



FEMA GRAS assessment of natural flavor complexes: Lemongrass oil, chamomile oils, citronella oil and related flavoring ingredients

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ABSTRACT

In 2015, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) initiated a program for the re-evaluation of the safety of over 250 natural flavor complexes (NFCs) used as flavor ingredients. This publication, eleventh in the series, evaluates the safety of NFCs characterized by primary alcohol, aldehyde, carboxylic acid, ester and lactone constituents derived from terpenoid biosynthetic pathways and/or lipid metabolism. The scientific-based evaluation procedure published in 2005 and updated in 2018 that relies on a complete constituent characterization of the NFC and organization of the constituents into congeneric groups. The safety of the NFCs is evaluated using the threshold of toxicological concern (TTC) concept in addition to data on estimated intake, metabolism and toxicology of members of the congeneric groups and for the NFC under evaluation. The scope of the safety evaluation does not include added use in dietary supplements or any products other than food. Twenty-three NFCs, derived from the *Hibiscus*, *Melissa*, *Ricinus*, *Anthemis*, *Matricaria*, *Cymbopogon*, *Saussurea*, *Spartium*, *Pelargonium*, *Levisticum*, *Rosa*, *Santalum*, *Viola*, *Cryptocarya* and *Litsea* 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.

Abbreviations: ADI, Acceptable daily intake; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; CAC, Codex Alimentarius Commission; CBPI, Cytokinesis-block proliferation index; CF, Correction factor; CFR, Code of Federal Regulations (USA); CHO, Chinese hamster ovary (cells); DTC, Decision tree class; ECHA, European Chemicals Agency; ECHA, European Flavour Association; EFSA, European Food Safety Authority; 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; GD, Gestation day; GLP, Good laboratory practices; GRAS, Generally recognized as safe; GST, Glutathione S-transferase; HPBL, Human peripheral blood lymphocytes; HRPT, Hypoxanthine (guanine) phosphoribosyltransferase (assay); IFEAT, International Federation of Essential Oils and Aroma Trades; IOFI, International Organization of the Flavor Industry; JECFA, Joint FAO/WHO Expert Committee on Food Additives; JFFMA, Japan Fragrance and Flavor Materials Association; LOD, Limit of detection; MoS, Margin of safety; NFC, Natural flavor complex; NTP, National Toxicology Program; NOAEL, No observed adverse effect level; OECD, Organization for Economic Co-Operation and Development; OFAS, Office of Food Additive Safety (FDA); PCE, Polychromatic erythrocytes; PCI, Per capita intake; PHA, Phytohaemagglutinin; PPD, Postpartum day; RBC, Red blood cell; SCE, Sister chromatid exchange (assay); TD₅₀, Dose giving a 50% tumor incidence; TTC, Threshold of toxicological concern; WHO, World Health Organization.

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1. Introduction

For six decades, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) has been the primary, independent body evaluating the safety of flavoring ingredients for use in human foods in the United States. Flavor ingredients are evaluated for “generally recognized as safe” (GRAS) status for intended use consistent with the 1958 Food Additive Amendment to the Federal Food Drug and Cosmetic Act (Hallagan and Hall, 1995, 2009; Hallagan et al., 2020). To date, the FEMA Expert Panel has determined that over 2700 flavoring ingredients have met the GRAS criteria for their intended uses.

A key part of FEMA’s GRAS program is the cyclical re-evaluation of FEMA GRAS flavoring ingredients. FEMA GRAS flavoring ingredients may be described as chemically defined or as complex mixtures known as natural flavor complexes (NFCs). The FEMA Expert Panel is in the process of re-evaluating the safety and GRAS status for NFC flavoring ingredients previously evaluated and determined to be GRAS by the Panel (Hall and Oser, 1965; Newberne et al., 1998; Oser et al., 1985). The scientifically-based procedure used in the safety evaluation by the Panel was first published in 2005 (Smith et al., 2005) and was reviewed and updated in 2018 (Cohen et al., 2018a). This re-evaluation, initiated in 2015, began with the evaluation of 54 Citrus-derived NFCs (Cohen et al., 2019) followed by several other groups: *Mentha*, dill, buchu and caraway-derived NFCs (Cohen et al., 2020), *Cassia*, *Cinnamomum* and *Myroxylon*-derived NFCs (Rietjens et al., 2020), clove, cinnamon leaf and West Indian bay leave-derived NFCs (Gooderham et al., 2020), lavender, guaiac, coriander-derived and related NFCs (Fukushima et al., 2020), *Eucalyptus*-derived and other eucalyptol containing NFCs (Eisenbrand et al., 2021), *Origanum* oil, thyme oils and related phenol derivative-containing NFCs (Cohen et al., 2021), asafetida, onion and garlic oils (Davidsen et al., 2023a), allspice, anise fennel-derived and related NFCs (Rietjens et al., 2023) and basil, nutmeg, parsley, tarragon and related allylalkoxybenzene-containing natural flavor complexes (Davidsen et al., 2023b). The identity of all FEMA GRAS flavor ingredients and the safety information supporting FEMA GRAS status have been submitted by FEMA to the U.S. Food and Drug Administration for inclusion in the Substances Added to Food Inventory. The inventory is maintained by the FDA Office of Food Additive Safety (OFAS) (<https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus>), and is also listed in the Flavor Ingredient Library on the FEMA Flavor website (<https://www.femaflavor.org/flavor-library>).

The procedure for the safety evaluation requires a complete constituent characterization and usage data for each NFC. The constituents are organized into congeneric groups based on their structure. Information is gathered on the annual usage, metabolism and toxicology, including genotoxicity, for each constituent congeneric group and the threshold of toxicological concern (TTC) approach is applied (Kroes et al., 2000). The TTC approach is used by both the European Food Safety Authority (EFSA) and the World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) in their evaluation of the safety of flavoring ingredients used in food (EFSA, 2016; JECFA, 1997, 2016). In addition, the potential toxicity and intake of the fraction of unidentified constituents is evaluated within the procedure. The constituent profiles of the NFCs under consideration in this manuscript are generally described by the presence of saturated and unsaturated primary alcohols, aldehydes, acids and related compounds such as citral, citronellal, citronellol, geraniol and santalol and lactones such as masoia lactone, *cis/trans*-ligustilide and dehydrocostus lactone.

In 2018, the FEMA Expert Panel issued a call for data requesting detailed chemical analysis for the NFCs listed in Table 1. Members from the International Organization of the Flavor Industry (IOFI), including FEMA, the Japan Fragrance and Flavor Materials Association (JFFMA), the European Flavour Association (EFFA) and the International Federation of Essential Oils and Aroma Trades (IFEAT), provided data on these NFCs derived from the *Hibiscus*, *Melissa*, *Ricinus*, *Anthemis*, *Matricaria*, *Cymbopogon*, *Saussurea*, *Spartium*, *Pelargonium*, *Levisticum*, *Rosa*,

Table 1

NFCs evaluated by the FEMA Expert Panel.

Name	FEMA No.	Estimated Intake (µg/person/day) ^a	Most Recent Annual Volume (kg) ^b
Ambrette Absolute Oil (<i>Hibiscus abelmoschus</i> L.)	2050	1.2	12
Balm Oil (<i>Melissa officinalis</i> L.) – Balm lemon oil, Melissa oil	2113	1.7	17
Castor Oil (<i>Ricinus communis</i> L.) – Ricinus oil, Palma Christi oil, Tangan-tangan oil	2263	690	6690
Chamomile Flower English Oil (<i>Anthemis nobilis</i> L.) ^c	2272	0.7	7
Chamomile Flower Hungarian Oil (<i>Matricaria chamomilla</i> L.)	2273	50	480
Chamomile Flower Roman Extract (<i>Anthemis nobilis</i> L.)	2274	50	460
Chamomile Flower Roman Oil (<i>Anthemis nobilis</i> L.) ^c	2275	60	650
Citronella Oil (<i>Cymbopogon nardus</i> Rendle and <i>Cymbopogon winterianus</i> Jowitt)	2308	190	1830
Wine Lees Green Oil	2331	10	100
Wine Lees White Oil	2332	6	61
Costus Root Oil (<i>Saussurea lappa</i> Clarke, <i>Aplotaxis lappa</i> Dec., <i>A. suriculata</i> DC, <i>Auklandia costus</i> Falc.) – Spiral flag oil	2336	1.1	11
Genet Absolute (<i>Spartium junceum</i> L.)	2504	4	47
Genet Extract (<i>Spartium junceum</i> L.) – Broom extract	2505	0.4	4 ^d
Geranium Rose Oil (<i>Pelargonium graveolens</i> L’Her)	2508	190	1810
Lemongrass Oil (<i>Cymbopogon citratus</i> DC. and <i>C. flexuosus</i> Stapf)	2624	410	3980
Lovage Root Extract (<i>Levisticum officinale</i> Koch) – Smellage extract, Smallage extract	2650	54	520
Lovage Root Oil (<i>Levisticum officinale</i> Koch) – Smellage oil, Smallage oil	2651	120	1170
Palmarosa Oil (<i>Cymbopogon martini</i> (Roxb.) Stapf)	2831	62	600
Rose Absolute (<i>Rosa alba</i> L., <i>Rosa centifolia</i> L. and varieties of these spp.)	2988	9	91
Rose Bulgarian True Otto Oil (<i>Rosa damascena</i> Mill.) – Attar of roses	2989	4	43
Sandalwood Yellow Oil (<i>Santalum album</i> L.) – Sandalwood East Indian oil, Saunders white oil	3005	17	160
Violet Leaves Absolute (<i>Viola odorata</i> L.)	3110	17	160
Masaoia Bark Oil (<i>Cryptocarya masoio</i>)	3747	0.04	0.4
<i>Litsea Cubeba</i> Berry Oil (<i>Litsea cubeba</i> Pers.) – May chang	3846	8	85

^a All NFCs have a surveyed volume less than 22,700 kg/year. The PCI x 10 (“eaters only”) calculation is shown.

^b Harman, C.L. and Murray, I.J., 2018. 2015 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association of the United States (FEMA), Washington DC, USA.

^c Chamomile Flower English Oil (FEMA 2272) and Chamomile Flower Roman Oil (FEMA 2275), derived from *Anthemis nobilis* L., are the same NFC. They will be combined under FEMA 2275 in Appendix A and for the safety evaluation.

^d Gavin, C.L., Williams, M.C., Hallagan, J.B., 2008. 2005 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association, Washington, DC, USA.

Santalum, *Viola*, *Cryptocarya* and *Litsea* genera currently used for flavoring food and beverage products.

2. History of food use

Most of the botanicals in this evaluation have a long history of use and for many of these botanicals, their essential oils and extractives have been used in the flavoring of foods and beverages and perfumery since the essential oils became available in commerce in the mid-to late eighteenth century. Several NFCs under consideration, such as Balm Oil (FEMA 2113), Citronella Oil (FEMA 2308) and Lemongrass Oil (FEMA 2624), have a lemon-like character. The herbs and grasses from which they are derived are rich in citral, citronellal, citronellol and geraniol. For over 2000 years, balm (*Melissa officinalis* L.) has been used as a sweetening herb. Its generic name is derived from the Greek for “bee” in reference to the use of flowers to attract bees for honey production (Kains, 1920; Tucker and DeBaggio, 2000). Balm is often grown in kitchen gardens and used to flavor salads, eggs, vegetables, poultry, fish, dressings, soups, stuffings, stews, sauces, desserts and beverages in addition to its use in teas, medicines and perfumes (Freeman, 1943; Guenther, 1950; Kains, 1920; Khan and Abourashed, 2010; Ravindran et al., 2006). Citronella oil is derived from steam distillation of *Cymbopogon nardus* Rendle (Ceylon type) or *Cymbopogon winterianus* Jowitt (Java type) (Baser and Buchbauer, 2010). In addition to the use of its leaves in various medicinal remedies, the oil of citronella is used as an insect repellent and in soaps and perfumes (Guenther, 1950; Khan and Abourashed, 2010). Citronella oil is used in alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings and breakfast cereals and fresh citronella leaves are used to garnish South and Southeast Asian rice and meat dishes (Ravindran, 2017).

Lemongrass oil is derived from *Cymbopogon flexuosus* (D.C.) Stapf (East Indian type), native to Southern India or *Cymbopogon citratus* (D. C.) Stapf. (West Indian type) (Khan and Abourashed, 2010). In addition to its use in teas, wines, sauces, stews, beverages and sherbets, the leaves and stem of lemongrass are popular in Caribbean, South Asian and Southeast Asian cuisines to flavor pork, beef and fish dishes. Leaves of young lemongrass are also used to garnish rice and curry dishes (Ravindran, 2017). Consumption of this herb in the United States has increased with the increasing popularity of Southeast Asian cuisine (Ravindran, 2017). The West Indian variety was commonly used in traditional Chinese medicine and is also currently used as a fragrant addition to soaps, perfumes and lotions. In food, it is mainly used to flavor candy and baked goods but can also be found in alcoholic and non-alcoholic beverages, frozen dairy desserts, gelatins and puddings, meat and meat products and fats and oils (Khan and Abourashed, 2010). Another natural source of citral is the oil derived from the berries of *Litsea cubeba* Pers that is primarily cultivated and produced in China (Lawrence, 1985, 2011; Robbins, 1983). The botanical has been used in traditional Chinese medicine for various ailments (Kong et al., 2015).

Ambrette (*Hibiscus abelmoschus* L.), also known as abel-musk, is native to India and cultivated in tropical climates (Guenther, 1952). Its essential oil and tincture are produced in Europe and North America for perfumery and cosmetic applications in pomades, extracts, bouquets, oils and powders and to flavor wines, bitters, non-alcoholic beverages, sweets, baked goods and desserts (Arctander, 1960; Guenther, 1952).

Historically, the rose-scented oils and waters have been used as flavoring ingredients and in fragrance applications. Rose oil (*Rosa damascena* Mill), otherwise known as “Otto of Rose” or “Attar of Rose”, obtained its name when a consort of the Indian Mughal emperor studied the thin membrane of oil on the surface of the rose water that filled the canals of the palace gardens and termed it “Atr-i-Jehangiri” (Guenther, 1952a). Distillation of roses dates to the eighth and ninth century Arabic civilization, and later, the city of Shiraz in Persia became the center for the rose industry during the tenth to seventeenth centuries (Guenther, 1952a). The increasing availability of East – West trade routes allowed

for the use of these materials to spread from Persia to India, China, North Africa and western Europe (Guenther, 1952a). Although rose flowers, rose oil and rose water are commonly thought of as ingredients for perfumery, rose water has also been used for flavoring cakes, cookies and pastries in Europe since the medieval period (Kirker and Newman, 2016). By the nineteenth century, Bulgaria became the leading producer of rose oil after introduction of the botanical by Ottoman Turkish merchants (Guenther, 1952a; Kovacheva et al., 2010). During the mid-nineteenth century when rose oil became rare and expensive due to reduced production in Bulgaria after nationalization of its rose industry, use of rose geranium oil (*Pelargonium graveolens* L’Her) became more prevalent (Demarne, 2002; Kovacheva et al., 2010). Rose geranium originates from South Africa and was introduced to Europe in the early seventeenth century through shipping expeditions and trade at the Cape of Good Hope (Lis-Balchin, 2002). The *graveolens* variety has a mint-like aroma (Blerot et al., 2016; Lis-Balchin, 2002) and *P. graveolens* L’Herit. ex Ait is regarded as the parent plant for all varieties used for production of geranium oil (Guenther, 1950; Seidemann, 2005). Palmarosa oil derived from *Cymbopogon martini* (Roxb.) Stapf, otherwise known as Indian or Turkish geranium oil, has a rose-like aroma. This essential oil has historically been used to impart a sweet, floral character in perfumes, cosmetics and flavorings (Akhila, 2010, de Groot and Schmidt, 2016).

The chamomile essential oils and extracts, like the rose-derived NFCs, are derived from the flowers of the botanical. As early as the 4th and 5th centuries, chamomile has been used medicinally as infusions to treat various ailments. The name “chamomile” is derived from Greek, noting a low (*kamai-*) flowering plant smelling of apples (*melon*) (Franke, 2005b; Grieve, 1970). *Anthemis nobilis* L. is referred to mainly as Roman chamomile or English chamomile. German or Hungarian chamomile (*Matricaria chamomilla*) oil is also known as “blue” chamomile (Lawless, 1995). Aside from its widely known use in teas, chamomile (specifically its essential oil) has been historically used to flavor liqueurs (Grieve, 1970).

Flowers are also the source material for genet absolute and genet extracts. Genet is known as Spanish broom because its branches were once commonly used in basket and broom making. The genet plant is native to the Mediterranean region and is currently cultivated in southern France and Italy for the production of genet absolute and genet extracts (Anonis, 1991).

The costus plant is indigenous to the Himalayan region of India, Pakistan and China where the root of the plant has historically been an ingredient in traditional Tibetan, Chinese and ayurvedic medicines. Costus root oil, an essential oil, is also used as incense and as a perfumery ingredient (Guenther, 1952a; Zahara et al., 2014).

East Indian sandalwood (*Santalum album* L.) originates from India and has spiritual, medicinal and commercial significance in the Hindu and Parsi communities (Guenther, 1952a; Kumar et al., 2012). Due to its popularity, sandalwood is also now cultivated in Asia and Australia (Arunkumar et al., 2012, 2019). Although sandalwood oil is used in the flavoring of foods and beverages, the unprocessed leaves and wood of the plant are not typically used as a food ingredient.

Massoia (*Cryptocarya massoia*) is an evergreen tree that is native to Papua. The bark of the tree has been used to prepare an essential oil that has historically been used as medicine, perfume and flavoring for food. It is often used as an ingredient in curry and rice-based foods (Rali et al., 2007).

Castor (*Ricinus communis* L.) is known as *Kiki* by the Greeks and *Palma Christi* by the Romans for the palm-like shape of its leaves (Gaginella et al., 1998). Production of castor oil from the bean of the plant started in the mid-nineteenth century with over twenty operational mills in the United States (Brigham, 1993). The demand and value of castor oil increased in the early 20th century due to its use in hydraulic fluids and lubricants for military equipment during the World Wars and other conflicts in the aftermath of World War II (Brigham, 1993). Production decreased in the 1970s due to low demand and high

competition between manufacturers (Brigham, 1993). Castor oil is a laxative when consumed at very high doses. It has medicinal uses as a drug delivery vehicle and as an excipient in pharmaceuticals (Patel et al., 2016). Castor oil is also used as a vegetable oil for cooking as well as to provide flavor to food (Patel et al., 2016).

Lovage, a leafy plant, is a member of the *Apiaceae* family. The lovage plant can be grown in kitchen gardens and is cultivated in Europe. It adds a celery-like flavor when used in soups, salads and sauces (Tucker and DeBaggio, 2000). Lovage root oil and extracts are used to flavor a variety of foods and are also used in perfumery applications (Grieve, 1970; Guenther, 1950).

Wine lees oil is produced from the particles of autolyzed yeast and grape skins, called wine lees, a by-product in the manufacture of cognac (*eau-de-vie de vin*). Traditionally, cognac, developed in the early seventeenth century, is obtained by double distillation of wine from the Cognac region of France (Lurton et al., 2012). Both green and white wine lees oils are obtained from steam distillation of wine lees, with the color dependent on the processing conditions.

3. Current usage

The estimated intake and annual usage in the United States in 2015 for each NFC under consideration is provided in Table 1. Because all the NFCs have a surveyed annual usage of less than 22,700 kg/year, the estimated intake is calculated using the $PCI \times 10$ ("eaters only") method in which it is assumed that annual usage of the NFC is consumed by 10% of the population. Castor Oil (FEMA 2263) has the largest reported annual usage, 6690 kg, of the NFCs listed in Table 1. NFCs with lemon-like characteristics have annual usage ranging from 3980 kg for Lemongrass Oil (FEMA 2624) and 1830 kg for Citronella Oil (FEMA 2308) to less than 100 kg for *Litsea Cubeba* Berry Oil (FEMA 3846) and Balm Oil (FEMA 2113). The NFCs with a rose-like characteristic have annual usages ranging from 1810 kg for Geranium Rose Oil (FEMA 2508) to 43 kg for Rose Bulgarian True Otto Oil (FEMA 2989) with intermediate usage reported for Palmarosa Oil (FEMA 2831) and Rose Absolute (FEMA 2988). Table 1 lists four NFCs derived from chamomile flowers, Chamomile Flower English Oil (FEMA 2272), Chamomile Flower Hungarian Oil (FEMA 2273), Chamomile Flower Roman Extract (FEMA 2274) and Chamomile Flower Roman Oil (FEMA 2275), with annual usage ranging from 7 to 650 kg. Chamomile Flower English Oil (FEMA 2272) and Chamomile Flower Roman Oil (FEMA 2275) have been determined to be identical NFCs. These NFCs are combined in the safety evaluation under FEMA 2275 with a combined annual usage of 660 kg and combined estimated intake of 70 $\mu\text{g/kg bw/day}$. The annual usage for Lovage Root Oil (FEMA 2651) and Lovage Root Extract (FEMA 2650), which are derived from a culinary herb popular in Europe, was 1170 kg and 520 kg, respectively. For the remaining NFCs listed in Table 1, the annual reported usage in the USA in 2015 was less than 200 kg.

Several NFCs are derived from botanicals that are used as a spice, herb or food. These botanicals include balm, chamomile, lemongrass, lovage root, rose and violet leaves. As part of the safety evaluation, the consumption of the NFCs of these botanicals from food compared to their consumption as flavoring added to food is evaluated. However, currently, data are not available on the annual usage of balm, chamomile, lemongrass, lovage root, rose and violet leaves despite their widespread use. Balm and lemongrass are popular in South Asian cuisine and lovage root is a widely used culinary herb in Europe. Chamomile teas are widely available and rose water and violet leaves are commonly used in baked goods. Because of the lack of quantitative data on these uses, however, a consumption ratio cannot be calculated.

4. Manufacturing methodology

Several of the NFCs are prepared using distillation techniques. The essential oils of the *Cymbopogon* genus, Citronella Oil (FEMA 2308),

Lemongrass Oil (FEMA 2624) and Palmarosa Oil (FEMA 2831) are distilled from the leaves of the plant. Citronella Oil (FEMA 2308) is produced by the steam distillation of the leaves of the *Cymbopogon nardus* Rendle plant (Ceylon type) or the *C. winterianus* Jowitt plant (Java type) (Akhila, 2010; Guenther, 1950). The Ceylon variety is native to Sri Lanka and is cultivated in other South Asian countries (Bhattacharya, 2015; Guenther, 1950). Typically, a freshly cut grass yields approximately 0.5–1% essential oil (Akhila, 2010). The dried grass material is harvested from the field and cut into smaller sizes to obtain a higher essential oil yield before steam distillation (Akhila, 2010; Guenther, 1950). Lemongrass is cultivated in the Southwestern region of India (*C. flexuosus* Stapf.) and in the West Indies and Central and South America (*C. citratus* DC). Distillation of fresh lemongrass yields 0.25–0.6% of essential oil, depending on the cultivar (Akhila, 2010). Palmarosa (*C. martini*) oil is manufactured by a steam or hydro-distillation process of its flowering shoots, leaves and stems, with yields in the range of 0.4–0.45% essential oil (Akhila, 2010). Up to 92% of the essential oil present in the starting material is obtained in the distillation process (Akhila, 2010). A hexane extraction of the distillate may be performed to obtain the remaining 8% lost during steam distillation (Akhila, 2010).

Balm (*Melissa officinalis* L.), also called lemon balm, is native to Europe, the Mediterranean, central Asia and Iran and is cultivated worldwide (Guenther, 1949; The Herb Society of America, 2007). The leaves are added to foods and beverages for flavor or used in teas (Singh et al., 2014). Balm essential oil is obtained by distillation of the aerial plant parts, with yields up to 0.1% essential oil (Lawrence, 1989; Singh et al., 2014). The rhizomes of lovage are used in the preparation of lovage root extract and are steam distilled to produce lovage essential oil (FCC, 2021c; Guenther, 1950). Similarly, costus roots are dried and subsequently ground into finer particulate matter prior to steam distillation to yield its essential oil (Guenther, 1952a; Lawrence, 1978). Alternatively, a resinoid can be isolated from the roots, which can also be extracted with solvents followed by vacuum distillation to obtain the essential oil (FCC, 2021b; Guenther, 1952a).

Two NFCs in Table 1 are derived from tree woods. Sandalwood (*Santalum album* L.) is native to India and the Malay peninsula and is currently cultivated in Australia and other Southeast Asian countries (Arunkumar et al., 2019; Arunkumar et al., 2012; Guenther, 1952a; Perfumer & Flavorist, 2015). Sandalwood oil is manufactured by water distillation of the soaked and fire-heated wood or steam distillation of the chopped wood in copper stills for 48–72 h (Guenther, 1952a). Massoia bark oil is obtained by the steam distillation or hydro-distillation of the bark of the tree native to Indonesia (Garnero and Joulain, 1983; Lawrence, 1984).

Several NFCs are derived from the flowers of botanicals. Rose oil is obtained from *Rosa damascena* Mill. by water distillation of the flowers of the plant, predominately in Turkey and Bulgaria (Baser et al., 2003; Bleimann, 2019). The initial product that separates from the still is the crude "first oil". The distillate is then redistilled to obtain the "second oil" also known as the "cooked oil". These two oil products are mixed to obtain the final commercial product (Baser et al., 2003). Rose geranium oil is produced from steam distillation or primitive direct-fire distillation of the leaves of *Pelargonium roseum* mainly in China and also in Egypt, Reunion Island and India (Blerot et al., 2016; Lis-Balchin, 2002).

Chamomile is native to Europe but can also be found growing in other temperate climates. Harvested flowers are collected when the majority of the flowers have bloomed and are passed through sieves to separate the flowers from other seeds and wing petals (Böttcher et al., 2005; Franke, 2005a). Chamomile oils are prepared by the steam distillation of fresh or dried flowering heads, with the highest concentration of the volatile oil collected just prior to blooming (Franke, 2005b; Schilcher et al., 2005). Flower heads can be air dried or dried with the use of mechanical assistance (Böttcher et al., 2005). Chamomile extracts are prepared from the flowers by solvent extraction (Carson et al., 2013).

The flowers of genet, commonly known as broom, are the source of the absolute and other extracts. Genet extract can be obtained by alcoholic extraction of the freshly picked genet flowers. Genet flowers may also be extracted with a non-polar solvent, followed by evaporation of the solvent to yield an extract known as a concrete. Genet absolute, like other absolutes, are prepared from the concrete by mixing with ethanol, heating the solution, followed by a cooling step, filtering of the mixture to remove waxes and finishing by evaporating off the ethanol (Anonis, 1991; Guenther, 1952a).

Three NFCs are derived from the seeds or fruits of the plant. Castor (*Ricinus communis* L.) is predominately cultivated in India, Africa, South America, Brazil and China (Patel et al., 2016). The seeds of the plant contain approximately 30–50% fixed oil, which is extracted using mechanical pressing, solvent extraction or a combination of both methods. In mechanical pressing, the seeds are cooked in a steam-jacket to remove moisture (Patel et al., 2016). Next, during prepressing, a screw or hydraulic press crushes the seeds to remove the oil. Up to 80% of the oil present can be obtained through this process. The extracted oil is filtered, mixed with another batch of seeds undergoing further extraction to maximize product yield. A press-cake of crushed castor beans containing 10% of oil is ejected from the press and is subject to solvent extraction using hexane, heptane or petroleum ether to obtain the remaining oil. Ricin, a protein found in castor beans that is toxic to humans and animals, remains in the seed cake. Any minor amounts extracted with the oil are removed in the refining of the extracted oil (Patel et al., 2016).

Steam distillation of crushed ambrette seeds (*Hibiscus abelmoschus* L.) produces a crude solid, concrete-like oil that is then extracted with alcohol or treated with calcium, lithium or magnesium salts to remove fatty acids, to yield 0.2–0.6% essential oil (Arctander, 1960; Guenther, 1952). Ambrette absolute oil is also the essential oil of ambrette seeds produced by removal of fatty acids from the crude oil. Alternatively, the absolute oil may be steam-distilled from the concrete produced by hydrocarbon extraction of the crushed seeds (Arctander, 1960). Lastly, white and green wine lees oils are obtained from the steam distillation of the lees, which are yeast and grape skin particulates that are a by-product in the production of cognac (Lawrence, 1979).

5. Chemical composition

The NFCs presented in Table 1, with the exception of Castor Oil (FEMA 2263), were analyzed, and their volatile constituents were identified using gas chromatography-mass spectrometry (GC-MS) and quantified using a flame ionization detector (FID). Identified and unidentified GC peaks were reported as the percent area of the chromatogram. For Castor Oil (FEMA 2263), a description of the constituent profile was obtained from the Food Chemical Codex (FCC, 2021a). For each NFC, the constituent data were collected and analyzed as shown in Appendix A. In Appendix A, the constituents present at levels greater or equal to 1% are listed by their respective congeneric groups. The chemical structures of some common constituents of these NFCs are shown in Fig. 1. For each NFC, there is some small inherent variability in the constituent composition due to their botanical origin and differences in growth, harvesting and processing conditions.

The constituent profiles are summarized for each NFC, with the exception of Castor Oil (FEMA 2263), in the pie charts shown in Fig. 2, and show a large percentage of Group 3 (Aliphatic linear and branched chain α,β -unsaturated aldehydes and related alcohols, acids and esters) compounds in Ambrette Absolute Oil (FEMA 2050), Balm Oil (FEMA 2113), Chamomile Flower English/Roman Oil (FEMA 2275), Chamomile Flower Roman Extract (FEMA 2274), Citronella Oil (FEMA 2308), Geranium Rose Oil (FEMA 2508), Lemongrass Oil (FEMA 2624), Palmarosa Oil (FEMA 2831), Rose Bulgarian True Otto Oil (FEMA 2989)

and *Litsea Cubeba* Berry Oil (FEMA 3846). As noted earlier, Chamomile Flower English Oil (FEMA 2272) and Chamomile Flower Roman Oil (FEMA 2275) have been determined to be identical NFCs and have been combined under FEMA 2275. Group 5 (Unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters) constituents are major components of Citronella Oil (FEMA 2308), Rose Bulgarian True Otto Oil (FEMA 2989), Geranium Rose Oil (FEMA 2508) and Violet Leaves Absolute (FEMA 3110). Group 7 (Saturated alicyclic primary alcohols, aldehydes, acids and related esters) constituents noted in Fig. 1 are present in Sandalwood Yellow Oil (FEMA 3005). Constituents of Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters) are present in Wine Lees Green Oil (FEMA 2331) and Wine Lees White Oil (FEMA 2332), as well as in Genet Absolute (FEMA 2504) and Lovage Root Extract (FEMA 2650). The constituents of Group 13 (Aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones) are characteristic of Costus Root Oil (FEMA 2336), Lovage Root Extract (FEMA 2650), Lovage Root Oil (FEMA 2651) and Massoia Bark Oil (FEMA 3747). Ricinoleic acid from Group 8 (Saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and related esters) is the primary fatty acid component of the triglycerides of Castor Oil (FEMA 2263).

6. Safety evaluation

The procedure for the safety evaluation for NFCs is guided by a set of criteria as outlined in two publications (Smith et al., 2004, 2005) with a recent update (Cohen et al., 2018a). The updated procedure with a more detailed evaluation of unidentified constituents inherent in NFCs is summarized in Fig. 3. Briefly, the NFCs pass through a 14-step process; Step 1 requires the gathering of data and assesses the consumption of the NFC as a flavor ingredient relative to intake from the natural source when consumed as food; Steps 2 through 6 evaluate the exposure and potential toxicity of the identified constituents (organized by congeneric group) based on available data on metabolism and toxicity and on the application of the TTC (Kroes et al., 2000). Steps 7–12 address the potential toxicity, including genotoxicity, of the unidentified constituents; in Step 13 the overall safety is evaluated along with considerations of safety for use by children, given their lower body weights; lastly in Step 14, the final determination of GRAS status is made. Below, the safety evaluation is presented in which each step of the procedure (Cohen et al., 2018a) (provided in italics) is considered and answered for the NFCs under consideration.

Step 1

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

a. Consumption of foods from which the NFCs are derived

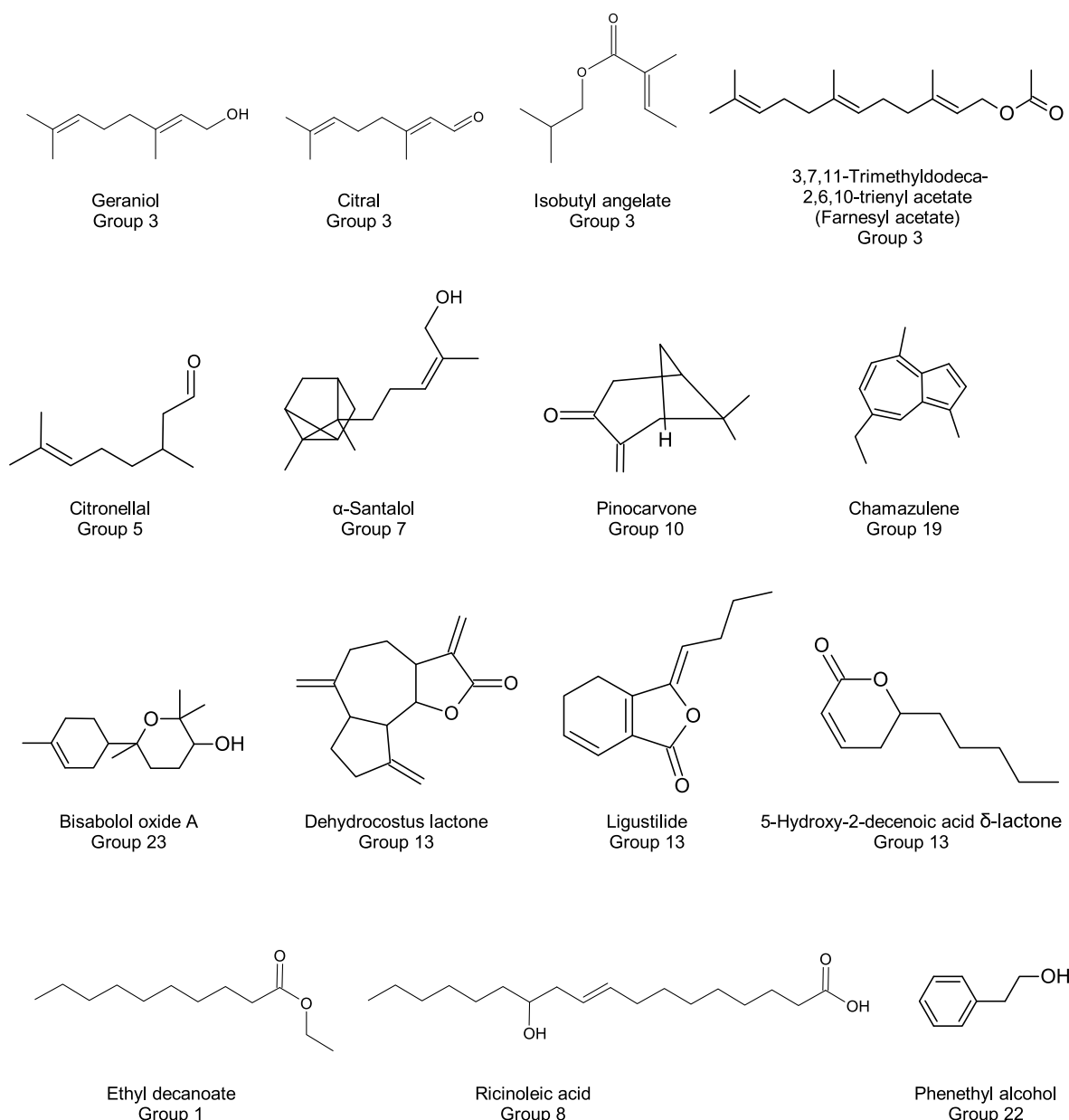


Fig. 1. Structures of commonly found constituents of the NFCs under evaluation and their congeneric group.

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:

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

where:

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

response rate from the most recently reported flavor industry volume-of-use surveys.

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

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

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

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

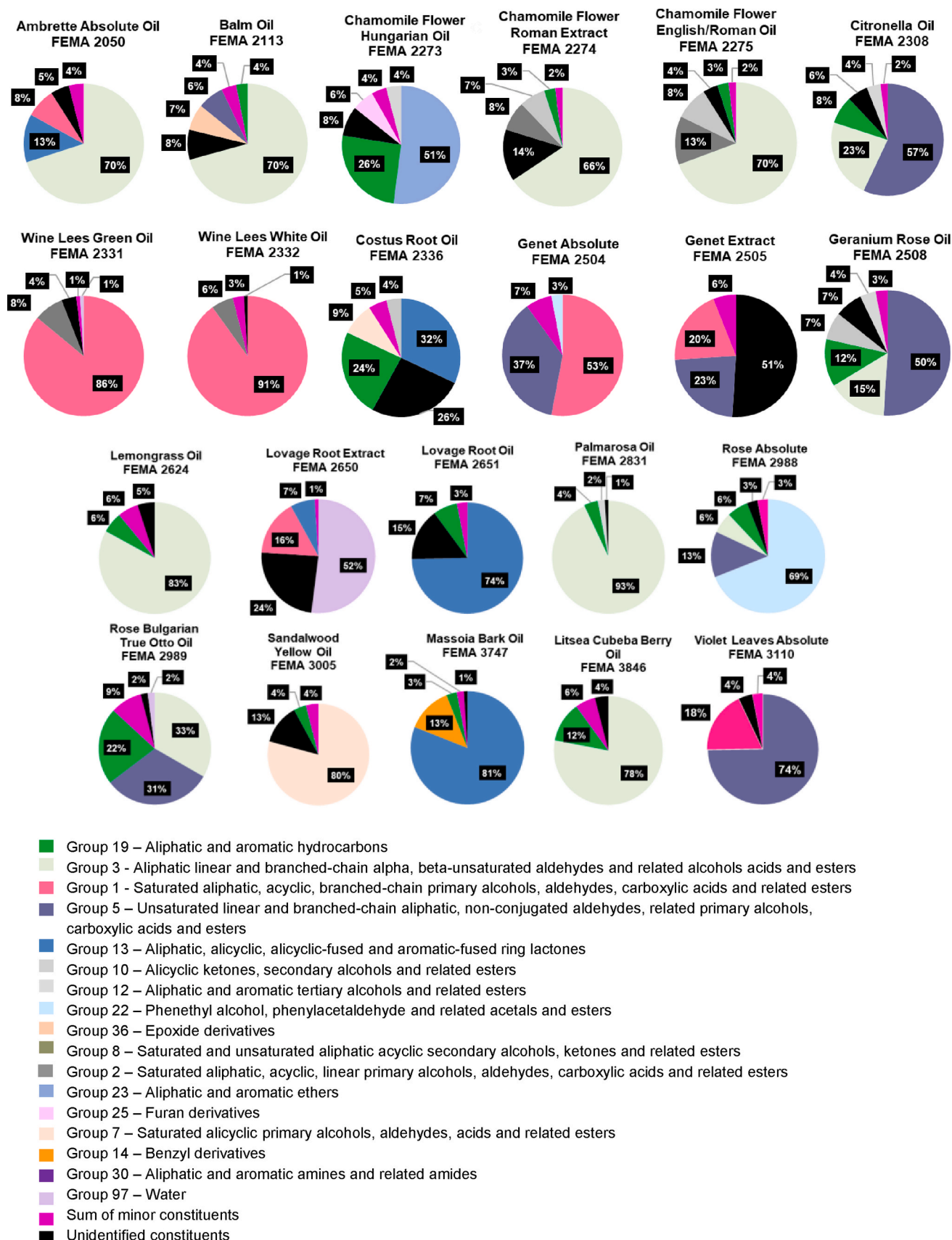
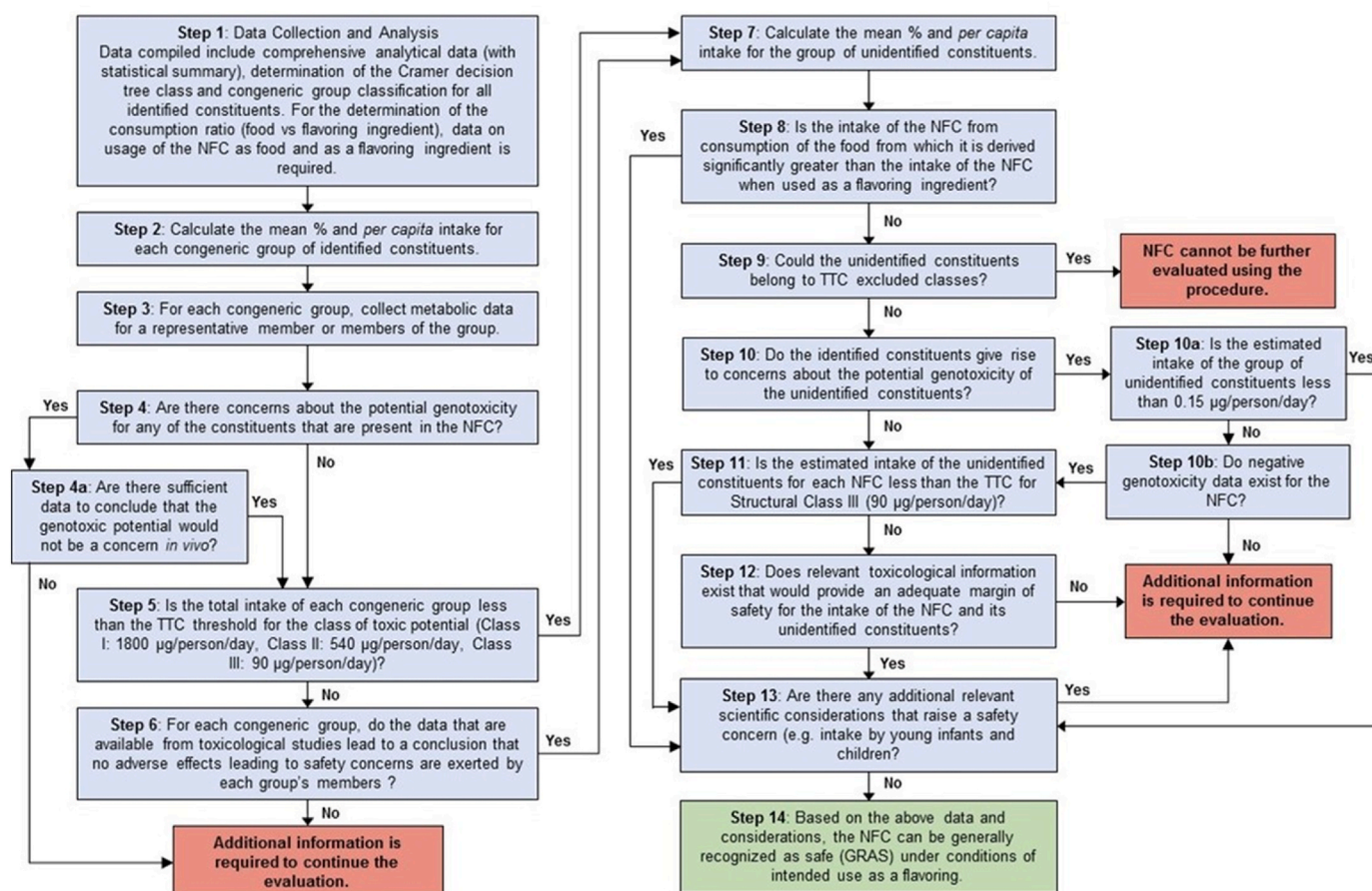


Fig. 2. Constituent congeneric group profiles for the NFCs under consideration. Castor Oil (FEMA 2263), which is composed of only triglycerides, is not shown in this figure.



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).

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).¹ At this Step, if the conditions of use² for the NFC result in levels that differ from intake of the same constituents in the food source, it should be reported.

For each NFC under consideration, the estimated *per capita* intake has been calculated and is presented in Table 1 and Appendix A. Several of the botanicals from which these NFCs are derived are commonly consumed as food. Lemongrass is widely used in Southeast Asian cuisine and teas (Ravindran, 2017), balm (lemon balm) and chamomile are consumed as herbal teas (Franke, 2005b; Kowalski et al., 2015), lovage root is used to flavor soups and salads (Venskutonis, 2016) and massoia bark is an ingredient in Indonesian curry (Rali et al., 2007). However, despite these common uses as food, quantitative data on their annual volumes of use are not available for the calculation of a consumption ratio.

b. Identification of all known constituents and assignment of Cramer Decision Tree Class

¹ See Stofberg and Grundschober (1987) for data on the consumption of NFCs from commonly consumed foods.

² 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.

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., 1976).

For each NFC under evaluation, the constituents were sorted by congeneric group and are presented in Appendix A in order from highest to lowest mean % (only constituents with a mean % greater than or equal to 1% are reported). Minor constituents of each congeneric group are summed and reported. Minor constituent congeneric groups are also aggregated and reported in the constituent table.

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). The congeneric groups are listed in Appendix A.

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.

The DTC for each congeneric group, determined by the most

conservative constituent in that group, is provided in [Appendix A](#).

Step 2

Determine (a) the mean percentage (%) of each congeneric group in NFCs, and (b) the daily per capita intake³ of each congeneric group. The value (a) is calculated by summing the mean percentage of each of the constituents within a congeneric group, and the value (b) is calculated from consumption of the NFC and the mean percentage.

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

Intake of congeneric group (μg / person / day)

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

where:

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

The intake of NFC (μg /person/day) is calculated using the $\text{PCI} \times 10$ or PCI equation as appropriate.

Proceed to Step 3.

In the summary report for each NFC provided in [Appendix A](#), the total mean% for each congeneric group is subtotaled and reported with the DTC and estimated intake (calculated using $\text{PCI} \times 10$ method) for each 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 metabolism of the members of each congeneric group would require additional considerations at Step 13 of the procedure.

Proceed to Step 4.

The constituent congeneric groups for each NFC are presented in [Appendix A](#). Sufficient metabolic data exist for each congeneric group or related compounds that indicate constituents of these groups are expected to be metabolized to innocuous products. The use of metabolic data in the safety evaluation of flavoring compounds and a summary of the expected metabolism of flavoring compounds by congeneric group is described in a FEMA Expert Panel publication ([Smith et al., 2018](#)). For this evaluation, the metabolism of Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters), Group 3 (Aliphatic linear and branched-chain α,β -unsaturated aldehydes and related alcohols, acids and esters), Group 5 (Unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters) and Group 7 (Saturated alicyclic primary alcohols, aldehydes, acids and related esters) constituents were reviewed. Monographs on flavoring ingredients of these groups, which include data on the metabolism of these substances, have been published by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) ([JECFA, 1997, 1998, 1999b, 2003, 2004b, 2006, 2007, 2011, 2012](#)). For other constituent congeneric groups such as Group 13 (Aliphatic and aromatic lactones), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 19 (Aliphatic and aromatic hydrocarbons) and Group 22 (Phenethyl alcohol, phenylacetaldehyde and related acetals and esters), the FEMA Expert Panel has published its safety evaluation of flavoring ingredients of these groups including reviews of metabolic studies ([Adams et al., 1998, 2005c, 2011; Marnett et al., 2014](#)). The Panel has also published assessments of other groups or individual constituents relevant for the evaluation of the NFCs under

consideration ([Adams et al., 1996, 2004, 2005b, 2005a, 2007, 2008](#)).

Step 4

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

If Yes, proceed to Step 4a.

If No, proceed to Step 5.

The FEMA Expert Panel has reviewed genotoxicity data on members of the constituent congeneric groups and concluded that these groups lack genotoxic potential with the exception of the allylalkoxybenzene constituents of Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives), methyl eugenol, estragole and myristicin that were reported to be present in Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989). Both *in vitro* and *in vivo* genotoxicity studies have been compiled and analyzed by JECFA for flavoring ingredients of Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters), Group 3 (Aliphatic linear and branched-chain α,β -unsaturated aldehydes and related alcohols, acids and esters), Group 5 (Unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters) and Group 7 (Saturated alicyclic primary alcohols, aldehydes, acids and related esters) ([JECFA, 1997, 1998, 1999b, 2003, 2004b, 2006, 2007, 2011, 2012](#)). In its review of the flavoring ingredients of these groups, JECFA identified no concern for genotoxic potential for the constituents of these groups. The FEMA Expert Panel has reviewed the data published by JECFA and the constituents listed in [Appendix A](#) and concurs that these compounds are not genotoxic. The FEMA Expert Panel has published safety evaluations of chemically defined flavoring ingredients for the following congeneric groups: Group 13 (Aliphatic and aromatic lactones), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 19 (Aliphatic and aromatic hydrocarbons) and Group 22 (Phenethyl alcohol, phenylacetaldehyde and related acetals and esters) and concluded that these groups lack genotoxic potential ([Adams et al., 1998, 2005c, 2011; Marnett et al., 2014](#)). Group 19 constituents reviewed in the safety evaluation of Citrus-derived NFCs were determined to be not genotoxic ([Cohen et al., 2019](#)) as well as Group 12 constituents reviewed in the safety evaluation of lavender, guaiac and coriander-derived and related NFCs ([Fukushima et al., 2020](#)) and Group 10 constituents reviewed in the safety evaluation of mint-derived and related NFCs ([Cohen et al., 2020](#)). The Panel has also published assessments of other groups of individual constituents relevant for the NFCs under consideration ([Adams et al., 1996, 2004, 2005b, 2005a, 2007, 2008](#)). A review of the minor constituents within the compositional profile of the NFCs under consideration also indicates the lack of genotoxic potential for the congeneric groups present. In addition, genotoxicity studies on individual constituents that have not been previously reviewed by JECFA that are relevant to the safety evaluation and on the NFCs are presented in the "Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation" section of this publication. Ambrette Absolute Oil (FEMA 2050), Balm Oil (FEMA 2113), Castor Oil (FEMA 2263), Chamomile Flower Hungarian Oil (FEMA 2273), Chamomile Flower English/Roman Oil (FEMA 2275), Citronella Oil (FEMA 2308), Wine Lees Green Oil (FEMA 2331), Wine Lees White Oil (FEMA 2332), Costus Root Oil (FEMA 2336), Genet Absolute (FEMA 2504), Genet Extract (FEMA 2505), Geranium Rose Oil (FEMA 2508), Lemongrass Oil (FEMA 2624), Lovage Root Oil (FEMA 2651), Palmarosa Oil (FEMA 2831), Sandalwood Yellow Oil (FEMA 3005), Violet Leaves Absolute (FEMA 3110), Massoia Bark Oil (FEMA 3747) and Litsea Cubeba Berry Oil (FEMA 3846) proceed to Step 5.

Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989) contain minor constituents belonging to

³ See [Smith et al., 2005](#) for a discussion on the use of $\text{PCI} \times 10$ for exposure calculations in the procedure.

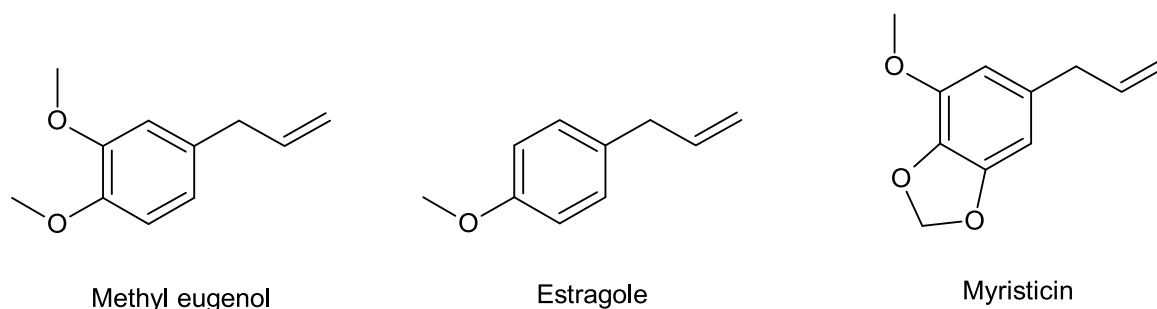


Fig. 4. Structures of methyl eugenol, estragole and myristicin.

Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives). The constituents, methyl eugenol, estragole and myristicin, possess an allylalkoxybenzene structural motif (see Fig. 4) that raises a genotoxicity concern (Rietjens et al., 2014). Estragole and myristicin are both naturally occurring in Chamomile Flower Roman Extract (FEMA 2274) at levels of 0.03% and 0.04%, respectively, and methyl eugenol is naturally occurring in Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989) at levels of 0.001%, 0.1% and 2%, respectively. The natural occurrence and estimated intakes for these Group 21 constituents are presented in Table 2. Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989) 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.

The chemical structures of methyl eugenol, estragole and myristicin (Fig. 4) share the structural motif of a benzene ring containing an alkoxy group located *para* to a 2-propenyl functional group. Cytochrome P450s catalyze the formation of 1'-hydroxy metabolites of these allylalkoxybenzene compounds which may then be sulfated by a sulfotransferase. The elimination of sulfate creates a DNA reactive species (Herrmann et al., 2012, 2014; Jeurissen et al., 2007; Phillips et al., 1984; Randerath et al., 1984; Rietjens et al., 2005, 2014; Wiseman et al., 1987; Zhou et al., 2007). Studies in rodents conducted on methyl eugenol and estragole and the related compound safrole indicate that these compounds are hepatocarcinogens at high dose levels (Drinkwater et al., 1976; Miller et al., 1983; NTP, 2000; Zangouras et al., 1981).

The direct addition of methyl eugenol, estragole and the related

allylalkoxybenzene, safrole, 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 2016, 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 a synthetic flavoring substance and adjuvant for use in food (83 Fed. Reg. 50490. 9 October 2018) in response to a food additive petition. The FDA explained that it had based its decision "as a matter of law" on the "extraordinarily rigid" Delaney Clause of the Federal Food, Drug, and Cosmetic Act and further noted that based on the data evaluated, that "it is unlikely that consumption of methyl eugenol presents a risk to the public health from use as a flavoring substance" (83 Fed. Reg. 50490. 9 October 2018).

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

As shown in Table 2, the estimated intakes of methyl eugenol, estragole and myristicin present in Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989) range from 0.0005 to 0.04 µg/person/day, which are below the TTC for compounds with structural alerts for genotoxicity of 0.15 µg/person/day. This TTC is determined based on an analysis of the dose-response data for carcinogenic compounds (Kroes et al., 2004), provided by the Gold database of carcinogens,⁴ presenting the dose giving a 50% tumor incidence (TD₅₀) (Gold et al., 1984). By linear extrapolation of these TD₅₀ data to a dose resulting in a 1 in 10⁶ tumor incidence, an exposure level or TTC at which the lifetime risk of cancer was 1 in 10⁶ was determined to be 0.15 µg/person/day used as the TTC for compounds with structural alerts for genotoxicity (Kroes et al., 2004). In a recent European Food Safety Authority (EFSA)/World Health Organization (WHO) review of the TTC

Table 2

Natural occurrence and estimated intake of methyl eugenol, estragole or myristicin from Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989).

FEMA No.	NFC	Constituent of Concern	Mean %	Estimated Intake (µg/person/day)
2274	Chamomile Flower Roman Extract	Estragole	0.03	0.02
2274	Chamomile Flower Roman Extract	Myristicin	0.04	0.02
2650	Lovage Root Extract	Methyl eugenol	0.001	0.0005
2988	Rose Absolute	Methyl eugenol	0.2	0.02
2989	Rose Bulgarian True Otto Oil	Methyl eugenol	1	0.04

⁴ Gold database currently maintained by Lhasa Ltd. <https://www.lhasalimited.org/products/lhasa-carcinogenicity-database.htm>.

approach, a 0.15 µg/person/day threshold was considered sufficiently protective for compounds with structural alerts for genotoxicity with the exclusion of high potency carcinogens (the Cohort of Concern) specified by Kroes and co-workers (EFSA, 2016; Kroes et al., 2004; Nohmi, 2018). Since the estimated intakes for methyl eugenol, estragole and/or myristicin in Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989) are below the 0.15 µg/person/day threshold for compounds with structural alerts for genotoxicity, these constituents do not raise a safety concern and these NFCs proceed to Step 5.

Step 5

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

If Yes, proceed to Step 7.

If No, proceed to Step 6.

Yes, the estimated intakes of the congeneric groups for each NFC were below the TTC for their respective decision tree classes, as shown in [Appendix A](#). All NFCs proceed to Step 7.

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.

Not required.

Step 7

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

Proceed to Step 8.

[Appendix A](#) details the mean % for the group of unidentified constituents and the estimated intake of this group for each NFC. These data are also summarized below in [Table 3](#).

Step 8

Table 3

Estimated intake of unidentified constituents.

Name	FEMA No.	Estimated Intake (µg/person/day)
Ambrette Absolute Oil	2050	0.05
Balm Oil	2113	0.1
Castor Oil	2263	<7
Chamomile Flower Hungarian Oil	2273	4
Chamomile Flower Roman Extract	2274	6
Chamomile Flower English/Roman Oil	2275	3
Citronella Oil	2308	11
Wine Lees Green Oil	2331	0.4
Wine Lees White Oil	2332	0.04
Costus Root Oil	2336	0.2
Genet Absolute	2504	<0.01
Genet Extract	2505	0.2
Geranium Rose Oil	2508	14
Lemongrass Oil	2624	21
Lovage Root Extract	2650	13
Lovage Root Oil	2651	19
Palmarosa Oil	2831	0.7
Rose Absolute	2988	0.2
Rose Bulgarian True Otto Oil	2989	0.07
Sandalwood Yellow Oil	3005	2
Violet Leaves Absolute	3110	0.7
Mossoia Bark Oil	3747	0.0005
Litsea Cubeba Berry Oil	3846	0.3

Using the data from Step 1, is the intake of the NFC from consumption of the food⁵ 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. While Balm Oil (FEMA 2113), Chamomile Flower Hungarian Oil (FEMA 2273), Chamomile Flower Roman Extract (FEMA 2274), Chamomile Flower English/Roman Oil (FEMA 2275), Lemongrass Oil (FEMA 2624), Lovage Root Extract (FEMA 2650), Lovage Root Oil (FEMA 2651) and Massoia Bark Oil (FEMA 3747) are derived from the parts of plants that are used in culinary applications, there are no data currently available on their annual consumption for the calculation of a consumption ratio. The remaining NFCs are derived from botanicals that are not typically consumed as food. As a result, the safety evaluation for all the NFCs under consideration 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. TTC-excluded compounds are not present in the constituent profiles ([Appendix A](#)) for the NFCs under consideration and TTC-excluded compounds are not expected to be present in the unidentified fraction of the NFCs under consideration. Based on the identified constituents, the unidentified constituents are most likely terpenoid compounds or products derived from lipid metabolism. For the materials

⁵ Provided the intake of the unidentified constituents is greater from consumption of the food itself, the intake of unidentified constituents from the added essential oil is considered trivial.

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

that are prepared by distillation in which only the volatile fraction is used, the presence of the substances from the TTC-excluded classes is unlikely. The presence of substances from the TTC-excluded classes is also unlikely for the NFCs prepared by extraction of the botanical with non-aqueous solvent systems which will exclude proteins and other hydrophilic substances. Finally, over the long history of use of these substances, there have not been any reports of constituents or contaminants of these NFCs from the 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 3, with the exception of Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989), the identified constituent profile does not give rise to concern about the potential genotoxicity of the unidentified constituents. Furthermore, a review of available genotoxicity studies on the NFCs presented later in this manuscript indicates a lack of genotoxic potential for these NFCs. These NFCs proceed to Step 11.

In Step 4, the natural occurrence of constituents of concern, methyl eugenol, estragole and/or myristicin, were reported in low amounts in Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989). The estimated intake for these constituents was less than the TTC of 0.15 µg/person/day for compounds with structural alerts 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. These compounds may be part of the unidentified fraction at concentrations below the respective limit of detection (LOD). Depending on the analytical method employed to collect the data contributed to this safety evaluation, the LOD is estimated to be 0.01–0.1% of the NFC. At the upper value of this range, 0.1%, the estimated intakes for an unidentified allylalkoxybenzene constituent in Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989) would fall below the TTC of 0.15 µg/person/day for compounds with structural alerts for genotoxicity. Based on this reasoning, in addition to a lack of reports of the occurrence of other allylalkoxybenzenes in Chamomile Flower Roman Extract (FEMA 2274), Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989), the FEMA Expert Panel determined that these related allylalkoxybenzenes are unlikely to be present in the unidentified constituent fraction and there is not a genotoxic concern for the unidentified constituents. Proceed to Step 11.

Step 10a

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

If Yes, proceed to Step 13.

If No, proceed to Step 10b.

Not required.

Step 10b

Do negative genotoxicity data exist for the NFC?

If Yes, proceed to Step 11.

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

Not required.

Step 11

Is the estimated intake of the unidentified constituents (calculated in Step 7) less than the TTC (Kroes et al., 2004; 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 3, the estimated intake of the unidentified constituents for all NFCs is below the TTC of 90 µg/person/day for Structural Class III, and all 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.

The FEMA Expert Panel concurs with other food ingredient safety evaluation bodies that the TTC is applicable to the entire population (EFSA, 2012, 2016; EFSA Scientific Committee et al., 2019), when taking the lower body weight of children into account. An evaluation to consider possible exposure of children, given their lower body weights, and the potential for toxicokinetic and toxicodynamic differences as compared to adults, was conducted for each NFC under consideration. The NFCs under consideration would not be added to foods specifically consumed by infants (CAC, 2007; CAC, 2017; CAC, 2019), indicating that exposure of infants is not expected. With the exception of Castor Oil (FEMA 2263) and Lovage Root Oil (FEMA 2651), the estimated intakes

⁷ The human exposure threshold of 90 µg/person/day is determined from a database of NOAELs obtained from 448 subchronic and chronic studies of substances of the highest toxic potential (structural class III) mainly herbicides, pesticides and pharmacologically active substances (Munro et al., 1996). The 5th percentile NOAEL (lowest 5%) was determined to be 0.15 mg/kg bw/day which upon incorporation of a 100-fold safety factor for a 60 kg person yielded a human exposure threshold of 90 µg/person/day. However, no flavoring substance or food additive in this structural class exhibited a NOAEL less than 25 mg/kg bw/d. Therefore the 90 µg/person/day threshold is an extremely conservative threshold for the types of substances expected in natural flavoring complexes. Additional data on other specific toxic endpoints (e.g. neurotoxicity, reproductive, and endocrine disruption) support the use of this threshold value (Kroes et al., 2000).

for the constituent congeneric groups of each NFC under consideration are at least 4-fold below the corresponding TTC for the group, with none close to the TTC threshold, and therefore do not raise a safety concern for exposure to children under conditions of intended use as flavoring.

For Castor Oil (FEMA 2263), the estimated intake of the congeneric group of triglycerides is 680 µg/person/day and is less than 3-fold below the TTC for Structural Class I. A MoS of greater than 860,000 was calculated for the estimated intake of Castor Oil (FEMA 2263) used as a flavoring ingredient based on the no-observed-adverse-effect level (NOAEL) of 10,000 mg/kg bw/day determined in a 13-week dietary study in rats (NTP and Irwin, 1992), which is protective for children considering their lower body weights. For Lovage Root Oil (FEMA 2651), the estimated intake for Congeneric Group 13 (Aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones) constituents, 89 µg/person/day, is less than 3-fold below the TTC for Structural Class III. The major constituents of Group 13 were *cis*- and *trans*-ligustilide and 3-butyridenephthalide (FEMA 3333). A MoS of greater than 6700 was calculated for the combined intake of Group 13 constituents in Lovage Root Oil (FEMA 2651) based on a NOAEL of 10 mg/kg bw/day determined in a 13-week dietary study in rats (Voss, 1985) for dehydromenthofuro lactone (FEMA 3755), which is protective for children considering their lowered body weights. Dehydromenthofuro lactone (FEMA 3755) is structurally similar to *cis*- and *trans*-ligustilide, with both having an alicyclic-fused lactone with an α/β unsaturated carbonyl group, and likely to have similar innocuous metabolic outcomes. In summary, the MoS for Castor Oil (FEMA 2263) and Group 13 constituents of Lovage Root Oil (FEMA 2651) are adequately protective for children.

In consideration of Chamomile Flower Roman Extract (FEMA 2274), in which low naturally occurring amounts of estragole and myristicin were reported, and Lovage Root Extract (FEMA 2650), Rose Absolute (FEMA 2988) and Rose Bulgarian True Otto Oil (FEMA 2989), in which low naturally occurring amounts of methyl eugenol were reported, these estimated intakes are at least 3-fold below the TTC for compounds with structural alerts for genotoxicity of 0.15 µg/person/day (see Step 4a) indicating no safety concern for children.

The FEMA Expert Panel conducted an additional analysis, identifying sources of uncertainty in Appendix B. Within the safety evaluation and the discussions below, the limitations in data and methodology applied are discussed. As discussed throughout the safety evaluation procedure, conservatism is incorporated into the evaluation with the use of the TTC approach (Kroes et al., 2000; Munro et al., 1996), safety factors to account for inter-species and intra-individual variability in the calculation of MoS and MOE and in the use of the TTC and MOE approaches to evaluate potential genotoxic constituents.

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.

Based on the above assessment the FEMA Expert Panel concluded that the current FEMA GRAS NFCs, Ambrette Absolute Oil (FEMA 2050), Balm Oil (FEMA 2113), Castor Oil (FEMA 2263), Chamomile Flower Hungarian Oil (FEMA 2273), Chamomile Flower Roman Extract (FEMA 2274), Chamomile Flower English/Roman Oil (FEMA 2275), Citronella Oil (FEMA 2308), Wine Lees Green Oil (FEMA 2331), Wine Lees White Oil (FEMA 2332), Costus Root Oil (FEMA 2336), Genet Absolute (FEMA 2504), Genet Extract (FEMA 2505), Geranium Rose Oil (FEMA 2508), Lemongrass Oil (FEMA 2624), Lovage Root Extract (FEMA 2650), Lovage Root Oil (FEMA 2651), Palmarosa Oil (FEMA 2831), Rose Absolute (FEMA 2988), Rose Bulgarian True Otto Oil (FEMA 2989), Sandalwood Yellow Oil (FEMA 3005), Violet Leaves Absolute (FEMA 3110), Massoia Bark Oil (FEMA 3747) and *Litsea Cubeba* Berry Oil (FEMA 3846) are affirmed as “generally recognized as safe (GRAS)” under conditions

of intended use as a flavoring substance. Chamomile Flower English Oil (FEMA 2272) and Chamomile Flower Roman Oil (FEMA 2275) have been determined to be identical NFCs and have been combined under FEMA 2275 as Chamomile Flower English/Roman Oil.

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

The constituent profiles for several of the NFCs are characterized by primary alcohols, acids and esters of Group 1 (Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters), Group 2 (Saturated aliphatic, acyclic, branched chain primary alcohols, aldehydes, carboxylic acids and related esters), Group 3 (Aliphatic linear and branched-chain α,β-unsaturated aldehydes and related alcohols, acids and esters), Group 5 (Unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters) and Group 7 (Saturated alicyclic primary alcohols, aldehydes, acids and related esters). Monographs on flavoring ingredients of these groups, which include data on the metabolism of these substances, have been published by JECFA (JECFA, 1997, 1998, 1999b, 2003, 2004b, 2006, 2007, 2011, 2012). The safety of Group 7 and α,β-unsaturated aldehydes and related substances used as flavor ingredients was also reviewed by the FEMA Expert Panel (Adams et al., 2008). In 1980, JECFA allocated a group ‘Acceptable daily intake’ (ADI) of 0–0.5 mg/kg bw/day, expressed as citral for citral, citronellol, geranyl acetate, linalool and linalyl acetate which was maintained in subsequent meetings in 1999, 2000 and 2004 (JECFA, 1980b, 1999a, 2000, 2004a) and an ADI of 0–0.7 mg/kg bw/day for castor oil (JECFA, 1980a).

For other NFCs, the primary constituent congeneric groups are Group 13 (Aliphatic and aromatic lactones), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 19 (Aliphatic and aromatic hydrocarbons), Group 10 (Alicyclic ketones, secondary alcohols and related esters) and/or Group 22 (Phenethyl alcohol, phenylacetaldehyde and related acetals and esters). The FEMA Expert Panel has published its safety evaluation of flavoring ingredients of these groups that include reviews of metabolic and toxicological studies (Adams et al., 1996, 1998, 2005c, 2011; Marnett et al., 2014). In addition, Group 19 constituents were further discussed in the safety evaluation of *Citrus*-derived NFCs (Cohen et al., 2019), Group 10 constituents were further discussed in the safety evaluation of mint-derived and related NFCs (Cohen et al., 2020) and Group 12 constituents were further discussed in the safety evaluation of lavender-derived and other tertiary alcohol containing NFCs (Fukushima et al., 2020). These publications in addition to studies on the NFCs and the discussion presented below, provide the basis for the safety evaluation of the NFCs under consideration. The additional information presented here includes studies on the NFCs themselves and newly available studies on constituents not considered within the reviews mentioned above. Studies concerning genotoxicity for the NFCs are summarized in Table 4.

7.1. Group 1 constituents: genotoxicity studies for decyl acetate (FEMA 2367), ethyl octanoate (FEMA 2449), ethyl decanoate (FEMA 2432), ethyl laurate (FEMA 2441) and palmitic acid (FEMA 2832)

In a GLP guideline-compliant Ames assay, no evidence of mutagenicity was observed when *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 were incubated with decyl acetate at concentrations up to 5000 µg/plate in the presence of an Aroclor 1254-induced rat liver S9 metabolic activation system. In the absence of S9 metabolic activation, no evidence of mutagenicity was observed when decyl acetate was tested at concentrations up to 1500 µg/plate (King, 2000). In another Ames assay, no evidence of mutagenicity was observed when *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were incubated with decyl acetate at concentrations of 4–17 µg/plate in the absence or presence of liver microsomal fraction, S9

Table 4
Summary of genotoxicity study results for NFCs under consideration.

Name	Test Type (System)	Test Concentrations	Results	Reference
<i>In vitro</i>				
Balm oil	Reverse mutation in <i>S. typhimurium</i> 1	88, 177, 442 µg/plate	Negative ^a	De Martino et al. (2009)
Castor oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	100–5000 µg/plate	Negative ^a	Hachiya (1987)
Castor oil	Reverse mutation in <i>S. typhimurium</i> ^a	100–10,000 µg/plate	Negative ^a	Zeiger et al. (1988)
Castor oil	<i>In vitro</i> sister chromatid exchange (SCE) in Chinese hamster ovary cells ^a	160–5000 µg/plate	Negative ^a	(NTP and Irwin, 1992)
Castor oil	<i>In vitro</i> chromosomal aberration in Chinese hamster ovary cells ^a	1600–5000 µg/plate	Negative ^a	(NTP and Irwin, 1992)
Chamomile oil (Roman/English)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	2–1600 µg/plate	Negative ^a	Mee (2017b)
Chamomile oil (Roman/English)	Reverse mutation in <i>S. typhimurium</i> ^a	225, 450, 900 µg/plate	Negative ^a	Zani et al. (1991)
Chamomile oil (Roman/English)	Rec assay in <i>B. subtilis</i>	9, 27 mg/disk	Negative	Zani et al. (1991)
Chamomile oil (Hungarian)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	2–500 µg/plate	Negative ^a	Mee (2017a)
		<i>S. typhimurium</i> 2–1600 µg/plate <i>E. coli</i>		
Chamomile oil (Hungarian)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	3–5000 µg/plate (test 1) 0.3–2500 µg/plate ^b (test 1) 1–2500 µg/plate ^c (test 2) 10–5000 µg/plate ^b (test 2)	Negative ^a	ECHA (2018a)
Chamomile oil (Hungarian)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	3–5000 µg/plate (test 1) 33–5000 µg/plate ^d (test 2) 0.3–1000 µg/plate ^e (test 2)	Negative ^a	ECHA (2018b)
Chamomile oil (Hungarian)	<i>In vitro</i> micronucleus induction in human lymphocytes ^a	52–890 µg/mL ^{f,g} 326–435 µg/mL ^{g,h} 52–395 µg/mL ^{f,i}	Negative ^a	Clare (2017)
Citronella oil (Java type)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	1.5–5000 µg/plate (test 1) 5–5000 µg/plate (test 2)	Negative ^a	Dakoulas (2014a)
Citronella oil (Java type)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	5–5000 µg/plate (test 1) 1.5–1500 µg/plate (test 2)	Negative ^a	ECHA (2014a)
Citronella oil (Ceylon type)	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	5–512 µg/plate ^a (test 1) 0.6–1600 µg/plate ^a (test 2)	Negative ^a	ECHA (2018b)
Citronella oil (Java type)	Reverse mutation in <i>S. typhimurium</i>	4.5, 9 ng/plate	Positive	Sivaswamy et al. (1991) ^j
Citronella oil	Rec assay in <i>B. subtilis</i> ^a	280–2250 µg	Negative ^f Positive ^h	Kuroda et al. (1989)
Citronella oil (Java type)	Forward mutation in mouse lymphoma L5178Y cells ^a	2–78 µg/mL ^{f,g} 5–156 µg/mL ^{g,h} 0.6–20 µg/mL ^{f,i}	Negative ^a	ECHA (2013a)
Citronella oil (Java type)	<i>In vitro</i> chromosomal aberration in human lymphocytes ^a	10–160 µg/mL ^{f,g} 20–320 µg/mL ^{g,h} 20–240 µg/mL ^{f,i}	Negative ^a	ECHA (2014b)
Citronella oil	<i>In vitro</i> comet assay in human lymphocytes	0, 50, 100, 200 µg/mL	Negative	Sinha et al. (2011)
Citronella oil	<i>In vitro</i> comet assay in human lymphocytes	100–2000 µg/mL	Positive	Sinha et al. (2014)
Citronella oil (Java type)	<i>In vitro</i> micronucleus induction in human lymphocytes ^a	10–200 µg/mL ^{f,g} 25–450 µg/mL ^{g,h} 10–135 µg/mL ^{f,i}	Negative ^a	Roy (2015a)
Geranium oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	1.5–5000 µg/plate (test 1) 5–5000 µg/plate (test 2)	Negative ^a	ECHA (2014c)
Geranium oil	Reverse mutation in <i>S. typhimurium</i>	4.5, 9 ng/plate	Positive	Sivaswamy et al. (1991) ^j
Lemongrass oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	1.5–5000 µg/plate (test 1) 0.5–1500 µg/plate (test 2)	Negative ^a	ECHA (2015)
Lemongrass oil	Reverse mutation in <i>S. typhimurium</i>	4.4, 8.9 ng/plate	Positive	Sivaswamy et al. (1991) ^j
Lemongrass oil	<i>In vitro</i> comet assay in human peripheral blood lymphocytes	25–800 µg/mL	Positive	Sinha et al. (2014)
Lovage root oil	Reverse mutation in <i>S. typhimurium</i> ^a	10–2600 µg/plate	Negative ^a	Jagannath (1983)
Lovage root oil	Unscheduled DNA synthesis in male rat hepatocytes	2580 µg/mL	Negative	Heck et al. (1989)
Lovage root extract	Reverse mutation in <i>S. typhimurium</i> ^a	1 to 10,000 µg/plate	Negative ^a	Jagannath (1985)
Palmarosa oil	<i>In vitro</i> comet assay in human lymphocytes	0, 50, 100, 200 µg/mL	Negative	Sinha et al. (2011)
Palmarosa oil	<i>In vitro</i> comet assay in human lymphocytes	100–2000 µg/mL	Positive	Sinha et al. (2014)
Rose absolute	<i>In vitro</i> chromosomal aberration in human lymphocytes	10 µg/mL	Negative	Hagag et al. (2014)
Rose oil, Bulgarian	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	1.5–5000 µg/plate (test 1) 5–5000 µg/plate (test 2)	Negative ^a	Dakoulas (2014b)
Rose oil, Bulgarian	<i>In vitro</i> micronucleus induction in human lymphocytes ^a	50–450 µg/mL ^{a,g} 5–125 µg/mL ^{f,i}	Negative ^a	Roy (2015c)
Rose oil	<i>In vitro</i> micronucleus induction in human lymphocytes	1–200 µg/mL	Positive	Shokrzadeh et al. (2017)
Sandalwood oil (type I)	Reverse mutation in <i>S. typhimurium</i>	5, 10 ng/plate	Positive	Sivaswamy et al. (1991) ^j
Sandalwood oil (type II)	Reverse mutation in <i>S. typhimurium</i>	5, 10 ng/plate	Positive	Sivaswamy et al. (1991) ^j
Sandalwood yellow oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	5–500 µg/plate in <i>S. typhimurium</i> 50–5000 µg/plate in <i>E. coli</i>	Negative ^a	ECHA (2013b)
Sandalwood yellow oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	1.6–1600 µg/plate	Negative ^a	Mee (2017c)
Sandalwood yellow oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	3–5000 µg/plate	Negative ^a	Sokolowski (2020)
<i>Litsea cubeba</i> oil	Reverse mutation in <i>S. typhimurium</i> ^a	0.8, 4, 20, 100, 500 µg/plate	Negative ^a	Luo et al. (2005)

(continued on next page)

Table 4 (continued)

Name	Test Type (System)	Test Concentrations	Results	Reference
<i>Litsea cubeba</i> oil	Reverse mutation in <i>S. typhimurium</i> and <i>E. coli</i> ^a	5–5000 µg/plate (test 1) 1.5–1500 µg/plate (test 2)	Negative ^a	ECHA (2014d)
In vivo				
Castor oil	<i>In vivo</i> micronucleus in B6C3F ₁ mice (peripheral blood)	900 to 15,000 mg/kg bw/day	Negative	(NTP and Irwin, 1992; Witt et al., 2000)
Chamomile oil (Hungarian)	<i>In vivo</i> sister chromatid exchange (SCE) in male NIH mice	10, 100, 500, 1000 mg/kg bw	Negative	Hernández-Ceruelos et al. (2002)
Chamomile oil (Hungarian)	<i>In vivo</i> sister chromatid exchange (SCE) in male NIH mice	500 mg/kg bw	Negative	Hernández-Ceruelos et al. (2010)
Lemongrass oil	<i>In vivo</i> comet assay in male Swiss mice (peripheral blood)	100 mg/kg bw	Negative	Costa et al. (2011)
<i>Litsea cubeba</i> oil	<i>In vivo</i> chromosomal aberration in male ICR mice (spermatocytes)	460, 925, 1850 mg/kg bw	Negative	Luo et al. (2005)
<i>Litsea cubeba</i> oil	<i>In vivo</i> micronucleus induction in male ICR mice (bone marrow)	185, 740, 1850 mg/kg bw	Negative	Luo et al. (2005)

^a In the absence and presence of an exogenous S9 metabolic activation system.

^b In *S. typhimurium* TA100 without S9 activation.

^c In *S. typhimurium* TA100 and TA1537 with and without S9 activation.

^d In all tester strains unless specified.

^e In *S. typhimurium* TA100.

^f In the absence of S9.

^g Short duration treatment (e.g., 3 or 4 h).

^h In the presence of S9.

ⁱ Continuous duration treatment (e.g. 24 or 48 h).

^j The Expert Panel has evaluated this study in a previous safety assessment and concluded that the study has numerous deficiencies and that the results of this study do not inform the safety evaluation (Rietjens et al., 2020).

(Richold, 1980).

In an OECD and GLP guideline-compliant Ames assay, *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA were incubated with ethyl octanoate at concentrations of 3–5000 µg/plate in the absence and presence of an S9 metabolic activation system derived from phenobarbital/β-naphthoflavone induced rat liver (Chang, 2016a). There were two experiments performed and in both there was no increase in revertant colonies. There was toxicity observed as a reduction of colonies in TA100 at ≥100 µg/plate in the absence of S9 and ≥2500 µg/plate in the presence of S9, in TA1537 at ≥1000 µg/plate without S9 and *E. coli* WP2uvrA at 5000 µg/plate with S9.

In an OECD and GLP guideline-compliant Ames assay, no evidence of mutagenicity was observed when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA were incubated with ethyl decanoate at concentrations of 5–5000 µg/plate in the absence and presence of an S9 metabolic activation system derived from the liver of Aroclor 1254-induced male rats (Bhalli, 2017a). In strains TA98 and TA100, toxicity was observed at 5000 µg/plate. In a confirmatory assay, the same test strains were incubated with ethyl decanoate at concentrations of 16–5000 µg/plate in the presence or absence of S9. In strain TA1535 in the presence of S9 and strain TA1537 in the absence of S9, toxicity was observed at the 5000 µg/plate. There were no increased reverse mutations with ethyl decanoate under the test conditions.

In an OECD and GLP guideline-compliant Ames assay, no increase in reverse mutations was observed when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA were incubated with ethyl laurate at concentrations of 1.5–5000 µg/plate in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Dakoulas, 2013). In the initial assay, there was precipitation observed beginning at concentrations of 1500 µg/plate in incubations with strains TA98, TA100 and TA1535 in the presence of S9. In the confirmatory assay, precipitation was observed beginning at concentrations of 1500 µg/plate. Although there was no toxicity observed based on background lawn reduction, there were revertant count reductions at 1500 or 5000 µg/plate in tester strains TA98, TA100 and TA1535 in the presence of S9.

In a GLP and OECD guideline-compliant *in vitro* micronucleus induction assay in human peripheral blood lymphocytes (HPBL), ethyl laurate (purity 99.3%) was tested at concentrations ranging from 0.228 to 2280 µg/mL in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Roy, 2014). In an initial

toxicity assay, HPBL were incubated with 0.228–2280 µg/mL ethyl laurate for a 4 h exposure period with a 20 h recovery period with and without S9 and in a continuous exposure experiment in the absence of S9 for 24 h. Based on cytotoxicity in the cytokinesis-blocked proliferation index (CPBI), the ranges for non-activated assays were identified as 10–70 µg/mL in the 4 h activated and non-activated assays and 20–225 µg/mL in the 24 h non-activated assay. In the 4 h exposure group with S9, cytotoxicity was observed at ≥100 µg/mL and in the continuous exposure group without S9, cytotoxicity was observed at ≥50 µg/mL. Under the test conditions, ethyl laurate did not induce micronucleated binucleated cells in the presence or absence of S9 metabolic activation in HPBL.

In a GLP and OECD guideline-compliant *in vitro* micronucleus induction assay, HPBL were incubated with palmitic acid in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Bhalli, 2014a). Palmitic acid was tested at concentrations of 43.1–2565 µg/mL in a continuous exposure for 24 h in the absence of S9 and concentrations of 120–2565 µg/mL in the absence and presence of S9 for a 3 h exposure period with a 20 h recovery period and harvest prior to 24 h. Precipitation was observed at the end of the exposure period for most tested concentrations ≥322 µg/mL in the absence of S9 and ≥199 µg/mL in the presence of S9. The assay was repeated with concentrations of 30.1–350 µg/mL for a continuous 24 h exposure in the absence of S9 and concentrations of 47–350 µg/mL for the 3 h exposure in the absence or presence of S9 with a 20 h recovery period. In the second experiment, precipitates were observed at concentrations ≥179 µg/mL in the absence of S9 and at concentrations ≥143 µg/mL in the presence of S9 (53% cytotoxicity). Under the conditions of the assay there was no increase in the induction of binucleated micronucleated cells in the presence of palmitic acid with and without S9 metabolic activation system.

7.2. Group 3 constituents: genotoxicity studies for isobutyl angelate (FEMA 2180), citronellol (FEMA 2309), citronellal (FEMA 2307), geraniol (FEMA 2507) and citral (FEMA 2303)

In an OECD and GLP guideline-compliant Ames assay, isobutyl angelate was tested in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA at concentrations of 5–5000 µg/plate in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Bhalli, 2017c). Toxicity was observed at

concentrations ≥ 500 $\mu\text{g}/\text{plate}$ in all tester strains in the absence of S9 with the exception of strain TA100, which showed toxicity at concentrations ≥ 160 $\mu\text{g}/\text{plate}$. In the presence of S9, toxicity was reported at concentrations ≥ 1600 $\mu\text{g}/\text{plate}$ in all test strains with exception of TA100 which could not be analyzed due to an abnormally low number of background revertants in the vehicle control. Based on toxicity observed in the first experiment, a second experiment was performed with 16–5000 $\mu\text{g}/\text{plate}$ of isobutyl angelate in *S. typhimurium* and *E. coli* strains in the presence of S9 and at 5–1600 $\mu\text{g}/\text{plate}$ in the absence of S9. No increase in mutations was observed.

In an OECD and GLP guideline-compliant *in vitro* micronucleus induction assay, HPBL were incubated with concentrations of isobutyl angelate of 19–90 $\mu\text{g}/\text{mL}$ continuously for 24 h in the absence of metabolic activation and 65.5–169 $\mu\text{g}/\text{mL}$ with an exposure of 3 h and a recovery period of 20 h both in the absence of metabolic activation and in the presence of an Aroclor 1254-induced rat liver S9 metabolic activation system at concentrations of 111–270 $\mu\text{g}/\text{mL}$ (Bhalli, 2017d). For this assay, the OECD guideline recommends that when the maximum test concentration is based on cytotoxicity, the highest concentration should achieve $55 \pm 5\%$ cytotoxicity (OECD, 2014). Since the experimental parameters in this first assay did not meet the OECD guideline for cytotoxicity, the assay was repeated. In a repeat assay, HPBL were continuously exposed to concentrations of 40.5, 52.3 and 78.8 $\mu\text{g}/\text{mL}$ isobutyl angelate for 24 h in the absence of S9 and 113, 125 and 146 $\mu\text{g}/\text{mL}$ with exposure for 3 h, in the absence of S9. The cytotoxicity at the highest concentrations tested was 55% and 53%, respectively. No statistically significant increase in the frequency of micronucleated binucleated cells was observed in the 24 h and 3 h experiments in the absence of S9 metabolic activation. An additional experiment for the 3 h exposure in the presence of S9 metabolic activation that was outside the OECD guideline based on the cytotoxicity measurements were also performed. In the fourth experiment for the 3 h exposure in the presence of S9 metabolic activation, HPBL from a female donor (previous experiments employed a male donor), were treated with concentrations of 150, 214 and 226 $\mu\text{g}/\text{mL}$ isobutyl angelate, where 48% cytotoxicity was observed at the highest concentration. There was no increased incidence of micronucleated binucleated cells observed in this experiment. Based on the results of this assay, isobutyl angelate was negative for the induction of micronucleated binucleated cells in HPBL when evaluated up to the limit of cytotoxicity.

An OECD and GLP guideline-compliant *in vitro* chromosomal aberration assay, V79 Chinese hamster cells were treated with citronellal with and without S9 metabolic activation derived from phenobarbital/ β -naphthoflavone-treated male Wistar rats (Schulz, 2014). In the first experiment the V79 cells were exposed to 12.5, 25 and 50 $\mu\text{g}/\text{mL}$ and 25, 50 and 100 $\mu\text{g}/\text{mL}$ of citronellal in the absence or presence of S9, respectively, with a recovery period of 24 h. In the second experiment the V79 cells were exposed to 6.25, 12.5 and 25 $\mu\text{g}/\text{mL}$ in the absence of S9 with an exposure period of 24 h and a recovery period of 24 h and 12.5, 25, 50 and 100 $\mu\text{g}/\text{mL}$ in the presence of S9 with an exposure period of 4 h and a recovery period of 40 h. Under the testing conditions, citronellal showed neither clastogenic nor aneugenic effects in the absence or presence of S9 metabolic activation.

In an OECD and GLP guideline-compliant *in vitro* mutation assay at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells, citronellal was negative for mutagenicity in experiments performed both in the presence and absence of S9 metabolic activation derived from the livers of Aroclor 1254-induced rats (Schulz, 2008b). In experiments in which CHO cells were exposed to 0.5–40 $\mu\text{g}/\text{mL}$ citronellal, in the absence of S9 metabolic activation and 1–128 $\mu\text{g}/\text{mL}$ citronellal in the presence of S9 metabolic activation, no increase in mutant frequencies was observed.

In an OECD and GLP guideline-compliant *in vitro* micronucleus assay, HPBL were incubated with citronellal in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Roy, 2016). The HPBL were exposed to the test agent for 4 h in the presence

and absence of S9 at concentrations of 25, 50 and 100 $\mu\text{g}/\text{mL}$ with a 20 h recovery period and a continuous 24 h exposure period at concentrations of 10, 25 and 50 $\mu\text{g}/\text{mL}$ in the absence of S9. The 4 h exposure experiment with and without S9 showed no increased induction of micronuclei, however the 24 h continuous exposure arm without S9 showed a statistically significant increase in the induction of micronuclei when compared to the experimental controls, but not the historical controls. A second assay was performed with a 24 h continuous exposure period in the absence of S9 at concentrations of 10, 30 and 55 $\mu\text{g}/\text{mL}$ of citronellal. In this second assay there was no indication of increased induction of micronuclei when compared to concurrent or historical controls. Appropriate levels of cytotoxicity were obtained in both experiments with levels above 50% and below 60%. The study director determined that the results of the first continuous exposure assay were not biologically relevant. It was concluded that under the conditions of the micronucleus assay, citronellal did not induce formation of micronuclei in HPBL.

In an OECD and GLP guideline-compliant HRPT locus assay, citral was incubated with CHO cells at concentrations of 0.63–100 $\mu\text{g}/\text{mL}$ in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Schulz, 2008a). In the first experiment, citral was tested at concentrations of 3.1–100 $\mu\text{g}/\text{mL}$ for a 4 h exposure period with a recovery period of 20–24 h in the absence and presence of S9. In the second experiment, citral was tested at concentrations of 0.63–20 $\mu\text{g}/\text{mL}$ for a 4 h exposure period and a 20–24 h recovery period in the absence of S9. In the third experiment, citral was incubated with CHO cells with an exposure period of 4 h and a recovery period of 20–24 h in the absence of S9 at concentrations of 2.5–20 $\mu\text{g}/\text{mL}$, and in the presence of S9 at concentrations of 2.5–80 $\mu\text{g}/\text{mL}$. Under the conditions of the study there were no increases in mutagenic activity at the HRPT locus when citral was incubated with CHO cells in the absence and presence of a S9 metabolic activation system.

7.3. Group 3 constituents: developmental/reproductive toxicity studies for citronellol (FEMA 2309), geraniol (FEMA 2507) and citral (FEMA 2303)

In an OECD guideline 414 and GLP guideline-compliant developmental assay, groups of 25 time-mated female Wistar rats were administered 0 (vehicle), 75, 250 or 750 mg/kg bw/day citronellol by corn oil gavage on gestation day (GD) 6 through 19 (Schneider, 2016b). Food consumption and body weights were recorded regularly throughout the study. The overall welfare and health of the animals were checked daily. On GD 20, blood samples were collected from all females and this was followed by euthanasia and gross examination (including liver, unopened uterus and placenta weights). Each dam was assessed for the number of corpora lutea and the distribution of implantation sites (differentiated between resorptions, live and dead fetuses). The fetuses were removed from the uteruses, sexed, weighed and examined for external findings. Half of the fetuses from each litter were examined for skeletal malformations and the other half for potential soft tissue effects. There were no unscheduled deaths during the study. All high-dose and 24/25 mid-dose females and two females from the low dose group exhibited transient salivation, usually immediately after dosing. Four high-dose females showed piloerection on GD16–19. There were no other clinical signs or behavioral changes observed. Water consumption in the test groups were similar to controls. The mean food consumption for the high-dose dams was significantly decreased on GD 6–10. By GD 20 there was a full recovery. Overall mean body weights were comparable across groups. Hematology parameters were similar in control and test groups. In the high-dose dams, serum globulin levels were decreased, but within the historical control range, and total protein levels were also reduced to levels marginally below the historical control range. Serum urea levels and alanine aminotransferase (ALT) levels were increased in high-dose dams but were within historical controls. Similarly, a decrease in inorganic phosphate was noted but was also within historical controls. These findings were considered incidental

and not biologically relevant. In high-dose dams there was a decrease in the bilirubin levels which in the absence of indicators of anemia was attributed to increased conjugation of bilirubin associated with enzyme induction. This correlated with significantly increased absolute and relative liver weights in the high-dose dams. There were no notable differences in the uterus weights of any test group. The conception rate was between 96 and 100%. There were no effects on corpora lutea or implantation sites or in the pre- or post-implantation losses, the number of resorptions or viable fetuses. The sex distribution of fetuses among the litters was unremarkable. The placental weights were comparable to controls. The mean fetal weights of the high-dose group were significantly decreased but within the range of historical controls. There were no test substance-related external findings observed. Soft tissue and skeletal changes were observed in both control and test groups and were not considered treatment related. Under the conditions of this study, there was the suggestion of an effect on the liver at the high dose such that the NOAEL of citronellol for maternal toxicity was considered by the FEMA Expert Panel to be 250 mg/kg bw/day. However, there were no findings of reproductive or developmental toxicity at any dose and a NOAEL for citronellol was determined to be 750 mg/kg bw/day for reproductive and developmental toxicity.

In an OECD and GLP guideline-compliant developmental toxicity study, groups of 25 time-mated female Wistar rats were administered 0 (vehicle), 100, 300 or 1000 mg/kg bw/day of geraniol (purity 94% mixture of isomers) by corn oil gavage on GD 6–19 (Schneider, 2015). The dams were terminated on GD 20. At termination, 22–25 dams per group had implantation sites. Gross pathology was performed with specific attention to the unopened uterus and placenta. Corpora lutea were counted and the number of implantation sites with note of resorptions (early vs. late), and live and dead fetuses were recorded for each dam. The fetuses were removed from the uteri, sexed, weighed and examined for external findings. Half of the fetuses from each litter were examined for soft tissue findings and the other half were examined for skeletal malformations inclusive of cartilage. The 1000 and 300 mg/kg bw/day groups showed reductions in maternal body weight. The high-dose maternal group showed a significant decrease in food consumption during GD 6–10 with recovery on GD 11–14 and on GD 15–17 showing a significant increase. It was noted that the high-dose group fetuses also had a reduction in bodyweights and other indications of developmental delay. The uterine weights were comparable across test and control groups. There were no significant differences between conception rates across control and test groups. The mean counts for corpora lutea, implantation sites and the values for post-implantation loss, resorptions and viable fetuses were comparable. It was noted that one litter in the 100 mg/kg bw/day group had an unusual male/female ratio of 10/2. Mean placenta weights were comparable across all groups. The mean fetal weights of the high-dose group were significantly lower compared to controls. There were no external malformations at incidences that reached statistical significance and did not also occur in the concurrent controls. In the 300 and 1000 mg/kg bw/day fetuses, there was an increased incidence in short innominate, dilated renal pelvis and dilated ureter but these effects were not dose-related. There were statistically significant rates of incomplete ossification observed in the 1000 mg/kg bw/day group fetuses subject to skeletal examinations. Based on reductions in body weights, the NOAEL for maternal toxicity was identified as 100 mg/kg bw/day. Based on body weight reductions and delayed ossification in the offspring, the NOAEL for prenatal developmental toxicity was identified as 300 mg/kg bw/day.

In an OECD guideline 414 and GLP guideline-compliant prenatal development study, artificially inseminated groups of 25 pregnant New Zealand white rabbits were administered 0 (vehicle), 20, 60 or 200 mg/kg bw/day of citral in methylcarboxycellulose by gavage from GD 6 through 28 (Schneider, 2016a). Daily cage-side observations and clinical observations were recorded. Periodic body weights and food consumption were recorded. The animals were terminated on Day 29. At necropsy, gross examination was performed on the uteri and ovaries. The

weights of the unopened uteri were recorded. Upon dissection of the uteri, the number of corpora lutea, implantation sites, live fetuses and dead implantations, early resorptions, late resorptions and dead fetuses were recorded. The litters were examined for skeletal, soft tissue and head malformations. Each fetus was weighed and examined for external findings. The viability of fetuses and condition of the placentas, umbilical cords, fetal membranes and fluids were recorded. The rate of conception, pre-implantation loss and post-implantation loss were calculated based on the post-mortem examinations.

Food consumption over the test period was reduced for the high-dose group. The low- and mid-dose groups showed similar food consumption as the concurrent vehicle controls. There were three premature maternal deaths in study in the 200 mg/kg bw/day group. Two were found dead in the cage on Day 23 and 28 and showed reddening of the stomach mucosa. The third was euthanized, *in extremis*, after aborting her litter on Day 26 and had multiple ulcerations of the stomach. These stomach effects were associated with the irritating effect of bolus gavage doses of aldehydes on stomach tissues. One of the high-dose does had 4 dead fetuses at the time of Cesarean section and had a marked reduction in food consumption and reduction in body weight near term, Day 28. One high-dose doe had a litter with 5 instances of malrotated limbs and paw hyperflexion. This litter also had a pale discolored placenta. These fetuses were not alive at the time of Cesarean section and the doe had lost more body weight than the average for the high-dose group. Since the 5 cases of paw hyperflexion were observed in dead fetuses they may be an artefact of in utero death, which was attributed to malnutrition of the doe and maternal toxicity, not direct developmental toxicity.

The effects observed in the high-dose group are indicative of the ability of bolus doses of aldehydes to irritate the intestinal tract of rabbits leading to reduced food consumption, body weight loss, maternal mortality, abortion and fetal mortality. There were no differences observed in the test groups in conception rate, mean number of corpora lutea, total implantations, resorptions, live fetuses, fetal sex ratios or the values for pre- and post-implantation indices. There were no differences observed for placental and fetal body weights. Upon examination of the soft tissues and skeletal malformations the fetuses showed no significant effects. The numbers and types of findings were considered spontaneous and not biologically relevant. Based on the effects on body weight and food consumption, the NOAEL for maternal toxicity of citral in rabbits was determined to be 60 mg/kg bw/day. Based on the lack of direct effects attributable to citral on the development of rabbit fetuses, the fetal development NOAEL was determined to be 200 mg/kg bw/day.

7.4. Group 7 constituents: genotoxicity studies for santalol (FEMA 3006)

In an OECD and GLP guideline-compliant Ames assay, *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA were treated with santalol at concentrations up to 5000 µg/plate with and without an Aroclor 1254-induced rat liver S9. Toxicity was observed in all tester strains at concentrations ≥1600 µg/plate with S9 except TA98 and WP2uvrA, where toxicity was observed at concentrations ≥500 and 5000 µg/plate, respectively. Without S9, toxicity was observed at concentrations ≥160 µg/plate in all tester strains except TA1537 and WP2uvrA, where toxicity was observed at concentrations ≥50.0 µg/plate in TA1537, and no toxicity was observed in WP2uvrA. Under the conditions tested, there were no increases in the frequency of revertant colonies as a result of incubation with santalol (Bhalli, 2014b).

In an OECD and GLP guideline-compliant *in vitro* micronucleus study, HPBL were treated with santalol for 3 h with and without an Aroclor 1254-induced S9 metabolic activation system, at concentrations of 46.6–175 µg/mL and 46.6–128 µg/mL, respectively. A concentration range of 8.62–40.2 µg/mL was also evaluated in a 24 h treatment arm without S9. Santalol did not induce the formation of micronuclei in HPBL under the conditions tested (Bhalli, 2014c).

7.5. Group 11 constituent: short term toxicity and genotoxicity studies for isopulegol (FEMA 2962)

In an OECD and GLP guideline-compliant Ames assay, no mutagenic potential was observed when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA were incubated with isopulegol (purity 99%, sum of isomers) at concentrations of 3–5000 µg/plate and in a repeat experiment at concentrations of isopulegol of 10–5000 µg/plate in the absence and presence of phenobarbital/β-naphthoflavone induced rat liver S9 metabolic activation (Chang, 2016b). In another OECD and GLP guideline-compliant Ames assay, no mutagenic potential was observed when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA were incubated with L-isopulegol (purity >99%) at concentrations of 3–5000 µg/plate in the absence and presence of phenobarbital/β-naphthoflavone induced rat liver S9 metabolic activation (Schulz, 2010).

In a GLP and OECD guideline-compliant *in vitro* micronucleus induction assay in HPBL, isopulegol (purity 99.3%) was tested at concentrations ranging from 0.228 to 2280 µg/mL in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Roy, 2015b). Isopulegol was tested at concentrations of 250–700 µg/mL in both the absence and presence of S9 metabolic activation in 4 h incubation experiments and at concentrations of 25, 50 and 100 µg/mL in a 24 h non-activated experiment. Under the test conditions, isopulegol did not induce micronucleated binucleated cells in the presence or absence of S9 metabolic activation in HPBL.

In an OECD and GLP guideline 90-day toxicity study, groups of adult Crl: Sprague-Dawley® CD® IGS rats (10/sex/group) were provided feed delivering 0 (vehicle), 200, 1800 or 3600 mg/kg bw/day isopulegol microencapsulated in acacia gum (Koetzner, 2013). Dietary stability analysis showed that only 20% of the target dose was achieved for microencapsulated isopulegol admixed into the feed. Daily observations of viability, gross toxicity and behavior were recorded throughout the study period. Body weights were recorded prior to study initiation and weekly along with food consumption during the study up to termination. Urine and blood samples were collected on Day 85. Coagulation assessments were performed on Day 93 or Day 94 at the time of euthanasia. Complete necropsies were performed along with histopathology examination of selected organs. There were no mortalities, observations of clinical toxicity, ophthalmology or gross changes associated with microencapsulated isopulegol in the diet. There were increased relative kidney weights for the male rats at the two highest dietary levels that correlated with chronic progressive nephropathy and the occurrence of hyaline droplets at all dietary levels. Only the highest dietary level showed granular casts in the renal tubules. These observations were not seen in control group males or females in the control and test substance groups. These findings are consistent with α_{2u}-globulin nephropathy which has been extensively discussed by the FEMA Expert Panel in previous publications (Adams et al., 2011; Cohen et al., 2019). It has been established that α_{2u}-globulin nephropathy is a condition unique to the male rat and not biologically relevant to humans (Capen et al., 1999; United States Environmental Protection Agency, 1991). The nominal NOAEL for microencapsulated isopulegol in the diet was 3600 mg/kg bw/day, which corresponds to a calculated intake of 720 mg/kg bw/day isopulegol.

7.6. Group 13 constituent: genotoxicity study for ambrettolide (FEMA 2555)

In an OECD and GLP guideline-compliant Ames assay, there were no increases in reverse mutations when concentrations of ambrettolide of 33–5000 µg/plate were incubated with *S. typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 in the absence or presence of a phenobarbital/β-naphthoflavone-induced rat liver S9 metabolic activation system, using both the plate incorporation and pre-incubation methods (Poth, 2003).

7.7. Group 13 structural analog: genotoxicity study for dehydromenthofurolactone (FEMA 3755)

In a 90-day dietary study, groups of Sprague-Dawley rats (20/sex/dose) were fed dehydromenthofurolactone in the diet at levels calculated to provide an average daily intake of 0, 1, 10 or 100 mg/kg bw for 13 weeks (Voss, 1985). The rats were monitored daily for premature death, clinical signs of toxicity and behavioural changes. Individual body weights and food consumption were recorded prior to study initiation and weekly thereafter. Serum chemistry, hematology determinations and gross necropsy were performed on 5 randomly selected rats of each sex for each dietary level at 4 weeks of exposure to dehydromenthofurolactone and for all surviving animals at termination of the study at week 13. At week 13 urine analysis was also performed for all surviving animals. At necropsy, absolute and relative organ weights were determined for the adrenals, brain, gonads, liver and kidneys. Microscopic examinations were performed on these tissues from rats in the control and high dose groups. Based on the findings from the gross necropsies performed at week 4, microscopic examinations of the esophagus and stomach were also conducted for the animals in the 1 and 10 mg/kg bw/day dietary level groups.

Two male deaths were reported: one in week eight in the 10 mg/kg bw exposure group and one in week five in the 100 mg/kg bw/day exposure group, which were determined to be due to accidental trauma and unrelated to dehydromenthofurolactone exposure in the diet. Decreased food consumption and significantly lower body weights were recorded for animals of both sexes in the 100 mg/kg bw dose group compared to the control group throughout the study. The decreases in body weight were observed in males and females at the 10 mg/kg bw per day dose group but were not statistically significant compared to the control groups. The decreased body weights corresponded to decreased food consumption and the authors attributed these effects to decreased palatability of the dehydromenthofurolactone diet.

It was reported that male and female rats in the 10 and 100 mg/kg bw dose had significantly increased ($p \leq 0.05$) esophageal mucosal hyperkeratosis and epithelial thickening, with no evidence of inflammation, which were attributed to an irritating action of the test substance. No other lesions related to the exposure of the test substance in the diet were reported. Relative liver weights were increased in both sexes of the 10 and 100 mg/kg bw exposure groups at study termination, but not in the lowest exposure group (1 mg/kg bw/day). The authors state that since the overall body weights for the 10 and 100 mg/kg bw/day groups were decreased and the absolute liver weights were comparable for test diet and control groups, the increased relative liver weights were likely due to the decreased palatability of the test substance in the diet. Similarly, significantly lowered relative kidney, brain and ovary weights in high dose females and significantly lowered relative testes weights in high dose males were also due to the lowered overall body weights in these high dose groups. Since increased serum total protein and albumin were reported for only males in the 100 mg/kg bw/day exposure group without a change in the females at this exposure level or in the other dietary level groups of either sex, and there was no effect on the albumin/globulin ratio or clinical chemistry, hematology and urinalysis measurements, this was not considered to be toxicologically significant. Because the relative organ weight changes were considered secondary to decreased body weight since there were no absolute organ weight changes and were not accompanied by histopathological changes or clinical chemistry or hematologic changes, they were considered not to be toxicologically relevant. The FEMA Expert Panel determined a NOAEL of 10 mg/kg bw/day for both sexes based on decreased body weights in high dose females. Based on this NOAEL, a MoS of greater than 6700 was determined for the intake of Congeneric Group 13 (Aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones) constituents in Lovage Root Oil (FEMA 2651), which is adequately protective for children, as discussed in Step 13 of the safety evaluation.

7.8. Group 19 constituents: genotoxicity studies for farnesene (FEMA 3839) and germacrene D

In an OECD and GLP guideline-compliant Ames assay, no evidence of mutagenicity was observed when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA were incubated with a mixture of farnesene isomers at concentrations of 5–5000 µg/plate in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Bhalli, 2016). In the tester strains, precipitation was observed at concentrations ≥ 1600 µg/plate in the absence and presence of S9. In a confirmatory assay, the tester strains were incubated with farnesene isomers at concentrations of 16–5000 µg/plate, in the presence or absence of S9. Similarly, there was precipitation observed at concentrations ≥ 1600 µg/plate with no indication of toxicity in the absence or presence of S9. Under the test conditions, farnesene isomers showed no mutagenic potential in the presence or absence of metabolic activation in the reverse mutation assay.

In an OECD and GLP guideline-compliant *in vitro* micronucleus induction assay, a mixture of farnesene isomers was incubated with HPBL at concentrations of 40.4–1000 µg/mL with an exposure period of 3 h and a 21 h recovery period, both with and without an Aroclor 1254-induced rat liver S9 metabolic activation and for a continuous exposure period of 24 h without S9 (Bhalli, 2017b). Precipitation was observed at concentrations ≥ 82 µg/mL for the 24 h exposure period without S9 and ≥ 343 and ≥ 490 µg/mL in the 3 h exposure assays in the presence and absence of S9, respectively. Based on the above findings, HPBL were incubated with concentrations of 12–59 µg/mL farnesene isomers in the 24 h treatment arm without S9, 39–100 µg/mL in the 3 h exposure arm without S9 and 53–200 µg/mL in the 3 h exposure arm with S9. The 24 h continuous exposure arm did not meet cytotoxicity requirements and was repeated to refine the concentrations scored. In a second assay, HPBL were exposed to the farnesene isomeric mixture at concentrations of 26–75 µg/mL in the 24 h exposure arm without S9. In this repeat assay there was a statistically significant but not a concentration-dependent induction of micronucleated binucleated cells observed at 50 µg/mL after 24 h continuous exposure in the absence of S9. This was not observed in the 3 h treatments with or without S9. A confirmatory assay was performed for the 24 h exposure group in the absence of S9 with concentrations of 19–60 µg/mL farnesene isomers. Because this assay did not meet the cytotoxicity requirements, a second confirmatory assay was performed with 20–100 µg/mL farnesene isomers with a 24 h exposure period in the absence of S9. This assay met the cytotoxicity requirements for an OECD guideline study and there was no increase in the induction of micronucleated binucleated cells. In summary, a farnesene mixture of isomers was not clastogenic or aneugenic in cultured HPBL in the presence or absence of metabolic activation, under the conditions tested.

In an OECD and GLP guideline-compliant Ames assay, *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA were incubated with concentrations of germacrene D of 0.5–5000 µg/plate in the absence and presence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Sawant, 2017). There was precipitation observed at concentrations ≥ 1600 µg/plate in incubations with all test strains in the presence and absence of S9 metabolic activation. There were no increases in reverse mutations observed under any of the test conditions. In a confirmatory assay in the same test strains, 16–5000 µg/plate concentrations of germacrene D were assayed in the absence and presence of S9. Precipitation was again observed at concentrations ≥ 1600 µg/plate in all tester strains with and without S9. There was no increase in reverse mutations in any tester strains at any concentration of germacrene D in the absence and presence of S9.

In an OECD and GLP guideline-compliant *in vitro* micronucleus induction study, HPBL were incubated with germacrene D at concentrations of 39.5–2000 µg/mL for 3 h in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system with a 21 h recovery period and for 24 h in the absence of S9 (Bhalli, 2017b). In

the continuous 24 h exposure without S9 and 3 h exposure with S9, precipitation was observed at concentrations ≥ 115 µg/mL and also in the 3 h treatment without S9 at concentrations ≥ 81 µg/mL. Based on these findings, HPBL were incubated with 16.3–135 µg/mL germacrene D in the continuous 24 h exposure treatment and 16.3–100 µg/mL in the 3 h treatment in the absence of S9. In the 3 h treatment with metabolic activation, germacrene D was tested at concentrations of 29–150 µg/mL but appropriate levels of cytotoxicity were not achieved. In the repeat assay, the 3 h exposure assays were conducted at concentrations of 14.4–63 µg/mL germacrene D without S9 and 97–2000 µg/mL with S9. Appropriate cytotoxicity was achieved in the assays without S9. In a fourth 3 h exposure period assay with S9, appropriate levels of cytotoxicity were not achieved but in a fifth 3 h exposure assay at concentrations of 32–200 µg/mL in the presence of S9, the appropriate cytotoxicity for assay validity was reached. No statistically significant increases in the frequency of micronucleated binucleated cells was observed under the conditions tested with exception of the lowest concentration scored for the 3 h treatment in the absence of S9. This finding was within historical ranges and was not concentration-dependent and therefore judged as not biologically relevant. The study author found no evidence that germacrene D induces aneugenicity or clastogenicity in HPBL in the presence or absence of metabolic activation.

7.9. Group 22 constituent: genotoxicity studies for phenethyl alcohol (FEMA 2858)

Phenethyl alcohol was evaluated for its mutagenic potential in two *in vitro* assays. In *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2uvrA, phenethyl alcohol did not increase reverse mutations when tested at concentrations up to 5000 µg/plate in the presence and absence of a phenobarbital/β-naphthoflavone-induced S9 metabolic activation system (ECHA, 2012b). Similarly, phenethyl alcohol was negative for mutagenicity in the forward mutation assay using mouse lymphoma L5178Y cells. In this assay, cells were incubated for 4 h at 76–1220 µg/mL in the presence and absence of S9 or for 24 h at the same concentration range. No increases in mutant frequency were observed in the 4 h or 24 h incubations under the conditions tested (ECHA, 2012c).

In human lymphocytes, phenethyl alcohol was tested at a concentration range of 38–1220 µg/mL for the ability to induce chromosomal aberrations in the presence and absence of S9 metabolic activation (ECHA, 2012a). Cultured lymphocytes were exposed to the test substance for 4 h with and without S9 or 24 h without S9. After cell harvesting, the concentrations selected for metaphase analysis were 305, 610 and 1220 µg/mL for all three treatment conditions. Analyzed cultures did not show an increase in aberrant cells when compared to the concurrent vehicle control, and phenethyl alcohol was concluded to be non-genotoxic.

7.10. Group 23 constituent: genotoxicity and short-term toxicity studies for Tetrahydro-4-methyl-2-(2-methylpropen-1-yl) pyran (Rose oxide) (FEMA 3236)

In a study designed to investigate the potential to induce gene mutations at the HRPT locus of V79 cells, rose oxide was incubated with Chinese hamster V79 cells at concentrations of 24–770 µg/mL (Wollny, 2012). In the first experiment, the cells were incubated with rose oxide with an exposure period of 4 h and a recovery period of 20 h, in the absence or presence of phenobarbital/β-naphthoflavone-induced rat liver S9. In the second experiment, cells were exposed to rose oxide for 4 h with a 20 h recovery period with S9 and for 24 h (continuous) without S9. There were no increases in mutation frequency observed at any concentration in the absence or presence of S9 under the test conditions with rose oxide. Rose oxide was determined to be non-mutagenic in the HRPT assay.

In an OECD and GLP guideline-compliant *in vivo* mouse micronucleus

induction study, groups of 5 male NMRI mice were administered 0 (vehicle), 250, 500 or 1000 mg/kg bw of rose oxide (purity 99.1%) by corn oil gavage (Schulz and Landsiedel, 2012). At 24 h following treatment, these mice were terminated and femoral bone marrow was harvested for analysis. Two additional groups of mice, a vehicle control group and a high-dose group were terminated 48 h following treatment. In the 24 h experiment, there were some weak dose-related increases in the induction of micronuclei, attributed by the study author to inter-animal variability. No statistical significance was reached at any dose and some of the control animals had an increase in polychromatic erythrocytes (PCE). Based on large inter-animal variability and failing to achieve statistical significance, this was regarded as not biologically relevant. In the 48 h experiment, there was also a weak dose-related induction of micronuclei but without statistical significance. In the 48 h experiment there was again wide inter-animal variability and positives observed in the concurrent vehicle control groups. The findings of a weak dose-related induction without statistical significance were determined by the authors of the study as not biologically relevant. The authors concluded that under the experimental conditions of this study, rose oxide did not induce clastogenic or aneugenic damage in the bone marrow cells of NMRI mice *in vivo*.

In an OECD and GLP guideline-compliant short-term toxicity study, groups of Wistar rats (5/sex/dose) were administered 0 (vehicle), 100, 300 or 1000 mg/kg bw/day of rose oxide by corn oil gavage for 28 days (Buesen et al., 2012). Body weights and food consumption were measured, and detailed clinical examinations were performed weekly. Functional observational battery and measurement of motor activity were performed at the end of the study. Also, clinical chemistry, hematology and urine analyses were performed at the end of the administration period. At termination, gross and histopathological examinations were performed. There were no significant clinical signs of toxicity observed and there were no early deaths. Body weight gain and food consumption were comparable across the test and control groups. There were incidental, non-treatment-related effects observed in the functional observational battery and motor activity examinations. In the high-dose group, there were decreased red blood cell (RBC) counts and increased relative reticulocyte counts for both sexes. Males had a decrease in hematocrit levels and females had a decrease in mean hemoglobin and platelet counts. High-dose females had an increase in serum ALT activity, triglyceride and cholesterol levels. Males in the mid- and high dose groups had decreased sperm motility and sperm head counts in the right cauda epididymis in addition to increased abnormal sperm counts. The male reproductive observations corresponded with significant increases in the absolute and relative mean epididymis weights with slight to severe immature ducts, minimal to slight interstitial edema and intraductal granulocytic infiltration. Based on the clinical chemistry and hematology effects, the NOAEL for females was determined to be 300 mg/kg bw/day for rose oxide and based on the male reproductive effects observed, the NOAEL for males was determined to be 100 mg/kg bw/day for rose oxide.

7.11. Natural flavor complexes: short-term/sub-chronic toxicity and/or reproductive/developmental studies for castor oil, citronella oil, costus root oil, lemongrass oil, Rose oil, sandalwood oil and Litsea cubeba berry oil

7.11.1. Castor oil

In a 13-week dietary toxicity study, castor oil was administered at doses of 600, 1,300, 2,500, 5000 and 10,000 mg/kg bw/day to male and female rats or 900, 1,950, 3,750, 7500 or 15,000 mg/kg bw/day in male and female mice (NTP, 1992). At the end of the study, organ weights were determined for the liver, right kidney, right testicle, heart, thymus, and lungs. There were no significant changes in hematological parameters between the test and control groups. Significant dose-related increases in total bile acids for males at the highest dose levels were measured at days 5 and 21 but were not observed at the study termination. Significant dose-related increases in serum ALP for males were

observed at days 5 and 90 for the three highest dose levels. In females, significant dose-related increases in serum ALP were observed at days 5 for the three highest dose levels and days 21 and 90 for the two highest dose levels. The study authors associated the increases in bile acids and serum ALP with elevated metabolic activity resulting from increased lipid absorption and not as a result of a toxic response. Increased absolute liver weights were observed in male rats receiving the highest dose and in male and female mice receiving 7500 and 15,000 mg/kg bw/day of castor oil. There were corresponding increases in relative liver weights in rats and mice, but they were not statistically significant. Histopathological examination did not reveal lesions in the liver in either species and clinical chemistry did not show changes that would indicate hepatotoxicity. No significant histopathological and morphological changes were observed in any organs and tissues of male and female rats and mice. Based on the absence of adverse effects in any organ system of either species, the NOAEL for castor oil was determined by the Expert Panel to be 10,000 mg/kg bw/day for rats and 15,000 mg/kg bw/day for mice, the highest doses tested.

7.11.2. Citronella oil

In an OECD and GLP guideline-compliant combined repeat dose and reproductive/developmental toxicity study, groups of male and female Sprague Dawley rats were administered citronella oil at dietary intakes of 100, 300 or 1000 mg/kg bw/day in the feed (ECHA, 2017a, b). Additional groups of rats were administered the basal diet (control) or the high-intake level diet as part of the recovery group (females of the recovery groups were unmated). Male rats were administered the basal diet (control) or the diet containing citronella oil for two weeks prior to mating, throughout the mating period and for two weeks after mating until the scheduled study termination, for a total of 28 days. Female rats were maintained on a similar feeding schedule through the end of the mating period, after which the test substance was administered in the diet until postpartum day (PPD) 4, for a minimum of 41 days. For animals in the main and recovery groups, there were no treatment-related clinical observations or deaths. A single female of the low intake group and her litter were found dead on PPD 1 with a prolapse of the uterus, but this finding was not associated with administration of the test substance in the diet. Mean group body weights were comparable between all treatment and control groups. There were no differences in food consumption for the male groups at any dietary level. Periodic reductions in food consumption were observed in all female treatment groups throughout the study. Based on mean body weights and food consumption, the achieved dietary intakes for the nominal levels of 100, 300 and 1000 mg/kg bw/day were reported to be 73, 216 and 708 mg/kg bw/day in males and 88, 249 and 771 mg/kg bw/day in females, respectively. There were no adverse findings observed upon ophthalmological, behavioral (functional observational battery), hematological and urinalysis examinations. Clinical chemistry findings included decreased serum potassium in low and middle intake group males (8% and 9%, respectively); decreased creatinine in middle intake group males (25%), high intake group females (13%) and high intake recovery group males (23%); increased globulin in middle and high intake group males (10% and 15%, respectively), increased phosphorus concentrations in high intake group males (23%); decreased AST activity in high intake group females (39%); increased triglycerides in two middle intake group females and one high intake group female (3- and 4.1-fold); and increased bile acids in two high intake group females (>2.2-fold). These findings were not dose-dependent, were low in severity and were without histopathological correlates and, therefore, were concluded to be not toxicologically significant. Slight differences in organ weights were reported, including a reduction in absolute heart and spleen weights in high intake females and increased relative kidney weights in high intake males, but these were without histopathological correlation. There were no adverse treatment-related findings upon macroscopic or histopathological examination in any of the groups provided citronella oil in the diet. Based on these findings, the NOAEL for systemic toxicity

was determined to be the highest intake level tested, 708 mg/kg bw/day in males and 771 mg/kg bw/day in females (ECHA, 2017a). The evaluation of reproductive functionality noted no changes to estrus cyclicity or other reproductive parameters such as pre-coital intervals, copulatory or fertility indices. However, decreases in live and total litter size were observed in middle and high intake group females and were considered to be treatment-related. In the same groups, decreases in corpora lutea and implantation sites were observed but were not statistically significant. There were no skeletal malformations or other adverse developmental findings reported in pups born to groups administered citronella oil. The NOAEL for developmental toxicity was determined to be 88 mg/kg bw/day (ECHA, 2017b). Using the most conservative NOAEL as a point of reference and the estimated intake provided in Table 1, the margin of safety for Citronella Oil (FEMA 2308) is greater than 26,000.

7.11.3. *Costus root oil*

In a 90-day dietary toxicity study, costus root oil was provided in the feed to groups of male and female FDRL rats at an achieved dietary intake of 1.77 and 2.17 mg/kg bw/day in males and females, respectively (Oser et al., 1965). Body weights and food consumption were measured throughout the study. At weeks 6 and 12, blood samples were collected for hematology and blood chemistry. At the end of the study, all animals were necropsied, and the liver and kidney weights were reported. Additional organs and tissues of half of the animals were excised and fixed for histopathology. Findings included increased food efficiency in male rats, decreased hemoglobin levels in males, decreased neutrophil counts in females and decreased blood urea nitrogen in males. Furthermore, the liver and kidney weights of one treated male (1/15) were elevated compared to the controls, but the increases in organ weights did not correlate with any histopathological findings. None of the findings were considered toxicologically relevant, and the NOAEL was determined to be the only dose tested of 1.77 mg/kg bw/day in males and 2.17 mg/kg bw/day in females (Oser et al., 1965).

7.11.4. *Lemongrass oil*

In a 14-day sub-acute toxicity study, lemongrass oil (described as 34% neral, 32% geranial, 20% myrcene and 3% terpinene-4-ol) was evaluated in male and female Wistar albino rats (Fandohan et al., 2008). Groups of 6 rats were administered 5, 50, 500, 1000, 1500, 2000, 3000 or 3500 mg lemongrass oil/kg bw by gavage. The rats administered nominal doses of 2000, 3000 or 3500 mg/kg bw lemongrass oil died on day 2 while all animals administered doses at nominal levels up to 1500 mg/kg bw survived the entire study period. Based on histopathology conducted on the stomach and liver tissues that revealed no adverse findings at doses up to 1500 mg/kg bw, the FEMA Expert Panel determined a NOAEL of 1500 mg/kg bw for lemongrass oil. However, because the animals used in the study were maintained under non-standard conditions for room temperature (25–30 °C) and relative humidity (averaged 90%), and salt was added to the diet at 1.5%, this study is not relevant to the safety evaluation of Lemongrass Oil (FEMA 2624).

No adverse treatment-related findings were reported for lemongrass oil in a 21-day repeat-dose oral toxicity study in male Swiss mice. Groups of animals were administered lemongrass oil by oral gavage at dose levels of 0 (vehicle), 1, 10 and 100 mg/kg bw/day (Costa et al., 2011). Blood samples were collected at necropsy for serum biochemical analyses and organs and tissues were weighed and/or fixed for histopathology. Body weight gains and absolute and relative organ weights were comparable between treated and control groups. Furthermore, histopathological examinations did not reveal any adverse changes to the brain, heart, kidneys, liver, lungs, spleen, stomach or urinary bladder. No differences in biochemical parameters were observed, except a significant reduction in total cholesterol ($p < 0.05$) in high-dose animals relative to the saline control group. Based on these findings, lemongrass oil was considered well-tolerated at dose levels up to 100 mg/kg bw/day in male mice (Costa et al., 2011). Based on this NOAEL of 100 mg/kg bw/day and the estimated intake provided in Table 1, the

margin of safety for Lemongrass Oil (FEMA 2624) is greater than 14,000.

In an OECD guideline-compliant 21-day repeat dose oral toxicity study, lemongrass essential oil was administered to five male and female Swiss albino mice at dose levels of 0 (saline) or 2000 mg/kg bw/day (Lulekal et al., 2019). The essential oil, containing 71% citral, 19% myrcene and other minor constituents was obtained by steam distillation of the leaves and stalks of *C. citratus*. At the termination of the study, blood was obtained for hematological analysis and the kidneys and livers of treated mice were collected for gross and histopathological analyses. No mortalities or significant differences in signs of clinical toxicity, food consumption, body weight changes, relative organ weights or serum biochemistry parameters were observed between the control and treatment group. No adverse histopathological changes were observed in control and treated mice (Lulekal et al., 2019). Based on the single dose tested, 2000 mg/kg bw/day and the estimated intake provided in Table 1, the margin of safety for Lemongrass Oil (FEMA 2624) is greater than 290,000.

In a 60-day dietary toxicity study, male Charles Foster rats were administered feed containing lemongrass oil (*C. citratus*) at a dietary level of 1500 ppm which corresponds to approximately 150 mg/kg bw/day. Body weights were measured at the initiation of the study and on days 30 and 60. On day 60, blood samples were collected for hematology and serum clinical chemistry analyses. Gross and histopathological examinations were not performed. Higher body weight gains and food consumption were noted over the study period in animals provided lemongrass oil in the diet, but these values were not statistically significantly different from the control. Hematology and clinical chemistry parameters were comparable with the control group. Overall, there was no evidence of toxicity of lemongrass oil when fed at 150 mg/kg bw/day for up to 60 days (Mishra et al., 1992).

7.11.5. *Rose oil*

In an unconventional 30-day gavage study, groups of albino rats (10/sex/dose) were administered 0 (vehicle), 85 or 425 mg/kg bw/day rose oil (unspecified composition) dissolved in 1 mL ethanol/kg of water (Kirov et al., 1988a). The high-dose rats had clinical signs of toxicity including lethargy and slowed reflexes. The death of 4 females and 3 males from the high-dose group between Days 4 and 28 of the study was associated with the toxicity of the test substance. There was one unexplained death in the female low-dose group. Decreased body weights were reported in treated animals of both sexes at the high dose. Clinical chemistry analysis revealed statistically significant decreases ($p < 0.05$) in total lipids at both dose levels and in the vehicle controls which the authors associated with the ethanol in the vehicle. Hemoglobin and hematocrit levels were significantly decreased in female rats in the high-dose group. Increases in relative kidney and spleen weights were observed in the high-dose females and increases in relative testes weights were observed in the high-dose males. Neither macroscopic nor microscopic examinations were reported for these tissues. Impairment of motor activity was noted in all control and treated groups but may have been attributable to the ethanol used as a vehicle. In a subsequent 30-day repeat dose reproductive study, pregnant female Wistar rats were administered 0.1 and 0.35 mL/kg bw/day rose oil in a 10% sunflower oil solution, corresponding to concentrations of 9 and 30 mg/kg bw/day⁸ and 1.75 mL/kg bw/day undiluted rose oil (corresponds to 1500 mg/kg bw).⁸ A NOAEL for teratogenic effects of 9 mg/kg bw/day 10% rose oil solution in sunflower oil was established based on wide fontanelle and cranial structure development observed in fetuses of high-dose females (Kirov et al., 1988b).

7.11.6. *Sandalwood oil*

In a study designed to observe the effects of sandalwood oil on the

⁸ Based on a specific gravity of 0.856 (Source Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD USA).

induction of glutathione metabolizing enzymes, yellow sandalwood oil was administered to adult male Swiss Albino mice (8/dose) at doses of 5 and 15 $\mu\text{L}/\text{day}$ for 10 or 20 consecutive days by gavage. Based on the average weight of a mouse, 20 g, and the density of sandalwood oil, 0.97 g/mL, these doses correspond to daily intakes of 243 and 730 mg/kg bw/day, respectively. The mouse liver isolates showed increased glutathione S-transferase (GST) enzyme activity in the cytosol and increased levels of acid-soluble sulfhydryl levels in hepatic tissue (Banerjee et al., 1993). In a 14- and 21-day postnatal enzyme induction study, female Swiss Albino mice (10/dose/generation with 10 offspring/dam) were administered 5 and 10 $\mu\text{L}/\text{day}$ of yellow sandalwood oil by gavage. These dose levels correspond to estimated daily intakes of 243 and 487 mg/kg bw/day. There were treatment-related increases in sulfhydryl content, cytochrome b5, GST activity, glutathione reductase activity and glutathione peroxidase activity reported in the mice (Chhabra and Rao, 1993).

7.11.7. *Litsea cubeba* berry oil

In a 28-day repeat dose oral toxicity study, *Litsea cubeba* oil, obtained from the fruit of the plant, was administered to Sprague-Dawley rats (6/sex/dose) at 0 (vehicle), 1, 10 and 100 mg/kg bw/day by soybean oil gavage (Tubtim and Wasiksiri, 2007). General observations and cage-side observations were conducted daily and body weights were measured at the start of the study and weekly thereafter. Blood samples were collected prior to necropsy for hematological and clinical chemistry evaluation. All animals were subject to gross examinations at necropsy and the liver, kidneys, heart and spleen were weighed. These organs were also fixed for histopathological examination. During the study period, there were no deaths or adverse clinical observations. At the end of the study period, mean group body weights of treated males were lower than the controls ($p < 0.05$) at all dose levels. Mid- and high-dose females displayed lower body weights when compared to the control group during the first two weeks of treatment, but by the end of the treatment period, there were no notable differences in mean body weight. In male rats, there were no differences in hematology or clinical chemistry parameters when compared to the control rats. Hematological and clinical chemistry findings in females included a decrease in serum urea nitrogen concentrations ($p < 0.05$) in low-dose females that was not observed at higher dose levels; increases in alkaline phosphatase (ALP) activity ($p < 0.05$) in mid- and high-dose females; and statistically significant ($p < 0.05$) decreases in hematocrit, hemoglobin and red blood cell counts in only mid-dose females. Decreases in relative liver weight were observed in low- and high-dose males but not the mid-dose, while increases in relative liver weight were observed in high-dose females. Increased relative kidney weights were present in high-dose males and mid- and high-dose females. An increase in relative heart weight was observed in mid-dose females. Upon histopathological examination, there were no adverse treatment-related findings in the liver, kidneys, heart or spleen. Therefore, the changes in clinical chemistry parameters and differences in relative organ weight were not considered toxicologically relevant due to the lack of dose-dependence and/or histological changes. The NOAEL for this study was determined to be 100 mg/kg bw/day, the highest dose tested, for *Litsea cubeba* oil in both males and females (Tubtim and Wasiksiri, 2007). Based on this NOAEL and the estimated intake provided in Table 1, the margin of safety for *Litsea Cubeba* Berry Oil (FEMA 3846) is greater than 750,000.

7.12. Natural flavor complexes: genotoxicity studies for balm oil, castor oil, chamomile oils, chamomile extract, citronella oil, geranium rose oil, lemongrass oil, lovage root extract, lovage root oil, palmarosa oil, Rose absolute

In vitro and *in vivo* genotoxicity studies are available for the NFC evaluations, which are summarized in Table 4 and are described in detail below.

7.12.1. Balm oil

Balm oil (*Melissa officinalis*) was not mutagenic when tested at concentrations of 88, 177 and 442 $\mu\text{g}/\text{plate}$ in an Ames assay conducted in *S. typhimurium* strains TA98 and TA100 in the presence and absence of S9 metabolic activation. The sample tested was chemically characterized and reported to contain 40% (–)-citronellal, 13% carvacrol, 9% isomenthone and minor amounts of other terpene hydrocarbons and sesquiterpenes (De Martino et al., 2009).

7.12.2. Castor oil

In vitro

Castor oil did not induce mutagenicity in an Ames assay conducted in *S. typhimurium* strains TA97, TA98, TA100 and TA102, as well as in *E. coli* WP2/pKM102 in the presence and absence of an S9 metabolic activation system at concentrations of 100–5000 $\mu\text{g}/\text{plate}$ (Hachiya, 1987). Castor oil was also non-mutagenic in an Ames assay conducted in *S. typhimurium* strains TA97, TA98, TA100 and TA1535 in the presence and absence of S9 at concentrations ranging from 100 to 10,000 $\mu\text{g}/\text{plate}$ (Zeiger et al., 1988).

No evidence of induction of sister-chromatid exchanges (SCE) in Chinese hamster ovarian (CHO) cells was observed in the presence and absence of an S9 metabolic activation system at concentrations of 160–5000 $\mu\text{g}/\text{mL}$ castor oil (NTP and Irwin, 1992). At concentrations of 1600–5000 $\mu\text{g}/\text{mL}$, no chromosomal aberrations in CHO cells were observed in the presence and absence of S9 (NTP and Irwin, 1992). Due to a lack of understanding of the underlying mechanism(s) of action of the SCE assay, it was removed from the OECD library of standardized assays in 2014 (OECD, 2015) and its relevance to the safety evaluation cannot be assessed (Gooderham et al., 2020).

In vivo

In an *in vivo* micronucleus assay conducted in peripheral blood erythrocytes of B6C3F₁ mice, no genotoxicity was observed for castor oil administered at 900, 1950, 3750, 7500 or 15,000 mg/kg bw/day in a 13-week dietary study. No difference in the percentage of normochromatic versus polychromatic micronuclei were observed for all test groups compared to the control group (NTP and Irwin, 1992; Witt et al., 2000).

7.12.3. Chamomile oil (Roman/English)

In an OECD and GLP guideline-compliant Ames assay, the essential oil of steam-distilled Roman/English chamomile flowers (*Anthemis nobilis* L.) was tested at up to 1600 $\mu\text{g}/\text{plate}$ using the plate incorporation method in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *E. coli* strain WP2uvrA/pKM101 in the presence and absence of an S9 metabolic activation system prepared from the liver of Aroclor 1254-treated rats. Chamomile oil (Roman/English type) did not induce mutations in the presence and absence of metabolic activation (Mee, 2017b). The essential oil of *A. nobilis* was also negative for mutagenicity in a reverse mutation assay in four strains of *S. typhimurium* and in a rec assay in *B. subtilis* (Zani et al., 1991). The components of the tested oil were determined to be 35% *n*-butyl angelate, 16% isoamyl angelate, 5% borneol and other minor constituents. In the reverse mutation assay, *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were incubated with chamomile oil in treatments with and without S9 metabolic activation system, prepared from Aroclor-treated Sprague-Dawley rats. At the tested concentrations of 225, 450 and 900 $\mu\text{g}/\text{plate}$,⁹ chamomile oil did not increase the number of revertant colonies in any of the tester strains. Similarly, in the rec assay, incubation of chamomile oil in

⁹ Based on a specific gravity of 0.90 (Source Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD USA).

B. subtilis cultures at 9 and 27 mg/disk⁹ did not result in growth inhibition (Zani et al., 1991).

7.12.4. Chamomile oil (Hungarian)

In vitro

Three independent OECD and GLP guideline-compliant Ames assays on blue chamomile oil (*Matricaria recutita* or *M. chamomilla*) were conducted in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *E. coli* strain WP2uvrA or WP2uvrA/pKM101. Each assay was conducted using concentrations up to 5000 µg/plate blue chamomile oil in the presence and absence of S9 metabolic activation (ECHA, 2018b, a; Mee, 2017a). When tested up to the limit of cytotoxicity, blue chamomile oil was non-mutagenic in all the strains tested in both the presence and absence of metabolic activation.

In an OECD and GLP guideline-compliant *in vitro* micronucleus induction assay, blue Egyptian type chamomile oil was non-genotoxic in human lymphocytes (Clare, 2017). Human lymphocytes were incubated with the essential oil in 3 h treatments with and without an Aroclor 1254-induced rat liver S9 metabolic activation system at concentration ranges of 326–435 µg/mL and 52–890 µg/mL, respectively and in a 24 h treatment without S9 at concentrations of 52–395 µg/mL. None of the treatments with blue chamomile oil increased the frequency of micronucleated cells under the conditions tested (Clare, 2017).

In vivo

To evaluate the potential to induce SCEs *in vivo*, chamomile oil (*M. chamomilla*) was orally administered to groups of male NIH mice at levels of 10, 100 and 1000 mg/kg bw. Composition analysis indicated that the tested oil contained 51% α-bisabolol oxide (various isomers), 30% farnesene (α- and β-isomers), and other minor constituents. Femoral bone marrow was collected for SCE evaluation 24 h following administration of the test substance. None of the dose levels tested resulted in SCE frequencies that differed from the control (Hernández-Ceruelos et al., 2002). The same chamomile oil was administered to groups of male NIH mice in two separate studies at a single dose level of 500 mg/kg bw. In both studies, chamomile oil did not induce SCE in the bone marrow and was concluded to be non-genotoxic (Hernández-Ceruelos et al., 2010). As discussed above, the SCE assay was removed from the OECD library of standardized assays in 2014 (OECD, 2015) and its relevance to the safety evaluation cannot be assessed (Gooderham et al., 2020).

7.12.5. Citronella oil

In vitro

In two OECD and GLP guideline-compliant Ames assay studies, Java-type citronella oil (*Cymbopogon winterianus*) was not mutagenic when tested at concentrations up to 5000 µg/plate in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA using the plate incorporation method (Dakoulas, 2014a) and using both plate incorporation and preincubation methods and another assay (ECHA, 2014a). In another OECD guideline-compliant Ames assay study, Ceylon-type citronella oil (*C. nardus*) was non-mutagenic in the presence and absence of S9 at concentrations up to 512 µg/plate using the plate incorporation method and up to 1600 µg/plate using the pre-incubation method (ECHA, 2018b). In a non-standard Ames assay, evidence of mutagenicity was reported in *S. typhimurium* strains with remarkably low concentrations (ng/plate) of citronella oil (Sivaswamy et al., 1991). The Expert Panel has evaluated this study in their previous safety assessment of *Cinnamomum* and *Myroxylon*-derived flavoring ingredients and concluded that the study has numerous deficiencies and

that the results of this study do not inform the safety evaluation (Rietjens et al., 2020). A rec assay was conducted in which citronella oil was incubated with *B. subtilis* using the spore plate method. Citronella oil, tested at 280–2250 µg¹⁰ was positive for mutagenicity with S9 metabolic activation and negative in the absence of S9 (Kuroda et al., 1989). The rec assay is limited by the lack of testing guidelines and the OECD notes that the results of indicator tests, including the rec assay, should be correlated to the results of other genotoxicity or mutagenicity assays (OECD, 2015).

In an OECD guideline forward mutation assay, citronella oil (Java type) was tested in mouse lymphoma L5178YTk^{+/−}± cells to evaluate its potential to induce mutations at the *hprt* locus (ECHA, 2013a). In 4 h treatments, the test concentrations were 2–78 µg/mL in the absence of S9 metabolic activation system and 5 to 156 µg/mL in the presence of S9. A 24 h continuous exposure treatment was done in the absence of S9 at concentrations of 0.6–20 µg/mL. Under the conditions tested, citronella oil did not increase the frequency of forward mutations in mouse lymphoma L5178Y cells (ECHA, 2013a).

An OECD and GLP guideline-compliant *in vitro* mammalian chromosome aberration (CA) assay in human lymphocytes showed that citronella oil (Java type) was non-clastogenic in the presence and absence of an S9 metabolic activation system (ECHA, 2014b). Human lymphocytes were incubated with citronella oil for 4 h at concentrations of 10–160 µg/mL without S9, 20–320 µg/mL with S9 and for 24 h at concentrations of 20–240 µg/mL without S9.

In two *in vitro* comet assays, HPBL were treated with citronella oil in the absence of S9 metabolic activation up to a maximum concentration of 2000 µg/mL (Sinha et al., 2011, 2014). At concentrations of 1000 µg/mL and greater, citronella oil increased the % tail DNA, indicative of DNA damage (Sinha et al., 2011, 2014). Due to the lack of a standardized procedure for the performance and evaluation of results of the *in vitro* comet assay, the relevance of the positive results cannot be assessed (Gooderham et al., 2020).

In an OECD and GLP guideline-compliant *in vitro* micronucleus assay conducted in HPBL, no induction of micronuclei was observed when citronella oil was tested at concentrations up to 200 µg/mL for 4 h and up to 135 µg/mL for 24 h in the absence of S9 metabolic activation, as well as up to 450 µg/mL for 4 h in the presence of S9 metabolic activation derived from the liver of Aroclor 1254-induced rats (Roy, 2015a).

7.12.6. Geranium rose oil

In an OECD and GLP guideline-compliant Ames assay using the plate incorporation method, rose geranium oil was non-mutagenic in the presence and absence of S9 at concentrations up to 5000 µg/plate in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, as well as in *E. coli* WP2uvrA (ECHA, 2014c). In Ames assays performed without metabolic activation, weakly positive mutagenicity was reported at concentrations of 4.5 ng/plate of rose geranium oil *S. typhimurium* strain TA1535 and TA1537 and evidence of mutagenicity was observed in *S. typhimurium* strain TA1538 at 4.5 ng/plate. No mutagenicity was observed at 9 ng/plate in *S. typhimurium* strain TA98 (Sivaswamy et al., 1991). As stated above, the Expert Panel has previously evaluated this study and concluded that because of numerous deficiencies, this study does not inform the safety evaluation (Rietjens et al., 2020).

7.12.7. Lemongrass oil

In an OECD and GLP guideline-compliant Ames assay, no mutagenicity was observed for lemongrass oil in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA (ECHA, 2015). Lemongrass oil was tested in the presence and absence of S9 metabolic activation at concentrations of 1.5–5000 µg/plate in an initial assay using the plate incorporate method, followed by a confirmatory assay at

¹⁰ Based on a relative density of 0.90 g/mL (ISO 3849:2016 Citronella oil (Java type) and ISO 3849:2003 Citronella oil (Sri Lanka type)).

concentrations of 0.5–1500 µg/plate. No increases in the number of revertant colonies in any of the test strains were reported under the conditions tested. Lemongrass oil was considered weakly mutagenic in *S. typhimurium* strains TA1537 and TA1538 at a concentration of 4 ng/plate and strongly mutagenic in *S. typhimurium* strain TA98 at 9 ng/plate in the absence of metabolic activation. No mutagenicity was observed at 9 ng/plate in *S. typhimurium* strain TA1537 in the absence of metabolic activation (Sivaswamy et al., 1991). The Expert Panel has evaluated this study in their previous safety assessment of *Cinnamomum* and *Myroxylon*-derived flavoring ingredients and concluded the study has numerous deficiencies and that the results of this study do not inform the safety evaluation (Rietjens et al., 2020).

In an *in vitro* comet assay conducted in HPBL, lemongrass oil was tested at concentrations of 25–800 µg/mL. Evidence of DNA damage, based on the measurement of an increase in the % tail DNA, was observed at concentrations of 100 µg/mL and higher. Decreases in cell viability, measured by a trypan blue exclusion test, were observed at 200 µg/mL and higher, reaching statistical significance at 400 and 800 µg/mL (13% and 20% decrease, respectively) (Sinha et al., 2014). The study authors attribute the positive genotoxicity observed at high concentrations to significant cytotoxicity present at these concentrations (Sinha et al., 2014). An *in vivo* comet assay in male Swiss mice was negative for DNA damage. Animals were administered a single dose of lemongrass oil at a level of 100 mg/kg bw by oral gavage, after which peripheral blood leukocytes were evaluated for DNA damage. The % tail intensity of animals treated with lemongrass oil was comparable to values for control groups (Costa et al., 2011). Because there is not a standardized procedure for the performance and evaluation of results of the *in vitro* comet assay, the relevance of the results of these assays cannot be assessed (Gooderham et al., 2020).

7.12.8. Lovage root oil

In a GLP guideline-compliant Ames assay, *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were treated with lovage oil at concentrations of 10–2600 µg/plate. All concentrations were tested in the presence and absence of an S9 metabolic activation system derived from Aroclor 1254-induced rat livers. Based on the conditions tested, lovage oil was found to be non-mutagenic in both the presence or absence of an S9 metabolic activation system (Jagannath, 1983). Additionally, lovage oil was negative in an unscheduled DNA synthesis assay in male rat hepatocytes at a concentration of 2580 µg/mL (Heck et al., 1989).

7.12.9. Lovage root extract

An extract of lovage root was non-mutagenic at concentrations up to 1000 µg/plate in an Ames assay conducted in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 using the plate incorporation method in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Jagannath, 1985).

7.12.10. Palmarosa oil

No evidence of genotoxicity was observed at concentrations of 50, 100 and 200 µg/mL of palmarosa oil incubated with HPBL for 3 h in an *in vitro* comet assay conducted in the absence of metabolic activation (Sinha et al., 2011). In a follow-up *in vitro* comet assay, HPBL were incubated with 100, 200, 500, 1000, 1500 and 2000 µg/mL palmarosa oil in the absence of exogenous metabolic activation for 3 h. An increase in % tail was observed at concentrations of ≥1000 µg/mL (Sinha et al., 2014). While no cytotoxicity was observed in the Trypan blue assay, a significant decrease in cell viability of 15 and 23% was observed in lymphocytes treated with 1500 and 2000 µg/mL of palmarosa oil. Due to a lack of a standardized procedure for the performance and evaluation of results of the *in vitro* comet assay, the relevance of the results of these assays cannot be assessed (Gooderham et al., 2020).

7.12.11. Rose absolute

Rose absolute, prepared from the flowers of *Rosa damascena trigintipetala* Dieck, was evaluated in an *in vitro* chromosomal aberration assay in human lymphocytes. The composition of the volatile fraction of the test substance was determined to be 33% phenethyl alcohol, 12% citronellol, 6% geraniol, 2% eugenol and 1% methyl eugenol by GC-MS analysis. At a single concentration of 10 µg/mL, rose absolute did not induce an increase in the frequency of aberrant cells and was concluded to be non-genotoxic (Hagag et al., 2014).

7.12.12. Rose oil

Rose oil was non-mutagenic in an OECD and GLP guideline-compliant Ames assay. When tested up to 5000 µg/plate in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system, rose oil did not increase the number of revertant colonies in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA (Dakoulas, 2014b).

In an OECD and GLP guideline-compliant *in vitro* micronucleus assay, induction of micronuclei was not observed when HPBL were treated with rose oil (Roy, 2015c). Rose oil was tested at concentrations of 50–450 µg/mL in the 4 h exposure tests in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system and at concentrations of 5–125 µg/mL in the non-activated 24 h exposure test. There were no increased incidences of micronucleated binucleated cells when compared to the concurrent vehicle controls. Another *in vitro* micronucleus assay in HPBL concluded that rose oil was positive for genotoxicity when tested at dose levels of 1–200 µg/mL (Shokrzaheh et al., 2017). The concentrations that showed statistically significant higher levels of micronucleated binucleated cells (greater than 10 µg/mL) also showed significant cytotoxicity based on reductions observed in the mitotic indices. Additionally, the OECD guideline for the *in vitro* micronucleus assay indicates that lymphocytes should be exposed to a mitogenic agent, phytohaemagglutinin (PHA), prior to test substance exposure to ensure induction of cell division (OECD, 2016). In this study, the test substance was added concomitantly with PHA and an appropriate positive control for clastogenicity was not evaluated. Therefore, the results reported in this non-standard study are not considered relevant to this safety evaluation of the FEMA GRAS material, Rose Bulgarian True Otto Oil (FEMA 2989).

7.12.13. Sandalwood yellow oil

Sandalwood yellow oil was found to be non-mutagenic in three separately conducted OECD and GLP guideline-compliant reverse mutation assays. In an OECD and GLP guideline-compliant study, mutagenicity in *S. typhimurium* strains TA97a, TA98, TA100, TA1535 and *E. coli* WP2uvrA was not observed when sandalwood oil was tested at 5–500 µg/plate¹¹ in the *S. typhimurium* strains and up to 5000 µg/plate¹¹ in *E. coli* in the presence and absence of an S9 metabolic activation system (ECHA, 2013b). In a second OECD and GLP guideline-compliant assay, no evidence of mutagenicity was reported in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA when yellow sandalwood oil was tested at concentrations of 1.6–1600 µg/plate, in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Mee, 2017c). In the third OECD and GLP guideline assay, yellow sandalwood oil was non-mutagenic when tested at concentrations of 3–5000 µg/plate in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA, in the presence and absence of a phenobarbital/β-naphthoflavone-induced rat liver S9 metabolic activation system (Sokolowski, 2020). In a non-standard Ames assay, two sandalwood oil varieties (termed Sandalwood I and Sandalwood II of unreported species) were assessed for mutagenicity in the Ames assay (Sivaswamy et al., 1991). The Expert Panel has

¹¹ Based on a specific gravity of 0.97 (Source Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD USA).

evaluated this study in a previous safety assessment and concluded the study has numerous deficiencies and that the results of this study do not inform the safety evaluation (Rietjens et al., 2020).

7.12.14. *Litsea cubeba* berry oil

No increases in reverse mutations were reported when *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were tested with concentrations of 0.8, 4, 20, 100 and 500 µg/plate *Litsea cubeba* berry oil in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system (Luo et al., 2005). In an OECD guideline-compliant Ames assay, no mutagenicity was observed using both the plate incorporation method at up to 5000 µg/plate and the pre-incubation method at up to 1500 µg/plate of *Litsea cubeba* berry oil in the presence and absence of S9 using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA (ECHA, 2014d).

Two *in vivo* cytogenetic studies were conducted on *Litsea cubeba* berry oil in male ICR mice to measure chromosome aberrations in spermatogenic cells or micronuclei in bone marrow erythrocytes (Luo et al., 2005). In the chromosomal aberration assay, mice (5/dose) were administered *Litsea cubeba* berry oil at doses of 460, 925 or 1850 mg/kg bw by gavage. There were no indications of chromosome aberrations in spermatogenous cells in the groups treated with *Litsea cubeba* oil when compared to the control concurrent group (Luo et al., 2005). When male ICR mice (5/dose) were administered the oil at 185, 740 or 1850 mg/kg bw by gavage, there was no evidence of micronucleus induction in the polychromatic erythrocytes of the bone marrow (Luo et al., 2005).

7.12.15. Summary of NFC genotoxicity studies

The large number of *in vitro* and *in vivo* studies conducted in accordance with widely accepted testing guidelines were negative for mutagenicity and other genotoxicity endpoints. Furthermore, there are no genotoxicity or toxicity concerns regarding the constituents that make up the compositions of these NFCs. Based on the available genotoxicity studies described above, there are no genotoxicity concerns for the NFCs under evaluation.

Table 5
FEMA GRAS flavoring materials affirmed.

FEMA No.	Name
2050	Ambrette Absolute Oil (<i>Hibiscus abelmoschus</i> L.)
2113	Balm Oil (<i>Melissa officinalis</i> L.) – Balm lemon oil, Melissa oil
2263	Castor Oil (<i>Ricinus communis</i> L.) – Ricinus oil, Palma Christi oil, Tantan-tangan oil
2273	Chamomile Flower Hungarian Oil (<i>Matricaria chamomilla</i> L.)
2274	Chamomile Flower Roman Extract (<i>Anthemis nobilis</i> L.)
2275	Chamomile Flower English/Roman Oil (<i>Anthemis nobilis</i> L.)
2308	Citronella Oil (<i>Cymbopogon nardus</i> Rendle and <i>Cymbopogon winterianus</i> Jowitt)
2331	Wine Lees Green Oil
2332	Wine Lees White Oil
2336	Costus Root Oil (<i>Saussurea lappa</i> Clarke, <i>Aplotaxis lappa</i> Dec., <i>A. suriculata</i> DC., <i>Auklandia costus</i> Falc.) – Spiral flag oil
2504	Genet Absolute (<i>Spartium junceum</i> L.)
2505	Genet Extract (<i>Spartium junceum</i> L.) – Broom extract
2508	Geranium Rose Oil (<i>Pelargonium graveolens</i> L'Her)
2624	Lemongrass Oil (<i>Cymbopogon citratus</i> DC. and <i>C. flexuosus</i> Stapf)
2650	Lovage Root Extract (<i>Levisticum officinale</i> Koch) – Smellage extract, Smallage extract
2651	Lovage Root Oil (<i>Levisticum officinale</i> Koch) – Smellage oil, Smallage oil
2831	Palmarosa Oil (<i>Cymbopogon martini</i> (Roxb.) Stapf)
2988	Rose Absolute (<i>Rosa alba</i> L., <i>Rosa centifolia</i> L. and vars. of these spp.)
2989	Rose Bulgarian True Otto Oil (<i>Rosa damascena</i> Mill.) – Attar of roses
3005	Sandalwood Yellow Oil (<i>Santalum album</i> L.) – Sandalwood East Indian oil, Saunders white oil
3110	Violet Leaves Absolute (<i>Viola odorata</i> L.)
3747	Massoia Bark Oil (<i>Cryptocarya massoia</i>)
3846	<i>Litsea Cubeba</i> Berry Oil (<i>Litsea cubeba</i> Pers.) – May chang

8. Recognition of GRAS status

The NFCs listed in Table 5 were previously determined to be GRAS under conditions of intended use by the FEMA Expert Panel (Newberne et al., 1998; Oser and Hall, 1965; Oser et al., 1985). The FEMA Expert Panel has presented a review on the history of use, chemical constituents, manufacturing methodology and the metabolic and toxicological data for these NFCs (Table 5). Chamomile Flower English Oil (FEMA 2272) and Chamomile Flower Roman Oil (FEMA 2275) have been determined to be identical NFCs and have been combined under FEMA 2275 as Chamomile Flower English/Roman Oil. The estimated intakes for the constituent congeneric groups for each NFC are below the TTC for their respective structural classes, indicating no safety concern. Adequate margins of safety specifically for children were determined for Group 13 constituents of Lovage Root Oil (FEMA 2651) the congeneric group of triglycerides in Castor Oil (FEMA 2263) where the estimated intakes of these groups are higher than their respective TTC thresholds when considering the lower body weight of children. A review of genotoxicity studies for the constituent congeneric groups, the constituents and the NFCs indicates a lack of genotoxic potential for these flavorings. By application of the safety procedure, the FEMA Expert Panel has affirmed the GRAS status for the NFCs listed in Table 5 when used as flavoring ingredients in food under conditions of intended use.

CRedit authorship contribution statement

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

Declaration of competing interest

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

et al., 2013) and are available on the FEMA website at <https://www.femaflavor.org/gras#conflict>.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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