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FEMA GRAS assessment of natural flavor complexes: Lavender, Guaiac Coriander-derived and related flavoring ingredients

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A B S T R A C T

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, fifth in the series, evaluates the safety of NFCs containing linalool and/or other characteristic mono- and sesquiterpenoid tertiary alcohols and esters using the safety evaluation procedure published by the FEMA Expert Panel in 2005 and updated in 2018. The procedure relies on a complete chemical characterization of the NFC intended for commerce and organization of the chemical constituents of each NFC into well-defined congeneric groups. The safety of each 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 both the constituent congeneric groups and the NFCs. Sixteen NFCs, derived from the *Lavandula, Aniba, Elettaria, Daucus, Salvia, Coriandrum, Ribes, Guaiacum/Bulnesia, Citrus, Pogostemon, Melaleuca* and *Michelia* genera, were affirmed as generally recognized as safe (GRAS) under their conditions of intended use as flavor ingredients based on an evaluation of each NFC and the constituents and congeneric groups therein.

1. Introduction

The Expert Panel of the Flavor and Extract Manufacturers Association (FEMA), formed in 1960, 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 (Hallagan and Hall, 1995, 2009; Hallagan et al., 2020). Flavoring ingredients can be pure chemically defined compounds or complex mixtures, known as natural flavor complexes (NFCs). To date, the FEMA Expert Panel has determined that over 2,700 flavoring ingredients have met the GRAS criteria for their intended uses.

The FEMA Expert Panel published its first list of GRAS flavoring ingredients that included both chemically defined and NFC flavoring ingredients in 1965 (Hall and Oser, 1965). A key part of the FEMA GRAS program is the re-evaluation of GRAS flavoring ingredients. The FEMA Expert Panel has completed two re-evaluations of FEMA GRAS chemically defined flavor ingredients and in 2015 expanded the re-evaluation program to encompass FEMA GRAS NFCs. For the safety evaluation of NFCs, the FEMA Expert Panel developed a scientifically-based procedure based on the chemical composition of the NFC (Smith et al., 2005). This procedure was reviewed and updated in 2018 (Cohen 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 the safety evaluation of each NFC, information is gathered

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Abbreviations

BfR	German Bundesinstitut für Risikobewertung
CA	Chromosomal Aberration
CF	Correction Factor
CFR	Code of Federal Regulations
CG	Congeneric Group
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
FAO	Food and Agriculture Organization of the United Nations
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
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
LC-MS	Liquid Chromatography-Mass Spectrometry
LOAEL	Lowest-Observed-Adverse-Effect-Level
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
PCI	Per Capita Intake
TD50	Dose giving a 50% tumor incidence
TDI	Tolerable Daily Intake
TTC	Threshold of Toxicological Concern
UDS	Unscheduled DNA Synthesis
US-EPA	United States Environmental Protection Agency
WHO	World Health Organization

on the estimated intake, metabolism and toxicology for each constituent congeneric group. The Threshold of Toxicological Concern (TTC) approach is applied to evaluate the estimated intake of each constituent congeneric group (Kroes et al., 2000; Munro et al., 1996). In addition, the potential toxicity of the unidentified constituent fraction is also evaluated in the updated procedure.

Beginning in 2015, the FEMA Expert Panel has issued a series of calls for data requesting detailed chemical analyses for over 250 NFCs with FEMA GRAS status. Members from the International Organization of the Flavor Industry (IOFI), including FEMA, the Japan Fragrance and Flavor Materials Association (JFFMA) and the European Flavour Association (EFFA), in addition to the International Federation of Essential Oils and Aroma Trades (IFEAT) have provided information in response to these data requests. NFC flavoring ingredients are often derived from botanical plants that are also sources of familiar foods and spices. Due to the large number of NFCs to be evaluated, the NFCs were parsed into groups based on their constituent congeneric group profile. The congeneric groups used for NFC analysis by the FEMA Expert Panel are provided in an appendix to the safety evaluation procedure (Cohen et al., 2018a) The first group of NFCs reviewed by the FEMA Expert Panel were derived from the *Citrus* genus and included orange, lemon, lime and grapefruit-derived NFCs (Cohen et al., 2019). The second group of NFCs evaluated were several mint, dill, caraway and buchu-derived NFCs for which Group 10 (Alicyclic ketones, secondary alcohols and related esters) constituents were a major fraction of their composition profile (Cohen et al., 2020). The Panel's third publication outlined the safety evaluation of cinnamon and cassia-derived NFCs whose composition profiles contained Group 16 (Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters) constituents (Rietjens et al., 2020). In its 4th publication in the series, the safety of eugenol-rich clove, cinnamon leaf and West Indian bay leaf-derived NFCs was evaluated (Gooderham et al., 2020). This publication, the fifth in the series, continues the re-evaluations by the FEMA Expert Panel on a set of NFCs which are characterized by the presence of Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) constituents such as linalool, α-terpineol and patchouli alcohol. These NFCs, listed in Table 1, include essential oils and extracts derived from botanicals of the *Lavandula, Aniba, Elettaria, Daucus, Salvia, Coriandrum, Ribes, Guaiacum/Bulnesia, Citrus, Pogostemon, Melaleuca* and *Michelia* genera that are used as flavoring ingredients.

2. History of food use

Lavender, spike lavender, lavandin and related perennial flowering plants in the *Lavandula* genus are herbs of the *Lamiaceae* family native to the Mediterranean region. Southern France is one of the largest past and present producers of lavender oil (Guenther, 1949) and Bulgaria and China are now also major producers (Giray, 2018). Considered hardy herbs, the different species of *Lavandula* are now cultivated around the world, including in the United States, Australia, Russia and several European countries (Guenther, 1949; Lis-Balchin, 2002a). Lavandin, a hybrid between true lavender and spike lavender, has a flowering period between that of true lavender (August) and spike lavender (September) during which it is harvested for use (Guenther, 1949). Lavender gets its name from the Latin *lavare* meaning "to wash" due to its use by ancient Romans to perfume bath water (Castle and Lis-Balchin, 2002). Users of lavender in Victorian England alternatively derived the name from the Latin *livere* meaning bluish (Festing, 1989). Lavender is used as a culinary herb in foods and beverages and is a component in 'Herbes de Provence' blends that are popular for the flavoring of savory foods (Grieve, 1970; Kehler and Schooley, 2006; Laget, 2005). Clary sage, another plant of the *Lamiaceae* family, has historically been used as a culinary herb, a substitute for hops in brewing and as a flavoring for wines, particularly wines originating from the Rhine region of Germany (Grieve, 1970; Guenther, 1949). Another member of the *Lamiaceae* family, the patchouly plant, is the source of an essential oil with distinctive aromatic and flavoring properties. Patchouly is cultivated extensively in Malaysia, Indonesia and other tropical climates and its leaves were used in traditional medicine (Murugan and Livingstone, 2010; Swamy and Sinniah, 2015). Patchouly oil, produced from the steam distillation of the leaves of the plant, is used as both a perfumery and a flavor ingredient (Guenther, 1949; van Beek and Joulain, 2018).

In addition to lavender, flowers from other botanicals are sources for several other NFCs used for flavoring food. The fragrant flowers of the bitter orange tree (*Citrus aurantium* L) can be distilled to collect its essential oil, known as neroli oil, or can be extracted sequentially with a non-polar solvent and ethanol to yield an absolute, both of which are used as flavor ingredients. In addition, orange blossom water from the distillation of neroli oil is commonly used as an ingredient in Moroccan cuisine. The fruit of this *Citrus* species is acidic in character and less palatable than sweet oranges, but the fruit peels are used in the preparation of marmalades and the essential oil from the peel is used to flavor

Table 1
NFCs evaluated by the Expert Panel.

Name	FEMA No.	Estimated Intake (μg/person/day)[a]	Most recent surveyed annual volume (kg)[b]
Bois De Rose Oil (*Aniba rosaeodore* Ducke)	2156	6	63
Cardamom Seed Oil (*Elettaria cardamomum* (L.) Maton)	2241	340	3,310
Carrot Oil (*Daucus carota* L.)	2244	24	240
Clary Oil (*Salvia sclarea* L.)	2321	11	110
Coriander Seed Oil (*Coriandrum sativum* L.)	2334	1,190	11,500
Currant Buds Black Absolute (*Ribes nigrum* L.)	2346	12	120
Guaiac Wood Extract (*Guaiacum officinale* L.; *G. sanctum* L.; *Bulnesia sarmienti* Lorentz)	2533	1	11[c]
Guaiac Wood Oil (Guaiacum officinale L.; G. sanctum L.; *Bulnesia sarmienti* Lorentz)	2534	150	1440
Lavandin Oil (Hybrids between *Lavandula offincinalis* Chaix *and L. latifolia* Viil.)	2618	1,010	9,780
Lavender Absolute (*Lavandula officinalis* Chaix)	2620	0.01	0.1
Lavender Oil (*Lavandula officinalis* Chaix)	2622	810	7,840
Orange Blossoms Absolute (*Citrus aurantium* L.)	2818	1	11
Patchouly Oil (*Pogostemon cablin* Benth. and *P. heyneanus* Benth.)	2838	220	2,120
Spike Lavender Oil (*Lavandula latifolia* Vill. (L. spica DC.))	3033	4	43[d]
Tea Tree Oil (*Melaleuca alternifolia*)	3902	330	32,300
Michelia Alba Oil (*Michelia alba* D.C.)	3950	2	18

[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., Murray, I.J., 2018.2015 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association, Washington, DC, USA.

[c] Gavin, C.L., Williams, M.C. and Hallagan, J.B., 2008.2005 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association of the United States (FEMA), Washington, DC, USA.

[d] Source: Harman, C.L., Lipman, M.D. and Hallagan, J.B. 2013. Flavor and Extract Manufacturers Association of the United States (FEMA) 2010 Poundage and Technical Effects Survey, Washington DC, USA.

food (Boelens and Oporto, 1991). Another example of the use of flowers to flavor food are the flowers of the *Michelia alba* tree. These flowers are distilled to produce an essential oil that is commonly used to flavor teas (Cohen et al., 2019; Ueyama et al., 1992). Blackcurrant is grown in the colder regions of Europe and its fruits are harvested for use in liqueurs such as crème de cassis, the preparation of juice concentrates and purees (Duponcel, 2007). The flower buds of the blackcurrant are extracted to produce an absolute that imparts a blackcurrant-like flavor in the flavoring of foods and alcoholic and non-alcoholic beverages (Fenaroli, 1975; Wytenhove, 1984).

Carrots are a well-recognized root vegetable of the *Apiaceae* family. Like other members of this family, such as coriander, dill and fennel, the carrot plant forms umbels which are groups of flowers that produce seeds. Historically, wild carrot varieties were found in Europe, Asia and Africa and carrot seeds were used as a traditional medicine by cultures that lived around the Mediterranean basin and carrot seed oil has a history of use in the preparation of alcoholic liquors in France (Guenther, 1950). Cultivation of carrot for harvesting of its roots began approximately five thousand years ago in the Iranian Plateau and in the Persian Empire (Stolarczyk and Janick, 2011) and continues to the present time.

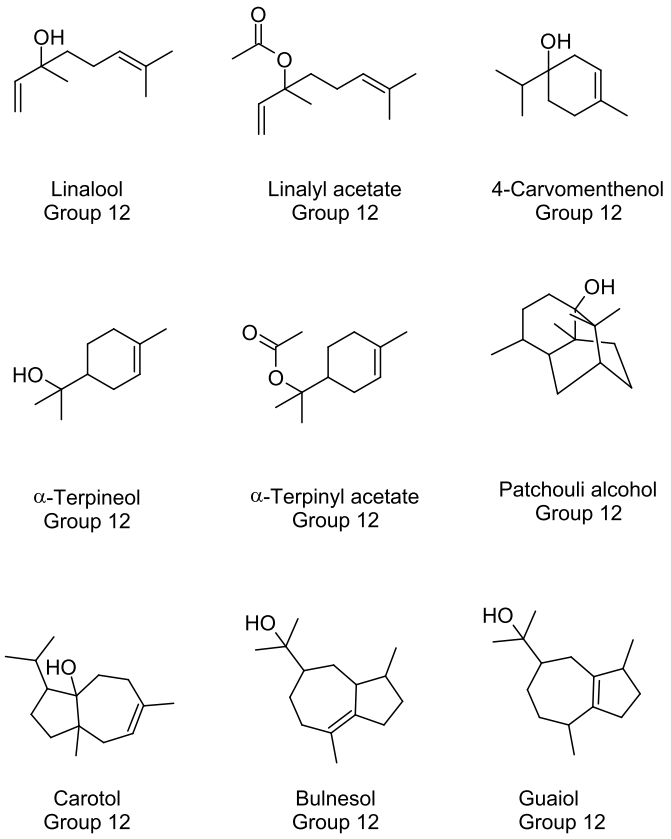


Fig. 1. Structures of commonly found Group 12 (Aliphatic and aromatic tertiary alcohols) constituents in the NFCs.

Another member of the *Apiaceae* family, coriander, also has a long history of food use, dating back to ancient India and Greece (Nadeem et al., 2013). In the Middle Ages, coriander was used as flavoring for meats and soups (Guenther, 1950). It is used in alcoholic beverages and is particularly important to the production of gin. While mainly cultivated in the eastern hemisphere, the herb was first introduced to North America by British colonists, and it later spread to South America (Guenther, 1950). In the United States, "coriander' typically refers to the seed of the plant that is used as a spice while the green leaves are commonly known as cilantro, while in Europe, the seed of the plant is known as coriander seed and the herby leaves are commonly known as coriander leaves.

A native of Asia, cardamom, a member of the *Zingiberaceae* or ginger family, was introduced to western civilizations via the spice trade. There are several varieties of cardamom that have historically been used as spices, but cardamom oil is derived from the species *Elettaria cardamomum*, or "true cardamom". Cardamom pods are the fruit of the plant and each pod encases brown and black seeds. It is an essential ingredient in many Indian dishes including garam masala and chai tea, and is frequently used in baked goods in Scandinavia (Korikanthimathum et al., 2001).

Several NFCs listed in Table 1 are derived from woody plants. Tea tree oil, produced by the distillation of the terminal branches and leaves of the plant, has historically been used as both flavoring and traditional medicine. In his voyage to Australia in the 18th century, Captain James Cook noted a shrub from which the leaves were used by his crew to brew tea. This species can be cultivated in sub-tropical areas of the world, including in its native Australia, as well as the United States, Zimbabwe, New Zealand, China and India (Colton and Murtagh, 1999; Southwell, 1999). *Bulnesia sarmietoi*, the tree species from which guaiac wood oil is derived, is native to South America, specifically the Chaco region of

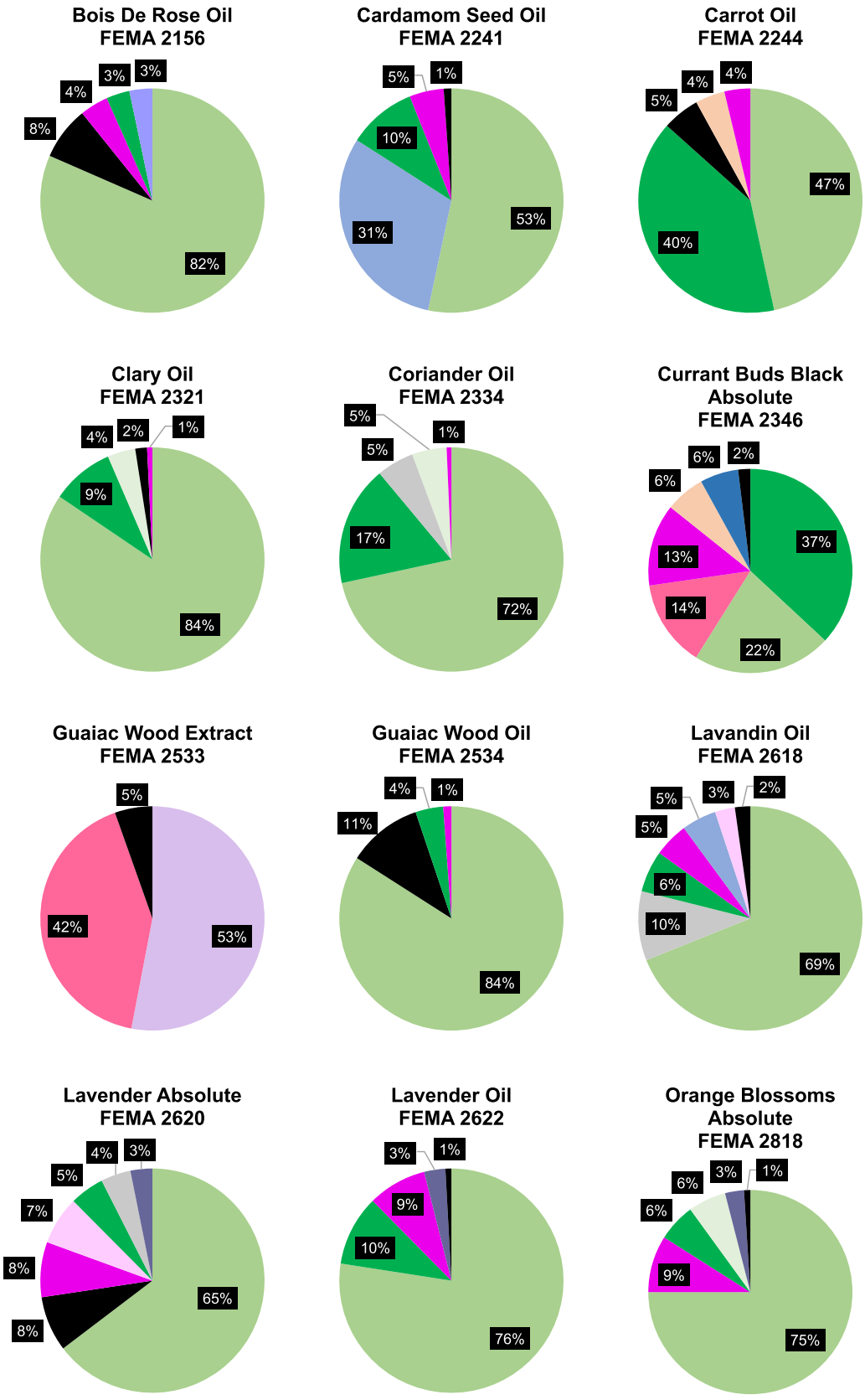


Fig. 2. Constituent congeneric group profiles for the NFCs under consideration.

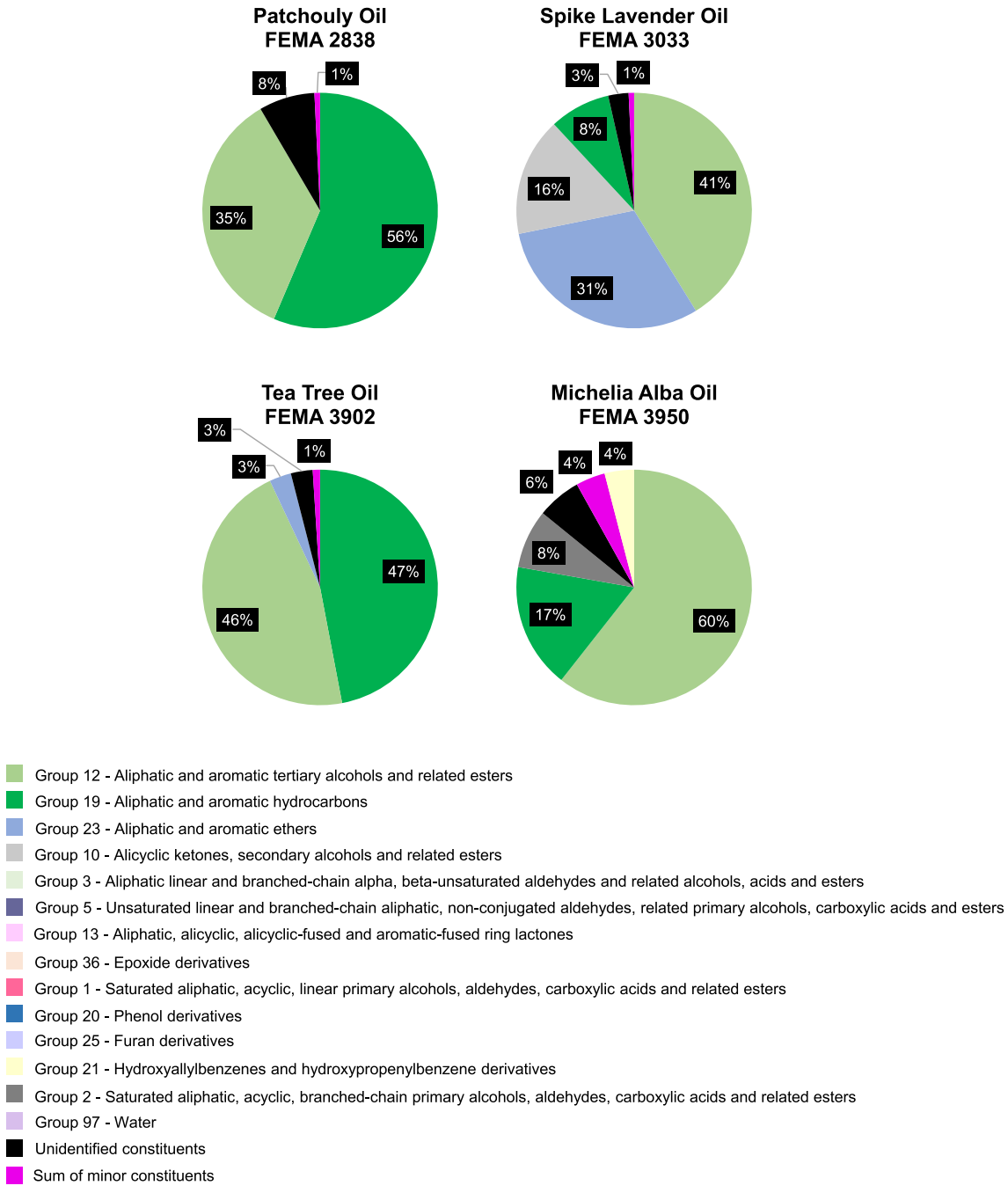


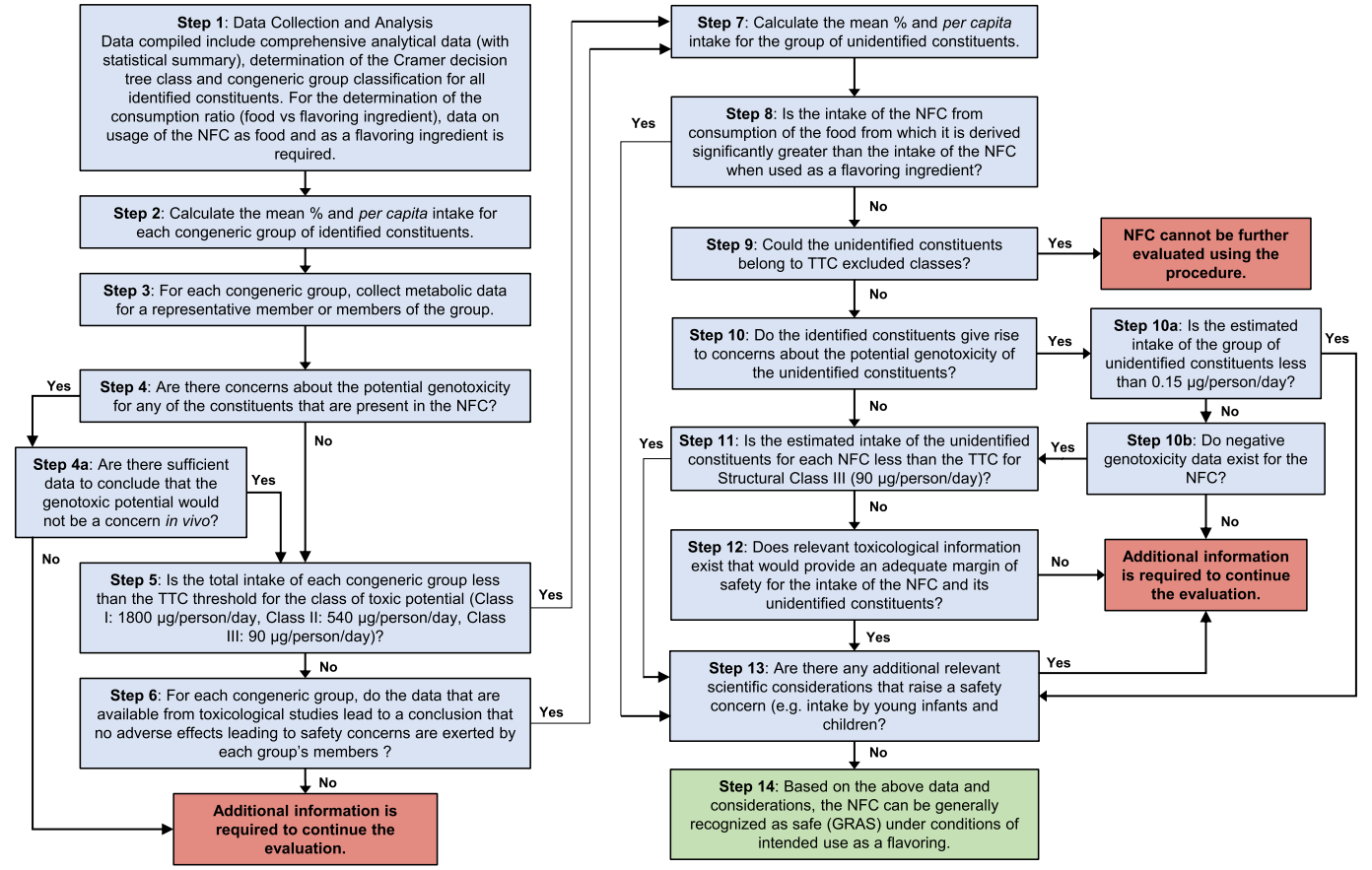
Fig. 2. (*continued*).

Argentina and Paraguay (Rodilla et al., 2011). The wood of the *Bulnesia* tree is very similar to that of the *Guaiacum* species, which is less available due to over-harvesting. The heartwood of rosewood trees, *Aniba rosaeodore,* is the source of bois de rose oil. (FAO, 1995; Fenaroli, 1975). *Aniba rosaeodore* originates in Brazil and is now cultivated in other regions.

3. Current usage

The NFCs listed in Table 1 are used in a variety of foods including beverages (both alcoholic and non-alcoholic), condiments, gravies, confectionary and others. Within this group of NFCs, Tea Tree Oil (FEMA 3902) has the highest annual reported volume of 32,300 kg. In contrast, NFCs of the *Lavandula* genus have more moderate annual usage ranging from 0.1 kg for Lavender Absolute (FEMA 2620) to 9780 kg for Lavandin Oil (FEMA 2618). Patchouly Oil (FEMA 2838), which is known for its distinctive aroma, had a reported use of 2120 kg in 2015. NFCs such as Currant Buds Black Absolute (FEMA 2346), Bois De Rose Oil (FEMA 2156), Carrot Oil (FEMA 2244), Clary Oil (FEMA 2321), *Michelia Alba* Oil (FEMA 3950), Guaiac Wood Extract (FEMA 2533), Guaiac Wood Oil (FEMA 2534) and Orange Blossoms Absolute (FEMA 2818) show low to moderate usage with annual volumes ranging from 11 to 1440 kg.

Both Coriander Seed Oil (FEMA 2334) and Cardamom Seed Oil



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

(FEMA 2241) are derived from botanicals commonly used as spices/food and have annual volumes of 11,500 and 3310 kg, respectively. The Economic Research Service (ERS) of the United States Department of Agriculture (USDA) compiles data on the yearly import of spices and reports that 5,850,000 kg of coriander seed was imported into the USA in 2015 (ERS/USDA, 2019). The essential oil content of coriander seed has been reported to be 0.3% (Nejad Ebrahimi et al., 2010), resulting in an estimated consumption of 17,550 kg of coriander seed oil from coriander seed in the USA. Because import data on cardamom seed are aggregated with other spices, a similar estimation of intake of the essential oil from the whole seed used as a spice cannot be made.

4. Manufacturing methodology

Species of the *Lavandula* genus, including lavender and lavandin, are cultivated from seed and are typically harvested following flowering (Denny, 2002). For the preparation of lavender essential oil, the freshly cut flowering tops, containing the ripe flowers and adjacent stems, are subsequently steam distilled, collecting the essential oil (Denny, 2002; Di Sotto et al., 2011; Lis-Balchin, 2002b). For another *Lavandula* species, spike lavender, the oil can also be prepared from the dried flowering tops of the plants (Boelens, 1986). Steam distillation in the fields limits handling and prevents exposure of the oils to air, which can lead to evaporation and loss of product (Denny, 2002). The essential oil of the fragrant flowers of the *Michelia alba* tree, cultivated in Southeast Asia, is also extracted by steam distillation (Pensuk et al., 2007).

Several other NFCs listed in Table 1 are prepared using distillation technology. Clary plants are harvested at an early maturation period, in which the plants are mechanically cut, and the flower heads, stems and select leaves are immediately chopped and collected in a tub. The contents of the collection tub are steam distilled to obtain the oil (Lawrence, 1994). The dried leaves of patchouly and of tea trees are both steam distillated to yield their respective oils (Southwell, 1999; Surburg and Panten, 2006). While carrot oil can be obtained from aerial parts of the plant after flowering, the flowering umbels are not used commercially for oil production (Jasicka-Misiak et al., 2004; Lawrence, 2003; Tawil et al., 2015). The seeds obtained from the umbels are crushed and steam distilled to extract the oil (Surburg and Panten, 2006). Cardamom seed oil is distilled from the seeds of the plant that are removed from the outer hull of the cardamom pod and crushed shortly before distillation (Menon and Sreekumar, 1994). Similarly, the partially dried fruits (seeds) of coriander are ground just prior to steam distillation or hydrodistillation to yield coriander seed oil (Anitescu et al., 1997; Fenaroli, 1975). Guaiac wood extract and guaiac wood oil, which come from either *Guaiacum* or *Bulnesia sarmientoi* trees, are derived from chipped wood or sawdust through solvent extraction and distillation, respectively (Rodilla et al., 2011). Bois de rose oil is similarly obtained from steam distillation of the chipped heartwood from *Aniba rosaeodora* variations (Farooqi and Sreeramu, 2004; Fenaroli, 1975; Ohashi et al., 1997).

Absolutes prepared from the flowers or buds of *L. officinalis*, *C. aurantium and Ribes nigrum* are also valuable flavoring ingredients. A "concrete" is prepared by the extraction of botanical material with a non-polar solvent such as hexane, toluene or petroleum ether (Surburg and Panten, 2006). Following this extraction, the solvent is removed resulting in a waxy material known as a concrete. Absolutes are

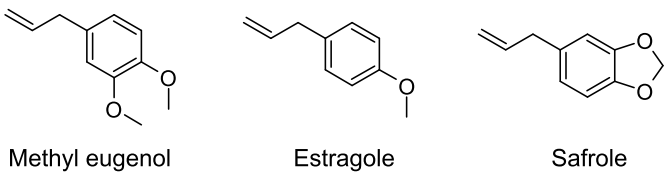


Fig. 4. Structures of methyl eugenol, estragole and safrole.

prepared from the concrete by mixing the concrete with ethanol, heating the solution, followed by a cooling step, filtering of the mixture to remove waxes and finally evaporating off the ethanol (Fenaroli, 1975; Lis-Balchin, 2002b). While botanically derived concretes and absolutes are used more commonly as perfumery ingredients, a few, such as lavender absolute, orange blossoms absolute and black currant buds absolute also have been historically used as flavor ingredients. Absolutes from orange blossoms and black currant buds are prepared from the carefully harvested flowers or dormant leaf buds, respectively, using this process (Boelens and Oporto, 1991; Fenaroli, 1975; Lawrence, 1997; Surburg and Panten, 2006).

5. Chemical composition

The compositions of the NFCs presented in Table 1 were determined by gas chromatography using mass spectrometry (GC-MS) to identify volatile constituents and a flame ionization (FID) or other general detector for quantitation. Identified and unidentified GC peaks were reported as the percent area of the chromatogram. For each NFC, the constituent data were collected and analyzed (Appendix A). In Appendix A, the constituents present at greater or equal to 1% are listed by their respective congeneric groups. The sum of the minor constituents is reported for each congeneric group and minor constituents (less than 1%) reported for other congeneric groups are summed and reported on the last line of the constituent table. The chemical structure of some common constituents of these NFCs are shown in Fig. 1.

The constituent profile for each NFC, summarized in the pie charts shown in Fig. 2, all show a large percentage of Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) and Group 19 (Aliphatic and aromatic hydrocarbons) constituents, except for Guaiac Wood Extract which is an aqueous ethanolic solution. The primary Group 12 constituents for Coriander Seed Oil (FEMA 2334), *Michelia Alba* Oil (FEMA 3950), Clary Oil (FEMA 2321), Orange Blossoms Absolute (FEMA 2818) and the *Lavandula*-derived NFCs were linalool and linalyl acetate. Other Group 12 constituents include carotol in Carrot Oil (FEMA 2244), guaiol and bulnesol in Guaiac Wood Oil (FEMA 2534), patchouli alcohol in Patchouly Oil (FEMA 2838) and 4-carvomenthenol in Tea Tree Oil (FEMA 3902). Other congeneric groups represented in the constituent profiles of the NFCs under consideration include Group 23 (Aliphatic and aromatic ethers), Group 10 (Alicyclic ketones, secondary alcohols and related esters), Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters) and Group 3 (Aliphatic linear and branched-chain α,β-unsaturated aldehydes and related alcohols acids and esters).

6. Safety evaluation

The safety evaluation for NFCs was first described in two publications (Smith et al., 2004, 2005) and has been recently updated (Cohen et al., 2018a). The updated procedure is summarized in Fig. 3. 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 by application of the Threshold of Toxicological Concern (TTC) approach (Kroes et al., 2000)[1] and scientific data on metabolism and toxicity for each congeneric group; Steps 7-12 address the potential toxicity, including genotoxicity of the unidentified constituents; Step 13 evaluates the overall safety 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 of the procedure, as stated in Cohen et al. (2018a) and 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 (*Gavin et al., 2008; Harman et al., 2013, 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*

[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).

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:*

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

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

*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.*

Although several botanicals from which the NFCs in this set are derived have historically been used as spices or ingredients in food, quantitative data on their usage are generally not available, except for coriander seeds. For coriander seeds, the United States Department of Agriculture's Economic Research Service reports that 5,850,000 kg was imported into the USA in 2015 (ERS/USDA, 2019). Coriander seeds have an average essential oil content of 0.3% (Nejad Ebrahimi et al., 2010) resulting in an estimated 17,550 kg of coriander seed oil consumed from the consumption of coriander seed as a spice in the USA in 2015. This annual usage is higher than the 11,500 kg annual usage reported for Coriander Seed Oil (FEMA 2334) used as flavoring, reported in FEMA's 2015 survey (Harman et al., 2018).

Table 2
Natural occurrence and estimated intake of methyl eugenol, estragole or safrole from Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950).

NFC FEMA No.	NFC Description	Constituent of Concern	Mean %	Estimated Intake (μg/person/day)
2533	Guaiac Wood Extract	Estragole	0.001	0.001
2533	Guaiac Wood Extract	Methyl eugenol	0.001	0.001
3902	Tea Tree Oil	Methyl eugenol	0.01	0.03
3950	*Michelia Alba* Oil	Methyl eugenol	3	0.06
3950	*Michelia Alba* Oil	Estragole	0.3	0.006
3950	*Michelia Alba* Oil	Safrole	0.05	0.001

[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.

b. Identification of all known constituents and assignment of Cramer 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*).*

All constituents identified in each NFC were sorted by congeneric group and a summary report for each NFC is provided in Appendix A.

Congeneric groups are recorded in order from highest to lowest mean %, with only mean % greater than or equal to 1% of the total NFC reported. Minor constituent percentages (<1% of the total NFC) are summed for the listed congeneric groups and the total mean % of each congeneric group is shown.

c. Assignment of the constituents to 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.

For each NFC, the DTC for each identified congeneric group was determined and reported in Appendix A.

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.

The summary report for each NFC, provided in Appendix A, provides the subtotal mean % and estimated intake values (PCI × 10 or PCI, where appropriate) for each constituent congeneric group.

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

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

Table 3
Data on Group 12 and 19 constituents for NFCs where the estimated intake of the congeneric group exceeds the relevant TTC.

Name (FEMA No.)	DTC[a]	Estimated Intake of CG (μg/p/day)	Estimated Intake of CG (mg/kg bw/day)	NOAEL (mg/kg bw/day)	MoS[b]
Congeneric Group 12 - Aliphatic and aromatic tertiary alcohols and related esters					
Lavandin Oil (FEMA 2618)	II	690	0.01	50	>4,300
Lavender Oil (FEMA 2622)	III	610	0.01	50	>4,900
Tea Tree Oil (FEMA 3902)	III	120	0.002	50	>25,000
Congeneric Group 19 - Aliphatic and aromatic hydrocarbons					
Patchouly Oil (FEMA 2838)	III	120	0.002	41	>20,000

[a] The DTC for each congeneric group is determined to be the most conservative DTC of the constituents reported in the respective group. Although CG12 is reported for both Lavandin Oil (FEMA 2618) and Tea Tree Oil (FEMA 3902), the reported CG12 constituents and their respective DTCs are different for these two NFCs.

[b] The MoS for Group 12 constituents is based the NOAEL determined from an 84-day study in which linalool was administered to rats of both sexes in the diet at 50 mg/kg bw/day (Oser, 1958). The MoS for Group 19 constituents in Patchouly Oil is based on a NOAEL of 41 mg/kg bw/day for patchouli oil, determined for both sexes, in the repeated dose portion of an OECD-compliant combined 28-day dietary and reproductive/developmental toxicity study in rats (Liwska, 2013b).

metabolism of the members of each congeneric group would require additional considerations in step 13 of the procedure.

Proceed to Step 4.

Appendix A lists the identified constituent congeneric groups for each NFC. A recent FEMA Expert Panel publication outlined the use of metabolic data in the safety evaluation of flavoring substances and provided a summary of the expected metabolism for each congeneric group (Smith et al., 2018). For the congeneric groups present in these NFCs, data exist on the constituents of the group or related compounds to conclude that the members of these respective congeneric groups are expected to be metabolized to innocuous products. Safety assessments, including metabolic data, for flavoring ingredients of several of the congeneric groups represented in the NFCs under consideration have been published by the FEMA Expert Panel, including assessments for Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 19 (Aliphatic and aromatic hydrocarbons) and Group 10 (Alicyclic ketones, secondary alcohols and related esters) flavoring ingredients (Adams et al., 1996, 2011; Marnett et al., 2014). In addition, the Panel has also published evaluations of other groups or individual constituents (Adams et al., 2004; Adams et al., 2005a, b, c; Adams et al., 2002; Adams et al., 1997; Adams et al., 2008; Adams et al., 1998; Adams et al., 2007; Newberne et al., 1999).

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.

With the exception of Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950), the identified constituents of the NFCs do not present a genotoxic concern. In its review of *in vitro* and *in vivo* genotoxicity studies for Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) flavoring ingredients, the primary congeneric group constituent of the NFCs under consideration, the FEMA Expert Panel determined a lack of genotoxic potential for these and related compounds (Marnett et al., 2014). A lack of genotoxic potential was also determined for the other major constituent groups reported in the NFCs under consideration, Group 19 (Aliphatic and aromatic hydrocarbons) and Group 10 (Alicyclic ketones, secondary alcohols and related esters) flavoring ingredients (Adams et al., 1996, 2011). More recent genotoxicity studies on Group 12 constituents and the NFCs are summarized in Table 6 and described later under "Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation" section of this manuscript. A review of the minor constituent profile of Bois de Rose Oil (FEMA 2156), Cardamom Seed Oil (FEMA 2241), Carrot Oil (FEMA 2244), Clary Oil (FEMA 2321), Coriander Seed Oil (FEMA 2334), Currant Buds Black Absolute (FEMA 2346), Guaiac Wood Oil (FEMA 2534), Lavandin Oil (FEMA 2618), Lavender Absolute (FEMA 2620), Lavender Oil (FEMA 2622), Orange Blossoms Absolute (FEMA 2818), Patchouly Oil (FEMA 2838), and Spike Lavender Oil (FEMA 3033) also indicates no genotoxic concern for the congeneric groups presented. These NFCs proceed to Step 5.

Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950), contain Group 21 (Hydroxy- and alkoxy-substituted propenyl benzenes) constituents methyl eugenol, estragole and safrole which have an allylalkoxybenzene structural motif (see Fig. 4), raising a genotoxicity concern (Rietjens et al., 2014a). All three can be found naturally occurring in *Michelia Alba Oil* (FEMA 3950) at concentrations of 3, 0.3 and 0.05%, respectively, estragole and methyl eugenol are reported in Guaiac Wood Extract (FEMA 2533) (0.001%), while only methyl eugenol is found naturally to occur in Tea Tree Oil (FEMA 3902) at a low concentration (0.01%). The natural occurrence and estimated intakes for the constituents of concern in these NFCs are shown in Table 2. Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950) proceed 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.

Table 4
Estimated Intake of unidentified constituents.

Name	FEMA No.	Estimated Intake(μg/person/day)
Bois De Rose Oil	2156	0.5
Cardamom Seed Oil	2241	3
Carrot Oil	2244	1
Clary Oil	2321	0.2
Coriander Seed Oil	2334	0
Currant Buds Black Absolute	2346	0.2
Guaiac Wood Extract	2533	0.05
Guaiac Wood Oil	2534	16
Lavandin Oil	2618	20
Lavender Absolute	2620	0.0008
Lavender Oil	2622	8
Orange Blossoms Absolute	2818	0.07
Patchouly Oil	2838	17
Spike Lavender Oil	3033	0.1
Tea Tree Oil	3902	9
Michelia Alba Oil	3950	0.1

The structures of methyl eugenol, estragole and safrole (see Fig. 4) share a motif of a benzene ring substituted with an alkoxy group located *para* to a 2-propenyl substituent. Cytochrome P450s catalyze the formation of 1′-hydroxy metabolites of these allylalkoxybenzene compounds which may be sulfated by a sulfotransferase. The subsequent elimination of sulfate creates a DNA reactive species (Daimon et al., 1997; Herrmann et al., 2012, 2014; Jeurissen et al., 2004, 2007; Phillips

et al., 1984; Randerath et al., 1984; Rietjens et al., 2005, 2014b; Ueng et al., 2004; Wiseman et al., 1987). Rodent studies have indicated that estragole, 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; 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 safrole, estragole and methyl eugenol as such to food is prohibited in the European Union and limits have been set for the presence of each in finished food categories (European Commission, 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).

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 as well as Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950). 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 has 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).

As presented in Table 2, the estimated intakes of methyl eugenol, estragole and safrole from the consumption of Guaiac Wood Extract (FEMA 2533), Tea Tree Oil (FEMA 3902) and *Michelia Alba* Oil (FEMA 3950) are low, ranging from 0.001 to 0.06 μg/person/day. These values 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 on carcinogens presenting the dose giving a 50% tumor incidence (TD_{50}) (Gold et al., 1984; Kroes et al., 2004). By linear extrapolation of these TD_{50} data to a 1 in 10^6 tumor incidence, an exposure level or TTC at which the lifetime risk of cancer was 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). Because the estimated intake for each of the constituents of concern for Guaiac Wood Extract (FEMA 2533), *Michelia Alb*a Oil (FEMA 3950) and Tea Tree Oil (FEMA 3902) listed in Table 2 is below the 0.15 μg/person/day TTC for compounds with structural alerts for genotoxicity, the constituents do not raise a safety concern and these NFCs proceed to Step 5.

Table 5
Estimated Intake and mean % of coumarin in NFCs.

FEMA No.	NFC	Mean %	Estimated Intake of Coumarin (μg/person/day)
2618	Lavandin Oil	3	30
2620	Lavender Absolute	4	0.0004
2622	Lavender Oil	0.01	0.08

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.

The estimated intakes for all reported congeneric groups present in Bois De Rose Oil (FEMA 2156), Cardamom Seed Oil (FEMA 2241), Carrot Oil (FEMA 2244), Clary Oil (FEMA 2321), Coriander Seed Oil (FEMA 2334), Currant Buds Black Absolute (FEMA 2346), Guaiac Wood Extract (FEMA 2533), Guaiac Wood Oil (FEMA 2534), Lavender Absolute (FEMA 2620), Orange Blossoms Absolute (FEMA 2818), Spike Lavender Oil (FEMA 3033) and *Michelia Alba* Oil (FEMA 3950) are below the TTC for their respective structural classes. These NFCs proceed to Step 7. The remaining NFCs, Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622), Patchouly Oil (FEMA 2838) and Tea Tree Oil (FEMA 3902), each have one congeneric group for which the estimated intake exceeds the relevant TTC (see Table 3), and the evaluation of these NFCs proceeds to Step 6.

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 Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622) and Tea Tree Oil (FEMA 3902), the margin of safety (MoS) is calculated for Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) constituents and shown in Table 3. The MoS for Group 12 constituents is based on the NOAEL derived from an 84-day study in which linalool was administered to rats of both sexes in the diet at 50 mg/kg bw/day (Oser, 1958). With the calculation of adequate MoS values for the NFCs with estimated intakes above the TTC for Congeneric Group 12, these NFCs proceed to Step 7.

The estimated intake of congeneric Group 19 (Aliphatic and aromatic hydrocarbons) exceeds the TTC in Patchouly Oil (FEMA 2838). A

review of toxicological studies was conducted for the GRAS re-affirmation of flavoring materials of this group (Adams et al., 2011) and more recently for the GRAS affirmation of *Citrus*-derived NFCs (Cohen et al., 2019). For Patchouly Oil (FEMA 2838), an adequate MoS was calculated based on a NOAEL of 41 mg/kg bw/day, determined for both sexes, in the repeated dose portion of an OECD-compliant combined 28-day dietary and reproductive/developmental toxicity study for patchouli oil in rats (Liwska, 2013b), as shown in Table 3. With the determination of an adequate MoS, this NFC proceeds to Step 7.

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.

The daily *per capita* intakes for the group of unidentified constituents reported for each NFC are listed below in Table 4 and in Appendix A.

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.

No. For the NFCs under consideration, except for Coriander Seed Oil (FEMA 2334), consumption as food/spice cannot be determined or is unlikely and therefore all the NFCs proceed to Step 9. In the case of Coriander Seed Oil (FEMA 2334), it is estimated that consumption of coriander seed oil from food is less than two times greater than the volume of Coriander Seed Oil (FEMA 2334). Based on this ratio, the consumption of coriander seed oil from food cannot be considered significantly higher than consumption as added flavoring and as a result, Coriander Seed Oil (FEMA 2334) also proceeds 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.

No. As previously discussed, this group of NFCs are collected from various flowers, seeds, leaves and woody plant fibers by either steam distillation of solvent extraction. The oils are primarily composed of low molecular weight monoterpenoid and sesquiterpenoid alcohols, esters and hydrocarbons. Based on the identified constituents, production methods and current literature, it is not expected that the unidentified constituents would belong to TTC-excluded classes. 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.

For the NFCs listed in Table 4, with the exception of Guaiac Wood Extract (FEMA 2533), *Michelia Alba* Oil (FEMA 3950) and Tea Tree Oil (FEMA 3902), the identified constituent profile does not give rise to concern about the potential genotoxicity of the unidentified constituents. These NFCs are primarily composed of linalool, linalyl acetate, 4-carvomenthenol and other Group 12 constituents, Group 19 (Aliphatic and aromatic hydrocarbons) constituents and other terpenoid pathway products that lack genotoxic potential (Adams et al., 2011; Marnett et al., 2014). The unidentified constituents are likely to belong to these groups and to not exhibit genotoxic potential. A review of available genotoxicity studies on the NFCs are presented later in this manuscript. These studies reported no evidence of genotoxic potential for these NFCs. These NFCs proceed to Step 11.

In Step 4, the occurrence of genotoxins estragole, methyl eugenol and safrole were reported in small amounts in *Michelia Alba* Oil (FEMA 3950), estragole and methyl eugenol were reported in small amounts in Guaiac Wood Extract (FEMA 2533), and a small amount of methyl eugenol was reported in Tea Tree Oil (FEMA 3902). The intake for these constituents was estimated to be less than the TTC of 0.15 μg/person/day for compounds with a structural alert for genotoxicity and thus do not raise a safety concern. 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, in addition to a lack of other reports of the occurrence of allylalkoxybenzenes in Guaiac Wood Extract (FEMA 2533), *Michelia Alba* Oil (FEMA 3950) and Tea Tree Oil (FEMA 3902), 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 (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 (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

[5] 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.

[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) and EFSA (2016)).

Table 6
Summary of genotoxicity study results.

Name of Substance Tested	Test Type (System)	Doses Tested	Results	Reference
a. Tertiary Alcohol Constituents				
Linalool	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–1580 μg/plate	Negative[a]	Slonina (2019)
Linalool	Reverse mutation in *S. typhimurium* and *E. coli*[a]	90–170 μg/plate	Negative[a]	Di Sotto et al. (2008)
Linalool	Reverse mutation in *S. typhimurium*[a]	0.23–1.8 mg/plate	Negative[a]	Beric et al. (2008)
Linalool	*In vitro* micronucleus in human lymphocytes	0.5–300 μL/mL	Negative	Di Sotto et al. (2011)
Linalool	*In vitro* chromosomal aberration in Chinese hamster ovary cells[a]	100–400 nL/mL[b] 100–500 nL/mL[c]	Negative[a]	Galloway (1983)
Linalool	Forward mutation in L5178Y mouse lymphoma cells[a]	12.5–500 μL/mL (test 1) 25–399 μL/mL (test 2)	Negative[a]	Cifone (1994)
Linalool	*In vivo* comet assay – forebrain tissue and peripheral blood of mice	10, 50, 100 and 200 mg/kg i.p.	Negative	Coelho et al. (2013)
Linalyl acetate	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.7–9000 μg/plate	Negative[a] (*S. typhimurium*) Positive at conc. greater than 3200 μg/plate[a] (*E. coli*)	Di Sotto et al. (2008)
Linalyl acetate	Reverse mutation in *S. typhimurium*[a]	20–5000 μg/plate (test 1) 20–2000 μg/plate (test 2) 3–50 μg/plate (test 3)	Negative[a]	ECHA (2019a)
Linalyl acetate	*In vitro* micronucleus in human lymphocytes	0.5–300 μL/mL	Positive	Di Sotto et al. (2011)
4-Carvomenthenol	Reverse mutation in *S. typhimurium*[a]	16–5000 μg/plate (test 1, 2)	Negative[a]	Scheerbaum (2001)
4-Carvomenthenol	*In vitro* micronucleus in human lymphocytes[a]	10–90 μg/mL[a,e] 0.5–1540 μg/mL[b,f]	Negative[a]	Roy, 2015
α-Terpineol	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.58–5000 μg/plate (test 1, 2)	Negative[a]	Rao (2019)
α-Terpineol	Reverse mutation in *S. typhimurium*[a]	10–1000 μg/mL	Negative[a]	Seifried et al. (2006)
α-Terpineol	Forward mutation in L5178Y mouse lymphoma cells[a]	0.14–0.65 μg/mL	Negative[a]	Seifried et al. (2006)
Terpineol (isomeric mixture)	Reverse mutation in *S. typhimurium and E. coli*[a]	9.77–1250 μg/plate	Negative[a]	ECHA (2013b)
Terpineol (isomeric mixture)	*In vitro* chromosomal aberration in human lymphocytes[a]	350, 425, 450 μg/mL[c,e] 300–650 μg/mL[b,e] 75, 200, 225 μg/mL[b,f]	Negative[a]	ECHA (2010)
Terpineol (isomeric mixture)	*In vitro* chromosomal aberration in Chinese hamster lung cells[a]	100–400 μg/mL[c,e] 100–500 μg/mL[b,e] 100–400 μg/mL[b,f]	Negative[a]	ECHA (2013a)
Terpinyl acetate (isomeric mixture)	*In vitro* micronucleus in human lymphocytes[a]	103–300 μg/mL[c,e] 49.4–175 μg/mL[b,e] 17.2–83.7 μg/mL[b,f]	Negative[a]	Bhalli (2015)
α-Terpinyl acetate	Reverse mutation in *S. typhimurium* and *E. coli*[a]	21–5000 μg/plate (test 1) 3.13–250 μg/plate (test 1)	Negative[a]	van den Wijngaard (2012)
α-Terpinyl acetate	Reverse mutation in *S. typhimurium* and *E. coli*[a]	5–5000 μg/plate (test 1) 1.6–5000 μg/plate (test 2)	Negative[a]	Bhalli (2014a)
α-Terpinyl acetate	*In vitro* micronucleus in human lymphocytes[a]	96.9–225 μg/mL[a,e] (test 1) 27.9–80 μg/mL[b,f] (test 1) 50–225 μg/mL[c,e] (test 2)	Negative[a]	Bhalli (2014b)
Patchouli alcohol	Reverse mutation in *S. typhimurium*[a] and *E. coli*[c]	1.6–500 μg/plate[a] (*S. typhimurium*) 16–5000 μg/plate[c] (*E. coli*)	Negative[a]	Bhalli (2014c)
Patchouli alcohol	*In vitro* micronucleus in human lymphocytes[a]	113–550 μg/mL[a,e] (test 1) 20.3–150 μg/mL[a,e] (test 2,3) 39.5–192 μg/mL[b,f]	Negative[a]	Bhalli (2014d)
a. Natural Flavor Complexes				
Bois de rose oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–5000 μg/plate (test 1) 5–1600 μg/plate (test 2)	Negative[a]	Mee (2017)
Bois de rose oil	*In vitro* micronucleus in human lymphocytes[a]	466.5–620.9 μg/mL[c,e] 491.1–568.5 μg/mL[b,e] 117.1–263.4 μg/mL[b,f]	Negative[a]	Clare (2017)
Cardamom oil	Reverse mutation in *S. typhimurium*[a]	0.005–2.5 μL/plate	Negative[a]	(DeGraff, 1983b; Heck et al., 1989)
Cardamom oil	Reverse mutation in *S. typhimurium*[a]	0.04–2.34 μL/plate	Negative[a]	Brusick (1982)
Cardamom oil	Forward mutation in L5178Y mouse lymphoma cells[a]	Up to 112 μg/mL[b] Up to 233 μg/mL[c]	Negative[a]	(Cifone, 1982; Heck et al., 1989)
Cardamom oil	Unscheduled DNA synthesis in rat hepatocytes	50.4 mg/mL[d]	Negative	Heck et al. (1989)
Clary oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–5000 μg/plate (test 1) 5–1600 μg/plate (test 2)	Negative[a]	Mee (2016a)
Clary oil	Reverse mutation in *S. typhimurium*[a]	5000 μg/plate[d]	Negative[a]	Heck et al. (1989)
Clary oil	*In vitro* micronucleus in human lymphocytes	263.4–888.9 μg/mL[c,e] 34.68–117.1 μg/mL[b,e] 59.97–117.1 μg/mL[b,f]	Negative[a]	Mee (2016b)
Clary oil	Unscheduled DNA synthesis in rat hepatocytes	101 μg/mL[d]	Negative	Heck et al. (1989)
Clary oil	Rec assay in *B. subtilis*	10, 30 μg/disk	Negative	Zani et al. (1991)

(*continued on next page*)

Table 6 (*continued*)

Name of Substance Tested	Test Type (System)	Doses Tested	Results	Reference
Coriander seed oil	Reverse mutation in *S. typhimurium*[a]	0.01–5 μL/plate	Negative[a]	(DeGraff, 1983a; Heck et al., 1989)
Coriander seed oil	Reverse mutation in *S. typhimurium*[a]	2, 7 mg/plate	Negative[a]	Marcus and Lichtenstein (1982)
Coriander seed oil	Unscheduled DNA synthesis in rat hepatocytes	300 μg/mL[d]	Negative	Heck et al. (1989)
Coriander seed oil	Forward mutation in L5178Y mouse lymphoma cells[a]	10–160 nL/mL[b] 50–300 nL/mL[c]	Negative[a]	(Cifone, 1983; Heck et al., 1989)
Coriander seed oil	*In vitro* chromosomal aberration in Chinese hamster fibroblasts	0.125 mg/mL[d]	Negative	(Ishidate Jr. et al., 1984)
Coriander seed oil	Rec assay in *B. subtilis*	8 mg/disk	Positive[b] Negative[c]	Ueno et al. (1984)
Guaiac wood oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.6–5000 μg/plate (test 1) 5–1600 μg/plate (test 2)	Negative[a]	Mee (2016b)
Guaiac wood oil	*In vitro* micronucleus in human lymphocytes[a]	117.1–395.1 μg/mL[c,e] 84.84–102.7 μg/mL[b,e] 63.74–77.13 μg/mL[b,f]	Negative[a]	Mee (2016c)
Lavender oil	Reverse mutation in *S. typhimurium*	4.4, 8.8 ng/plate	Positive	Sivaswamy et al. (1991)
Lavender oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	1.5–5000 μg/plate (test 1) 5–5000 μg/plate (test 2)	Negative[a]	Dakoulas (2014)
Lavender oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	Up to 2780 μg/plate[a] (*S. typhimurium*) Up to 2500 μg/plate[a] (*E. coli*)	Negative[a]	Evandri et al. (2005)
Lavender oil	Reverse mutation in *S. typhimurium*[a]	87, 177, 443 μg/plate	Negative	De Martino et al. (2009)
Lavender oil	*In vitro* micronucleus in human lymphocytes	0.5–300 μg/mL	Positive[d]	Di Sotto et al. (2011)
Lavender oil	*In vitro* micronucleus in human lymphocytes[a]	50–450 μg/mL[c,e] 10–150 μg/mL[b,e] 10–125 μg/mL[b,f]	Negative[a]	Roy (2015b)
Patchouly oil	Reverse mutation in *S. typhimurium*[a]	0.5–50 μg/plate (test 1, 2)	Negative[a]	Jones (1988)
Patchouly oil	*In vitro* chromosomal aberration in Chinese hamster ovary cells[a]	1.6–50 μg/mL[a] (test 1) 12.5–75 μg/mL[c] (test 2) 7.5–60 μg/mL[c] (test 3)	Negative[a]	Brooker (1989)
Patchouly oil	*In vitro* chromosomal aberration in Chinese hamster ovary cells[a]	6–60 μg/mL[b] 50–90 μg/mL[c]	Negative[a]	Song (2009)
Patchouly oil	Forward mutation in L5178Y mouse lymphoma cells[a]	20–275 μg/mL[c,e] 0.5–50 μg/mL[b,e] 6–36 μg/mL[b,f]	Negative[a]	Kirby (2009)
Tea tree oil	Reverse mutation in *S. typhimurium* and *E. coli*[a]	Up to 2000 μg/plate	Negative[a]	Evandri et al. (2005)
Tea tree oil	Reverse mutation in *S. typhimurium*[a]	Up to 5000 μg/mL	Negative[a]	Fletcher et al. (2005)
Tea tree oil	*In vitro* micronucleus in human lymphocytes[a]	95, 182, 365 μg/mL	Negative[a]	Pereira et al. (2014)
Tea tree oil	*In vitro* chromosomal aberration in human lymphocytes[a]	95, 182, 365 μg/mL	Negative[a]	Pereira et al. (2014)

[a] In the absence and presence of an exogenous metabolic activation system.
[b] In the absence of S9.
[c] In the presence of S9.
[d] Highest inactive dose tested or lowest active dose tested.
[e] 3h or 4 h treatment.
[f] 24 h treatment.

Table 7
NFCs affirmed FEMA GRAS

FEMA No.	Name
2156	Bois De Rose Oil (*Aniba rosaeodore* Ducke)
2241	Cardamom Seed Oil (*Elettaria cardamomum* (L.) Maton)
2244	Carrot Oil (*Daucus carota* L.)
2321	Clary Oil (*Salvia sclarea* L.)
2334	Coriander Seed Oil (*Coriandrum sativum* L.)
2346	Currant Buds Black Absolute (*Ribes nigrum* L.)
2533	Guaiac Wood Extract (*Guaiacum officinale* L.; *G. sanctum* L.; *Bulnesia sarmienti* Lorentz)
2534	Guaiac Wood Oil (*Guaiacum officinale* L.; *G. sanctum* L.; *Bulnesia sarmienti* Lorentz)
2618	Lavandin Oil (Hybrids between *Lavandula officinalis* Chaix *and L. latifolia* Viil.)
2620	Lavender Absolute (*Lavandula officinalis* Chaix)
2622	Lavender Oil (*Lavandula officinalis* Chaix)
2818	Orange Blossom Absolute (*Citrus aurantium* L.)
2838	Patchouly Oil (*Pogostemon cablin* Benth. and *P. heyneanus* Benth.)
3033	Spike Lavender Oil (*Lavandula latifolia* Vill. (*L. spica* DC.)
3902	Tea Tree Oil (*Melaleuca alternifolia*)
3950	*Michelia Alba* Oil (*Michelia alba* D.C.)

*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, as shown in Table 4, the estimated intake of the fraction of unidentified constituents for each of NFC under consideration does not exceed the TTC for Structural Class III, 90 μg/person/day. These NFCs

[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 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).

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.

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.

Small percentages of naturally occurring coumarin have been identified in Lavandin Oil (FEMA 2618), Lavender Absolute (FEMA 2620) and Lavender Oil (FEMA 2622). The intake of coumarin for each of these NFCs is presented in Table 5. In 1954, the US Food and Drug Administration prohibited coumarin, a naturally occurring constituent of tonka beans as well as *Cinnamomum cassia*, from use as an added flavor in foods (21 CFR 189.130). This restriction was implemented following the observation of hepatotoxic effects in dietary feeding studies of coumarin conducted in rats and dogs (Hazleton et al., 1956). Since the publication of the 1956 study, additional studies were performed and reported that investigate 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, which is also briefly summarized by the FEMA Expert Panel in a recent manuscript on *Cinnamomum* and *Myroxylon*-derived NFCs (Rietjens et al., 2020) the European Food Safety Authority (EFSA) has determined that coumarin is not an *in vivo* genotoxicant and that a threshold exists for the toxicity for coumarin (EFSA, 2004, 2008). Concurrently, EFSA established (and later maintained) a tolerable daily intake (TDI) of 0.1 mg/kg bw based on a NOAEL of 10 mg/kg bw/day for coumarin determined from a two-year feeding study in dogs and a safety factor of 100, in consideration of the potentially more vulnerable CYP2A6-deficient subpopulation that cannot metabolize coumarin efficiently (EFSA, 2004, 2008). In an expert opinion report commissioned by the German Federal Institute for Drugs and Medical Devices, the German Bundesinstitut für Risikobewertung (BfR, Federal Institute for Risk Assessment) concurred with EFSA's opinion that coumarin-induced hepatotoxicity occurs by a non-genotoxic mechanism and has a threshold. The risk assessment by the BfR was based on the lowest hepatotoxic exposure of coumarin reported in humans, 25 mg/day, and an uncertainty factor of 5 to derive an intake level at which no adverse effects would be observed, even in sensitive populations (Abraham et al., 2010; Bergmann, 1999). Using these parameters, a safe level of 5 mg/person/day was established and a rounded TDI of 0.1 mg/kg bw for a 60 kg adult was determined (Abraham et al., 2010). As presented in Table 5, the estimated intake of coumarin found naturally Lavandin Oil (FEMA 2618), Lavender Absolute (FEMA 2620) and Lavender Oil (FEMA 2622) is substantially below the TDI established by both EFSA and the German BfR. The TDI of 0.1 mg/kg bw 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. Therefore, the levels of coumarin in these NFCs do not raise a safety concern.

In addition, two furocoumarins, bergaptene and psoralen, were reported in Orange Blossom Absolute (FEMA 2818) at mean % values of 0.3 and 0.1%, respectively. Furocoumarins are a well-known group of natural food constituents known to occur in *Citrus* peel oils and foods, such as parsnips, carrots, parsley and celery (Dolan et al., 2010). Furocoumarins have both phototoxic and photomutagenic properties following exposure to UV light and thus the use of furocoumarin-containing materials in skin care and cosmetic products is regulated (Cosmetic Ingredient Review Expert Panel, 2016; Scientific Committee on Consumer Products, 2005). In consideration of the limited information on the typical intake of furocoumarins from food and their potential effects, regulatory bodies have not regulated dietary exposure to furocoumarin content from food. Opinions published by regulatory groups on the dietary exposure to furocoumarin were reviewed by the FEMA Expert Panel in its review of over 50 *Citrus* NFCs (Cohen et al., 2019). The Panel concurs with these opinions and concludes that the potential additional safety concerns arising from the extremely low level of furocoumarins present in *Citrus*-derived NFCs such as Orange Blossom Absolute (FEMA 2818) used as flavor ingredients does not present a safety concern under conditions of intended use.

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. With the exception of Group 12 constituents of Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622) and Patchouly Oil (FEMA 2838) and Group 19 constituents of Tea Tree Oil (FEMA 3902), the estimated intakes are substantially below the corresponding TTC for their respective groups, with none close to the TTC. For the congeneric groups that exceed the TTC in Lavandin Oil (FEMA 2618), Lavender Oil (FEMA 2622), Patchouly Oil (FEMA 2838) and Tea Tree Oil (FEMA 3902) listed in Table 3, adequate margins of safety were established that are protective at lower body weights. Table 4 lists the intake of the unknown constituent fraction, of which, none are close to the TTC value for Class III. Intakes well below the TTC for compounds with a structural alert for genotoxicity result from the reported low naturally occurring concentrations of methyl eugenol, estragole and safrole in *Michelia Alba* Oil (FEMA 3950) of estragole and methyl eugenol in Guaiac Wood Extract (FEMA 2533) and of methyl eugenol in Tea Tree Oil (FEMA 3902) as presented in Step 4a. Together, these results indicate the approach to be protective for consumption by children.

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 listed in Table 7 are affirmed as GRAS under conditions of intended use as flavoring substances.

7. Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation

Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) dominates the constituent profiles of the NFCs described here. The FEMA Expert Panel has reviewed the safety of flavoring ingredients of this group (Marnett et al., 2014) as well as other dominant consitutent groups: Group 19 (Aliphatic and aromatic hydrocarbons) and Group 10 (Alicyclic ketones, secondary alcohols and related esters) (Adams et al., 1996, 2011). In addition, the Panel has also published evaluations of other groups or individual constituents (Adams et al., 2004; Adams et al., 2005a, b, c; Adams et al., 2002; Adams et al., 1997; Adams et al., 2008; Adams et al., 1998; Adams et al., 2007; Newberne et al., 1999).

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. Studies concerning genotoxicity are summarized in Table 6.

7.1. Tertiary alcohol constituents

7.1.1. Linalool

In an OECD-compliant Ames assay, mutagenicity was not observed when linalool was tested at concentrations up to 1580 μg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2 *uvrA* both in the presence and absence of an S9 metabolic activation system derived from the liver of phenobarbital and benzoflavone treated rats (Slonina, 2019). In a bacterial reverse mutation assay in *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2 *uvrA*, linalool was non-mutagenic when tested at concentrations up to 170 μg/plate, with and without rat liver S9 obtained from phenobarbital/β-naphthoflavone-treated rats (Di Sotto et al., 2008). An additional mutagenicity assay tested linalool in *S. typhimurium* strains TA98, TA100 and TA102 in the presence and absence of S9 activation. When tested up to 1.8 mg/plate, linalool was negative for inducing revertant mutant colonies (Beric et al., 2008). Linalool was also negative in a human lymphocyte micronucleus assay, where micronucleus induction was not observed when linalool was incubated with human peripheral lymphocytes at concentrations ranging from 0.5 to 300 μg/mL (Di Sotto et al., 2011). In a chromosomal aberration (CA) assay in Chinese hamster ovary (CHO) cells, linalool did not induce chromosomal aberrations in CHO cells treated with up to 340 μg/mL of linalool in the presence of S9 and up to 430 μg/mL in the absence of S9, which was derived from the liver of rats treated with Aroclor 1254 (Galloway, 1983). When tested up to an overall maximum of 500 μg/mL, linalool did not cause forward mutations in mouse lymphoma L5178Y cells incubated with and without exogenous metabolic activation by S9 obtained from the liver of Aroclor 1254-treated rats (Cifone, 1994). An *in vivo* comet assay study in mice found no DNA damage in the forebrain tissue and peripheral blood sampled following the administration of a single 10, 50, 100 or 200 mg/kg dose of linalool by intraperitoneal injection (Coelho et al., 2013). In summary, linalool was negative for all measured endpoints.

In a short-term dietary study, a 1:1 mixture of linalool and citronellol, resulting in an average daily intake of 50 mg/kg bw/day of each, was incorporated into the diet of male and female rats treated for 12 weeks. A slight decrease in body weight gain was observed in the male rats but was concluded by the study author not to be of biological relevance (Oser, 1958). This study was used to calculate a MoS for the intake of Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) constituents for Lavadin Oil (FEMA 2618), Lavender Oil (FEMA 2622) and Tea Tree Oil (FEMA 3902) in Step 6 of the safety evaluation.

In a reproductive/developmental toxicity study, there were no deaths or signs of gross toxicity in female Sprague-Dawley rats administered linalool at doses of 0 (control), 250, 500 or 1000 mg/kg bw/day for 11 days after confirmed gestation (Politano et al., 2008). There were no treatment-related deaths or gross signs of toxicity in the maternal animals. There were also no adverse developmental effects noted in the offspring. The no-observed-adverse-effect level (NOAEL) for maternal rats was determined to be 500 mg/kg bw/day due to treatment-related changes in motor function and fur stained with urine at the highest dose. The NOAEL for the development of offspring was determined to be 1000 mg/kg bw/day (Politano et al., 2008).

7.1.2. Linalyl acetate

In a bacterial reverse mutation assay, linalyl acetate did not increase the number of revertant mutants in either of the *S. typhimurium* strains TA98 and TA100 but did induce statistically significant, concentration-dependent increases in revertant colonies in *E. coli* WP2 *uvrA* up to the highest dose tested, 9000 μg/plate, with and without S9 activation (Di Sotto et al., 2008). A statistically significant increase in revertant colonies was only observed at concentrations at and above 3200 μg/plate. The two highest concentrations tested, 6400 and 9000 μg/plate, exceed the recommended 5000 μg/plate upper limit for this assay in the OECD guideline (Di Sotto and Mazzanti, 2016; OECD, 1997). The same authors, in a separate study, reported that linalyl acetate also yielded a significant concentration-dependent induction of micronuclei in a human lymphocyte micronucleus assay at concentrations from 10 to 300 μg/mL (Di Sotto et al., 2011). This study was considered further in a fragrance safety assessment (Api et al., 2015), to which a response from the study authors was received (Di Sotto and Mazzanti, 2016). The conditions of the *in vitro* micronucleus study on linalyl acetate did not comply with OECD testing guidelines since the test substance exposure period of 72 h greatly exceeded the OECD suggested 3- to 6 h exposure time to detect clastogens and aneugens (OECD, 2014). The authors noted that the study was performed according to a protocol for the cytokinesis-block micronucleus assay with only minor modifications (Di Sotto and Mazzanti, 2016; Fenech, 2007). The positive outcomes of these studies are inconsistent with previously reviewed studies on linalyl acetate (Marnett et al., 2014) that reported negative mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and absence of metabolic activation (Heck et al., 1989), lack of measurable DNA damage in the *Bacillus subtilis* rec assay (Oda et al., 1978), lack of induction of chromosomal aberrations in human peripheral blood lymphocytes (Bertens, 2000) and lack of unscheduled DNA synthesis (UDS) in rat hepatocytes (Heck et al., 1989). In addition, in a recent report, linalyl acetate was reported not to be mutagenic in *S. typhimurium* strains TA98, TA100, TA1535 and TA1538 at concentrations up to 5000 μg/plate in the presence and absence of Aroclor 1254-induced rat liver S9 (ECHA, 2019a). In addition to these studies, linalyl acetate is expected to be hydrolyzed to linalool upon oral consumption, which has been shown to be non-genotoxic. In summary, the weight-of-evidence indicates that linalyl acetate is not genotoxic despite the positive result reported in a non-OECD-compliant *in vitro* micronucleus study.

7.1.3. 4-Carvomenthenol

In an OECD-compliant Ames assay, mutagenicity was not observed when 4-carvomenthenol was tested at concentrations between 16 and 5000 μg/plate in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535 both in the presence and absence of an S9 metabolic activation system derived from the liver of phenobarbital/*β*-naphthoflavone-treated rats. Cytotoxicity was noted at 1600 μg/plate (Scheerbaum, 2001). In an OECD-compliant *in vitro* micronucleus assay, human peripheral blood lymphocytes were treated with 4-carvomenthenol for 4 and 24 h, with and without metabolic activation. Up to the maximum tested concentration of 1540 μg/mL, 4-carvomenthenol tested negative for the induction of micronuclei (Roy, 2015). In conclusion, two guideline studies on 4-carvomenthenol were both found to be negative, leading to the conclusion that the substance is not of genotoxic concern.

In a 28-day toxicity study, 4-carvomenthenol was administered by oral gavage to male Sprague-Dawley rats at 400 mg/kg bw/day to investigate its nephrotoxic potential. The study concluded that 4-carvomenthenol did not induce any treatment-related renal changes (Schilcher and Leuschner, 1997).

7.1.4. α-Terpineol

A recently conducted OECD-compliant reverse mutation assay tested α-terpineol in *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvr*A. α-Terpineol did not increase the frequency of revertants when tested up to 5000 μg/plate in the presence and absence of an S9 metabolic activation system prepared from phenobarbital/5,6-benzoflavone-treated rats. Therefore, α-terpineol was considered negative for genotoxicity under the conditions tested (Rao, 2019). In a second reverse mutation assay using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, α-terpineol was not mutagenic at concentrations of 10–1000 μg/plate in the presence and absence of S9 from Aroclor 1254-treated male Syrian hamsters or Sprague-Dawley rats

(Seifried et al., 2006). α-Terpineol was non-mutagenic in a mouse lymphoma L5178Y forward mutation assay when tested at ranges of 0.14–0.38 μg/mL or 0.17–0.56 μg/mL in the absence or presence of liver S9 from male rats treated with Aroclor 1254, respectively (Seifried et al., 2006).

7.1.5. Terpineol (Isomeric mixture)

The isomeric mixture of terpineol was tested at concentrations up to 1250 μg/plate in an OECD-compliant reverse mutation assay. In *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvr*A, terpineol did not increase the frequency of mutant colonies in treatments with or without S9 metabolic activation (ECHA, 2013b). In addition, two independent *in vitro* chromosomal aberrations assays were conducted on terpineol using cultured human lymphocytes or Chinese hamster lung cells (ECHA, 2010, 2013a). In human lymphocytes, concentrations up to 450 or 625 μg/mL were tested for induction of structural aberrations in the short-term 3 h treatments with and without Aroclor 1254-induced S9, respectively. In continuous 24 h treatments without S9 metabolic activation, concentrations up to 225 μg/mL were tested for induction of aberrations. Based on the conditions tested, terpineol was non-clastogenic in the human lymphocytes in the presence and absence of S9 metabolic activation (ECHA, 2010). In Chinese hamster lung cells, concentrations ranging from 100 to 400 μg/mL were tested in the absence of S9 (6 h or continuous 24 h treatment). In the presence of S9 (6 h treatment only), a range of 100–500 μg/mL was tested. Terpineol did not induce chromosomal aberrations in Chinese hamster lung cells when tested at concentrations similar to the first assay using lymphocytes (ECHA, 2013a).

In an OECD-compliant combined repeated dose and reproductive/development toxicity screening study, groups of male and female Sprague-Dawley rats were administered terpineol in corn oil by oral gavage at dose levels of 100, 300 or 1000 mg/kg bw/day (ECHA, 2019b). Males were treated for a total of 44 days, including the two-week pre-mating through Day 30 after mating. Females were treated during the pre-mating period through Day 4 of lactation for a total of 41–51 days. Additionally, a group of non-mating females was treated with 1000 mg/kg bw/day terpineol on the same schedule as the males, and groups of males and non-mating females were maintained for a two-week recovery period. Six females belonging to the high-dose group were found dead or moribund; moribund animals displayed poor health, including significant clinical observations, decreased body weights and lower food consumption. Necropsy and histopathology of these dead or moribund females showed weight reduction of the spleen and thymus and adverse changes in the kidneys, urinary bladder and liver.

For surviving animals, no treatment related clinical signs, body weight changes, changes in food consumption, hematological, clinical biochemistry or behavior findings were reported. Increased water consumption was observed in high-dose males and non-mating females and corresponded to increased urine volume and low urinary osmotic pressure in these animals. Upon necropsy, significantly higher liver and kidney weights and lower testes and epididymis weights were observed in high-dose males and were also reported following the recovery period. Non-mating females of the 1000 mg/kg bw/day group had increased liver, kidney and adrenal gland weights; only the increased liver weights were present after the recovery period. An increase in relative and absolute liver weights was observed in mid-dose mating females, but not in high-dose mating females. No gross abnormalities were observed in mating females; however, females of the high-dose group were infertile (9/12), which was considered a result of the smaller testes in the males of the group.

Histopathology indicated minimal to mild vacuolation of adrenal cortical cells in mating females dosed with 300 mg/kg bw/day or higher and in high-dose non-mating females. Smaller testes and epididymis in high-dose males (including recovery group males) correlated with moderate atrophy of the seminiferous tubules and other findings reported in these organs. Findings in the kidneys of mid- and high-dose males and of high-dose mating and non-mating females were correlated to higher relative and absolute kidney weights in these groups. Eosinophilic droplets in mid- and high-dose males were positive for α_{2u}-globulin immunochemistry and negative for periodic acid-Schiff staining. Renal effects related to α_{2u}-globulin are widely considered non-relevant to human risk assessment, as this effect is considered unique to male rats (Capen et al., 1999; Flamm and Lehman-McKeeman, 1991; Swenberg and Lehman-McKeeman, 1999; US-EPA, 1991). High-dose animals of both sexes also displayed umbrella cell atrophy and hypertrophy or hyperplasia of transitional epithelial cells of the urinary bladder. Minimal to moderate umbrella cell vacuolation was also observed in the urinary bladder of mid-dose males and mating females. A higher incidence of decreased zymogen granules in the pancreas was reported in mid-dose mating females and high-dose non-mating females. Assessment of reproductive functionality reported no changes to sexual cycles in any of the treatment groups and no significant differences in gestation ratio, pregnancy period, number of corpora lutea, implantation number or ratio, delivery ratio or number of stillborn or live pups. Lower insemination index ($p < 0.01$) and fertility index ($p < 0.01$) were reported in high-dose males and mating females, respectively, and were a result of the histopathological changes observed in male reproductive organs. Live pups born to treatment groups did not present any morphological abnormities or gross pathological findings upon necropsy. Based on these findings, the systemic toxicity NOAEL obtained from the repeated dose portion of the study was determined to be 100 mg/kg bw/day. The reproductive NOAEL was considered 300 mg/kg bw/day in males and 100 mg/kg bw/day in females, while the developmental NOAEL was the highest dose tested, 1000 mg/kg bw/day (ECHA, 2019b).

A second OECD-compliant combined repeated dose toxicity and reproductive/developmental toxicity study was conducted on terpineol (isomeric mixture) via gavage administration at dose levels of 60, 250 or 750 mg/kg bw/day (ECHA, 2019c). The dosing for main (non-recovery group) animals lasted a minimum of five weeks for males and non-mating females, which included the two-week pre-mating period. Mating females were dosed for the pre-mating period and throughout mating and gestation, until Day 6 of lactation. One low-dose male and one low-dose mating female were found dead or morbid but this was not considered a result of test substance administration. Slightly reduced, but not statistically significant, body weight gains were observed in high-dose males and mating females of all dose levels. No adverse changes to body weight, food consumption or hematological parameters were observed for any of the treatment groups. There were no dose-related trends and the effects were minimal in degree. At necropsy, increased liver weights for high-dose males and females were reported, in addition to increased kidney weights in high-dose males. These differences in liver and kidneys weights were not observed in the recovery group. Markedly lower testes and epididymis weights were observed in most high-dose males and in two mid-dose males; high-dose males of the recovery group also displayed the lower testes and epididymis weights. Gross pathology presented a range of testicular findings in high-dose males and recovery group males, including small, flaccid testes and the presence of masses in some epididymides that were correlated to histopathological findings of spermatocele granuloma, a benign cyst-like growth that occurs spontaneously in rats. No gross lesions were reported in any females. Histopathological examinations revealed minimal centrilobular hepatocyte hypertrophy in three high-dose females that showed complete recovery after two weeks. In male rats, examination of the kidneys revealed hyaline droplet formation, characteristic of α_{2u}-globin nephropathy, at doses of 250 and 750 mg/kg bw/day, that persisted in the recovery group high-dose males. Reproductive parameters, such as estrous cyclicity, mating performance and fertility, were generally unaffected by test substance administration. Offspring did not display any differences in body weight or gross findings, and there were no clinical signs of toxicity as a result of maternal exposure. Based on

these findings, the repeated dose toxicity and fertility NOAEL was determined to be 250 mg/kg bw/day for males and 750 mg/kg bw/day for females. The developmental toxicity NOAEL was greater than 750 mg/kg bw/day, the highest dose tested (ECHA, 2019c).

7.1.6. Terpinyl acetate (Isomeric mixture)

Human peripheral blood lymphocytes were exposed to terpinyl acetate in an OECD-compliant *in vitro* micronucleus assay at concentrations up to 36.0 μg/mL for a 24 h exposure period without metabolic activation, concentrations up to 103 μg/mL for the 3 h exposure period without metabolic activation and concentrations up to 158 μg/mL with metabolic activation with S9, prepared from the liver of Aroclor 1254-treated rats. The study concluded that terpinyl acetate did not induce significant increases in micronuclei under the conditions tested (Bhalli, 2015).

7.1.7. α-Terpinyl acetate

Two OECD-guideline bacterial reverse mutation assays were conducted on α-terpinyl acetate in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 *uvrA*. In both studies that employed the plate incorporation method, treatment with α-terpinyl acetate up to a concentration of 5000 μg/plate in the presence and absence of Aroclor 1254-induced rat liver S9 did not result in any increases in revertant colony frequencies (Bhalli, 2014a; van den Wijngaard, 2012).

An *in vitro* micronucleus induction assay was conducted in accordance with OECD testing guidelines, in which human peripheral blood lymphocytes were treated with α-terpinyl acetate in the presence and absence of S9 metabolic activation derived from Aroclor 1254-treated rats (Bhalli, 2014b). Based on a preliminary cytotoxicity assay, α-terpinyl acetate was tested up to 225 μg/mL for the 3 h exposure periods with and without S9 and up to 80 μg/mL for the 24 h exposure period without S9. At a single scored concentration of 58.3 μg/mL in the 24 h treatment arm, there was a statistically significant increase in micronuclei frequency, but it was within the laboratory's historical values for the vehicle control and therefore was determined to be not biologically relevant. The overall conclusion was that α-terpinyl acetate was not genotoxic under the conditions tested (Bhalli, 2014b).

7.1.8. Patchouli alcohol

In an OECD-compliant reverse mutation study, patchouli alcohol was not mutagenic when tested in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA* at concentrations up to 5000 μg/plate, with and without S9 metabolic activation obtained from the liver of Aroclor 1254-treated male Sprague-Dawley rats (Bhalli, 2014c). In an OECD-compliant *in vitro* micronucleus assay, patchouli alcohol was tested in human peripheral blood lymphocytes for 3 h at concentrations up to 89 μg/mL without S9 metabolic activation and 150 μg/mL with S9 metabolic activation. Concentrations up to 83 μg/mL were tested for the 24 h exposure in the absence of S9 metabolic activation, prepared from the liver of Aroclor 1254-treated rats. The study concluded that patchouli alcohol did not induce an increase in micronuclei in binucleated cells under the conditions tested (Bhalli, 2014d).

7.2. Natural flavor complexes

7.2.1. Bois de rose oil

In an OECD-compliant reverse mutation assay, bois de rose oil was not mutagenic when tested at concentrations up to 5000 μg/plate in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2, in the absence and presence of S9 metabolic activation prepared from the liver of Aroclor 1254-treated rats (Mee, 2017). In an OECD-compliant *in vitro* micronucleus study, bois de rose oil was tested at concentrations up to 621 μg/mL in the 3 h treatment in the presence of S9 metabolic activation, 570 μg/mL in the 3 h treatment in the absence of S9 metabolic activation and concentrations up to 176 μg/mL in the 24 h treatment in the absence of S9 metabolic activation. The S9 metabolic activation system was prepared from the liver of Aroclor 1254-treated male rats. Bois de rose oil did not induce the formation of micronuclei in human lymphocytes under the conditions tested (Clare, 2017).

7.2.2. Cardamom Seed Oil

In a GLP guideline study, cardamom seed oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 both in the presence and absence of an Aroclor 1254-treated rat liver metabolic activation system at concentrations up to 2325 μg/plate[8] (DeGraff, 1983b; Heck et al., 1989). Another reverse mutation assay with the same *S. typhimurium* strains tested cardamom seed oil at a concentration range of 40–2200 μg/mL.[9] Under the conditions of the study, cardamom seed oil was negative for mutagenic potential with and without S9 prepared from the liver of Aroclor 1254-treated rats (Brusick, 1982). When cardamom seed oil was incubated with L5178Y mouse lymphoma cells in a GLP-compliant assay, an increase in the mutant frequency at the TK locus was not observed in the presence or absence of S9 metabolic activation. Concentrations up to 112 μg/mL[10] were tested in the absence of metabolic activation and up to 233 μg/mL in the presence of Aroclor 1254-treated rat liver S9 metabolic activation (Cifone, 1982; Heck et al., 1989). Cardamom seed oil was negative in an UDS assay in rat hepatocytes at a dose of 50 mg/mL (Heck et al., 1989).

In a GLP-compliant reproductive/developmental toxicity study, cardamom seed oil was administered by oral gavage to virgin female rats (10/group) at doses of 0 (corn oil control), 375, 750 or 1500 mg/kg bw/day for seven days prior to cohabitation, gestation, delivery and a four-day lactation/post-parturition period (Hoberman, 1989a). There was one death in the mid-dose group (750 mg/kg bw/day) on Day 22 of gestation due to pronounced weight loss and petechial hemorrhaging in the gastric mucosa and one moribund sacrifice in the high-dose group (1500 mg/kg bw/day) due to clinical signs of toxicity and pronounced weight loss. Statistically significant numbers of rats with clinical observations such as salivation, decreased motor function, emaciated appearance or tremors and twitches were noted at all dose levels. The onset was dose-related and attributed to the administration of the test substance.

Dose-dependent decreases in maternal body weight gain were observed with significant decreases observed in the middle and high dose groups during the pre-cohabitation period and in all groups during the gestation period. However, significant reduction in feed consumption was only observed in the high dose group. Significant enlargement of the liver was observed in the middle and high dose groups and attributed to hepatic enzyme induction.

Except for the one mortality in the middle dose group during gestation, all dams delivered one or more live pups. There was no dose-related effect observed for implantation incidences or live litter sizes. Decreased pup body weights were observed at the middle and high doses and a significant increase in pup mortality was observed at the high dose. Based on clinical observations and decreased body weight gains and feed consumption in the dams, a maternal NOAEL for toxicity could not be determined. Based on the lack of adverse effects of cardamom oil on mating, fertility, duration of gestation or duration of parturition, a NOAEL of 1500 mg/kg bw/day was determined for reproductive effects. The NOAEL for the offspring was determined to be 375 mg/kg bw/day based on the decreased body weights (Hoberman, 1989a).

[8] Based on a density of 0.93 g/mL (Source: Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD, USA).

[9] The mean achieved dose is calculated for each treatment group based on the food consumption per day and the mean body weight over each measurement period. Differences in calculated mean achieved dose and the nominal concentration between studies is due to a greater mean body weight (per measurement period) in the longer duration (28-day) study.

In a 28-day repeat-dose study, cardamom oil was administered by oral gavage to male and female Sprague-Dawley rats at 0 (control), 240, 600 or 1500 mg/kg bw/day (Serota, 1991). The constituent analysis reported a composition of 36% terpinyl acetate, 38% eucalyptol, 6% linalool, 6% α-terpineol and several minor constituents for the cardamom oil used in the study. The vehicle control was a 1% methyl cellulose solution. Examination for clinical signs and measurement of food consumption failed to reveal any differences between test and control groups. A significant decrease in body weight was reported in males in the high dose group. Females showed no changes in body weight gain at any dose level. Clinical hematology values were normal. Clinical chemistry evaluation showed elevated total protein and albumin in the high-dose males and females and decreased glucose in high-dose males. There were no significant changes in any parameter related to liver or kidney function. Morbidity was observed for a single high-dose male which was considered treatment-related, although the cause of death could not be determined by macroscopic or microscopic examinations. Clinical findings were noted for this animal prior to its sacrifice, including dyspnea, urine stains and lacrimation of both eyes.

Enlarged livers were reported in high-dose males and mid- and high-dose female treatment groups and increased incidence of pale livers was observed in all treatment groups except the low-dose male group. Significant increases in absolute and relative liver weight were recorded for at all dose levels in males and females. Histopathological examination of males revealed periportal cytoplasmic vacuolization in the low (7/10), mid- (5/10) and high-dose (5/10) groups. Females showed a similar non-dose-related trend in cytoplasmic vacuolization [low (7/10), mid (8/10) and high (6/10)]. The vacuolization was graded as slight. The authors noted that the vacuoles seemed to be fat-like deposits and were considered to have little effect on the function or health of the animals. Since changes in bilirubin and liver enzymes were not observed in females, the relevance of the lesion to the health of the animals is unknown.

Increases in absolute and relative kidney weights were noted in the mid- and high-dose females and relative kidney weights in mid- and high-dose males. There was no evidence of histopathologic abnormalities in any female group. However, histopathological examination of treated males revealed renal tubule regeneration (hyaline droplet degeneration) and necrosis, likely related to the lysosomal handling of α_{2u}-globulin, an effect specific to the male rat and not of toxicological relevance to humans (Capen et al., 1999; Flamm and Lehman-McKeeman, 1991; Swenberg and Lehman-McKeeman, 1999; US-EPA, 1991). High-dose males showed an increase in absolute and relative testes weights with a significant increase in epididymis weights in the high dose group of male rats. Histopathology revealed testicular giant cell degeneration with associated hypospermia in the epididymis. There was no evidence of histopathology of the testes or epididymis in the mid- and low-dose males and no evidence of any adverse effects to female reproductive organs (ovaries and uterus) at any dose level. Changes in clinical chemistry in high-dose males and females and increased absolute and relative adrenal weights in mid- and high-dose females were noted but did not correlate with microscopic findings, nor were the observations dose-related. Based on the findings of the study, the lowest-observed-adverse-effect-level (LOAEL) was determined to be 240 mg/kg bw per day for both male and female rats (Serota, 1991).

7.2.3. Clary Oil

In an OECD-compliant study, clary oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvrA*/pKM101 both in the presence and absence of an Aroclor 1254-treated rat liver metabolic activation system at concentrations up to 5000 μg/plate (Mee, 2016a). In a separate study, clary oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of S9 metabolic activation at concentrations up to 5000 μg/plate (Heck et al., 1989). Clary oil was also negative in an OECD-compliant *in vitro* micronucleus test in human lymphocytes in both the presence and absence of S9 at concentrations up to 888.9 μg/mL, the lowest concentration in which cytotoxicity was observed (Mee, 2016b). Genotoxicity was not observed in an UDS assay in rat hepatocytes up to 101 μg/mL or in a rec assay at 10 and 30 μg/disk (Heck et al., 1989; Zani et al., 1991). In summary, the results of these assays demonstrate a lack of genotoxic potential for clary oil.

7.2.4. Coriander Seed Oil

Coriander seed oil was not mutagenic in a GLP study with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of an S9 activation system derived from Aroclor 1254-treated rat liver, at concentrations ranging from 9 to 4350 μg/plate[10] (DeGraff, 1983a; Heck et al., 1989). Similarly, when coriander seed oil was incorporated into *S. typhimurium* cultures TA98 and TA100 at 2 and 7 mg/plate, it was negative for mutagenic potential with and without rat liver S13 metabolic system (Marcus and Lichtenstein, 1982). Coriander seed oil was also negative in an UDS assay in rat hepatocytes up to 300 μg/mL; a CA study in Chinese hamster ovary cells up to 0.125 mg/mL; and a mouse lymphoma mutation assay in the presence and absence of S9 up to 160 nL/mL and 300 nL/mL, respectively (Cifone, 1983; Heck et al., 1989; Ishidate Jr. et al., 1984). In a rec assay, coriander seed oil tested at a concentration of 8 mg/disk was positive without S9 activation and negative with S9 activation (Ueno et al., 1984). The rec assay does not have an OECD testing guideline; the OECD guideline for genotoxicity testing notes that indicator tests such as the rec assay should be weighted relative to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015). In conclusion, the weight of evidence indicates a lack of genotoxic potential for coriander seed oil due to negative results in the standard Ames, CA and mouse lymphoma assays.

In a 28-day oral gavage toxicity study, Sprague-Dawley rats were administered coriander seed oil, containing 73% linalool, at 0 (control), 160, 400 or 1000 mg/kg bw/day (10/sex/group) with 1% methyl cellulose as the vehicle control (Serota, 1990). No treatment-related effects were observed based on mortality, clinical observations, body weight or food consumption. A significant increase in absolute and relative liver weights was observed in the mid- and high dose groups for both male and female rats and a significant increase in the absolute liver weight was observed in the female low dose group. This effect was accompanied by periportal hepatocellular cytoplasmic vacuolization in the liver of high-dose females with lower incidences in low- and mid-dose female rats but was not observed in any treatment groups in the male rats. For all treatment groups, no histopathological findings were reported in the liver of male rats. Hepatocyte vacuolation observed in the low- and mid-dose female rats was likely due to fatty degeneration, although this was not confirmed by special staining. Significant increases in absolute and relative kidney weight were observed in the high dose male and female groups and increases in relative kidney weight were seen in the middle dose group of male rats. For the male rats, treatment-related degenerative lesions of the renal cortex of the kidney in high-dose males were related to increased absolute and relative kidney weights. Lesions in the non-glandular stomach of the mid- and high-dose female groups were also found including erosion, inflammation and hyperplasia particularly in high-dose females. Increases in total protein and serum albumin were observed in the mid-dose male rats and high-dose male and female rats. Based on these observations, the NOAEL was determined to be 160 mg/kg bw/day for male rats. A NOAEL could not be determined for female rats (Serota, 1990).

In a GLP-compliant reproductive/development study, coriander seed oil, containing 73% linalool, was administered to Crl:CD(SD)BR virgin

[10] Based on a density of 0.87 g/mL (Source: Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD, USA).

female rats (10/group) at doses of 0 (control), 250, 500 or 1000 mg/kg bw/day through the 7-day pre-cohabitation period, cohabitation (7 days maximum), gestation, delivery and the 4-day lactation/post-parturition period. Significant decreases in body weight gain and feed consumption were observed in the highest dose group during the pre-mating period. During gestation, statistically significant, treatment-related increases in weight gain and feed consumption occurred in all test groups compared to the control group, that were considered biologically relevant. These changes were also observed during lactation with less severity. There were no dose-related or statistically significant changes in duration of cohabitation, pregnancy incidences or implementation averages in the treatment groups. A statistically significant increase in pup mortality was noted in the 1000 mg/kg bw/day treatment group. No differences in duration of gestation, pup sex ratios or pup body weights were found in any of the treatment groups compared to the control group. There were also no adverse developmental effects noted in the offspring. From these observations, the NOAEL for progeny was determined to be 500 mg/kg bw/day. A maternal NOAEL was not determined, based on clinical observations and altered body weights and food consumption at the lowest dose tested (Hoberman, 1989b).

7.2.5. Guaiac Wood Oil

In an OECD-compliant study, guaiac wood oil was negative for mutagenicity when incubated with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *E.coli* WP2 *uvrA*/pKM101 both in the presence and absence of an Aroclor-1254 treated rat liver metabolic activation system at concentrations up to 5000 μg/plate (Mee, 2016b). In an OECD-compliant *in vitro* micronucleus test in human blood lymphocytes, cells were exposed to up to 395 μg/mL of guaiac wood oil in the presence and absence of an S9 metabolic activation system obtained from Aroclor 1254-treated male Sprague-Dawley rat liver Guaiac wood oil was determined to be negative for the induction of micronuclei in the presence and absence of S9 metabolic activation (Mee, 2016c).

In a 90-day toxicity study, FDRL rats (15/sex/group) were administered guaiac wood oil in the diet at a single nominal dose of 31.8 mg/kg bw/day (equivalent to 30.7 and 36.0 mg/kg bw/day for male and female rats, respectively). The test substance was diluted in cotton seed oil at a concentration yielding a dose of 2% in the diet (Oser et al., 1965). During the study, body weight and food consumption were recorded. At Weeks 6 and 12, hematological and blood chemistry analyses were performed. At the end of the study, the animals were autopsied during which liver and kidney weights were measured and tissues were collected for histopathology. Observations included increased efficiency of food utilization, increased red blood cell count and decreased hemoglobin, lymphocytes and blood urea nitrogen. There were no significant adverse effects detected and no histopathological findings. The NOAEL was determined to be the only dose tested, 31.8 mg/kg bw/day (Oser et al., 1965).

7.2.6. Lavender Oil

In a reverse mutation assay conducted with lavender oil at concentrations of 4.4 and 8.8 ng/plate, it was found to be mutagenic in *S. typhimurium* strains TA1535 and TA1537 (concentration of 4.4 ng/plate) and TA98 (concentration of 8.8 ng/plate) (Sivaswamy et al., 1991). This study did neither report the chemical composition of the lavender oil tested nor indicate a dose-response to support its validity (OECD, 1997). Additionally, the concentrations tested were extraordinarily low. Based on these shortcomings, this study is not considered relevant to the safety evaluation of lavender oil. In an OECD-compliant reverse mutation test in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA*, treated with or without exogenous rat metabolic activation, lavender oil was not mutagenic up to the maximum tested concentration of 5000 μg/plate (Dakoulas, 2014). Another OECD-compliant reverse mutation test also showed no mutagenic potential, in *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2 *uvrA,* with and without S9 metabolic activation derived from phenobarbital/5,6-benzoflavone treated rats, up to 2780 and 2500 μg/plate for *Salmonella* and *Escherichia* strains, respectively (Evandri et al., 2005). In a separate study, lavender oil did not induce mutagenicity in *S. typhimurium* strains TA98 and TA100 both with and without liver S9 from Aroclor 1254-treated rats at concentrations up to 443 μg/plate (De Martino et al., 2009).

In an *in vitro* micronucleus study, lavender oil was tested in human peripheral blood lymphocytes at concentrations up to 100 μg/mL. While there was a small significant increase in the frequency of micronuclei observed at the highest concentration tested, a dose response was not observed. (Di Sotto et al., 2011). In a separate OECD-compliant study, lavender oil was tested in human peripheral blood lymphocytes, in the presence and absence of an exogenous metabolic system. In this study, no increase in the induction of micronuclei was observed up to the highest concentration tested, 450 μg/mL (Roy, 2015b). Altogether, the weight of evidence provided by the results of the three OECD-compliant Ames and *in vitro* micronucleus studies reported here, in addition to the negative genotoxicity reported for linalool and linalyl acetate, the major constituents of lavender oil, supports the conclusion that lavender oil is not of genotoxic concern.

7.2.7. Patchouly Oil

Patchouly oil was tested in a GLP-compliant Ames reverse mutation assay to evaluate its mutagenic potential in a dose range of 0.5–50 μg/plate (Jones, 1988). When incubated with and without an S9 metabolic activation system from the liver of Aroclor 1254-treated rats, patchouly oil did not increase the numbers of revertant colonies in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 (Jones, 1988). Two separate *in vitro* CA studies in Chinese hamster ovary cells had negative results in the presence and absence of metabolic activation; the highest concentration tested was 90 μg/mL (Brooker, 1989; Song, 2009). Patchouly oil was not mutagenic in an OECD-compliant forward mutation assay conducted with mouse lymphoma cells at concentrations up to 50 μg/mL in the absence of S9 metabolic activation or up to 275 μg/mL in the presence of S9 metabolic activation for 4 h and the results were confirmed in a separate assay testing up to 36 μg/mL for 24 h in the absence of S9 metabolic activation (Kirby, 2009).

In a 14-day pilot dietary study on patchouly oil, the highest tolerable dose was 12000 ppm (equivalent to a mean achieved dose[10] of 979 mg/kg bw/day) (Marr, 2011). Following the dose range-finding study, patchouly oil was tested in an OECD-compliant 28-day combined dietary and reproductive/developmental toxicity study in Wistar Han rats (10/sex/group) (Liwska, 2013a, b). For the repeated dose dietary application of the combined study, patchouly oil was incorporated into the diet at concentrations of 500, 4000 and 13000 ppm, corresponding to mean doses of 41, 323 and 977 mg/kg bw/day, respectively, for both male and female rats (Liwska, 2013b). There were no treatment-related deaths or abnormal clinical observations during the study. At the highest dose tested, lower food consumption and decreases in body weight gain compared to controls were reported in both male and female rats. A significant increase in relative and absolute liver weights was observed in middle and high dose groups for both male and females which was correlated to the observation of minimal to moderate centrilobular hepatocellular hypertrophy in these groups. This effect is considered a consequence of hepatocellular induction of enhanced hepatic metabolism. A significant increase in relative and absolute kidney weights was observed in middle and high dose male groups. Hyaline droplet nephropathy was observed in all the male treatment groups and demonstrated a dose-related severity consistent with the accumulation of α_{2u}-globulin, an effect specific to the male rat and of no toxicological relevance to humans (Capen et al., 1999; Flamm and Lehman-McKeeman, 1991; Swenberg and Lehman-McKeeman, 1999; US-EPA, 1991).

A significant reduction in absolute and relative thyroid weights was observed in all female dose groups. Histopathological analysis revealed an increased incidence and/or severity of follicular hypertrophy in the

thyroid for both male and female rats in the middle and high dose groups and males in the low dose group. This was related to an increased metabolism of the thyroid hormones (T3/T4) due to hepatocellular hypertrophy and were considered a secondary effect of treatment and not adverse. A significant decrease in relative and absolute brain and spleen weights was evident in the high dose male group and a significant reduction in relative and absolute heart weight was observed in the female high dose group. These findings had no histopathological correlation and were not considered to be of toxicological significance by the study authors (Liwska, 2013b). T The FEMA Expert Panel considered ed the findings and assigned a assigned a NOAEL of 41 mg/kg bw/day, the lowest dose tested.

For the assessment of reproductive/developmental toxicity, male and female rats were administered concentrations of 1300, 4000 or 13000 ppm of patchouly oil incorporated into the diet for up to 8 weeks corresponding to mean dose levels of 91.4, 277 or 810 mg/kg bw/day, respectively (Liwska, 2013a). On Day 15 of the study, animals were paired (1 male:1 female) for a maximum of 14 days. Following mating, males were returned to their original cage and the females were transferred to an individual cage. Pregnant females were allowed to give birth and maintain their offspring to Day 5 *post-partum* and were then euthanized. Male rats were euthanized on Day 43. Lower food consumption and food efficiency was observed at the high dose in both sexes which correlated with decreased body weight gain. Also, at the highest dose, there were differences in group mean corpora lutea counts compared with concurrent and historical control values, but there was no obvious effect upon reproductive performance or apparent impairment of estrous cyclicity in the majority of females. No increase in neonatal mortality was observed but there was an indication of reduced individual offspring body weight gain from Day 1 to Day 4 *post-partum*. Due to treatment-related effects observed in maternal rats and in offspring *pre-* and *post-partum* at the highest dose tested of 810 mg/kg bw/day, the NOAEL was determined to be 277 mg/kg bw/day for systemic toxicity in the adult rats and for reproduction and offspring development (Liwska, 2013a).

A 90-day dietary study on patchouli oil observed no adverse effects for male FDRL rats dosed at 11.9 mg/kg bw/day and female FDRL rats dosed at 14.5 mg/kg bw/day (Oser et al., 1965). The test substance was diluted in cotton seed oil at a concentration of 2% in the diet. During the study, observations of body weight and food consumption were taken. At Weeks 6 and 12, hematological and blood chemistry analyses were performed. At the end of the study, the animals were autopsied during which liver and kidney weights were measured and tissues were collected for histopathology. No adverse effects were observed for patchouli oil in this study (Oser et al., 1965).

7.2.8. Tea Tree Oil

In a reverse mutation assay, *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2 *uvrA,* were incubated with tea tree oil at concentrations up to 2000 μg/plate in both the presence and absence of S9 metabolic activation. The tea tree oil sample was characterized by gas chromatography and was composed of 39.1% 4-carvomenthenol (terpinen-4-ol), 20.4% *p*-mentha-1,4-diene (γ-terpinene), 9.2% *p*-mentha-1,3-diene (α-terpinene), 4.1% eucalyptol (1,8-cineole) and other minor components. This test material was not mutagenic under the conditions studied (Evandri et al., 2005). In a similar reverse mutation study, commercially available tea tree oil was tested in *S. typhimurium* strains TA98, TA100 and TA102 in the presence and absence of an S9 metabolic activation system, and there were no induced increases in revertant mutant colonies up to 5000 μg/mL (Fletcher et al., 2005). In human peripheral blood lymphocytes, tea tree oil neither increased the frequency of micronuclei nor the frequency of chromosomal aberrations at concentrations ranging from 95 to 365 μg/mL (Pereira et al., 2014). In all three *in vitro* genotoxicity studies, tea tree oil was non-genotoxic.

7.2.9. Summary of genotoxicity data

With the exception of the non-OECD compliant studies reported by Di Sotto and colleagues (Di Sotto et al., 2008, 2011), assays on the tertiary alcohols, linalool, 4-carvomenthenol, α-terpineol, terpineol (isomeric mixture) and patchouli alcohol and related esters, α-terpinyl acetate and the isomeric mixture of terpinyl acetate (summarized in Table 6) were negative for genotoxicity. Similarly, genotoxicity assays on bois de rose oil, cardamom seed oil, clary oil, coriander seed oil, guaiac wood oil, lavender oil, patchouly oil and tea tree oil were negative. A positive result reported for lavender oil tested in an *in vitro* micronucleus assay (also conducted by Di Sotto and colleagues) and a non-OECD compliant reverse mutation assay in *S. typhimurium* were not considered relevant to the safety evaluation of lavender oil. Overall, the weight of evidence indicates no concern for genotoxicity for the NFCs under consideration.

8. Recognition of GRAS status

The 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) Expert Panel in 1965 and in subsequent years. Upon application of the safety procedure, it was concluded that the NFCs listed in Table 7 do not present safety concerns. There are adequate margins of safety using conservative estimates of exposure and NOAEL values from short and long-term toxicity studies. Also, the weight of evidence indicates a lack of genotoxic potential for these flavorings. These data indicate that there is no significant safety concern and support the FEMA Expert Panel's affirmation of GRAS status for these NFCs as flavoring ingredients in food under conditions of intended use.

CRediT authorship contribution statement

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Declaration of competing interest

Drs. Fukushima, Cohen, Eisenbrand, Gooderham, Guengerich, Hecht, Rietjens and Rosol are members of the Expert Panel of the Flavor and Extract Manufacturers Association. The FEMA Expert Panel's

Statement on Conflict of Interest Protections and Procedures is available at https://www.femaflavor.org/gras#conflict. Authors Davidsen, Harman, Lu and Taylor are employed by Verto Solutions, which provides scientific and management support services to FEMA.

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Appendix A. Supplementary data

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