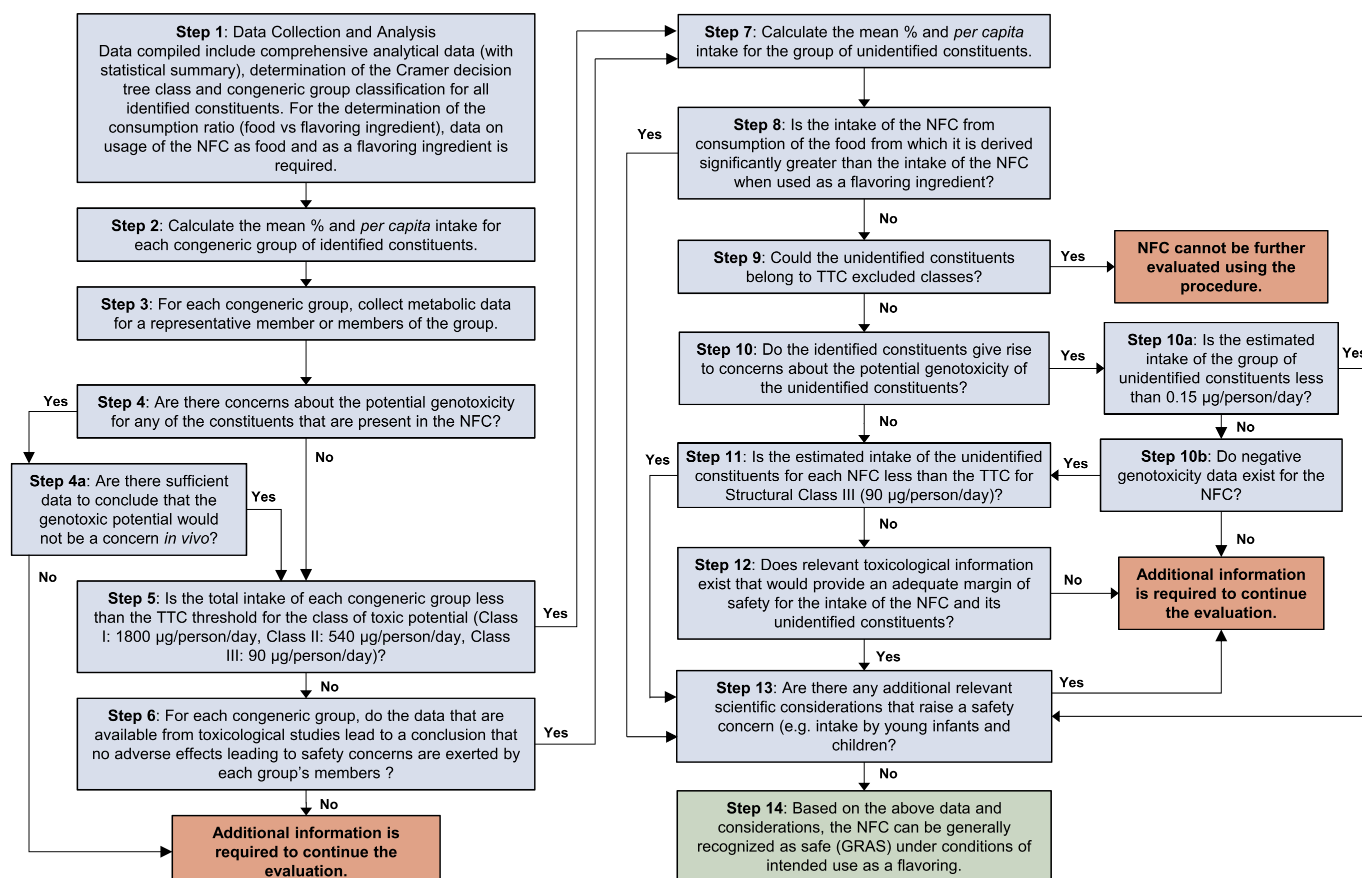

Fig. 3. Procedure for the safety evaluation of NFCs (Cohen et al., 2018a).

predominantly from the food of which it is derived.

The balsams from the *Myroxylon* tree, source of Balsam oil, Peru (FEMA 2117) and Tolu Balsam Extract (FEMA 3069), are not known to be used as food. Therefore, all consumption is assumed to be as flavoring added to food.

All reported constituents in the six NFCs were organized by congeneric group and a summary report for each NFC is shown in Appendix A. 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. The total mean % for each congeneric group is subtotaled and reported with the DTC for the group.

Step 2. *Determine (a) the mean percentage (%) of each congeneric group in the NFC, and (b) the daily per capita intake*[6] *of each congeneric group. The value (a) is calculated by summing the mean percentages of each of the constituents within a congeneric group, and the value (b) is calculated from consumption of the NFC and the mean percentage.*

Calculation of PCI for each constituent congeneric group of the NFC

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

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 report for each NFC provided in Appendix A, the total mean % for each congeneric group is subtotaled and reported with the DTC and the estimated intake (PCI × 10 or PCI, as appropriate) for each congeneric group listed.

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 in* Step 13 *of the procedure.*

Proceed to Step 4.

For each congeneric group listed in Appendix A, metabolic data exist for one or more representative members of the group that indicate 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). For more detailed descriptions of the studies and extensive discussion and interpretation of the findings see the related FEMA Expert Panel safety assessments for the primary two congeneric groups, Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters congeneric group) and Group 14 (Benzyl derivatives) (Adams et al., 2004, 2005a) and previously published assessments of other groups or individual constituents (Adams et al., 2005b, c; Adams et al., 2002; Adams et al., 1997; Adams et al., 2011a; Adams et al., 2008; Adams et al., 1998; Adams et al., 1996;

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

Table 2
Estimated Intake of Cinnamon and Cassia oils from food (in bold) and estimated intakes of NFCs used as flavoring.

	Estimated Intake (μg/person/day)
Cinnamon and Cassia Oil consumed from food (as spice) (ERS/USDA)	**5,000**
2257 - Cassia Bark Extract (*Cinnamomum cassia* Blume)	250
2258 - Cassia Bark Oil (*Cinnamomum cassia* Blume)	280[a]
2290 - Cinnamon Bark Extract (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)	690
2291 - Cinnamon Bark Oil (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)	440

[a] For high volume materials (greater than 22,500 kg/year), the PCI *per capita* is shown. For all other NFCs listed here, the estimated intake was calculated using the PCI × 10 method.

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 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) that are major constituents for the NFCs under consideration and determined a lack of genotoxic potential for these and related compounds (Adams et al., 2004). More recent genotoxicity studies are described later under "Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation". In addition, a review of the minor constituent profile of Balsam Oil, Peru (FEMA 2117), Tolu Balsam Extract (FEMA 3069), Cassia Bark Extract (FEMA 2257), Cassia Bark Oil (FEMA 2258) and Cinnamon Bark Extract (FEMA 2290) also indicates no genotoxic concern for the congeneric groups presented. These NFCs proceed to Step 5. Cinnamon Bark Oil (FEMA 2291), however, contains small amounts of the naturally occurring allylalkoxybenzene compounds safrole and methyl eugenol. These constituents share a structural motif which raises a genotoxicity concern (Rietjens et al., 2014). The occurrence and estimated intake for these constituents are shown for this NFC in Table 3. Cinnamon Bark Oil (FEMA 2291) proceeds to Step 4a.

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 safrole and methyl eugenol (see Fig. 4) share a motif of a benzene ring substituted with an alkoxygroup 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 sulfonation at this site by a sulfotransferase. The elimination of sulfate creates a DNA reactive carbocation species (Daimon et al., 1997; Herrmann et al., 2012, 2014; Jeurissen et al., 2004, 2007; Rietjens et al., 2014; Ueng et al., 2004). Rodent studies have indicated that safrole and methyl eugenol are hepatocarcinogens at high dose levels (Abbott et al., 1961; Homburger et al., 1965; Homburger et al., 1962; Long et al., 1963; NTP, 2000). The addition of safrole to food is prohibited in the USA (21 CFR §189.180) and both the addition of safrole and methyl eugenol to food is prohibited in Europe (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 to humans (Cohen et al., 2018b). Recognizing that naturally occurring methyl eugenol occurs in herbs, spices and the essential oils and extractives of these botanicals, the Expert Panel concluded that these flavorings continue to meet the criteria for FEMA GRAS under their conditions of intended use as flavorings (Cohen et al., 2018b). Later, in October 2018, addition of synthetic methyl eugenol to food in the USA was prohibited by the FDA (83 Fed. Reg. 50490.9 October 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. 50490.9 October 2018). In addition, 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. 50490.9 October 2018). Both methyl eugenol and safrole are naturally occurring constituents in herbs and spices such as basil, tarragon, allspice, cinnamon, anise, nutmeg and mace, and both were reported in Cinnamon Bark Oil (FEMA 2291) at low concentrations (Table 3).

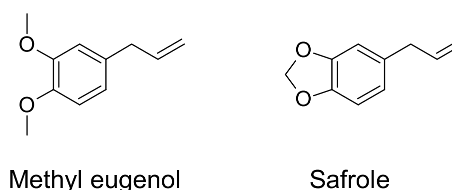

Fig. 4. Structures of methyl eugenol and safrole.

Naturally occurring methyl eugenol was reported at 0.005% in Cinnamon Bark Oil (FEMA 2291) resulting in an estimated intake of 0.02 μg/person/day. This value is below the TTC of 0.15 μg/person/day determined based on an analysis of the dose-response data for carcinogenic compounds (Kroes et al., 2004), provided by the Gold database of carcinogens presenting the dose giving a 50% tumor incidence (TD50) (Gold et al., 1984). 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;WHO, 2016; Kroes et al., 2004; Nohmi, 2018).

Safrole was reported in Cinnamon Bark Oil (FEMA 2291) at a mean of 0.1%, resulting in an estimated intake of 0.6 μg/person/day which is above the TTC for carcinogenic compounds. In cases where the intake of a naturally occurring carcinogen from food exceeds the TTC for genotoxic, carcinogenic substances, a Margin of Exposure (MOE) approach is applied (EFSA, 2009). The MOE is calculated based on the

Table 3
Estimated daily intake of methyl eugenol and safrole in Cinnamon Bark Oil.

Name (FEMA No.)	Constituent of Concern	Mean %	Estimated Intake (μg/person/day)
Cinnamon Bark Oil (FEMA 2291)	Safrole	0.1	0.6
Cinnamon Bark Oil (FEMA 2291)	Methyl eugenol	0.005	0.02

Table 4
MOE Analyses for naturally occurring safrole in Cinnamon Bark Oil (FEMA 2291) (van den Berg et al., 2011).

Name (FEMA No.)	Constituent of Concern	Estimated Intake (mg/kg bw/day)	$BMDL_{10}$ (mg/kg bw/day)	MOE
Cinnamon Bark Oil (FEMA 2291)	Safrole	1×10^{-5}	1.9	> 190,000

Table 5
Mean % and Estimated Intake for Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) for each NFC.

Name (FEMA No.)	DTC[a]	Mean %	Estimated Intake (µg/person/day)	TTC (µg/person/day)
Balsam Oil, Peru (FEMA 2117)	I	22	42	1800
Tolu Balsam Extract (FEMA 3069)	I	46	0.9	1800
Cassia Bark Extract (FEMA 2257)	I	89	210	1800
Cassia Bark Oil (FEMA 2258)	I	93	260	1800
Cinnamon Bark Extract (FEMA 2290)	I	2	8	1800
Cinnamon Bark Oil (FEMA 2291)	I	76	330	1800

[a] The DTC for each congeneric group is determined to be the most conservative DTC of the constituents reported in the respective group.

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). 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 value resulting from the estimated daily intake of safrole via use of Cinnamon Bark Oil (FEMA 2291). The MOE is much greater than 10,000 indicating a low concern. Cinnamon Bark Oil (FEMA 2291) proceeds to Step 5.

Step 5. *Is the total intake of each 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.

Yes, for the NFCs under consideration, the total estimated intake for each of the congeneric groups present in each NFC is below the corresponding TTC when applied to the appropriate class of toxic potential (Cramer et al., 1978). These thresholds do not apply for constituents with genotoxic potential such as methyl eugenol and safrole, which were analyzed in Steps 4 and 4a. The data summarized in Appendix A for each NFC report the mean % and estimated intake for all constituent groups. In addition, the estimated intake of Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) constituents, the dominant constituent group for these NFCs, is summarized in Table 5 below. For each NFC listed, all Group 16 constituents reported belong to structural Class I and the estimated intake for Group 16 constituents is less than the TTC for structural Class I, 1800 µg/person/day. Proceed to Step 7 of the evaluation procedure.

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.

This step is not required.

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 estimated intake for the group of unidentified constituents of each NFC. These data are also summarized in Table 6.

Step 8. *Using the data from* Step 1*, is the intake of the NFC from consumption of the food*[7] *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.

Yes. For the *Cinnamomum* and *Cassia* NFCs, Cassia Bark Extract (FEMA 2257), Cassia Bark Oil (FEMA 2258), Cinnamon Bark Extract (FEMA 2290) and Cinnamon Bark Oil (FEMA 2291), the estimated intake as a spice/food is significantly higher than intake as flavoring added to food (see Table 2). For these NFCs, proceed to Step 13.

No. Since Balsam oil, Peru (FEMA 2117) and Tolu Balsam Extract are derived from a natural tree resin that is not used as food, intake of these NFCs is expected to only come from use as flavoring added to food. Proceed to Step 9.

Step 9. *Could the unidentified constituents belong to TTC excluded classes? 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;WHO, 2016; Kroes et al., 2004).

If Yes, the NFC is not appropriate for consideration via this procedure.
If No, proceed to Step 10.

No. Unidentified constituents are not suspected to belong to TTC

[7] Provided the intake of the unidentified constituents is greater from consumption of the food itself, the intake of unidentified constituents from the added NFC is considered trivial.

Table 6
Estimated intake of unidentified constituents.

Name	FEMA No.	Estimated Intake µg/person/day
Balsam Oil, Peru	2117	2
Tolu Balsam Extract	3069	0.07
Cassia Bark Extract	2257	2
Cassia Bark Oil	2258	3
Cinnamon Bark Extract	2290	20
Cinnamon Bark Oil	2291	7

excluded classes. Based on the identified constituents, the unidentified constituents are most likely cinnamic products of the shikimate pathway or monoterpenoid and sesquiterpenoid products of the isoprene pathway. Based on the known constituents and the isolation procedure, the presence of constituents from TTC excluded classes in the unidentified constituents is unlikely. Proceed to Step 10.

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.

No. Based on the composition of the identified constituents Balsam oil, Peru (FEMA 2117) and Tolu Balsam Extract (FEMA 3069), there is no indication that the unidentified substances would have structural alerts for genotoxicity. Steps 10a and 10b are not required. Proceed to Step 11.

Step 10a. *Is the estimated intake of the group of unidentified constituents less than 0.15 µg/person/day* (Koster et al., 2011; Rulis, 1989)*? A TTC of 0.15 µg/person/day has been proposed for potentially genotoxic substances that are not from the TTC excluded classes materials* (Kroes et al., 2004*;* Rulis, 1989)*?*

If Yes, proceed to Step 13.

If No, proceed to Step 10b.

This step is 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.

This step is not required.

Step 11. *Is the estimated intake of the unidentified constituents (calculated in* Step 7*) for each NFC less than the TTC (*Kroes et al., 2000; Munro et al., 1996*) for Structural Class III (*90 µg*/person/day)?*[8]

If Yes, proceed to Step 13.

If No, proceed to Step 12.

[8] 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 the 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).

Yes, as calculated in Appendix A and summarized in Table 6, for all NFCs listed, the estimated intake of the unidentified constituent fraction is less than 90 µg/person/day. Proceed to Step 13.

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.

This step is 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 the additional data required to address the concern before proceeding to Step 14.

If No, proceed to Step 14.

In 1954, coumarin, a substance known to naturally occur in tonka beans, was banned for use as an added flavor in food in the USA by the US Food and Drug Administration (FDA, 1954). This action was based on dietary feeding studies of coumarin in rats and dogs that showed hepatotoxic effects (Hazleton et al., 1956). Coumarin has also been found in *C. cassia* which is commonly used as a ground spice. Since 1956, numerous studies have been conducted to further understand the hepatotoxicity observed in experimental animals, the metabolic pathways of coumarin and whether study findings in rodents are relevant to humans. Based on this work, summarized later in this manuscript, the EFSA has determined that coumarin is not an *in vivo* genotoxicant and that a threshold exists for the toxicity for coumarin. The EFSA chose a no-adverse-effect-level (NOAEL) from a 2-year study in dogs of 10 mg/kg bw/day. Using a safety factor of 100 in consideration of the CYP2A6 – deficient subpopulation that may not efficiently metabolize coumarin, a tolerable daily intake (TDI) of 0.1 mg/kg bw was established in a 2004 opinion and maintained in their 2008 opinion (EFSA, 2004, 2008). In an expert opinion commissioned by the German Federal Institute for Drugs and Medical Devices, it was found that the lowest hepatoxic dose of coumarin reported in humans was 25 mg/day (Abraham et al., 2010; Bergmann, 1999). This was used as point of departure for risk assessment of human dietary exposure in an expert opinion of the German Federal Institute for Risk Assessment (BfR). Using a factor of five to determine a dose at which no adverse effects, even in sensitive populations, would be expected, a level of 5 mg coumarin per day per person was determined. A rounded TDI of 0.1 mg/kg bw was derived based on a 60 kg adult (Abraham et al., 2010).

In this study, very low levels of naturally occurring coumarin were reported in Balsam oil, Peru (FEMA 2117), Cassia Bark Extract (FEMA 2257), Cassia Bark Oil (FEMA 2258), Cinnamon Bark Extract (FEMA 2290), and Cinnamon Bark Oil (FEMA 2291). As shown in Table 7, the estimated intake of coumarin from each NFC is significantly lower than the TDI of 0.1 mg/kg bw/day which is equivalent to an intake of 6000 µg/person/day for a 60 kg adult and 2000 µg/person/day for a 20 kg child, and therefore not a safety concern.

A further evaluation to consider possible exposure to 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. For the NFCs under consideration, the total estimated intake for each of the congeneric groups present in each NFC are substantially below the corresponding TTC for the group, with none close to the TTC threshold, indicating the approach to be protective for consumption by children. Table 6 lists the estimated intake

Table 7
Estimated intake and mean % of coumarin in NFCs.

Name (FEMA No.)	Mean %	Estimated Intake of coumarin (µg/person/day)
Balsam Oil, Peru (FEMA 2117)	0.01	2×10^{-6}
Cassia Bark Extract (FEMA 2257)	2	5
Cassia Bark Oil (FEMA 2258)	2	5
Cinnamon Bark Extract (FEMA 2290)	0.2	1
Cinnamon Bark Oil (FEMA 2291)	0.05	0.2

of the unknown constituent fraction for the NFCs evaluated, none of which is close to or exceeds the TTC thresholds for Class 3.

Step 14. *Based on the above data and considerations, the NFC can be generally recognized as safe (GRAS) under conditions of intended use as a flavoring ingredient.*

Yes. Based on the above assessment the FEMA Expert Panel concluded that the current FEMA-GRAS NFCs can be affirmed as "generally recognized as safe" under conditions of intended use as a flavoring substance.

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

The constituent profiles of the *Cinnamomum* NFCs are dominated by cinnamaldehyde with smaller amounts of other Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters congeneric group) constituents such as *o*-methoxycinnamaldehyde and cinnamyl acetate. The safety of these constituents for use as flavoring ingredients has been reviewed by the FEMA Expert Panel (Adams et al., 2004). The FEMA Expert Panel has also reviewed flavoring ingredients from Group 19 (Aliphatic and aromatic hydrocarbons) (Adams et al., 2011a) and Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) (Marnett et al., 2014) as well as other groups that are present in minor amounts (Adams et al., 2005a, b; Adams et al., 2002; Adams et al., 1997; Adams et al., 2008; Adams et al., 1998; Adams et al., 1996; Adams et al., 2007). The toxicity of eugenol and other naturally occurring Group 21 (Hydroxy- and alkoxy-substituted propenyl benzenes) constituents reported in Cinnamon Bark Oil (FEMA 2291) has also been reviewed by the Panel (Rietjens et al., 2014) in addition to Group 14 (Benzyl derivatives) constituents in Balsam Oil, Peru (FEMA 2117) (Adams et al., 2005a).

The additional information presented here includes studies on the NFCs themselves, studies on the principal constituents of these NFCs and newly available studies on constituents not considered within the reviews mentioned above.

7.1. Group 16: Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters

Several flavorings within Group 16 are also used as flavoring raw materials and/or are present as components of spices. For flavorings such as cinnamaldehyde, a significant human exposure was considered possible and for this reason, it was nominated for studies at the National Toxicology Program.

7.1.1. Cinnamaldehyde

Several repeated dose studies for cinnamaldehyde were extensively reviewed (Adams et al., 2004). In a two-year carcinogenicity study (Hooth et al., 2004), groups of male and female F344/N rats (50/sex/group) and B6C3F$_1$ mice (50/sex/group) were administered diets with microencapsulated cinnamaldehyde at concentrations of 1000, 2100 or 4100 ppm, or a vehicle control diet. These doses of cinnamaldehyde resulted in average daily exposures of 50, 100 or 200 mg/kg bw per day in both sexes of rats, 125, 270 or 540 mg/kg bw per day in male mice, and 125, 270 or 570 mg/kg bw per day in female mice (Hooth et al., 2004). Food consumption and body weights were monitored during the study. In the rat study, urine from 10 rats/sex/group was collected at week 2, week 3, month 12 and month 18 for the analysis of creatinine and hippuric acid concentrations. Upon study termination, complete necropsies, macroscopic observations and complete histopathological examinations were conducted on all animals.

The survival of male rats in the highest dose group, 200 mg/kg bw per day, was higher than the vehicle control while survival of the male rats in the low and middle dose groups and all dose groups of female rats were not significantly different from the control group. Mean body weights in the highest dose group were generally lower than those of the vehicle controls throughout the study for males, and after week 18 for females and in the middle dose group of males after week 94. Feed consumption by male and female rats in the highest dose group and male rats in the middle dose group was lower than the vehicle control group at the beginning and at the end of the study. The ratio of hippuric acid to creatine in the urine was found to be proportional to the administered dose, indicating that the absorption, metabolism and excretion pathways for cinnamaldehyde were not saturated. There was no evidence of increases in malignant or non-malignant lesion incidences at any dose level in both sexes. Neoplastic findings were either determined to be random across dose groups or similar in incidences compared to the vehicle control group.

The survival rates for all treatment groups of male and female mice were comparable to the control group, except for slightly lower survival in males in the middle dose group. There were no clinical findings reported in any of the treatment groups. Mean body weights were reduced in the middle and high dose groups of both sexes as well as in male mice in the low dose group after week 74. Feed consumption in all treatment groups was comparable to the control group. Sporadic occurrence of both squamous cell papilloma and squamous cell carcinoma was observed in middle dose male (2/50) and female mice (4/50), as well as in one high dose male mouse. These findings were determined to not be statistically significant. Incidence of hepatocellular adenoma and carcinoma were significantly decreased in both the high and middle dose male mice and a negative trend in incidence of hepatocellular adenoma and carcinoma was observed in female mice. Neoplastic findings were either determined to be random across dose groups or similar in incidences compared to the vehicle control group. Due to the lack of directly correlated tumor incidences at all dose levels in both species, cinnamaldehyde was determined to be not carcinogenic in rats and mice (Hooth et al., 2004).

In Ames assays, no mutagenicity was detected when cinnamaldehyde was incubated with *Salmonella typhimurium* strains TA98, TA102, TA104, TA1535, TA1537 and TA1538, both with and without metabolic activation at concentrations up to 10,000 µg/plate (Azizan and Blevins, 1995; Eder et al., 1982a, 1982b, 1991; Kasamaki et al., 1982; Kato et al., 1989; Lijinsky and Andrews, 1980; Lutz et al., 1982; Neudecker et al., 1983; Prival et al., 1982; Sasaki and Endo, 1978; Sekizawa and Shibamoto, 1982; Shoeibi et al., 2009). In strain TA100, some positive or weakly positive results were obtained for cinnamaldehyde (Dillon et al., 1992; Ishidate et al., 1984; NTP, 2004) but the majority of results found no mutagenicity for cinnamaldehyde in this strain. Cinnamaldehyde was determined to be negative for mutagenicity when incubated with *Escherichia coli* WR2 *uvrA* without metabolic activation (Sekizawa and Shibamoto, 1982; Yoo, 1986). Rec assays in

Bacillus subtilis for cinnamaldehyde were positive in three of four studies reviewed (Kuroda et al., 1984; Oda et al., 1978; Sekizawa and Shibamoto, 1982; Yoo, 1986).

Mixed results were also reported when cinnamaldehyde was tested using *in vitro* mammalian cell assays. In the sister chromatid exchange (SCE) assay performed with Chinese hamster ovary (CHO) cells, a low concentration of cinnamaldehyde gave negative results but weakly positive results were reported for cinnamaldehyde at concentrations approaching cytotoxic levels (Galloway et al., 1987; Sasaki et al., 1987). In a forward mutation assay in mouse L1210 lymphoma cells, cinnamaldehyde gave equivocal to positive results, however, insufficient information on methodology, test concentrations and cytotoxic testing provided for this study do not allow for further interpretation of the results (Eder et al., 1993; Moon and Pack, 1983). No evidence of mutagenicity was reported for cinnamaldehyde in a mutation assay in Chinese hamster V79 cells (Fiorio and Bronzetti, 1994). Cinnamaldehyde was reported to induce chromosomal aberrations in Chinese hamster fibroblasts and B241 cells at relatively low concentrations in both the presence and absence of metabolic activation (Ishidate et al., 1984; Kasamaki et al., 1982; Kasamaki and Urasawa, 1985) but not in CHO cell lines, both with and without metabolic activation (Galloway et al., 1987). Weakly positive results were observed in a micronucleus assay with the incubation of cinnamaldehyde with HepG2 cells (Sanyal et al., 1997).

In addition, several *in vivo* genotoxicity assays for cinnamaldehyde have been reported. In an unscheduled DNA synthesis assay, cinnamaldehyde administered to rats and mice at a dose of 1000 mg/kg bw by oral gavage resulted in no evidence of unscheduled DNA synthesis in the liver (Mirsalis et al., 1989). There was no increase in micronuclei in the bone marrow of mice after administration of cinnamaldehyde by oral gavage in two separate experiments at doses 1700 mg/kg bw or 1100 mg/kg bw or in a third experiment in which cinnamaldehyde was administered by intraperitoneal injection at a dose of 500 mg/kg bw (Hayashi et al., 1984, 1988; Mereto et al., 1994). In another study, no increase in the induction of micronuclei in peripheral blood cells was observed in a 3-month dietary study in B6C3F1 mice at levels up to 4950 mg/kg bw/day (NTP, 2004). An increase in the frequency of micronuclei in the liver and the forestomach mucosa was observed following administration of 1700 mg/kg bw cinnamaldehyde to rats by oral gavage and an increase in the frequency of micronuclei in the liver was observed following administration of 1100 mg/kg bw cinnamaldehyde to mice by oral gavage, but these effects were not observed at the lower doses tested, 850 and 550 mg/kg bw in rats and mice, respectively. The increase in micronuclei in the forestomach mucosa of high dose rats was accompanied by karyorrhexis and pyknosis which are indicative of toxicity rather than clastogenicity. In the liver, the induction of micronuclei was only observed at the highest treatment dose and may be indicative of greater exposure of the liver to the cinnamaldehyde treatment compared to the bone marrow, which was negative for micronuclei induction at all levels tested in mice and rats (Adams et al., 2004; Mereto et al., 1994). This observation is also consistent with the depletion of hepatocellular glutathione levels reported with the oral administration or intraperitoneal injection of greater or equal to 500 mg cinnamaldehyde/kg bw in rats (Swales and Caldwell, 1993).

In summary, cinnamaldehyde was non-mutagenic in the Ames and *E. coli* bacterial assays but some positive results were reported the *B. subtilis* rec assay. However, the rec assay has not been standardized in an OECD guideline for genotoxicity testing, and 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). In mammalian assays (SCE, CA, comet and mouse lymphoma assays), mixed results were obtained for cinnamaldehyde but the reported *in vitro* activity did not translate into mutagenic, clastogenic or genotoxic activity *in vivo,* and cinnamaldehyde did not show carcinogenic activity in rats or mice.

7.1.2. *o-Methoxycinnamaldehyde*

In Ames assays, *o*-methoxycinnamaldehyde did not induce mutagenicity in *S. typhimurium* strains TA98, TA1535 and TA1537 and *E. coli* WR2 *uvrA* (Mortelmans et al., 1986; Thompson, 2013) in the presence or absence of metabolic activation. Positive results in TA100 at concentrations of 100 μg/plate and 333 μg/plate in the presence of metabolic systems of Aroclor-treated rat liver and hamster liver, respectively, reported by Mortelmans et al. (1986) were not replicated in a later OECD compliant study performed at concentrations up to 150 μg/plate without metabolic activation and 500 μg/plate with metabolic activation derived from rat livers induced with a phenobarbitone/β-naphthoflavone mixture. In an *in vivo* study in CD-1 mice, induction of micronuclei in the bone marrow was not observed following the administration of single oral doses of 0, 250, 500 or 1000 mg/kg bw *o*-methoxycinnamaldehyde by oral gavage (Flanders, 2012; Wall et al., 2015).

7.1.3. *Cinnamyl acetate*

Cinnamyl acetate did not induce mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA102, TA1535 or TA 1537 at concentrations up to 2000 μg/plate in both the presence and absence of metabolic activation (King, 2003; Veskeep-Rip, 2003). Cinnamic acetate was found not to induce sister chromatid exchange in CHO cells (Sasaki et al., 1989) and did not induce micronuclei formation in human peripheral lymphocytes in either the presence or absence of metabolic activation at concentrations up to 900 μg/mL (Bhalli, 2015).

7.1.4. *Cinnamic acid*

Cinnamic acid was not mutagenic when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 at concentrations up to 1000 μg/plate using both the plate incorporation and pre-incubation methods with and without S9 metabolic activation system derived from rat or hamster liver microsomal fractions (Lijinsky and Andrews, 1980). Negative results were also reported for cinnamic acid in the *B. subtilis* rec assay (Oda et al., 1978; Yoo, 1986) and in an *in vitro* comet assay in rat hepatoma cells at concentrations up to 1500 μM (Maistro et al., 2011). In an OECD compliant *in vitro* micronucleus study with human peripheral blood lymphocytes (HPBL), treatment with cinnamic acid showed no induction of micronuclei either with or without Aroclor 1254-induced rat liver S9 metabolic activating system (Roy, 2015). In an *in vitro* micronucleus assay in rat hepatoma cells, cinnamic acid induced micronuclei at all conditions tested (50–1500 μM) (Maistro et al., 2011). However, deviations within the study from the OECD guideline (OECD, 2014), including the use of a non-validated cell line, failure to use recommended methods for the determination of cytotoxicity and failure to use recommended exposure times for test substances raise questions regarding the relevance of this study to the safety assessment.

7.1.4.1. Summary on genotoxicity. Cinnamaldehyde, o-methoxycinnamaldehyde, cinnamyl acetate and cinnamic acid show a lack of mutagenic activity in the Ames and *E. coli* bacterial assays. Genotoxic activity was not observed for *in vitro* mammalian assays on cinnamyl acetate and in the OECD-compliant study on cinnamic acid. In addition, *in vivo* studies for cinnamaldehyde and *o*-methoxycinnamaldehyde also demonstrated a lack of genotoxicity up to the limit of cytotoxicity. In conclusion, there is no concern for the genotoxic potential of these compounds.

7.2. *Coumarin*

Several *in vitro* assays on the mutagenicity and genotoxicity of coumarin have been reported, with both positive and negative results. Coumarin did not induce mutagenicity when incubated with *S. typhimurium* strains TA98, TA1535, TA1537 or TA1538 at concentrations up to 3333 μg/plate in both the presence and absence of metabolic

activation systems from hamster livers and livers from Aroclor 1254-treated rats. A weakly positive result was reported in strain TA100 but only in the presence of the hamster S9 metabolic system (Haworth et al., 1983; NTP, 1993). Both positive and negative results have been reported in SCE and chromosomal aberration assays in CHO cells (Galloway et al., 1987; NTP, 1993). Coumarin was negative for the induction of micronucleus formation in rat hepatocytes (Muller-Tegethoff et al., 1995) but positive in human hepatoma HepG2 cells and human lymphocytes (Kevekordes et al., 2001; Sanyal et al., 1997). However, the positive results do not correlate with the lack of coumarin-induced genotoxicity observed *in vivo*. No evidence of genotoxicity has been found in *in vivo* assays including an unscheduled DNA synthesis assay (Edwards et al., 2000) and negative micronucleus assays in Swiss, IRC and B6C3F$_1$ mouse strains (Api, 2001; Morris and Ward, 1992; NTP, 1993). In addition, coumarin did not form detectable DNA adducts in the liver or kidneys of SD or F344 rats (Swenberg, 2003). Based on the available data, the weight of evidence indicates that coumarin is not a genotoxicant (EFSA, 2004; Lake, 1999).

Since the ban on the addition of coumarin to food in the United States in 1954, numerous additional studies have been conducted to examine its potential toxicity in various species, including rats, mice, hamsters, dogs, and baboons. These studies have been extensively summarized and reviewed (EFSA, 2004; IARC, 1976; Lake, 1999).

In rats, the onset and type of liver toxicity induced by coumarin were found to be dependent on the magnitude of the dose and the duration of the treatment (EFSA, 2004; IARC, 1976; Lake, 1999). A threshold for coumarin was demonstrated, below which toxicity and carcinogenicity were not observed (Carlton et al., 1996; Griepentrog, 1973; Hagan et al., 1967). When coumarin was administered orally at hepatotoxic doses to rats, centrilobular hepatocellular necrosis was observed after approximately 4 weeks. With longer treatment, these lesions regressed and bile duct hyperplasia developed in the periportal area of the liver lobule (Lake, 1999, and references within). A later study indicated that a combination of changes in coumarin pharmacokinetics and subcellular distribution of a key P450 enzyme contribute to resistance to coumarin-induced hepatic necrosis (Tanaka et al., 2016, 2017).

The hepatotoxic effects observed in rats, however, did not occur in CD-1 mice fed 0.05–0.5% coumarin in the diet for up to 13 weeks, in DBA/2J and C3H/HeJ mice fed 0.5–1.0% coumarin in the diet for 32 weeks or in CD-1 mice fed 0.03–0.3% coumarin in the diets for 52 weeks (Carlton et al., 1996; Lake and Grasso, 1996; Seidel and Kreuser, 1979). Hepatotoxicity was also not observed in a 2-year study in which Syrian hamsters were fed up to 0.5% coumarin in the diet (Ueno and Hirono, 1981). However, in a 2-year gavage study in B6C3F1 mice conducted by the National Toxicology Program (NTP) at doses of 0, 50, 100 and 200 mg/kg bw/day, coumarin induced a statistically significant increase in liver tumors in female mice in the 50 and 100 mg/kg bw/day dose groups that was not observed in the high dose group (200 mg/kg bw/day) and lung tumors in both sexes in the 200 mg/kg bw/day dose groups (NTP, 1993). The reason for the lack of a liver tumorigenic effect at the high dose could not be determined.

Studies in limited numbers of baboons found no evidence of liver damage histologically and only slight evidence of reversible biochemical, histochemical or ultrastructural changes after oral dosing at 50 and 100 mg/kg bw/day for 3 weeks or dietary administration of doses up to 67.5 mg/kg bw/day for 2 years (Cohen, 1979). In addition, only limited effects on liver function have been reported when coumarin was evaluated for the treatment of various clinical conditions in humans at doses several thousand times greater than the calculated dietary intake (Lake, 1999).

The metabolism of coumarin in various species has been further examined to understand the differences in toxicity observed between species and to determine the relevance of the effects observed in experimental animals to humans (Lake, 1999). In humans, coumarin is metabolized by CYP2A6 that catalyzes the formation of 7-hydroxycoumarin followed by conjugation with glucuronic acid or sulfation and excretion in the urine (see Fig. 5) (Rietjens et al., 2008). In mice and rats, coumarin is not significantly converted to 7-hydroxycoumarin, instead it is primarily oxidized to 3,4-coumarin epoxide which rearranges to form *o*-hydroxyphenyl-acetaldehyde (*o*-HPA). A similar low conversion rate of coumarin to 7-hydroxycoumarin has also been noted in dogs (Lake, 1999). *o*-HPA can undergo further oxidation to its corresponding carboxylic acid, *o*-hydroxyphenylacetic acid (*o*-HPAA) or reduction to *o*-hydroxyphenylethanol (*o*-HPE). Significant differences in the metabolism of *o*-HPA to *o*-HPAA and *o*-HPE in mouse, rat and human liver microsomes were observed leading the authors to conclude that species differences in the detoxication of *o*-HPA are a determining factor in coumarin toxicity (Vassallo et al., 2004). The formation of 3,4-coumarin epoxide in the club cells (formally referred to as Clara cells)

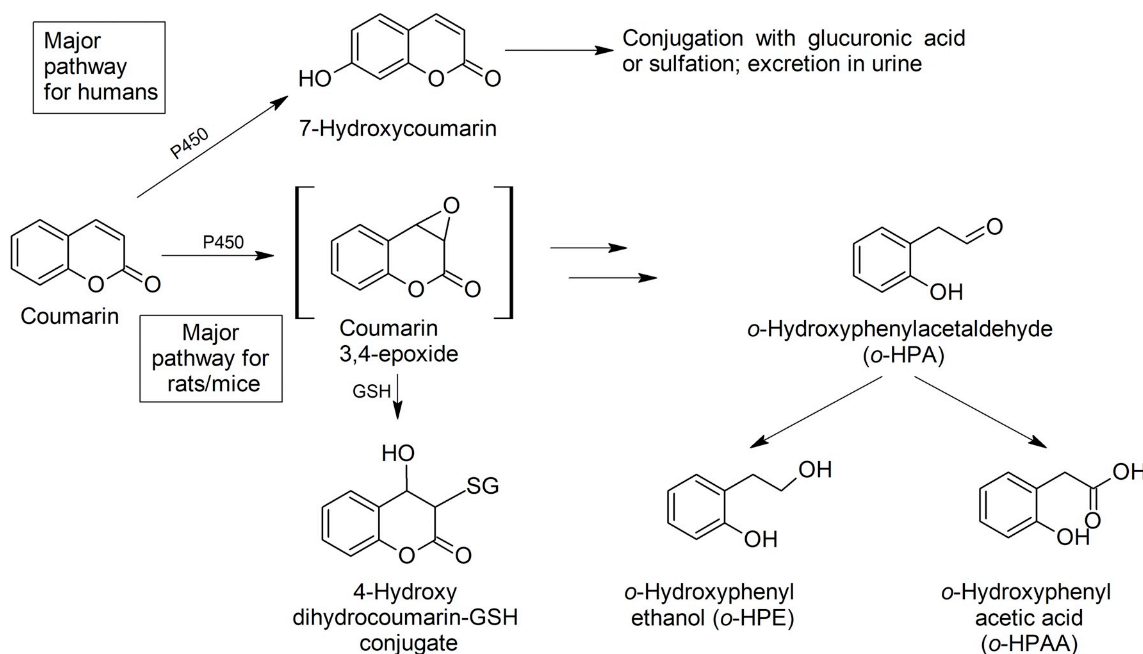

Fig. 5. Major metabolic pathways of coumarin in humans and rodents (Adapted from Lake, 1999 and Vassallo et al., 2004.).

Table 8
NFCs affirmed FEMA GRAS.

FEMA No.	Name
2117	Balsam Oil, Peru (*Myroxylon pereirae* Klotzsch)
3069	Tolu Balsam Extract (*Myroxylon balsamum* L. Harms (*M. toluiferum* HBK))
2257	Cassia Bark Extract (*Cinnamomum cassia* Blume)
2258	Cassia Oil (*Cinnamomum cassia* Blume)
2290	Cinnamon Bark Extract (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)
2291	Cinnamon Bark Oil (*Cinnamomum zeylanicum* Nees, *C. loureirii* Blume, *C. cassia* Blume)

of mice that are abundant in the terminal bronchiolar region of the lung is thought to be a major determinant for mouse susceptibility to lung toxicity and carcinogenicity of coumarin observed in B6C3F1 mice (Born et al., 2003; EFSA, 2004; NTP, 1993). For human subjects completely deficient in coumarin 7-hydroxylation, a physiologically based biokinetic (PBBK) model of rat and human liver microsomal reactions estimated that the formation of *o*-HPA was significantly lower in these humans than in the rat, indicating that liver toxicity due to *o*-HPA accumulation observed in the rat was not likely to occur in 7-hydroxylation deficient humans (Rietjens et al., 2008). In summary, studies on the metabolism of coumarin have revealed significant differences among the species tested and show that coumarin metabolism in the rat is not a good model for human metabolism, while also explaining why humans are less sensitive than the rat.

In summary, studies on coumarin indicate that it is a non-genotoxic carcinogen allowing for the determination of a mechanism of toxicity and a threshold below which toxicity would not be a concern. For the NFCs under consideration, small percentages of coumarin were reported in Balsam oil, Peru (FEMA 2117), Cassia Bark Extract (FEMA 2257), Cassia Bark Oil (FEMA 2258), Cinnamon Bark Extract (FEMA 2290), and Cinnamon Bark Oil (FEMA 2291) corresponding to estimated intakes ranging between 2×10^{-6} and 5 μg/person/day. These estimated intakes are significantly lower than the TDI of 0.1 mg/kg bw determined by both EFSA (EFSA, 2004, 2008) and the BfR (Abraham et al., 2010) for coumarin and therefore do not present a safety concern.

7.3. Cassia and Cinnamon essential oils and extracts

A water or methanol extract of *C. cassia* was tested in both Ames and rec assays. The extracts were prepared by the addition of 50 g of crushed material to 300 mL of water or methanol. Each mixture was heated at 40 °C for 5 h, then filtered and dried. The final yield was not reported. The dried extract was re-suspended in water for testing. The *C. cassia* extracts were negative in *S. typhimurium* strains TA98 and TA100 both with and without metabolic activation at concentrations up to 10 mg/mL of the prepared extracts. The *C. cassia* extracts also tested negative in *Bacillus subtilis* in a rec assay at concentrations up to 66 mg/mL of the prepared extracts (Morimoto et al., 1982).

C. cassia essential oil, prepared by steam distillation, was not mutagenic at concentrations up to 250 μg/plate in *S. typhimurium* strain TA100 with and without S9 metabolic activation prepared from the liver of Aroclor 1254-treated rats (Park, 2002). Cinnamon oil (botanical species not specified) showed a slight increase in the number of revertant colonies when tested at 5.1 ng/plate in TA98 without metabolic activation, but this increase was not observed at the higher concentration tested, 10.2 ng/plate. Cinnamon oil, in the absence of metabolic activation, also showed a slight (less than 2-fold) increase in revertant colonies at 10.2 ng/plate in strains TA1537 and TA1538 and no mutagenicity in strain TA1535. (Sivaswamy et al., 1991). Because the Sivaswamy et al., studies did not determine the chemical composition of the tested cinnamon oil, did not demonstrate a dose response, 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 this NFC.

Cassia oil and cinnamon oil tested positive in *Bacillus subtilis* in a rec assay at concentrations of 100 μg/disk. The constituent profile of the cassia oil tested was 92.3% cinnamaldehyde, 1.8% cinnamyl acetate and 0.3% benzaldehyde. The constituent profile of the cinnamon oil tested was 78.3% cinnamaldehyde, 4.5% cinnamyl acetate, 3.1% eugenol, 2.9% linalool and 2.1% benzaldehyde. In this study, rec^+ cells also showed a significantly higher growth rate compared to rec^- cells. Since the difference in cell counts between these 2 strains is the basis of determination of genetic toxicity (DNA repair), this difference in growth combined with the incomplete diffusion of the test compound into the agar may have contributed to a questionable result for both cassia oil and cinnamon oil (Sekizawa and Shibamoto, 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 considered with the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015).

An extract of Ceylon cinnamon (*Cinnamomum zeylanicum*) was prepared from the ground spice by serial Soxhlet extractions with petroleum ether, chloroform and ethanol. Following the Soxhlet procedure, the extract was concentrated, stripping the solvent, and the spice material was dried prior to subsequent Soxhlet extractions. The three extracts were evaluated using several variations of the rec assay, using *B. subtilis* strains H17 and M45 in standard streak, spore and "cold" methods, with mixed results. Because chemical analyses were not performed on the extract and their preparation differs considerably from the NFCs under consideration, this study is not considered relevant to this evaluation (Ungsurungsie et al., 1984). In a spore rec assay in *B. subtilis* M45 and H17, cinnamon oil tested positive in the absence of S9 metabolic activation and equivocal in the presence of S9 metabolic activation (Ueno et al., 1984). An equivocal result was obtained in the incidence of polyploid cells (6.0 vs. 3.0%) in a chromosomal aberration assay in which cinnamon bark oil was incubated with Chinese hamster fibroblast cells. (Ishidate et al., 1984).

Genotoxicity assays on *Cinnamomum* derived extracts and essential oils described above yielded mixed results. However, because many of the assays described were performed under non-standard conditions or were performed on undefined botanical samples or extracts not representative of the NFCs under consideration, they are not considered relevant to this evaluation. A lack of genotoxic potential is expected for the *Cinnamomum*-derived NFCs whose constituent profile is dominated by Group 16 compounds such as cinnamaldehyde, *o*-methoxycinnamaldehyde, cinnamic acetate and cinnamic acid which have been determined to have no genotoxic concern.

7.4. Balsam oil, Peru and Tolu Balsam

Balsam oil Peru and tolu balsam extract did not induce mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA102, TA1535 or TA 1537 at concentrations up to 5000 μg/plate (balsam oil peru) or 10,000 μg/plate (tolu balsam extract) in both the presence and absence of S9 metabolic activation prepared from the livers of Aroclor 1254-treated male rats (DeGraff, 1983; Jagannath, 1984). Based on these results and the lack of genotoxic concerns for the constituent profile of these materials, there is no genotoxic concern for *Myroxylon*-derived NFCs.

8. Recognition of GRAS status

Upon review of the relevant scientific data on the safety evaluation of the above listed NFCs used as flavoring ingredients, it was concluded that these NFCs do not present safety concerns under intended conditions of use as flavorings. The safety of these *Cinnamomum* and

Myroxylon derived NFCs is further supported by their self-limiting properties as flavoring ingredients in food resulting in use levels that do not saturate pathways of metabolism and excretion. It has been demonstrated that the constituents are rapidly absorbed, distributed, metabolized and excreted. The estimated intakes of the constituent congeneric groups for each NFC were below the TTC, giving adequate margins of safety. Additionally, based on the weight of evidence it is concluded that genotoxic potential is absent.

The *Cinnamomum* and *Myroxylon*-derived NFCs discussed here were determined to be generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients by the Flavor and Extract Manufacturers Association (FEMA) in 1965. Based on the safety evaluation described in this manuscript, the FEMA Expert Panel has affirmed the GRAS status for the materials listed in Table 8.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Drs. Rietjens, Cohen, Eisenbrand, Fukushima, Gooderham, Guengerich, Hecht, 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.

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Transparency document

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Appendix A. Supplementary data

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