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FEMA GRAS assessment of natural flavor complexes: Vanilla extract, Bitter almond oil, Wintergreen oil and related flavoring ingredients

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ABSTRACT

The Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) is conducting a program to re-evaluate the safety of over 250 natural flavor complexes (NFCs) used as flavoring ingredients. This publication, fourteenth in the series, evaluates the safety of NFCs composed primarily of benzaldehyde, methyl salicylate, vanillin and other benzyl derivative compounds. In 2018, the Expert Panel published an update of its safety evaluation procedure for NFCs that was first published in 2005. This procedure relies on a complete constituent characterization of the NFC and organization of the constituents of each NFC into defined congeneric groups. The safety of the NFC is evaluated using the threshold of toxicological concern (TTC) approach using updated estimates of exposure in addition to the evaluation of all relevant safety data on the NFC and its principal constituents. The scope of the safety evaluation contained herein does not include added use in dietary supplements or any products other than food. Eighteen (18) NFCs, derived from the *Vanilla*, *Prunus, Betula, Acacia, Cuminum, Jasminum, Gaultheria, Polianthes and Evernia* genera, were affirmed as generally recognized as safe (GRAS) under their conditions of intended use as flavor ingredients, based on an evaluation of each NFC and the constituents and congeneric groups therein.

1. Introduction

For over six decades, the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) has been the primary, independent body evaluating the "generally recognized as safe" (GRAS) status for substances intended for use as flavorings in human foods in the USA, consistent with the 1958 Food Additive Amendment to the Federal Food Drug and Cosmetic Act (Hallagan and Hall, 1995, 2009; Hallagan et al., 2020).

One element of the FEMA GRAS program is the cyclical re-evaluation of FEMA GRAS flavoring ingredients. Chemically defined flavoring ingredients are ordinarily single chemical substances whereas the NFCs are naturally occurring mixtures typically derived from botanical materials. In 2015, the FEMA Expert Panel initiated a program for the re-evaluation of FEMA GRAS NFCs and as a first step, updated its scientifically based procedure, previously introduced in 2005, for the safety evaluation of NFCs (Cohen et al., 2018a; Smith et al., 2005). This procedure organizes the constituents of an NFC into congeneric groups of similar chemical structure, reactivity and toxicity. Metabolism data for each constituent congeneric group are considered (Smith et al., 2018)

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Table 1
NFCs evaluated by the FEMA Expert Panel.

Name	FEMA No.	Most Recent Annual Volume of Use (kg)[a]	Estimated Intake (μg/person/day)[b]
Almonds Bitter Oil (FFPA)[c] (*Prunus amygdalus* Batsch var. *amara* (DC.) Focke, *P. armeniaca* L., *P. persica* (L.) Batch)	2046	5220	540
Apricot Kernel Oil (*Prunus armeniaca* L.) - Persic oil	2105	6660[d]	680
Birch Sweet Oil (*Betula lenta* L.)	2154	360	37
Cassie Absolute (*Acacia farnesiana* (L.) Willd)	2260	29	3
Cherry Bark Wild Extract (*Prunus serotina* Ehrh.)	2276	10,500	1080
Cherry Bark Wild Distillate (*Prunus serotina* Ehrh.)	5063	5	0.7
Cherry Laurel Oil (FFPA) (*Prunus laurocerasus* L.)	2277	1[e]	0.1
Cumin Oil (*Cuminum cyminum* L.)	2343	6480	670
Cumin Oleoresin (*Cuminum cyminum* L.)	5064	3640	370
Jasmine Absolute (*Jasminum grandiflorum* L.)	2598	100	10
Jasmine Concrete (*Jasminum grandiflorum* L.)	2599	0.4	0.04
Jasmine Oil (*Jasminum grandiflorum* L.)	2600	2	0.2
Oak Moss Absolute (*Evernia prunastri* (L.) Ach., *E. furfuracea* (L.) Mann, and other lichens)	2795	34	4
Sloe Berries Extract (*Prunus spinosa* L.)	3021	17	1
Tuberose Oil (*Polianthes tuberosa* L.)	3084	0.8[e]	0.08
Vanilla Extract[f] (*Vanilla planifolia* Andrews, *V. tahitensis* J.W. Moore, *V. pompona*[g])	3105	341,000	3,500[h]
Vanilla Oleoresin[f] (*Vanilla planifolia Andrews, V. tahitensis J.W. Moore, V. pompona*[g])	3106	1700	170
Wintergreen Oil (*Gaultheria procumbens* L.)	3113	700	72

[a] Harman, C.L. and Linman, M.J. 2023. Flavor and Extract Manufacturers Association of the United States (FEMA) 2020 Poundage and Technical Effects Survey, Washington DC, USA.

[b] For high volume materials (greater than 22,700 kg/year), the PCI per capita is shown. For materials with a lower surveyed volume (less than 22,700 kg/year, PCI × 10 (“eaters only’) calculation is shown. Slight variations may occur from rounding.

[c] Almond Oil, Bitter, FFPA. Food Chemicals Codex, The United States Pharmacopeial Convention (USP), Rockville, MD, USA. Effective date 1 June 2008, Date accessed:4 December 2025. FFPA - Free from Prussic Acid (also known as hydrogen cyanide or cyanide). Acceptance criteria for hydrocyanic acid test: No blue precipitate or color appears (about 0.015 %).

[d] Gavin, C.L., Williams, M.C., Hallagan, J.B., 2008. Flavor and Extract Manufacturers Association of the United States (FEMA) 2005 Poundage and Technical Effects Update Survey, Washington DC, USA.

[e] Harman and Murray, 2018. 2015 Poundage and Technical Effects Survey. Flavor and Extract Manufacturers Association, Washington, DC, USA.

[f] In the USA, vanilla extract and concentrated vanilla extract are subject to a federal standard of identity which describes the manufacture and identity of the products (21 CFR Secs. 169.3[3]; 175[4]; 176[5]).

[g] *V. pompona* is not a specified botanical species within the US Standard of Identity for vanilla extract (21 CFR Secs. 169.3[3]; 175[4]; 176[5]).

[h] For the safety evaluation, the FEMA Expert Panel estimated the intake of vanilla extract to be 300,000 μg/person/day, using the Single Portion Exposure Technique (SPET) method, developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2009).

and the estimated intake of each constituent congeneric group is evaluated using the Threshold of Toxicological Concern (TTC) approach (Kroes et al., 2000). In addition, the genotoxic potential of the constituent congeneric groups as well as the potential toxicity and estimated intake of the fraction of unidentified constituents are evaluated in the updated procedure (Cohen et al., 2018a). The re-evaluation program of FEMA GRAS NFCs began with the evaluation of 54 *Citrus*-derived NFCs (Cohen et al., 2019) and continued with the safety evaluations of NFCs derived from *Mentha,* dill, buchu and caraway botanicals (Cohen et al., 2020), NFCs derived from *Cassia, Cinnamomum* and *Myroxylon* botanicals (Rietjens et al., 2020), NFCs derived from clove, cinnamon leaf and West Indian bay (Gooderham et al., 2020b) as well as lavender, guaiac, coriander-derived and related NFCs (Fukushima et al., 2020). Additional safety evaluations have been published for an *Eucalyptus*-derived NFC and NFCs containing eucalyptol (Eisenbrand et al., 2021); NFCs characterized by the presence of the phenolic constituents carvacrol and thymol (Cohen et al., 2021); asafetida, onion and garlic oils (Davidsen et al., 2023a); allspice, anise fennel-derived and related NFCs (Rietjens et al., 2023); basil, nutmeg, parsley, tarragon and related allylalkoxybenzene-containing natural flavor complexes (Davidsen et al., 2023b); lemongrass, chamomille, citronella and related NFCs (Rosol et al., 2023); sage, orris, tagetes-derived and related NFCs (Gooderham et al., 2023) and pepper, ginger, coniferous-derived and related NFCs (Hecht et al., 2025). In the present publication, the safety evaluation of a group of NFCs that includes Vanilla Extract (FEMA 3105), Bitter Almond Oil (FEMA, 2046), Wintergreen Oil (FEMA 3113) and other NFCs containing benzaldehyde, vanillin, methyl salicylate and other benzyl derivative flavoring ingredients is presented.

Data describing the preparation and composition for the NFCs listed in Table 1 were requested in a call for data issued by the FEMA Expert Panel. 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 *Vanilla, Prunus, Betula, Acacia, Cuminum, Jasminum, Gaultheria, Polianthes and Evernia* genera currently used for flavoring food products.

2. History of food use

Several of the NFCs listed in Table 1 are derived from plants of the *Prunus* genus and impart a cherry and/or fruity flavor to foods: Almonds Bitter Oil (FEMA, 2046), Cherry Laurel Oil (FEMA 2277), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063) and Sloe Berries Extract (FEMA 3021). The three botanical sources for bitter almond oil are *P. amydalus* Batch (almond bitter oil), *P. armeniaca* L. (apricot kernel oil) and *P. persica* (L.) Batch (peach kernel oil). Bitter almond oil can be distilled from the partially de-oleated press cake of bitter almond kernels, or the partially de-oleated press cake of apricot kernels (*P. armeniaca*), cherries (*Cerasus* species), plums (*P. domestica*) or peach (*Amygdalus persica*), but it is noted that the latter sources are not currently used (Arctander, 1961). The triglyceride component of apricot kernels, known as apricot kernel oil or persic oil, was traditionally used as a cooking oil (Abd El-Aal et al., 1986). Cherry Laurel Oil (FEMA 2277) is derived from the leaves of *P. laurocerasus* L., and like bitter almond oil, imparts a cherry-like flavor to food. Cherry Bark Wild Extract (FEMA 2276) and Cherry Bark Wild Distillate (FEMA 5063) are derived from the bark of the North American wild black cherry tree, *P. serotina* (Gatchell, 1971) and were traditionally used in the preparation of cough medicines and as bittering agents in alcoholic beverages (Budavari et al., 1989;

Marquis, 1990; Parsons, 2011). Sloe Berries Extract (FEMA 3021) is prepared from the fruit of the sloe or blackthorn tree (*P. spinosa*), a shrub native to Europe, the Mediterranean region, Asia, and North Africa, and domesticated in North America (Popescu and Caudullo, 2016). Sloe berries are purplish drupes that are sour and astringent when ripened (Tardío and Pardo-de-Santayana, 2016). Extracts of sloe berries have been traditionally used in liqueurs such as sloe-flavored gin, wine, jams, jellies and desserts (Alarcón et al., 2015; Jarić et al., 2015; Kültür, 2007; Pardo-de-Santayana et al., 2007).

In the USA, extracts and essential oils of the bark and twigs of sweet birch (*Betula lenta* L.) and the leaves of wintergreen (*Gaultheria procumbens* L.) were often used as medicinal formulations and flavorings for food by Native Americans, and later, by colonial Americans (Betts, 1945; Duke, 2002; Urdang, 1948). Oil of wintergreen was one of the first essential oils produced in the United States and appears in the United States Pharmacopeia published in 1820 for use by apothecaries for medicinal, cosmetic and flavoring purposes (Urdang, 1948).

Flowers are the part of the plant used for the preparation of Cassie Absolute (FEMA 2260), Jasmine Absolute (FEMA 2598), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600) and Tuberose Oil (FEMA 3084). The jasmine flower is an edible flower and traditionally used as a flavoring for rice and teas (Hussain et al., 2019; Kirker and Newman, 2016). Similarly, cassie absolute is prepared from the flowers of *Acacia farnesiana* (L.) Willd native to the Mediterranean coast and is often used in raspberry flavors (De Rovira, 2017; Parrotta, 1992). Native to Mexico and Central America, the flowers of the tuberose (*Polianthes tuberosa* L.) are characterized by an intense sweet odor (Fenaroli et al., 1975; Mebakerlin and Chakravoty, 2015). In Indonesia, the flowers of tuberose are traditionally used as a food ingredient in vegetable soups and sauces (Mebakerlin and Chakravoty, 2015).

Vanilla extract is prepared from the cured and dried fruits of plants of the *Vanilla* genus which are members of the orchid family. In the USA, Vanilla Extract (FEMA 3105) is derived from *Vanilla planifolia* Andrews or *V. tahitensis* J.W. Moore, to conform to the USA federal standard of identity for vanilla extract (21 CFR Part 169).[4] Outside of the USA, cured fruits from *V. pompona* may also be used in preparations of vanilla extract. Vanilla extract is a familiar food ingredient that is widely used to impart the popular vanilla flavor to foods. Unlike other FEMA GRAS NFCs used as flavoring ingredients, Vanilla Extract (FEMA 3105) is also used directly in the preparation of various home-prepared foods, especially baked goods. The cultivation of vanilla for use as an ingredient in foods and beverages originated in Mesoamerica, an area that on a modern map would include northern Costa Rica, Nicaragua, Honduras, El Salvador, Guatemala, Belize and parts of central and southern Mexico (Lubinsky et al., 2008). Madagascar and Indonesia are major suppliers of vanilla beans imported into the United States, with smaller amounts imported from India, Papua New Guinea, Comoros, Uganda and Mexico.[2]

Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064) are derived from cumin seed, a common culinary spice. Cumin seed (*Cuminum cyminum* L.) likely originated in Egypt and was introduced to other cultures by the spice trade. Currently, India, Syria, Turkey and the United Arab Emirates are the major global suppliers of cumin (Rahman et al., 2020). Cumin is commonly found in spice blends in Asian, Middle Eastern, South American, Chinese, Cuban, Spanish and North American cuisines. In the USA, cumin is used as an ingredient in Mexican and "Tex-Mex" foods (Challancin, 2012).

3. Current usage

The NFCs listed in Table 1 are used to flavor numerous foods including hard and soft candies, baked goods, chewing gum, beverages, meats and sauces. Table 1 lists the annual usage in the USA (Gavin et al., 2008; Harman and Linman, 2023; Harman and Murray, 2018) and the estimated intake for each NFC under consideration. With the exception of Vanilla Extract (FEMA 3105), all the NFCs have reported usage less than 22,500 kg, thus the PCI × 10 'eaters only' method is used to calculate the estimated per capita intake, which assumes that the annual volume is consumed by only 10 % of the population. A correction factor of 0.8 is used in this calculation, based on the estimation that 80 % of total usage is reported in the FEMA usage surveys.

In 2020, a total usage of 341,000 kg of Vanilla Extract (FEMA 3105), with a corresponding estimated intake of 3500 μg/person/day, was reported in the 2020 FEMA Poundage and Technical Effects Survey (Harman and Linman, 2023). Because the reported usage of Vanilla Extract (FEMA 3105) was greater than 22,500 kg, the per capita intake was calculated using the PCI method and is reported in Table 1, which assumes that the annual volume is consumed by the entire population of the USA in 2020. In 2020, United States Department of Agriculture's (USDA) Foreign Agricultural Service Global Agricultural Trade System online database reports that 1470 metric tons of vanilla beans (includes whole and ground) were imported into the USA.[2] While whole vanilla beans are available in food markets, the use of vanilla beans for the preparation of extract far exceeds the use of vanilla beans used directly in food preparation.

The 2020 import volume of vanilla beans suggests that the 2020 annual usage of Vanilla Extract (FEMA 3105) as a flavoring to foods may be under reported in FEMA Poundage and Technical Effects Survey (Harman and Linman, 2023) due to its common addition as such to food and beverages. As a result, the Expert Panel estimated the intake of vanilla extract from use as a flavoring ingredient using the Single Portion Exposure Technique (SPET) method which is an alternative method used to estimate exposure to flavoring ingredients as developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2009). To calculate the estimated intake for Vanilla Extract (FEMA 3105) using SPET, the current average usual use level of Vanilla Extract (FEMA 3105) is multiplied by the standard portion size for each food category defined by CODEX Alimentarius's General Standard for Food Additives (GSFA) food categories. In an evaluation of 549 additional flavoring substances using three different sources of use level data: 2007–08 IOFI data, 1977 National Academy of Sciences (NAS) – National Research Council (NRC) survey data (National Research Council (U.S.) Committee on GRAS List Survey - Phase III Food and Nutrition Board, 1979) and 1965–2007 FEMA GRAS data by JECFA, exposure estimates calculated by the SPET method were higher than those calculated by the PCI method in 92 % of the cases studied (JECFA, 2009). While SPET is generally considered an overestimation of actual intake because it is based on the assumption that the flavoring is present in every food within a food category, it was used to estimate the dietary intake of vanilla extract, given it accounts for the broad usage of vanilla extract as a flavoring for foods by home cooks and other sources. Vanilla Extract (FEMA 3105), like most flavoring ingredients, is self-limiting, meaning that the addition of vanilla extract beyond a certain use level becomes unpalatable. The estimated intake of Vanilla Extract (FEMA 3105) calculated using the SPET method is 300,000 μg/person/day and this value is used in the safety evaluation presented below.

For the NFCs that impart cherry-like character, Cherry Bark Wild Extract (FEMA 2276) has the highest reported usage followed by Almonds Bitter Oil (FFPA) (FEMA, 2046). Cherry Laurel Oil (FFPA) (FEMA 2277) has limited current usage of only 1 kg in 2015, with no usage reported in 2020. Sloe Berries Extract (FEMA 3021) has a reported annual usage of 17 kg. For the NFCs derived from flowers and lichen, current usage in the USA for Jasmine Absolute (FEMA 2598) is 100 kg with lower usage reported for Cassie Absolute (FEMA 2260), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600), Oak Moss Absolute (FEMA 2795) and Tuberose Oil (FEMA 3084). For the NFCs with a minty character, the annual usage for Birch Sweet Oil (FEMA 2154) and Wintergreen Oil (FEMA 3113) were 360 and 700 kg, respectively.

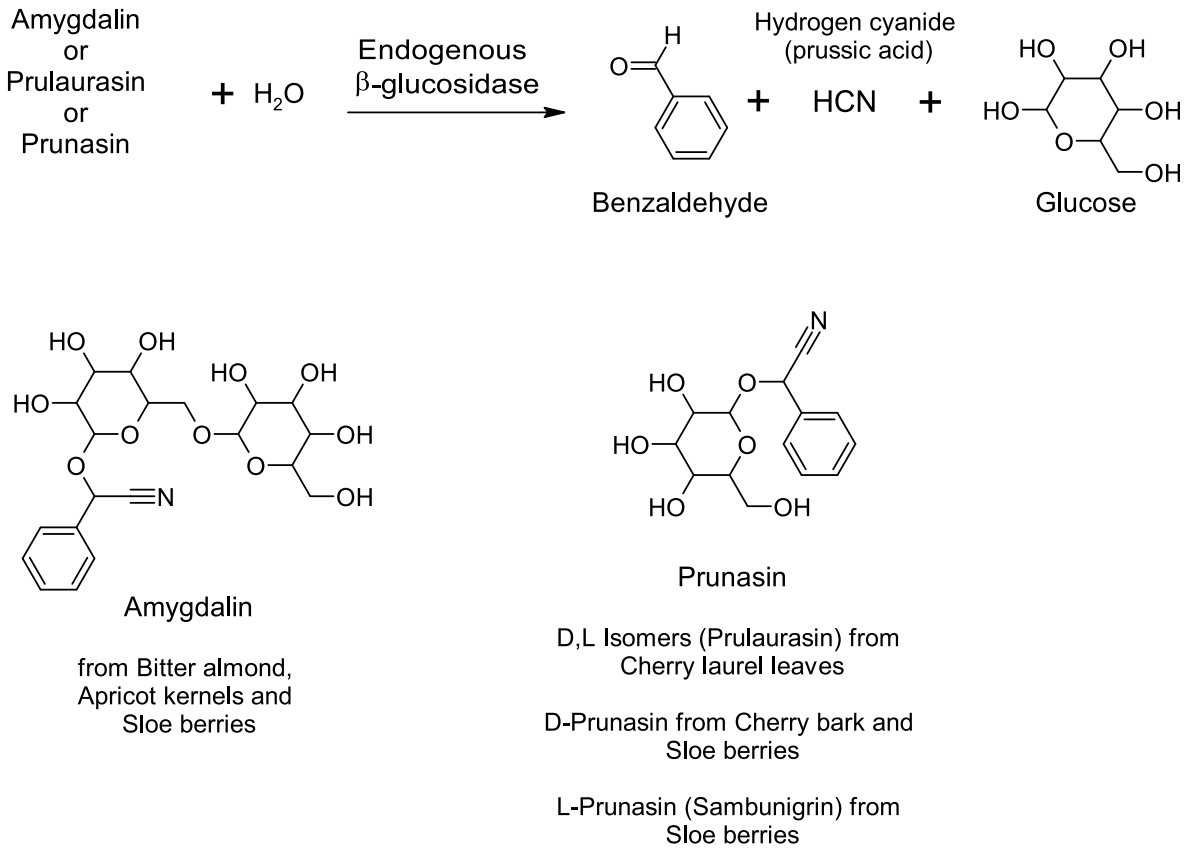

Fig. 1. The hydrolysis of cyanogenic glycosides by β-glucosidases naturally present in the kernels of almonds and apricots, sloe berries, cherry bark and the leaves of cherry laurel yields benzaldehyde, hydrogen cyanide and glucose, as a result of maceration in water.

Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064), derived from cumin seed, a common culinary spice, have annual usages of 6480 kg and 3640 kg, respectively, as flavoring ingredients. According to the USDA's FAS GATS online database,[1] 20 million kg (43 million lbs) of cumin seeds were imported into the USA in 2020. Cumin seeds contain about 2.5 % (v/w) volatile oil (American Spice Trade Association (ASTA), 2005; Peter, 2006) and 13–29 % fixed (triglyceride) oil (Khan and Abourashed, 2010; Merah et al., 2020). Assuming that the total import volume was consumed in food by use as a spice, and that cumin seeds, conservatively, contain 15 % oleoresin (consisting of approximately 2 % essential oil and 13 % fixed oil[2]), an estimated 2,900,000 kg of cumin oleoresin would be consumed in the USA per year by consuming cumin seed as a spice, with a per capita estimated intake of 24,700 μg/person/day. Cumin oleoresin consists of its essential oil, fixed oil and resin of the cumin seed. Of the estimated 2.9 million kg of cumin oleoresin consumed via consumption of cumin as a spice, 500,000 kg is cumin essential oil, that corresponds to an estimated intake of 4100 μg/person/day. The remaining fixed oil - resin fraction, 2,400,000 kg of the oleoresin, corresponds to an estimated intake of 20,600 μg/person/day. In summary, the consumption of cumin oil and cumin oleoresin from consumption of the spice form in 2020 is estimated to be much greater than the current usage as flavoring ingredients.

[1] FAS GATS, 2023. https://apps.fas.usda.gov/gats/United States Department of Agriculture, Date accessed: 29 March 2023.

[2] A fixed oil is a non-volatile, triglyceride-based oil.

4. Manufacturing Methodology

Almonds Bitter Oil (FFPA) (FEMA, 2046) is produced from the kernels of bitter almonds (*P. amygdalus* Batsch var. *amara* (DC.) Focke), apricot kernels (*P. armeniaca* L.) and peach kernels (*P. persica* (L.) Batch) which contain the cyanogenic glycoside amygdalin that releases benzaldehyde, glucose and hydrogen cyanide during the production process. In the production of these NFCs, the kernels or pits are crushed and the triglyceride-based oils (i.e. fixed oils) are expressed. Fixed oils such as sweet almond oil (*P. amydalus*) and apricot kernel oil, also known as persic oil (*P. armeniaca*), are the result of this process. Once the kernels are de-oleated, they are mixed with water and soaked for several hours, until the amygdalin has been hydrolyzed yielding benzaldehyde, hydrogen cyanide and glucose (Fig. 1). This reaction is catalyzed by emulsin, a β-glucosidase already present in the kernels. The solution is then distilled to yield the final essential oil that is rich in benzaldehyde (Guenther, 1952b; Rabak, 1908; Sievers, 1928). After distillation, the volatile oil, which contains approximately 2–4 % hydrogen cyanide, is mixed with calcium hydroxide and hydrous ferrous sulfate, forming a calcium ferrocyanide precipitate that is removed by filtration. The remaining benzaldehyde containing portion of the oil is steam distilled to obtain a benzaldehyde-rich essential oil. Alternatively, sodium sulfite can be distilled with the oil to remove hydrogen cyanide, yielding an oil 'free from prussic acid' (FFPA) (Guenther, 1952b). Bitter almonds yield 0.5–0.7 % essential oil content and apricot kernels yield 0.6–1.8 % essential oil (Guenther, 1952b). The absence of hydrogen cyanide is determined using the Prussian Blue test, in which the formation of a blue precipitate upon mixing the test sample with sodium hydroxide, ferrous sulfate and hydrochloric acid indicates the presence of hydrogen cyanide (Food Chemical Codex, 2008). The acceptance criteria for the

Prussian Blue Test, as described by the Food Chemical Codex (FCC) in its monograph for Almond Oil, Bitter, FFPA, implies no blue precipitate or color corresponding to a level of less than 0.015 % (150 ppm) hydrogen cyanide.

Similar processes are used in the preparation of Cherry Laurel Oil (FFPA) (FEMA 2105). The common cherry laurel (*Prunus laurocerasus* L. (fam. Rosaceae)) is an evergreen bush or small tree native to south-eastern Europe and Turkey, although it is also cultivated in many other temperate climates such as those of the Mediterranean region (Guenther, 1952b). The leaves of cherry laurel, which contain the monoglucoside prulaurasin, that is also known as D,L-prunasin (Fig. 1), are ground to a fine powder and macerated with warm water prior to distillation, thus favoring hydrolysis of the monoglucoside by the enzyme prunase, a β-glucosidase present in the leaves. This enzymatic reaction yields glucose, hydrogen cyanide and benzaldehyde (Fig. 1) (Arctander, 1961; Guenther, 1952b). Hydrogen cyanide is precipitated in a complex with iron and calcium upon treatment with calcium hydroxide and hydrous ferrous sulfate or sulfite, and the resulting product is redistilled to yield an oil free from hydrogen cyanide (FFPA). For the essential oil of cherry laurel, 21 CFR Sec. 172.510 sets a limit of 25 ppm hydrogen cyanide.

Cherry Bark Wild Extract (FEMA 2276) and Cherry Bark Wild Distillate (FEMA 5063) are derived from the dried stem bark from *Prunus serotina* prevalent in the northern and central parts of the continental U.S (Fenaroli et al., 1975; Merory, 1960). Sloe Berries Extract (FEMA 3021) is derived from the fruit of the sloe or blackthorn shrub that grows in Europe, Siberia, Caucasus, southwest Asia and northwest Africa. For the preparation of Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063) and Sloe Berries Extract (FEMA 3021), the macerated bark or berries are soaked in warm water allowing for the hydrolysis of amygdalin and/or prunasin to yield benzaldehyde as well as glucose and cyanide as side products. Sloe Berries Extract (FEMA 3021) is a dilute extract. Cherry Bark Wild Extract (FEMA 2276) may be used as a tincture or it may be concentrated to a solid form that is then standardized using combinations of ethanol, water, glycerin (glycerol), propylene glycol, corn syrup or caramel color. Cherry Bark Wild Distillate (FEMA 5063), a dilute aqueous ethanol solution, is prepared by collecting the distillate of the macerated bark solution (Heath, 1981).

A hydrolysis step is required in the production of Wintergreen Oil and Birch Sweet Oil. Leaves from the evergreen *Gaultheria procumbens* shrub or the bark of *Betula lenta* are macerated in water to convert the glucoside gaultherin to methyl salicylate and primeverose using the enzyme primeverosidase (Fig. 2). Upon completion of the hydrolysis reaction, the essential oil, primarily composed of methyl salicylate, is isolated by steam distillation (Ojha et al., 2022).

Cassie Absolute (FEMA 2260) and Jasmine Absolute (FEMA 2598) are derived from the flowers of *Acacia farnesiana*, more commonly known as sweet acacia, and *Jasminum grandiflorum*, respectively. Flowers are collected and extracted with hexane, petroleum ether or another non-polar solvent. Following extraction, the solvent is evaporated from the mixture, yielding a material called a concrete. Jasmine Concrete (FEMA 2599) is used as a flavoring material. Further processing of the concrete by washing with warm ethanol followed by cooling, removal of waxes by filtration, then removal of the ethanol solvent by distillation yields an extract called an "absolute" (IFEAT, 2015; Ranchana et al., 2017; Reverchon et al., 1995).

Evernia prunia (L.) Ach. and *E. furfuracea* (L.) Mann, from which oak moss absolute is derived, originate from southern and central Europe (Guenther, 1952c). True oak moss (*E. prunia* (L.) Ach.) can be found growing on oak trees, while tree moss (*E. furfuracea* (L). Mann) is found growing on spruces and pines in humid forests (Koga et al., 1992). These epiphytes are collected during the dry season and their concretes are extracted using a non-polar solvent. Oak Moss Absolute (FEMA 2795) is then derived from the concrete by further extraction with hot alcohol, followed by filtration and solvent removal (Guenther, 1952c; Koga et al., 1992). Some oak moss preparation methods include an additional step in which an "absolute oil" is prepared by vacuum distillation of the concrete, and subsequently, the absolute is obtained from the absolute oil by alcohol extraction (Lawrence, 1976).

Although native to Central America, tuberose is cultivated in Egypt and India. Formerly, tuberose oil was isolated using the enfleurage technique where the cut flowers were laid on plates coated with an odorless fat. The fat layer absorbed the volatile compounds from the flower and the fragrance saturated fat was gathered and called a pomade. A pomade might be further extracted with alcohol to produce the absolute (Guenther, 1952a; Lawless, 1995; Surburg and Panten, 2006). Currently, the oil is produced following the preparation of a concrete or absolute by non-polar solvent extraction of the flowers (Guenther, 1952a; Lawrence, 1995; Surburg and Panten, 2006). The concrete or absolute is then steam distilled to yield the essential oil (Fenaroli et al., 1975).

Vanilla extract is prepared from the fruit of the orchid plant. The

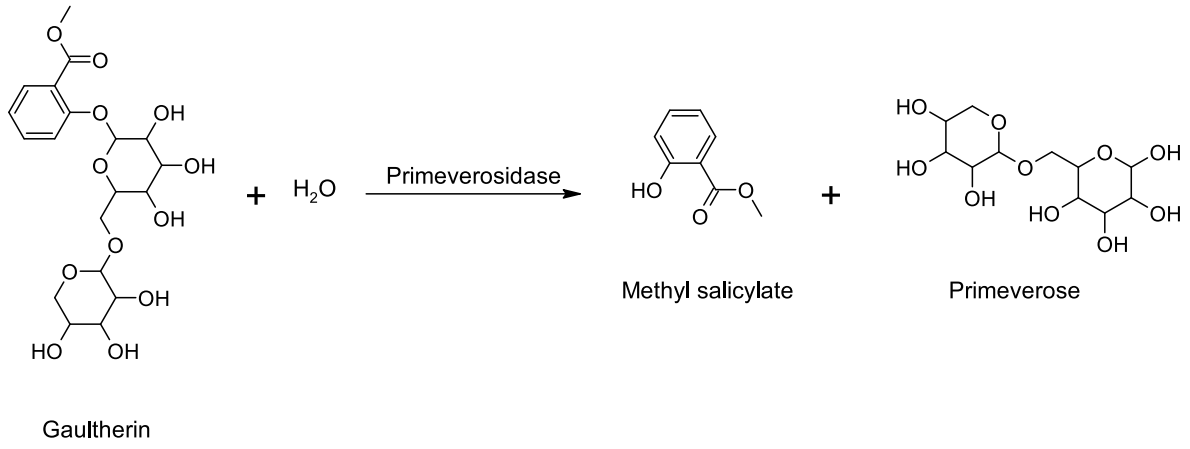

Fig. 2. The hydrolysis of gaultherin naturally present in the leaves of wintergreen (*Gaultheria procumbens*) and the bark of sweet birch (*Betula lenta*) followed by distillation yields wintergreen oil and sweet birch oil, respectively, for which methyl salicylate is the primary constituent.

fruit, also called pods or beans, undergoes a multistep curing process. Upon harvesting of the fresh green pods, they are blanched in hot water to prevent further ripening and fermentation, then placed in containers for a "sweating" step, where the pods begin to cure and turn brown. The pods are treated with several cycles involving drying and sweating steps, during which vanilla's characteristic flavor and aroma are developed (Frenkel et al., 2011). Different growers may use different curing methodologies (Sethi, 2017). In the USA, there is a federal standard of identity for vanilla extract, single fold, defined in 21 CFR Secs. 169.3[3] and 169.175[4] which indicates that it contains 100 g pods per L extract. Vanilla extract, in the USA, contains not less than 35 % ethyl alcohol and may contain glycerin (glycerol), propylene glycol, sugar, dextrose and/or corn syrup as described in 21 CFR Sec. 169.175.[4] Vanilla extracts are often concentrated or folded. In the USA, concentrated vanilla extract conforms to the definition and standard of identity except that it is concentrated to remove part of the solvent, as described in 21 CFR Sec. 169.176.[5] For example, 2-fold concentrated vanilla extract contains 200 g pods per L extract and not less than 35 percent ethanol by volume. Globally, vanilla extracts may be prepared using pods from *V. plantifolia* and *V. tahitensis* as well as *V. pompona* and may be of concentrations not based on the USA standard of identity in which single fold extract is defined as being prepared from 100 g pods per L extract. In the European Union, vanilla extracts conforming to the definition of flavoring preparations as described in Art. 3(2)(d) of the Flavoring Regulation (EC) 1334/2008 may be prepared using permitted solvents[6] other than ethanol/water and the ratio of pods to solvent used in the preparation of extract is not defined. Vanilla Oleoresin (FEMA 3106) is prepared by the solvent extraction of cut vanilla beans. Upon removal of the spent beans, the extract is concentrated then standardized with an approved food-grade solvent.

Cumin is native to northern Egypt and the surrounding Mediterranean regions, though India is now the largest producer of cumin seeds, followed by China and Turkey (Verghese, 1991). Cumin oil is prepared by steam distillation of the crushed seeds. Whole cumin has a volatile oil content of 2.5–5.0 % v/w (Guenther, 1950; Heath, 1981; Peter, 2006; Verghese, 1991). Spice oleoresins such as cumin oleoresin are usually prepared by the extraction of the spice with a volatile solvent such as acetone, isopropanol, ethanol or hexane followed by removal of the solvent from the extract by distillation. Alternatively, following the collection of the volatile oil of the spice by distillation, the non-volatile spice fraction is extracted with an approved solvent, concentrated by solvent removal then combined with the volatile portion collected earlier in the process. In addition, the FCC monograph on spice oleoresins requires that the essential oil of an oleoresin be similar in its physical and chemical properties, including its infrared spectrum, to that distilled from the spice of the same origin (Food Chemical Codex, 2023).

5. Chemical composition

In general, the constituent profiles of the NFCs listed in Table 1 were characterized by analysis of their volatile constituents by gas chromatography-mass spectrometry (GC-MS) where analytes were identified by comparison against a standardized library. A flame ionization detector (FID) was used for detection and quantitation of each constituent. Identified and unidentified GC peaks were reported as the area percent of the chromatogram. For the analysis of the *Vanilla*-derived NFCs, liquid chromatographic methods were applied and

[3] Code of Federal Regulations Title 21 Sec. 169.3 Definitions.

For the purposes of this part:

(a) The term vanilla beans means the properly cured and dried fruit pods of *Vanilla planifolia Andrews* and of *Vanilla tahitensis Moore*.

(b) The term unit weight of vanilla beans means, in the case of vanilla beans containing not more than 25 percent moisture, 13.35 ounces of such beans; and, in the case of vanilla beans containing more than 25 percent moisture, it means the weight of such beans equivalent in content of moisture-free vanilla-bean solids to 13.35 ounces of vanilla beans containing 25 percent moisture. (For example, one unit weight of vanilla beans containing 33.25 percent moisture amounts to 15 ounces.) The moisture content of vanilla beans is determined by the method prescribed in "Official Methods of Analysis of the Association of Official Analytical Chemists," 13th Ed. (1980), sections 7.004 and 7.005, which is incorporated by reference, except that the toluene used is blended with 20 percent by volume of benzene and the total distillation time is 4 h. Copies of the material incorporated by reference may be obtained from the AOAC INTERNATIONAL, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html. To prepare samples for analysis, the pods are chopped into pieces approximately 1⁄4-inch in longest dimension, using care to avoid moisture change.

(c) The term unit of vanilla constituent means the total sapid and odorous principles extractable from one unit weight of vanilla beans, as defined in paragraph (b) of this section, by an aqueous alcohol solution in which the content of ethyl alcohol by volume amounts to not less than 35 percent.

[4] Code of Federal Regulations Title 21 Sec. 169.175 Vanilla extract.

(a) Vanilla extract is the solution in aqueous ethyl alcohol of the sapid and odorous principles extractable from vanilla beans. In vanilla extract the content of ethyl alcohol is not less than 35 percent by volume and the content of vanilla constituent, as defined in Sec. 169.3(c), is not less than one unit per gallon. The vanilla constituent may be extracted directly from vanilla beans or it may be added in the form of concentrated vanilla extract or concentrated vanilla flavoring or vanilla flavoring concentrated to the semisolid form called vanilla oleo-resin. Vanilla extract may contain one or more of the following optional ingredients:

(1) Glycerin. (2) Propylene glycol. (3) Sugar (including invert sugar). (4) Dextrose. (5) Corn sirup (including dried corn sirup).

(b)(1) The specified name of the food is "Vanilla extract" or "Extract of vanilla".

(2) When the vanilla extract is made in whole or in part by dilution of vanilla oleoresin, concentrated vanilla extract, or concentrated vanilla flavoring, the label shall bear the statement "Made from _" or "Made in part from _", the blank being filled in with the name or names "vanilla oleoresin", "concentrated vanilla extract", or "concentrated vanilla flavoring", as appropriate. If the article contains two or more units of vanilla constituent, the name of the food shall include the designation "_-fold", the blank being filled in with the whole number (disregarding fractions) expressing the number of units of vanilla constituent per gallon of the article.

(3) Wherever the name of the food appears on the label so conspicuously as to be easily seen under customary conditions of purchase, the labeling required by paragraph (b)(2) of this section shall immediately and conspicuously precede or follow such name, without intervening written, printed, or graphic matter.

(c) Label declaration. Each of the ingredients used in the food shall be declared on the label as required by the applicable sections of parts 101 and 130 of this chapter.

[5] Code of Federal Regulations Title 21 Sec. 169.176 Concentrated vanilla extract.

(a) Concentrated vanilla extract conforms to the definition and standard of identity and is subject to any requirement for label statement of ingredients prescribed for vanilla extract by Sec. 169.175, except that it is concentrated to remove part of the solvent, and each gallon contains two or more units of vanilla constituent as defined in Sec. 169.3(c). The content of ethyl alcohol is not less than 35 percent by volume.

(b) The specified name of the food is "Concentrated vanilla extract _- fold" or "_-fold concentrated vanilla extract", the blank being filled in with the whole number (disregarding fractions) expressing the number of units of vanilla constituent per gallon of the article. (For example, "Concentrated vanilla extract 2-fold".)

[6] In accordance with Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients (OJ L 141/3, 6.6.2009), as amended.

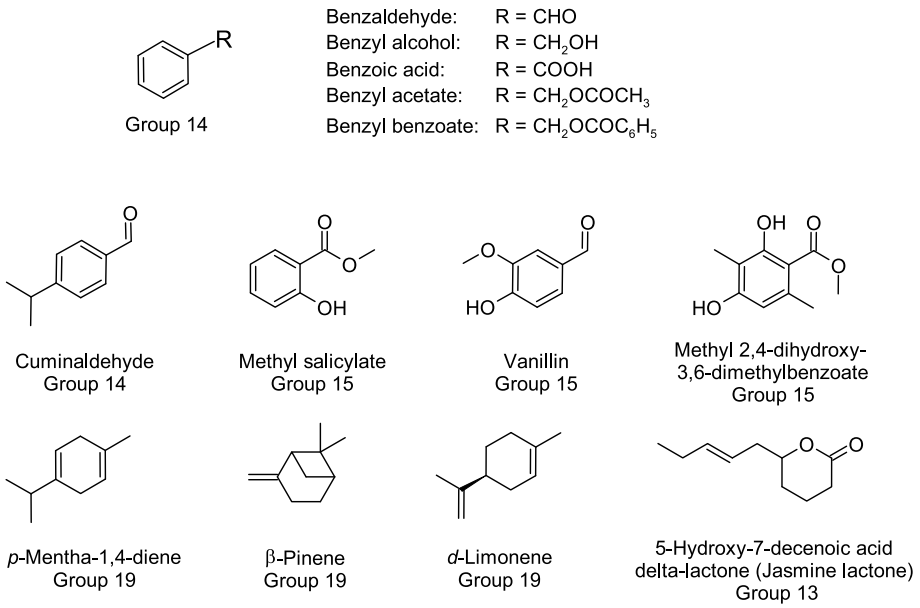

Fig. 3. Structures of commonly found constituents in the NFCs under consideration and their congeneric group.

analytes were quantified using reference standards. Constituent data, organized by congeneric group for each NFC, are reported in Appendix A where constituents present in most NFCs at a level greater or equal to 1 % are reported and minor constituents are summed and reported together. For Cherry Bark Wild Distillate (FEMA 5063) and Sloe Berries Extract (FEMA 3031), which are composed of greater than 99 % food-grade solvent, the most abundant volatile constituents are reported, although they account for less than 1 % of the NFC. For Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106), a range of values is provided for each constituent and congeneric group. For Vanilla Extract (FEMA 3105), the range of constituent values are inclusive for single fold extracts derived from pods of *V. planifolia* and *V. tahitensis* and result from its preparation from 100 g pods per L extract, as stated in the USA standard of identity (21 CFR Sec. 169.3[4]) for vanilla extract. These ranges, which are less than 1 %, are also generally inclusive for Vanilla Extract (FEMA 3105) prepared from pods of *V. pomona*, which is not currently included in the USA standard of identity but may be used outside of the USA. For Vanilla Oleoresin (FEMA 3106), a range of values is provided to be inclusive of the range of constituent values for different standardizations. The congeneric groups used in the safety evaluation are listed in Appendix A of the updated procedure (Cohen et al., 2018a). A further refinement of Group 21 (Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives) into two sub-groups, Group 21A (1′-Propenylalkoxybenzenes and 2′-Propenylhydroxybenzenes) and Group 21B (Selected Allylalkoxybenzenes) based on their metabolism, genotoxicity and carcinogenicity, was introduced in the ninth and tenth publications of the Panel's series of NFC safety evaluations (Davidsen et al., 2023b; Rietjens et al., 2023). The Cramer Decision Tree Class (DTC) was determined for each constituent by following the procedure outlined by Cramer and co-workers (Cramer et al., 1978). The DTC of the congeneric group is determined by assigning the most conservative class for the constituents evaluated within each group. The chemical structures of commonly reported constituents are shown in Fig. 3. The constituent profiles of the NFCs under consideration, organized by congeneric group, are depicted in pie charts shown in Fig. 4.

Most constituent profiles contain Group 14 (Benzyl derivatives) and/or Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives) constituents. For Cherry Bark Wild Distillate (FEMA 5063), Sloe Berries Extract (FEMA 3031), Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106), the constituent profile has been normalized to reflect the composition of these NFCs in the absence of solvents. The cherry flavored NFCs, Almonds Bitter Oil (FEMA, 2046) and Cherry Laurel Oil (FFPA) (FEMA 2277) are predominantly composed of benzaldehyde, a Group 14 (Benzyl derivatives) constituent. Apricot Kernel Oil (FEMA 2105) is a triglyceride-based fixed oil. Wintergreen Oil (FEMA 3113) and Birch Sweet Oil (FEMA 2154) contain high amounts of methyl salicylate, a Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives) flavoring ingredient. Because these NFCs are predominantly composed of one constituent, pie charts are not shown for these NFCs.

A major constituent of Cumin Oil (FEMA 2343) is cuminaldehyde, a Group 14 (Benzyl derivative) constituent. Cumin Oil also contains Group 19 (Aliphatic and aromatic hydrocarbons) and Group 7 (Saturated alicyclic primary alcohols, aldehydes, acids and related esters) constituents. Group 14 (Benzyl derivatives) constituents are also reported in the *Jasminum*-derived NFCs, which also contain constituents from Group 3 (Aliphatic linear and branched-chain α,β-unsaturated aldehydes and related alcohols, acids and esters), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters) and Group 19 (Aliphatic and aromatic hydrocarbons). Group 14 (Benzyl derivatives) and Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives) are reported in Cassie Absolute (FEMA 2260) and in Oak Moss Absolute (FEMA 2795) along with several Group 20 (Phenol derivatives) constituents. Additionally, Group 21A (1′-Propenylalkoxybenzenes and 2′-Propenylhydroxybenzenes) and Group 14 (Benzyl derivatives) are reported in Tuberose Oil (FEMA 3084).

Because of the variable nature of the constituent profile of spice oleoresins, they are characterized separately from the essential oil and extract NFCs. Raw spice oleoresins are highly concentrated and consequently they are often standardized by dilution with a food grade ingredient that also often provides an associated solubility profile for the standardized oleoresin. For oil-based applications, an oleoresin may be standardized with an edible vegetable oil. A raw oleoresin may be standardized with a polysorbate ester that results in a water-soluble standardized oleoresin. Oleoresins may be spray-dried with a modified starch or dispersed on a food grade carrier such as salt or dextrose (Reineccius, 1994). The customization of spice oleoresins for specific applications does not allow for the determination of a single chemical composition, although ranges for volatile oil contents for some standardized spice oleoresins are listed in the FCC. For example, cumin oleoresin may contain 10–30 % essential oil according to the acceptance

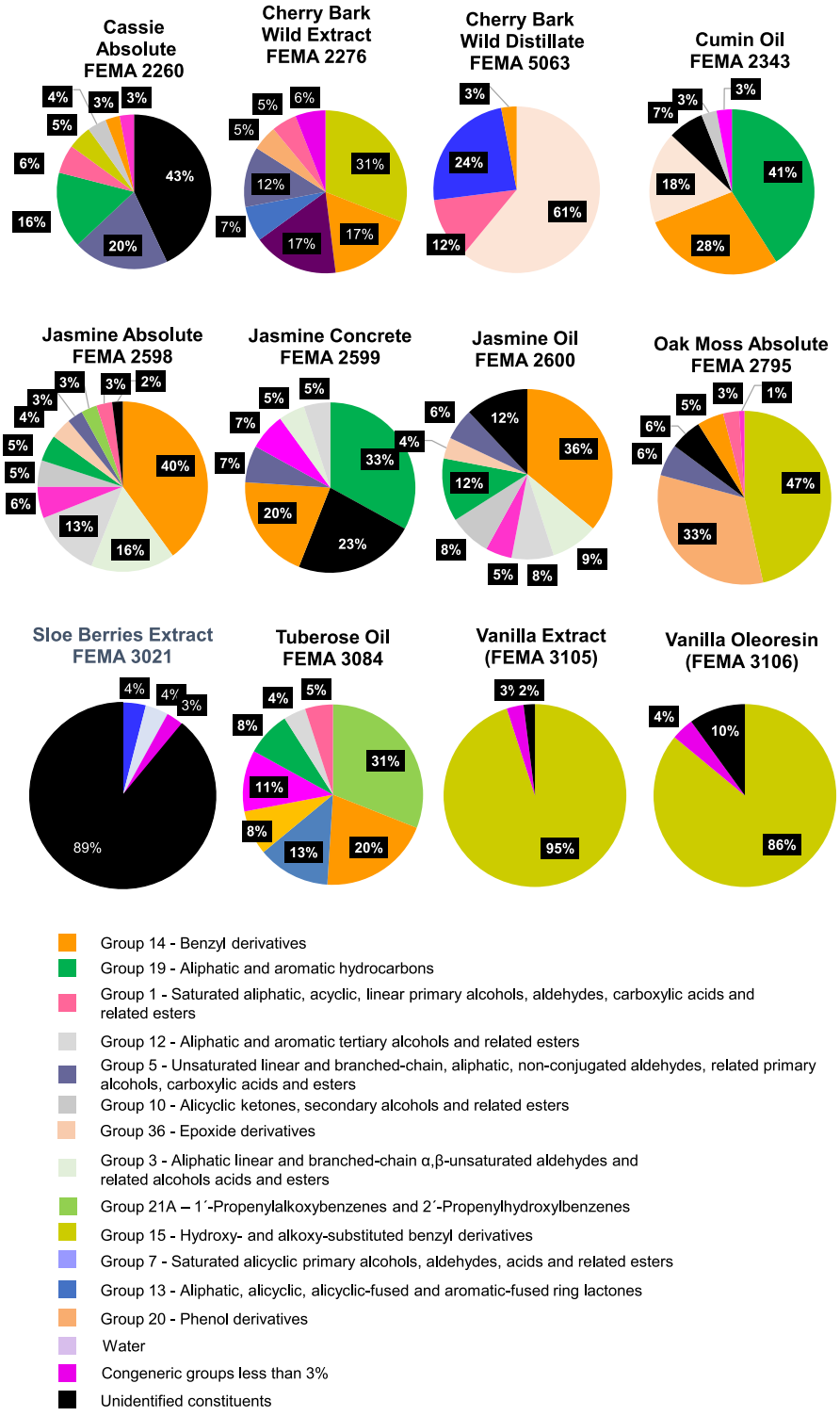

Fig. 4. Constituent congeneric group profiles for some of the NFCs under consideration. For Cherry Bark Wild Distillate (FEMA 5063), Sloe Berries Extract (FEMA 3031), Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106), the constituent profile has been normalized to reflect the composition of these NFCs in the absence of solvents. Almonds Bitter Oil (FEMA, 2046), Apricot Kernel Oil (FEMA 2105), Birch Sweet Oil (FEMA 2154), Cherry Laurel Oil (FEMA 2277), and Wintergreen Oil (FEMA 3113) are composed of >95 % of a single constituent, benzaldehyde, methyl salicylate or triglyceride; therefore, pie charts for these substances are not shown.

criterion of the FCC, with up to 90 % resinous material. However, this oleoresin may be further standardized with a food-grade diluent to contain a much lower percentage of essential oil. The range of the percentages of volatile oil, resin and food grade standardization agent in Cumin Oleoresin (FEMA 5064) is depicted in Fig. 5. The safety evaluation for Cumin Oleoresin (FEMA 5064) is based on volatile oil content ranging from 10 to 30 % (FCC, 2023).

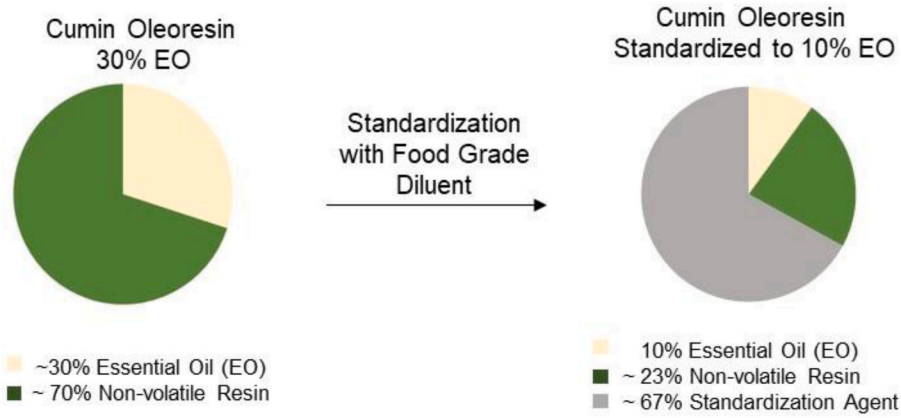

Fig. 5. Cumin oleoresin containing 30 % volatile oil, is standardized by dilution with a food grade standardization agent, such as vegetable oil or salt, resulting in Cumin Oleoresin (FEMA 5064) composed of 10 % essential oil, approximately 67 % standardization agent and 23 % non-volatile resins. Cumin Oleoresin (FEMA 5064) (standardized) containing 10–30 % essential oil is used as a flavoring ingredient.

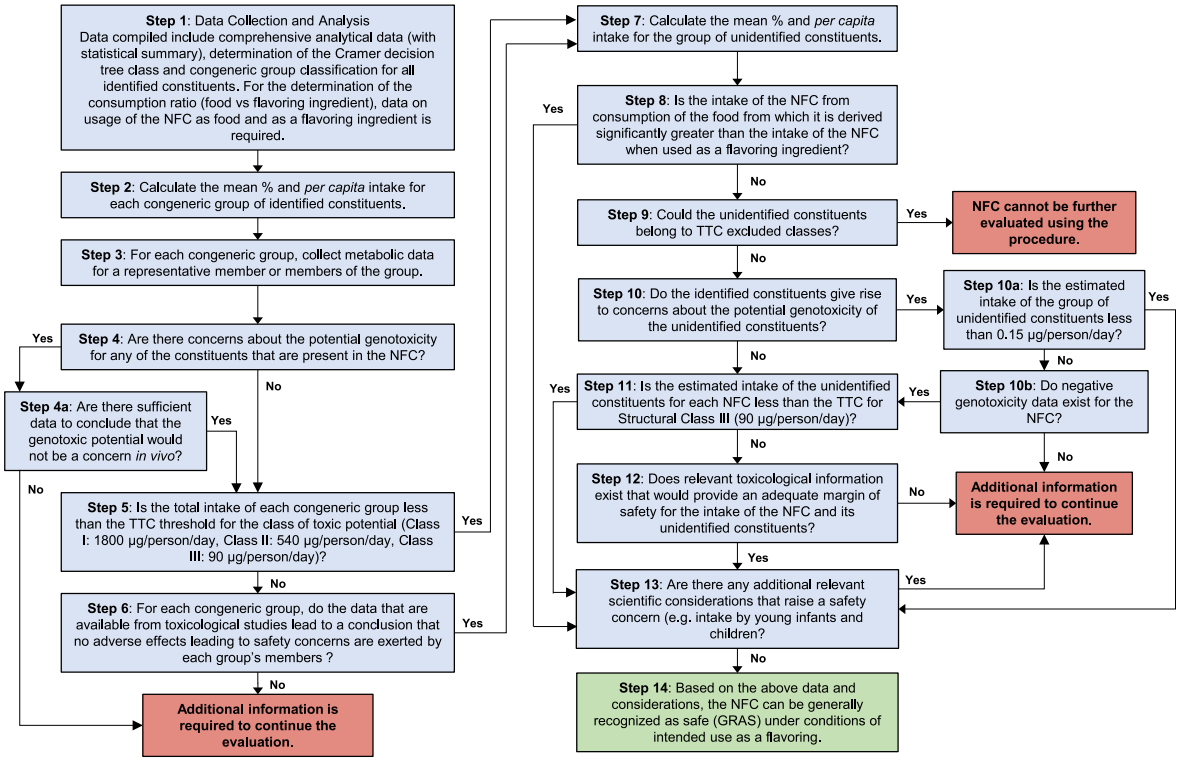

Fig. 6. Procedure for the safety evaluation of NFCs (Cohen et al., 2018a).

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). Briefly, as summarized in Fig. 6, the NFC passes through a 14-step process; Step 1 requires the gathering of data and assesses the consumption of the NFC as a flavor relative to intake from the natural source when consumed as food; Steps 2 through 6 evaluate the exposure and potential toxicity, including genotoxicity, of the identified constituents by the application of the threshold of toxicological concern (TTC) approach and scientific data on metabolism and toxicity for each congeneric group; 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 weight; 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 answered for the NFCs under consideration.

Step 1

To conduct a safety evaluation of an NFC, the Panel requires that

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

a. *Consumption of foods from which the NFCs are derived*

Calculate the per capita daily intake (PCI)[7] *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:

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

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 and Linman, 2023; Harman et al., 2013; Harman and Murray, 2018; Lucas et al., 1999). A correction factor (CF) is used in the calculation to correct for possible incompleteness of the annual volume survey. For flavorings, including NFCs, that are undergoing GRAS re-evaluation, the CF, currently 0.8, is established based on the response rate from the most recently reported flavor industry volume-of-use surveys.

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

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

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

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 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).[8] *At this Step, if the conditions of use*[9] *for the NFC result in levels that differ from intake of the same constituents in the food source, it should be reported.*

The estimated intake for each NFC under consideration, with the exception of Vanilla Extract (FEMA 3105), was calculated using the PCI × 10 method and is reported in Table 1 and Appendix A. As discussed in the "Current Usage" section above, the estimated intake calculated using the SPET method, 300 mg/person/day, will be used instead of the value calculated using the PCI method for the safety evaluation of Vanilla Extract (FEMA 3105). The SPET method, for which the estimated intake is based on use level data, was considered to be a better estimate for the estimated intake of Vanilla Extract (FEMA 3105) due to its high use. To calculate the estimated intake for Vanilla Extract (FEMA 3105) using SPET, the average usual use levels of Vanilla Extract (FEMA 3105) are multiplied by the standard portion size for each food category defined by CODEX Alimentarius's General Standard for Food Additives (GSFA) food categories. The SPET value, 300 mg/person/day for Vanilla Extract (FEMA 3105), is the maximum value calculated amongst the food categories. The safety evaluation for FEMA 3105 Vanilla Extract is inclusive of single fold extracts, as well as multiple-fold extracts, prepared as described in the "Manufacturing Methodology" section using the SPET method for calculating estimated intake. The use levels used to calculate SPET assume the use of single fold FEMA 3105 Vanilla Extract but for 2-fold and higher fold extracts, the amount used would be proportionally reduced to give the same flavor, so the overall exposure is the same. For example, for a 3.3-fold extract, the amount used in each food category would be a third of the amount of single fold extract to provide the same flavor impact. Finally, because Vanilla Extract (3105), in the USA, is prepared in accordance with the USA's standard of identity, using 100 g pods of *V. planifolia* and/or *V. tahitensis* per L extract (21 Sec. 169.3[4]) and the original concentrations of extractables may vary depending on qualities of the vanilla pods used, a range of values for each named constituent and each congeneric group is given in Appendix A. Finally, whole vanilla beans are available in food markets, the use of vanilla beans for the preparation of extract far exceeds the use of vanilla beans used directly in food preparation. As a result, the safety evaluation for the *Vanilla*-derived NFCs, the estimated intake of these NFCs from the consumption of whole beans is insignificant compared to the estimated intakes as flavoring.

In reviewing Table 1, the majority of the botanicals from which the NFCs considered here are derived are not typically consumed directly as foods. An exception is Cumin Oil (FEMA 2343) which is derived from cumin seeds (*Cuminum cyminum* L.), a common culinary spice. FAS GATS reports that 20 million kg (43 million lb) of cumin seed were imported into the USA in 2020.[2] Cumin seeds contain about 2.5 % (v/w) volatile oil (American Spice Trade Association (ASTA), 2005; Peter, 2006) and 13–29 % fixed (triglyceride-based) oil (Khan and Abourashed, 2010; Merah et al., 2020). Assuming that the import total was consumed in food by use as a spice and conservatively contains 15 % oleoresin (consisting of approximately 2 % essential oil and 13 % fixed oil), the estimated intake of cumin oleoresin, which consists of the essential oil and a resin fraction, from the consumption of the spice is 24,700 μg/person/day. The estimated intake of the essential oil fraction is 4100 μg/person/day and the estimated intake of the resin fraction is 20,600 μg/person/day. The estimated intake of cumin oil from the consumption of the spice is at least 6-fold higher than the estimated intake of Cumin Oil (FEMA 2343) used as flavoring in food, as shown in Table 2. The estimated intakes of the essential oil and resin fractions of cumin oleoresin from the consumption of the spice are also much greater than their estimated intakes from the consumption of Cumin Oleoresin (FEMA 5064) used as flavoring in food, as shown in Table 3.

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

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

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

[7] See Smith et al., 2005 and Hall and Ford (1999) for a discussion on the use of PCI and PCI × 10 for exposure calculations in the procedure.

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

[9] 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.

Table 2
Consumption ratio for Cumin Oil (FEMA 2243).

NFC	Estimated Intake from Food (μg/person/day)	Estimated Intake from Flavoring (μg/person/day)[a]	Consumption Ratio Food:Flavoring
Cumin Oil (FEMA 2343)	4100	670	6

[a] In this analysis, the estimated intake from flavoring is calculated using the PCI x 10 (eaters only) method that assumes consumption by 10 % of the population. The estimated intake for each NFC from food (spice) is calculated in a per capita basis, assuming consumption by the entire population.

Table 3
Consumption ratio for Cumin Oleoresin (FEMA 5064).

NFC	Fraction of Essential Oil	Essential oil Estimated Intake PCI x 10 (μg/person/day)[a]	Essential oil Consumption Ratio Food:Flavoring	Non-Essential oil Fraction of Oleoresin Estimated Intake PCI x 10 (μg/person/day)[a,b]	Non-Essential oil Fraction of Oleoresin Consumption Ratio Food:Flavoring
Cumin Oleoresin (FEMA 5064)	10–30 %	37–111	36–110	85–259	79–240

[a] In this analysis, the estimated intake from flavoring is calculated using the PCI x 10 (eaters only) method that assumes consumption by 10 % of the population. The estimated intake for each NFC from food (spice) is calculated in a per capita basis, assuming consumption by the entire population.
[b] For example, an unstandardized Cumin Oleoresin (FEMA 5064) containing 30 % essential oil, the resinous fraction will also be 70 % of the oleoresin. If this oleoresin is standardized, i.e. diluted, to contain 10 % essential oil, this standardized oleoresin will have a resinous fraction of 23 % and contain 67 % of a food grade diluent. The estimated intake for the resinous fraction of Cumin Oleoresin (FEMA 5064) is based on the compositional range of 10–30 %.

Congeneric groups are recorded in order from highest to lowest mean %, within which constituents with a mean % greater than or equal to 1 % of the total NFC are included, except for Cherry Bark Wild Distillate (FEMA 5063), Sloe Berries Extract (FEMA 3031) and Vanilla Extract (FEMA 3105). Because these extract NFCs are composed of greater than 99 % food-grade solvent, the most abundant volatile constituents are reported, although they comprise less than 1 % of the NFC. Minor constituent percentages are grouped together under each of the listed congeneric groups. The total mean % of each listed congeneric group is shown.

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

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

Step 2

Determine (a) the mean percentage (%) of each congeneric group in NFCs, and (b) the daily per capita intake[10] *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:

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

where:

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

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

Proceed to Step 3.

The summary report for each NFC, provided in Appendix A, provides the subtotal mean % and intake values, calculated using the PCI × 10 method, for each constituent congeneric group. For Vanilla Extract (FEMA 3105), the SPET method was used to determine the estimated intake for the NFC and this value was used to calculate the estimated intake of each constituent congeneric group based on the maximum % of the 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 use of metabolic data in the safety evaluation of flavoring compounds and a summary of the expected metabolism of flavoring compounds, by congeneric group, are discussed in a previous publication by the FEMA Expert Panel (Smith et al., 2018). For each congeneric

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

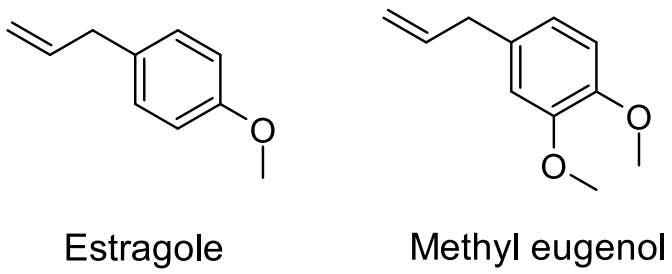

Fig. 7. Structures of estragole and methyl eugenol.

group listed in Appendix A, metabolic data exist for one or more representatives that indicate that members of the respective groups are metabolized to innocuous products. For the major constituent congeneric groups, Group 14 (Benzyl derivatives) and Group 15 (Hydroxy and alkoxy-substituted benzyl derivatives), the FEMA Expert Panel has published safety evaluations that discuss the metabolism and toxicity of flavoring ingredients of these congeneric groups (Adams et al., 2005a, 2005b). Safety assessments, including an evaluation of metabolic studies, for flavoring ingredients of several of the congeneric groups with a prominent presence in the NFCs under consideration including Group 10 (Alicyclic ketones, secondary alcohols and related esters), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 19 (Aliphatic and aromatic hydrocarbons), Group 3 (Aliphatic linear and branched-chain α,β-unsaturated aldehydes and related alcohols acids and esters), Group 13 (Aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones) and Group 22 (Phenethyl alcohol, phenylacetaldehyde and related acetals and esters) have been published by the FEMA Expert Panel (Adams et al., 1996, 1998, 2005c, 2008, 2011; Marnett et al., 2014). The metabolism of Group 23 (Aliphatic and aromatic ethers) was reviewed for the safety evaluation of *Eucalyptus* and related NFCs (Eisenbrand et al., 2021). The Panel has also reviewed the metabolism and toxicity of Group 21A (1′-Propenylalkoxybenzenes and 2′-Propenylhydroxybenzenes) and Group 21B (Selected Allylalkoxybenzenes) constituents (Rietjens et al., 2014). Additional information on 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), Group 7 (Saturated alicyclic primary alcohols, aldehydes, acids and related esters), Group 8 (Saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and related esters), Group 20 (Phenol derivatives), Group 24 (Furfuryl alcohol, furfural and related substances) and Group 29 (Aliphatic acyclic diols, triols and related substances) flavoring ingredients have been published by JECFA (JECFA, 1997, 1998, 1999, 2001, 2002, 2003, 2004, 2006, 2007, 2011, 2012). For the congeneric groups present in these NFCs, with the exception of the allylalkoxybenzenes discussed in Step 4, data on the constituents of the group or related compounds allow the conclusion that the members of these respective congeneric groups are metabolized to innocuous products.

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 applies a weight of evidence approach in the evaluation of genotoxicity data for flavoring ingredients (Gooderham et al., 2020a). With the exception of Cassie Absolute (FEMA 2260), Cumin Oil (FEMA 2343), Cumin Oleoresin (FEMA 5064) and Tuberose Oil (FEMA 3084), the identified constituents of the NFCs do not present a genotoxic concern. The FEMA Expert Panel has previously reviewed *in vitro* and *in vivo* genotoxicity studies on Group 14 (Benzyl derivatives) constituents, which includes benzaldehyde (FEMA 2127) and cuminaldehyde (FEMA 2341), and determined a lack of genotoxic potential for these and related compounds (Adams et al., 2005a). In addition, no potential genotoxicity concern was discerned in a review of genotoxicity studies on Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives) flavoring ingredients such as methyl salicylate and vanillin (FEMA 2745) (Adams et al., 2005b). Studies that have become available since the publications of the safety evaluations of Group 14 and Group 15 flavoring ingredients are described later under "Biochemical and Toxicological Supporting Information Relevant to the Safety Evaluation." A review of the minor constituent profile of Almonds Bitter Oil (FFPA) (FEMA, 2046), Apricot Kernel Oil (FEMA 2105), Sweet Birch Oil (FEMA 2154), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063), Cherry Laurel Oil (FFPA) (FEMA 2277), Jasmine Absolute (FEMA 2598), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600), Oak Moss Absolute (FEMA 2795), Sloe Berries Extract (FEMA 3021), Vanilla Extract (FEMA 3105), Vanilla Oleoresin (FEMA 3106) and Wintergreen Oil (FEMA 3113) also indicate no genotoxic concern for the congeneric groups presented. These NFCs proceed to Step 5.

The presence of a small percentage of naturally occurring methyl eugenol in Cassie Absolute (FEMA 2260) and Tuberose Oil (FEMA 3084) and estragole in Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064) was reported. Estragole and methyl eugenol, Group 21B (Selected Allylalkoxybenzenes) constituents, have an allylalkoxybenzene structural motif (see Fig. 7) that raises a genotoxicity concern (Rietjens et al., 2014).

The low concentrations of estragole and methyl eugenol in Cassie Absolute (FEMA 2260), Cumin Oil (FEMA 2343), Cumin Oleoresin (FEMA 5064) and Tuberose Oil (FEMA 3084) (Table) are further evaluated in Step 4a.

Step 4a

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

If Yes, proceed to Step 5.

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

The structures of estragole and methyl eugenol (see Fig. 7) have a motif of a benzene ring substituted with an alkoxy group located *para* to a 2-propenyl substituent. Cytochromes P450 catalyze the formation of 1-hydroxy metabolites of these allylalkoxybenzene compounds, which is followed by sulfation at this site by a sulfotransferase. Elimination of sulfate from the 1′-sulfoxy metabolites formed creates a DNA reactive species (Daimon et al., 1997; Herrmann et al., 2012, 2014; Jeurissen et al., 2007; Rietjens et al., 2005, 2014). Experimental carcinogenicity studies have indicated that methyl eugenol and estragole are hepatocarcinogenic at high dose levels in rats and mice (Miller et al., 1983; National Toxicology Program (NTP), 2000).

The direct addition of estragole and methyl eugenol, as well as 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 (Regulation EC No 1334/2008). 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,

Table 4
Natural occurrence and estimated intake of estragole and methyl eugenol for the NFCs under consideration.

Name (FEMA No.)	Constituent of Concern	Mean %	Estimated Intake (μg/person/day)[a]
Cassie Absolute (FEMA 2260)	Methyl eugenol	0.01	0.0004
Cumin Oil (FEMA 2343)	Estragole	0.0025	0.017
Cumin Oleoresin (FEMA 5064)	Estragole	0.00025–0.00075	0.0009–0.003
Tuberose Oil (FEMA 3084)	Methyl eugenol	2	0.002

[a] The TTC for compounds with structural alerts for genotoxicity is of 0.15 μg/person/day, as originally stated by Kroes et al., 2004.

in October 2018, FDA's food additive regulations were amended to no longer authorize the use of methyl eugenol as a synthetic flavoring substance or 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 and estragole, as well as safrole, 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).

The estimated intakes of methyl eugenol from consumption of Cassie Absolute (FEMA 2260) and Tuberose Oil (FEMA 3084) and estragole from the consumption of Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064), shown in Table 4, are less than 0.01 μg/person/day and below the TTC of 0.15 μg/person/day for compounds with structural alerts for genotoxicity, as originally stated by Kroes et al. (2004). This TTC value was determined based on an analysis of the dose-response data for carcinogenic compounds, provided by the Gold database of carcinogens,[11] presenting the dose giving a 50 % tumor incidence (TD50) (Gold et al., 1984; Kroes et al., 2004). By linear extrapolation of these TD50 data to a 1 in 10^6 tumor incidence, an exposure level or TTC at which the lifetime risk of cancer was less than 1 in 10^6 was determined to be 0.15 μg/person/day (Kroes et al., 2004). In an EFSA/WHO review of the TTC approach, a 0.15 μg/person/day threshold was proposed and considered sufficiently protective for compounds with structural alerts for genotoxicity with the exclusion of high potency carcinogens (the Cohort of Concern) specified by Kroes and co-workers (EFSA, 2016; Kroes et al., 2004; Nohmi, 2018). The safety evaluations of Cassie Absolute (FEMA 2260) Cumin Oil (FEMA 2343), Cumin Oleoresin (FEMA 5064) and Tuberose Oil (FEMA 3084) 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 total estimated intakes for each constituent congeneric group present in Almonds Bitter Oil (FFPA) (FEMA, 2046), Apricot Kernel Oil (FEMA 2105), Sweet Birch Oil (FEMA 2154), Cassie Absolute (FEMA 2260), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063), Cherry Laurel Oil (FFPA) (FEMA 2277), Cumin Oil (FEMA 2343), Cumin Oleoresin (FEMA 5064), Jasmine Absolute (FEMA 2598), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600), Oak Moss Absolute (FEMA 2795), Sloe Berries Extract (FEMA 3021), Tuberose Oil (FEMA 3084), Vanilla Extract (FEMA 3105), Vanilla Oleoresin (FEMA 3106) and Wintergreen Oil (FEMA 3113) are below the TTC for their structural classes, as reported in Appendix A. These 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

Table 5
Estimated Intake of unidentified constituents.

Name	FEMA No.	Estimated Intake (μg/person/day)
Almonds Bitter Oil (FFPA)	2046	0.05
Apricot Kernel Oil	2105	<7
Birch Sweet Oil	2154	0.6
Cassie Absolute	2260	1
Cherry Bark Wild Extract	2276	<3
Cherry Bark Wild Distillate	5063	0
Cherry Laurel Oil (FFPA)	2277	0.0001
Cumin Oil	2343	46
Cumin Oleoresin	5064	85–259
Jasmine Absolute	2598	0.2
Jasmine Concrete	2599	0.01
Jasmine Oil	2600	0.03
Oak Moss Absolute	2795	0.2
Sloe Berries Extract	3021	0.2
Tuberose Oil	3084	0.00009
Vanilla Extract	3105	30[a]
Vanilla Oleoresin	3106	17[b]
Wintergreen Oil	3113	0.8

[a] For Vanilla Extract (FEMA 3105) which is defined by the units of pods extracted into a specific volume of solvent, an estimation of the unknown fraction is not available. The unknown fraction is expected to contain very low levels (less than 0.01 %) of Groups 15, 20 and 24 constituents as well as a small amount of residual plant material which is expected to be dependent on variable filtration procedures used in production. Estimated intake of unidentified constituents, excluding residual plant material, would be 30 μg/person/day.

[b] For Vanilla Oleoresin (FEMA 3106) which is defined by the units of pods extracted into a specific volume of solvent, an estimation of the unknown fraction is not available. The unknown fraction is expected to contain very low levels (less than 2 %) of Groups 15, 20 and 24 constituents as well as a small amount of residual plant material which is expected to be dependent on variable filtration procedures used in production. Estimated intake of unidentified constituents assuming an conservative estimate of the unknown fraction of 10 % would be 17 μg/person/day.

[11] Gold database currently maintained by Llasa Ltd. https://www.lhasalimited.org/products/lhasa-carcinogenicity-database.htm.

Table 6
Estimated Intake of cyanide from the consumption of NFCs derived from amygdalin and/or prunasin containing raw materials, assuming a body weight of 60 kg.

Name	FEMA No.	NFC Estimated Intake (μg/person/day)	Maximum Concentration of cyanide in NFC (ppm)[a]	Maximum Estimated Intake of Cyanide (μg/kg bw/day) (60 kg bw)
Almonds Bitter Oil (FFPA)	2046	540	150	0.001
Cherry Bark Wild Extract	2276	1080	150	0.003
Cherry Bark Wild Distillate	5063	0.5	150	1×10^{-6}
Cherry Laurel Oil (FFPA)	2277	0.1	25	4×10^{-8}
Sloe Berries Extract	3021	1	150	4×10^{-6}

[a] For Almonds Bitter Oil (FFPA) (FEMA, 2046), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063) and Sloe Berries Extract (FEMA 3021), the concentration of hydrogen cyanide in each NFC is assumed to be 150 ppm hydrogen cyanide, which is the approximate limit estimated in the FCC monograph for Almond, Oil, Bitter, FFPA (FCC, 2008). For Cherry Laurel Oil (FFPA) (FEMA 2277), the concentration of hydrogen cyanide in this NFC is assumed to be 25 ppm hydrogen cyanide, the limit set in 21 CFR Sec. 172.510.

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 exposure (MOE) 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).

Not required.

Step 7

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

Proceed to Step 8.

Appendix A details the mean % for the group of unidentified constituents and the estimated per capita intake of the group of unidentified constituents for each NFC. These data are also summarized below in Table 5.

Step 8

Using the data from Step 1, is the intake of the NFC from consumption of the food[12] from which it is derived significantly greater than the intake of the NFC when used as a flavoring ingredient?

If Yes, proceed to Step 13.

If No, proceed to Step 9.

As discussed in Step 1, the estimated intakes of cumin oil and cumin oleoresin from consumption of cumin as a spice is significantly higher than the estimated intake of Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064) as added flavoring. The evaluation of Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064) proceeds to Step 13.

The estimated intakes for Almonds Bitter Oil (FFPA) (FEMA, 2046), Apricot Kernel Oil (FEMA 2105), Sweet Birch Oil (FEMA 2154), Cassie Absolute (FEMA 2260),Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063), Cherry Laurel Oil (FFPA) (FEMA 2277), Jasmine Absolute (FEMA 2598), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600), Oak Moss Absolute (FEMA 2795), Sloe Berries Extract (FEMA 3021), Tuberose Oil (FEMA 3084), Vanilla Extract (FEMA 3105), Vanilla Oleoresin (FEMA 3106) and Wintergreen Oil (FEMA 3113) are expected to be predominately from consumption of the added flavoring. These NFCs proceed to Step 9.

Step 9

Could the unidentified constituents belong to TTC excluded classes?[13] 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. Based on the analyses of the preparation method and identified constituents (see Appendix A) for each NFC, members of the TTC-excluded classes are not present in the NFCs under consideration (Koster et al., 2011). Almonds Bitter Oil (FFPA) (FEMA, 2046), Sweet Birch Oil (FEMA 2154), Cherry Bark Wild Distillate (FEMA 5063), Cherry Laurel Oil (FFPA) (FEMA 2277), Cumin Oil (FEMA 2343), Jasmine Oil (FEMA 2600), Tuberose Oil (FEMA 3084) and Wintergreen Oil (FEMA 3113) are produced by distillation of the botanical which precludes the presence of metals and non-volatiles such as aflatoxins, proteins and steroids in this type of NFC. All NFCs under consideration are produced under Good Manufacturing Practice (GMP) guidelines which control contaminants such as metals, aflatoxins and pesticide residues. For the other NFCs under consideration, which are prepared by an extraction process, non-aqueous solvents are used precluding the presence of a protein fraction. Because of their botanical origin, the presence of nanomaterials, radioactive constituents, *N*-nitroso compounds and azoxy compounds are not expected.

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.

Because food dominant consumption ratios were established for Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064) in Step 1,

[12] 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.

[13] 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)).

these NFCs are not evaluated in this step. For the remaining NFCs listed in Table 1, with the exception of Cassie Absolute (FEMA 2260) and Tuberose Oil (FEMA 3084), the identified constituent profile does not give rise to concern about the potential genotoxicity of the unidentified constituents. These NFCs are primarily composed of Group 14 (Benzyl derivatives), Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives), Group 19 (Aliphatic and aromatic hydrocarbons) and other minor constituents, as reported in Appendix A, that are not genotoxic (Adams et al., 2005a, 2005b, 2011; Cohen et al., 2019). The unidentified constituents are also likely to be products of the isoprene and/or shikimate biosynthetic pathways encoded in the botanicals from which they are derived and be constituents Groups 14, 15 and 19 and also not raise a concern for genotoxicity with the exception of allylalkoxybenzene compounds.

As discussed in Step 4, Cassie Absolute (FEMA 2260) and Tuberose Oil (FEMA 3084) contain a small amount of naturally occurring methyl eugenol which has suspected genotoxic potential. Since allylalkoxybenzene compounds with structural motifs similar to methyl eugenol such as safrole, estragole, myristicin and elemicin are represented in current mass spectral libraries and are readily detected and identified by GC-MS, 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 provided in this safety evaluation, the LOD is estimated to be 0.01–0.1 % of the NFC. The estimated intakes of an unidentified constituent occurring at the upper end of this range, at a concentration of 0.1 %, is 0.000008 μg/person/day for Tuberose Oil (FEMA 3084) and 0.003 μg/person/day for Cassie Absolute (FEMA 2260). For Tuberose Oil (FEMA 3084) and Cassie Absolute (FEMA 2260), these estimated intakes are below the TTC for compounds with structural alerts for genotoxicity of 0.15 μg/person/day. A review of available genotoxicity and toxicological studies on the NFCs under consideration is presented later in the manuscript. These studies reported no evidence of genotoxic potential. Based on these data, it is concluded that the unidentified constituents in the NFCs under consideration do not raise a concern for genotoxicity. 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 at Step 10.

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)?[14]

If Yes, proceed to Step 13.

If No, proceed to Step 12.

Yes, as shown in Table 5, the estimated intake of the fraction of unidentified constituents for each NFC under consideration is below the TTC for Structural Class III, 90 μg/person/day. These NFCs proceed to Step 13. Both Cumin Oil (FEMA 2343) and Cumin Oleoresin (FEMA 5064), which have food dominant consumption ratios (see Step 8), are not evaluated in this step.

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 at Step 11.

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.

In the production of Almonds Bitter Oil (FEMA, 2046) and Cherry Laurel Oil (FEMA 2277), in which the enzymatic degradation of amygdalin and prunasin releases benzaldehyde, hydrogen cyanide is a toxic by-product that must be removed. In the United States, the acceptance criteria for the Prussian Blue Test for hydrogen cyanide, as described by the Food Chemical Codex (FCC) in its monograph for Almond Oil, Bitter, FFPA, indicates no blue precipitate or color corresponding to a level of less than 0.015 % (150 ppm) hydrogen cyanide. The concentration of hydrogen cyanide in Cherry Laurel Oil (FFPA) (FEMA 2277) may not exceed 25 ppm (21 CFR 172.510). The level of hydrogen cyanide in food products is limited in Europe to a maximum of 50 mg/kg in nougat, marzipan or its substitutes or similar products, 5 mg/kg in canned stone fruits and 35 mg/kg in alcoholic beverages (European Commission, 2008). In addition, hydrogen cyanide is limited to a maximum of 7 g/hL (or 7 mg/100 mL) of 100 % volume alcohol in fruit marc spirits and fruit spirits (European Parliament and Council of 17, 2019). At the 74th meeting of JECFA, an Acute Reference Dose (ARfD) of 0.09 mg/kg bw/day cyanide equivalents was determined based on modeling of a developmental toxicity study of linamarin, a cyanogenic glycoside that naturally occurs in cassava. The Committee determined a preliminary tolerable daily intake (PMTDI) of 20 μg/kg bw/day as cyanide from a

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

13-week study of sodium cyanide in drinking water conducted by the National Toxicology Program (JECFA, 2012; NTP, 1993a). In 2016, EFSA considered the health risks related to the presence of cyanogenic glycosides in foods and established an ARfD of 20 μg/kg bw/day for cyanide equivalents from all food sources (EFSA Panel on Contaminants in the Food Chain, 2019).

In Table 6, the maximum estimated intakes of hydrogen cyanide calculated for a 60 kg adult are presented for the five NFCs derived from botanical sources containing the cyanogenic glycosides amygdalin and/or prunasin. For Cherry Laurel Oil (FFPA) (FEMA 2277), the maximum concentration of hydrogen cyanide in this NFC is assumed to be 25 ppm hydrogen cyanide, the limit set in 21 CFR Sec. 172.510 for cherry laurel oil. For Almonds Bitter Oil (FFPA) (FEMA, 2046), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063) and Sloe Berries Extract (FEMA 3021), the concentration of hydrogen cyanide in each NFC is assumed to be 150 ppm hydrogen cyanide, which is the approximate limit estimated in the FCC monograph for Almond, Oil, Bitter, FFPA (FCC, 2008). For each NFC, the maximum estimated intakes of hydrogen cyanide are well below the PMTDI determined by JECFA and the ARfD established by EFSA of 20 μg/kg bw/day and present no safety concern for consumption by children.

In the 57th meeting of JECFA, no safety concerns were found for benzyl derivatives used as flavoring ingredients. An acceptable daily intake (ADI) of 0–5 mg/kg bw/day was established for benzoic acid, calcium, potassium and sodium benzoate salts, benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, considered as benzoic acid equivalents based on previously reviewed hydrolysis data (JECFA, 2001). Based on the data gathered, the estimated intakes of these constituents from the consumption of Almonds Bitter Oil (FFPA) (FEMA, 2046), Apricot Kernel Oil (FEMA 2105), Cassie Absolute (FEMA 2260), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063), Cherry Laurel Oil (FFPA) (FEMA 2277), Cumin Oil (FEMA 2343), Cumin Oleoresin (FEMA 5064), Jasmine Absolute (FEMA 2598), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600), Oak Moss Extract (FEMA 2795), Sloe Berries Extract (FEMA 3021) and Tuberose Oil (FEMA 3084) are significantly below this limit. In addition, the estimated intakes of Group 14 (Benzyl derivatives) constituents for each of these NFC was below the TTC. At the same meeting, JECFA established an ADI of 0–0.5 mg/kg bw/day for methyl salicylate based on a no-observed-effect level (NOEL) of 50 mg/kg bw/day in a two-year study conducted in dogs (Webb and Hansen, 1963). Based on this NOEL of 50 mg/kg bw/day from the two-year study conducted in dogs, the estimated intakes of methyl salicylate from the NFCs under consideration, that range from 0.0001 μg/kg bw/day for Tuberose Oil (FEMA 3084) to), result in MOE values ranging from 420,000,000 to 41,000, based on a body weight of 60 kg. In 2020, the annual usage of methyl salicylate (FEMA 2745) as a flavoring ingredient, added as such, was 115,000 kg (Harman and Linman, 2023), corresponding to an estimated intake of 1190 μg/person/day (0.020 mg/kg bw/day based on a body weight of 60 kg). Considering the combined estimated intake of methyl salicylate used as flavoring, an MOE of greater than 2500 was calculated based on the NOEL of 50 mg/kg bw/day from the two-year study conducted in dogs (Webb and Hansen, 1963).

The FEMA Expert Panel concurs with other safety evaluation bodies that the TTC is applicable to the entire population (EFSA, 2012, 2016; EFSA Scientific Committee, 2019), however, a further 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 evaluated. The NFCs under consideration, except for Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106), would not be added to foods consumed by infants (Codex Alimentarius Commission, 2024; Codex Alimentarius Commission, 2023; Codex Alimentarius Commission, 2023a), indicating that exposure by infants is not expected. Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106), limited by Good Manufacturing Practices (GMP), are permitted in follow-up formula (for ages greater than 6 months), cereal-based foods for infants and young children and canned baby foods (Codex Alimentarius Commission, 2023; Codex Alimentarius Commission, 2023a, 2023). For intake by children, the estimated intakes of constituent congeneric groups were re-examined. For the NFCs under consideration, with three exceptions, the estimated intakes of the constituent congeneric groups of the NFCs under consideration are more than 3-fold below the TTC for their respective structural class and do not raise a safety concern for exposure to children, considering their lower body weights. Group 19 constituents of Cumin Oil (FEMA 2343), with an estimated intake of 270 μg/person/day which exceeds the TTC for Structural class II for children, considering their lower body weights, are mostly monoterpene hydrocarbons with structural similarity to D-limonene. An MOE of greater than 47,000 was calculated for the estimated intake of Group 19 constituents in Cumin Oil (FEMA 2343) based on a no-observed-adverse-effect-level (NOAEL) of 215 mg/kg bw/day for D-limonene, that is also protective for consumption by children. This NOAEL for limonene of 215 mg/kg bw/day (adjusted daily dose from 300 mg/kg bw/day administered 5 days/week) was reported for a two-year toxicity study of D-limonene by oral gavage in female F344 N rats (Cohen et al., 2019; NTP, 1990a). Apricot Kernel Oil (FEMA 2105) consists mainly of triglycerides, which is not a standard congeneric group, and thus the whole oil was evaluated against the TTC. The estimated intake of Apricot Kernel Oil (FEMA 2105) was above the TTC for Structural Class I for children considering their lower body weights. An MOE of greater than 800 was calculated based on its total estimated intake based on a single dose dietary study in which Wistar rats showed no adverse effects when apricot kernel oil was administered at 10 % in the diet, resulting in an estimated intake of 10 mg/kg bw/day (Gandhi et al., 1997). This MOE for Apricot Kernel Oil (FEMA 2105) is protective for consumption by children. In the safety evaluation of Vanilla Extract (FEMA 3105), the maximum estimated intake of Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives), 1200 μg/person/day, exceeds the TTC for Structural Class I for children considering their lower body weights. Vanillin was the major constituent of this group. An MOE of greater than 25,000 was calculated for the estimated intake of Group 15 constituents based on a NOAEL of 500 mg/kg bw/day determined for ethyl vanillin in an OECD 414 guideline compliant study in Wistar rats, which is also protective for children.

In Table 6, the maximum estimated intakes of hydrogen cyanide from the consumption of Almonds Bitter Oil (FFPA) (FEMA, 2046), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063) Cherry Laurel Oil (FFPA) (FEMA 2277) and Sloe Berries Extract (FEMA 3021) are reported, assuming the maximum allowable limit in each NFC. For these NFCs, the maximum estimated intakes of hydrogen cyanide are below the PMTDI determined by JECFA and the ARfD established by EFSA of 20 μg/kg bw/day when considering the lower body weights of children. In consideration of the presence of low concentrations of methyl eugenol in Cassie Absolute (FEMA 2260) and Tuberose Oil (FEMA 3084) and estragole in Cumin Oil (FEMA 2343) discussed in Steps 4 and 4a, these estimated intakes are at least 87-fold below the TTC for compounds with structural alerts for genotoxicity and thus do not raise a concern for consumption by children.

The FEMA Expert Panel conducted an additional analysis, identifying sources of uncertainty in Appendix C. 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 interindividual variability in the calculation of MOE values and in the use of the TTC 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.

The FEMA Expert Panel has determined that the following NFCs are affirmed as GRAS, under conditions of intended use as flavoring ingredients: Almonds Bitter Oil (FFPA) (FEMA, 2046), Apricot Kernel Oil (FEMA 2105), Sweet Birch Oil (FEMA 2154), Cassie Absolute (FEMA 2260), Cherry Bark Wild Extract (FEMA 2276), Cherry Bark Wild Distillate (FEMA 5063), Cherry Laurel Oil (FFPA) (FEMA 2277), Cumin Oil (FEMA 2343), Cumin Oleoresin (FEMA 5064), Jasmine Absolute (FEMA 2598), Jasmine Concrete (FEMA 2599), Jasmine Oil (FEMA 2600), Oak Moss Extract (FEMA 2795), Sloe Berries Extract (FEMA 3021), Tuberose Oil (FEMA 3084), single and multiple folds of Vanilla Extract (FEMA 3105), Vanilla Oleoresin (FEMA 3106) and Wintergreen Oil (FEMA 3113).

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

The FEMA Expert Panel has previously reviewed the safety of the flavoring ingredients of Group 14 (Benzyl derivatives) and Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives), the major constituent congeneric groups of the NFCs under consideration (Adams et al., 2005b, 2005a). Group 14 (Benzyl derivatives) includes benzyl alcohol (FEMA 2137), benzaldehyde (FEMA 2127), benzoic acid (FEMA 2131) and related esters such as benzyl benzoate (FEMA 2138), benzyl acetate (FEMA 2135) and cuminaldehyde (FEMA 2341). Group 15

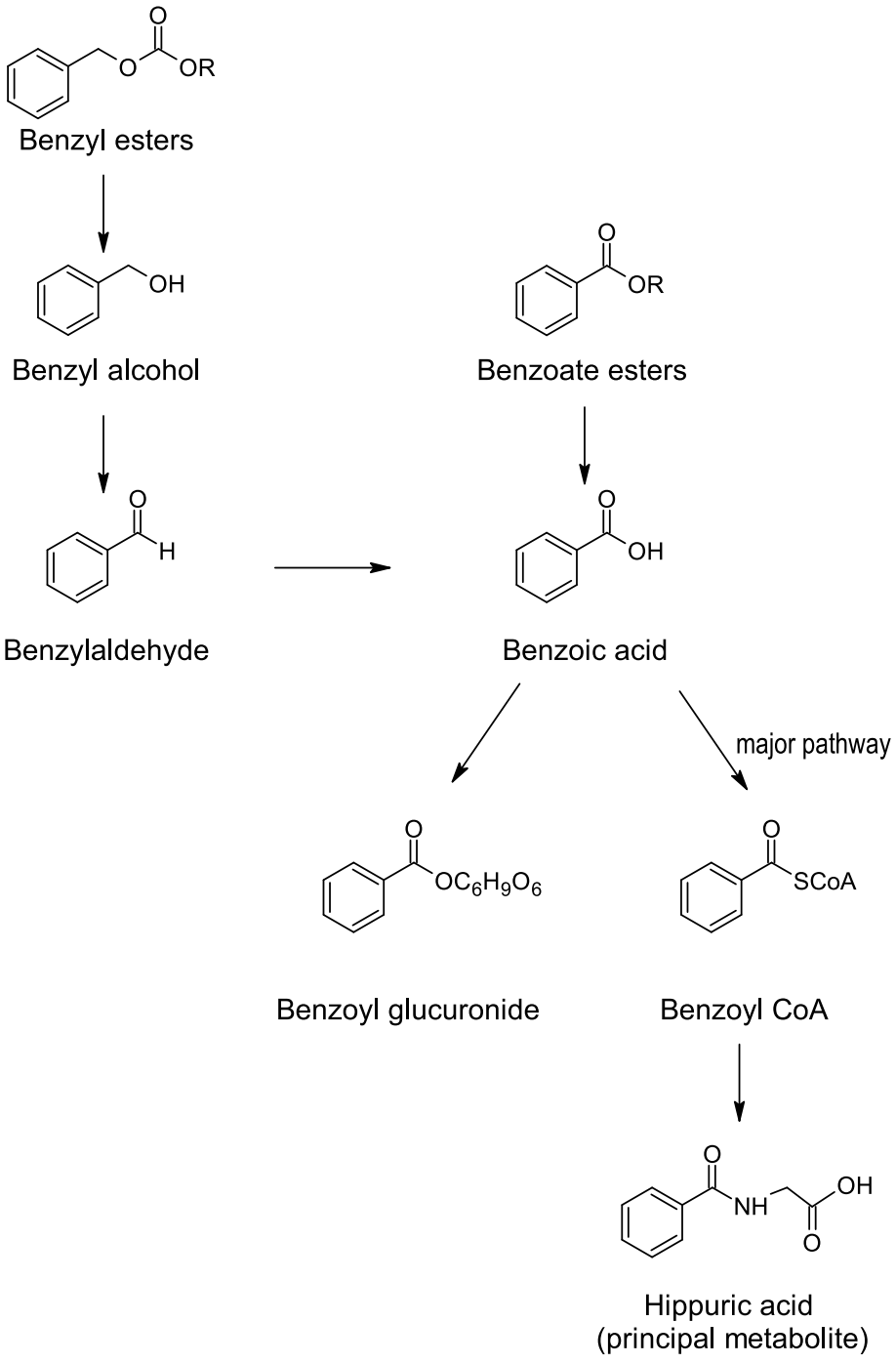

Fig. 8. Metabolism of benzoic acid and related benzyl derivatives in humans.

(Hydroxy- and alkoxy-substituted benzyl derivatives) flavoring ingredients include methyl salicylate (FEMA 2745), a major constituent of Birch Sweet Oil (FEMA 2154) and Wintergreen Oil (FEMA 3113) and vanillin, which is a key constituent in Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106). In addition, safety evaluations of flavoring ingredients of other congeneric groups represented in the constituent profiles including Group 19 (Aliphatic and aromatic hydrocarbons), Group 12 (Aliphatic and aromatic tertiary alcohols and related esters), Group 10 (Alicyclic ketones, secondary alcohols and related esters) and Group 3 (Aliphatic linear and branched-chain alpha, beta-unsaturated aldehydes and related alcohols, acids and esters) have been published by the FEMA Expert Panel (Adams et al., 1996, 2008, 2011; Marnett et al., 2014). The Panel has also published evaluations of other groups or individual constituents present in the NFCs under consideration (Adams et al., 1998, 2004, 2005c, 2007).

The discussion below presents newly available studies on the constituents of the primary constituent groups of these NFCs, Group 14 (Benzyl derivatives) and Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives) and studies for the NFCs under consideration. Previously evaluated and newly available genotoxicity studies for Group 14 (Benzyl derivatives) constituents are summarized in Appendix B.

7.1. Group 14 (benzyl derivatives)

7.1.1. Metabolic fate of benzyl derivatives

Benzyl derivatives such as benzyl esters, benzyl alcohol, benzaldehyde and benzoate esters are primarily metabolized to benzoic acid. In humans, the major pathway for the detoxication of benzoic acid is conjugation with glycine to form hippuric acid, which is excreted in the urine (Fig. 8) (Abdo et al., 1985; Chidgey and Caldwell, 1986; Kubota et al., 1988; Kubota and Ishizaki, 1991; McMahon et al., 1989; NTP, 1990a(NTP, 1990b); Temellini et al., 1993; Yuan et al., 1995). Benzoyl glucuronide, formed by the glucuronidation of benzoic acid, is a minor metabolite observed upon the administration of high dose levels of benzoic acid (Adams et al., 2005a; Amsel and Levy, 1969; Bridges et al., 1970).

7.1.2. Genotoxicity of benzyl derivatives: benzaldehyde, benzoic acid, benzoic salts, benzyl acetate, benzyl alcohol, benzyl benzoate cis-3-hexenyl benzoate and cuminaldehyde

7.1.2.1. In vitro. In an OECD and GLP guideline-compliant Ames assay, *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535 were treated with lithium benzoate (purity 98 %) in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system using both the preincubation and plate incorporation method (ECHA, 2017f). Concentrations tested were 50, 150, 500, 1500 and 5000 μg/plate both with and without metabolic activation. No cytotoxicity or precipitate was observed at any concentration level. There were no increases in revertants in any of the strains in the presence or absence of S9, indicating that lithium benzoate was non-mutagenic under the conditions tested. In another assay, benzoic acid was not mutagenic in an Ames assay in *S. typhimurium* strains TA97a and TA102 in the presence and absence of S9 metabolic activation when tested at concentrations up to 10 mg/plate (Fujita and Sasaki, 1986). Benzoic acid was also negative in a mutagenicity assay in *S. typhimurium* strains TA98 and TA102 at a concentration of 100 mg/disc in the absence and presence of several S9 metabolic activation systems derived from the livers of polychlorobiphenyl treated male rats, mice, guinea pigs and golden hamsters (Kuboyama and Fujii, 1992). Benzoic acid was negative for mutagenicity, in the absence of S9 metabolic activation in a rec assay conducted using *Bacillus subtilis* strains H17 and H45 (Kuboyama and Fujii, 1992). The rec assay has not been standardized in an OECD guideline for genotoxicity testing and OECD has noted that indicator tests such as the rec assay should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015, 2017).

In an OECD and GLP guideline-compliant Ames assay, *cis*-3-hexenyl benzoate was tested for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2*uvrA* in the presence and absence of an S9 metabolic activation system derived from the livers of phenobarbitone/β-naphthoflavone treated rats. Cytotoxicity was observed at 15 μg/plate in strain TA100 in the absence of S9 and 500 μg/plate with S9 metabolic activation. *cis*-3-Hexenyl benzoate was negative for mutagenicity at concentrations up to 50 μg/plate in the *S. typhimurium* strains in the absence of S9 and up to 1500 μg/plate in the presence of S9. *cis*-3-Hexenyl benzoate was negative for mutagenicity in the *E. coli* WP2*uvrA* strain at concentrations of up to 500 μg/plate in the absence of S9 and up to 5000 μg/plate in the presence of S9 (Thompson, 2013).

In a GLP guideline-compliant Ames assay, cuminaldehyde was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2*uvrA* in the presence and absence of an S9 metabolic activation system derived from the livers of phenobarbital/5,6-benzoflavone-treated male Sprague-Dawley (SD) rats. Based on a preliminary cytotoxicity experiment performed at concentrations up to 5000 μg/plate, the concentrations tested ranged from 3.9 to 250 μg/plate for TA100 and TA1535 in the absence of S9 and 15.6–500 μg/plate for TA98, TA1537 and WP2*uvrA* in the absence of S9, as well as for all tester strains in the presence of S9. Cytotoxicity, as evident by a reduction in the revertant colony counts or a reduction in the background lawn, was observed in all conditions at a concentration of 250 μg/plate and higher. There were no increases in revertants in any of the strains treated with cuminaldehyde, in the presence or absence of S9 metabolic activation (Japan Ministry of Health, Labour and Welfare (JMHLW), 2012a).

In an *in vitro* micronucleus assay conducted in Chinese hamster lung (CHL) cells and human TK6 lymphoblastoid cells, benzyl alcohol was negative for genotoxicity at concentrations up to 10 mM in the presence and absence of an Aroclor 1254-induced rat liver S9 metabolic activation system in a 3 h treatment with a 21 h recovery phase and in the absence of S9 under a 24 h treatment. At each dose, at least 1000 binucleated cells were scored per replicate culture for the presence of micronuclei (Fowler et al., 2012). Benzyl alcohol was also reported to be non-mutagenic in an Ames assay in *S. typhimurium* strains TA98 and TA100, however, the test concentrations were not reported (Rogan et al., 1986). In an *in vitro* micronucleus assay conducted in mouse L5178Y lymphoma cells, benzoic acid was negative for the induction of micronuclei in the experiments performed with and without metabolic activation at concentrations of 250–1000 μg/mL (ECHA, 1999).

In an OECD and GLP guideline-compliant *in vitro* mammalian cell gene mutation assay, mouse lymphoma L5178Y cells were incubated with benzaldehyde at concentrations up to 800 μg/mL for 3 h both with and without an S9 metabolic activation system derived from the livers of Aroclor-1254 induced rats (ECHA, 2019b). Under these experimental conditions, benzaldehyde was non-mutagenic at the *tk* locus in mouse lymphoma L5178Y cells.

In a study consistent with the OECD guideline study, CHL fibroblast cells (V79) were treated with benzyl benzoate at concentrations ranging from 50 to 500 μg/mL in the presence of an S9 metabolic activation system derived from the livers of Aroclor-1254 induced rats and at concentrations between 10 and 120 μg/mL in the absence of S9. Cytotoxicity was not observed in experiments with S9 but was observed at concentrations greater than 100 μg/mL in the absence of S9. Benzyl benzoate was not mutagenic at the hypoxanthine-guanine phosphoribosyltransferase (*hgprt*) locus in V79 cells under the conditions tested (ECHA, 1990).

In an OECD and GLP guideline-compliant *in vitro* chromosomal aberration (CA) assay in human lymphocytes, benzyl benzoate was negative for clastogenicity in the presence and absence of an S9 metabolic

activation system derived from the livers of Aroclor-1254 induced male Wistar rats. In experiments without S9, benzyl benzoate was tested at concentrations of 10–250 μg/mL, with a 24 h sampling time, and at 250 μg/mL, with a 48 h sampling time. In the presence of S9, benzyl benzoate was tested at concentrations of 30–500 μg/mL, with a 24 h sampling time, and at 500 μg/mL, with a 48 h sampling time. Benzyl benzoate was negative for clastogenicity under the conditions tested (ECHA, 1991a).

In an OECD and GLP guideline-compliant *in vitro* micronucleus assay, benzaldehyde was incubated with human peripheral blood lymphocytes (HPBL) at concentrations up to 800 μg/mL for 3 h both with and without an S9 metabolic activation system derived from the livers of Aroclor 1254-induced rats and 200 μg/mL for 24 h without metabolic activation (ECHA, 2019a). Under the conditions tested, there was no induction of micronuclei in HPBL cells upon treatment with benzaldehyde.

In an OECD and GLP guideline-compliant *in vitro* micronucleus assay, HPBL were incubated with *cis*-3-hexenyl benzoate at concentrations of 15–100 μg/mL in 4 h incubations in the absence of S9, 60–650 μg/mL for 4 h incubations in the presence of S9 and 7.5–85 μg/mL in 24 h incubation experiments. The S9 metabolic activation system was prepared from the livers of Aroclor 1254-induced rats. The percentage of cells with micronucleated binucleated cells in the non-activated and S9-activated 4 h experiments was statistically increased relative to vehicle control at a single dose level but these increases were considered biologically irrelevant since they were within the historical solvent control data and there was no dose related response. *cis*-3-Hexenyl benzoate was negative for the induction of micronuclei under the conditions tested (Roy, 2013).

In an *in vitro* alkaline comet assay, human lymphocytes were incubated with benzaldehyde at concentrations of 1, 5, 10, 25 and 50 mM (Demir et al., 2010). Distilled water was used as a negative control. A significant increase in % tail DNA was observed at 10, 25 and 50 mM compared to the solvent control, and a significant increase in tail moment was observed at 10 and 25 mM, but neither increase was concentration dependent. Benzyl alcohol and benzyl acetate were also tested using this assay under the same experimental conditions. For benzyl alcohol, significant increases in % tail DNA and tail moment compared to the control were observed at concentrations of 25 and 50 mM but these increases were not concentration dependent. For benzyl acetate, a statistically significant increase in % tail DNA and tail moment was observed at 50 mM, the highest concentration tested, compared to the control. Benzoic acid was also tested at lower concentrations of 0.05, 0.1, 0.5, 1 and 5 mM and was reported to have an apoptotic effect at concentrations higher than 5 mM. For benzoic acid, a statistically significant increase in % tail DNA and tail moment was observed at a concentration of 5 mM, however, the increase in the tail moment was small and similar to the values obtained for negative controls for the other substances in the study. In these experiments, cytotoxicity of benzaldehyde, benzyl acetate and benzyl alcohol in the test system was not assessed and the concentrations tested in these experiments exceeded the recommended 10 mM maximum concentration in some cases. In all four experiments, details on treatment and sampling times were not provided nor was there a full description of the type of human lymphocytes used. In addition, use of distilled water as a negative control is not recommended since it may cause a significant change in the osmolality of the testing solution. Due to these limitations and the lack of a standardized procedure and OECD guideline for the *in vitro* alkaline comet assay, the relevance of the positive results cannot be assessed (Gooderham et al., 2020a) and as a result, this study was not considered helpful to the safety evaluation of benzaldehyde, benzyl acetate, benzyl alcohol and benzoic acid.

In another *in vitro* alkaline comet assay, HPBL were incubated for 1 h at 37 °C with 0 (distilled water negative control), 50, 100, 200 and 500 μg/mL of benzoic acid (purity ≥99.5 %) (Yilmaz et al., 2014). Two slides, each containing 100 cells, were analyzed per test concentration for DNA damage. A statistically significant, but non-dose dependent increase in % mean tail intensity and mean tail length relative to the negative control was observed. This study, however, did not report historical negative control values and analyzed only 200 cells per test point compared to the 300 normally scored. In another study from the same laboratory, potassium and sodium salts of benzoic acid were also tested by the *in vitro* comet assay in HPBL (Zengin et al., 2011). Potassium benzoate was assayed at concentrations ranging from 62.5 to 1000 μg/mL and sodium benzoate was assayed at concentrations ranging from 6.25 to 100 μg/mL for 1 h in HPBL. There was a statistically significant but non-dose dependent increase in tail moment % observed with sodium benzoate while no statistically significant increase in tail moment % was observed for potassium benzoate. These results are difficult to interpret because both salts are expected to behave similarly in this assay and potassium benzoate, tested at 10-fold higher concentrations than sodium benzoate, did not induce DNA damage in this assay. In a third *in vitro* comet assay, human sperm cells were incubated with 0, 50, 100, 200 and 500 μg/mL of benzoic acid for 1 h at 32 °C (Pandir, 2016). A statistically significant increase in % tail DNA, tail length, and tail moment was observed only at the highest tested concentration (500 μg/mL) compared to the control group (Pandir, 2016). The study authors did not assess cytotoxic limits of the test substance, the response was not dose dependent and the use of human sperm has not been validated for *in vitro* comet assay tests. In addition to the limitations of these studies, as previously noted, the *in vitro* comet assay lacks a standardized procedure and as a result, the relevance of the positive results cannot be assessed (Gooderham et al., 2020a). For these reasons, these studies are not considered helpful in the evaluation of the genotoxicity of benzoic acid for this safety evaluation.

Benzoic acid, potassium benzoate and sodium benzoate were also evaluated in several other *in vitro* assays in HPBL including the CA assay, sister chromatid exchange (SCE) assay and the micronucleus assay (Yilmaz et al., 2009; Zengin et al., 2011). The concentration ranges used for these assays were 5–500 μg/mL for benzoic acid, 62.5–1000 μg/mL for potassium benzoate and 6.25–100 μg/mL for sodium benzoate. In the CA assay, significant increases in the frequency of chromosomal aberrations were detected in a dose-dependent manner for benzoic acid, sodium benzoate and potassium benzoate after 24 and 48 h of incubation with HPBL at all concentrations compared to the negative control. In the SCE assay, significant dose-dependent increases in SCEs/cell were observed for the 24 h incubation with benzoic acid and the 24 h and 48 h incubations with potassium and sodium benzoate. In the micronucleus assay, a non-dose dependent increase in the induction of micronuclei was observed at the two highest benzoic acid concentrations (200 and 500 μg/mL) in the 48 h incubation with HPBL, (Yilmaz et al., 2009), the three highest concentrations of sodium benzoate (≥25 μg/mL) and all but the lowest concentration of potassium benzoate (≥125 μg/mL) (Zengin et al., 2011). In these studies, the concentration range of potassium benzoate tested was 10-fold higher than that of sodium benzoate but the results of these similar salts of benzoic acid varied significantly. In addition, the treatment and sampling times used in the CA assay deviated from the OECD testing guideline (OECD, 2014b). The OECD guideline for the SCE assay was deleted in 2014 (OECD, 2015, 2017). Concentrations of benzoic acid (mM) were used in these tests which might be expected to appreciably lower the pH of the medium (1 mM benzoic acid ~ pH 3.6); low pH is well known to induce artifactual chromosomal damage in eukaryote cells (Scott et al., 1991) and OECD guidelines specify the need to control for such pH changes (OECD, 2014b). However, in both of these reports it is stated that the pH of the medium was not altered with treatment, but confirmatory data is not presented. Based on these considerations, these assays are not considered helpful in the safety evaluation of benzoic acid and its salts.

In an OECD and GLP guideline-compliant *in vitro* micronucleus assay, HPBL were treated with methyl benzoate at concentrations up to 1360 μg/mL in 4 h exposure experiments both with and without an S9 metabolic activation system derived from the livers of phenobarbital/β-naphthoflavone-induced male Wistar rats and in 20 h exposure

experiments in the absence of S9 (Bohnenberger, 2013). Under the conditions tested, methyl benzoate did not induce the formation of micronuclei in HPBL.

In a GLP guideline-compliant *in vitro* CA assay, CHL cells were treated with cuminaldehyde for 6 h in the presence and absence of S9 metabolic activation system or for 24 h in the absence of an S9 metabolic activation. Concentrations of 92, 140 and 210 μg/mL (6 h without S9); 140, 210 and 310 μg/mL (6 h with S9); or 69, 100 and 160 μg/mL (24 h treatment without S9) were analyzed for chromosomal aberrations. In the 6 h treatments with and without S9, there were no statistically significant increases in the frequency of structural aberrations. In the 24 h treatment without S9, a statistically significant increase ($p < 0.01$) in the frequency of cells with aberrations excluding gaps was observed at 100 μg/mL. No statistically significant increase was observed at the next highest analyzed concentration of 160 μg/mL, nor was a concentration-dependent trend demonstrated. Therefore, the observed increase at 100 μg/mL is not considered biologically relevant. Under the conditions tested, cuminaldehyde was negative for induction of chromosomal aberrations in cultured CHL cells (JMHLW, 2012b).

7.1.2.2. In vivo. In an OECD and GLP guideline-compliant *in vivo* micronucleus study, groups of NMRI mice (5/sex/group) were orally administered a single dose of 0 (corn oil vehicle control), 250, 500 or 1000 mg/kg bw benzaldehyde (purity 99.8 %) (Honarvar, 2009). A previous dose-range finding study established 1000 mg/kg bw benzaldehyde as the maximum tolerated dose. Samples of bone marrow were collected 24 h post-administration for all dose levels and at 48 h for an additional test for the 1000 mg/kg bw group dose level only. The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that benzaldehyde did not have any cytotoxic properties in the bone marrow. No significant increase or decrease of the frequency of micronuclei in any of the benzaldehyde test groups was observed compared to the vehicle control. Benzaldehyde was negative for micronuclei induction at doses up to 1000 mg/kg bw.

In an *in vivo* comet assay, male CD-1 mice were administered a single dose of 1000 mg/kg bw benzoic acid or a single dose of 1000 mg/kg bw sodium benzoate by gavage (Sasaki et al., 2002). Several organs were analyzed including the stomach, colon, liver, kidney, bladder, lung, brain and bone marrow at both 3 and 24 h after administration of the test substances. No death, morbidity, or clinical signs were observed after treatment. Neither benzoic acid nor its corresponding salt, sodium benzoate, induced statistically significant increases in DNA migration in the sampled organs and histopathological examination of these organs upon necropsy indicated no treatment related adverse effects. Twelve male ddY mice were administered a single dose of 1600 mg/kg bw benzyl acetate and 12 male Wistar rats were administered a single dose of 1200 mg/kg benzyl acetate by oral gavage, using olive oil as a vehicle, for *in vivo* comet assays (Sasaki et al., 2000; Sekihashi et al., 2002). In each study, four animals were sacrificed at three time points: 3, 8 and 24 h post administration of the test substance and DNA from stomach, colon, liver, kidney, bladder, lung, brain and bone marrow tissues were analyzed. Migration was reported as the average difference in the length of the whole comet and the diameter of the head observed in 50 nuclei per organ per animal. In the mouse study, the comet assay was positive in the stomach, colon, bladder and brain at the 3 h sampling point, the stomach, colon, kidney and bladder at the 8 h sampling point and negative for all organs at the 24 h sampling point. In the rat study, the comet assay was positive in the stomach, colon, bladder and lung at the 3 h sampling point, the stomach, liver, colon, kidney, bladder and lung at the 8 h sampling point and kidney and lung at the 24 h sampling point. A similar study was also conducted in which male ddY mice were administered a single dose of 400 mg/kg benzyl alcohol. In this study, the comet assay was negative in all organs at the 3 h sampling point and positive in the stomach, colon and bladder at the 24 h sampling point (Sasaki et al., 2000). These single dose studies are not considered helpful to the safety evaluation of benzyl acetate or benzyl alcohol due to the lack of data at lower doses that would indicate the presence or absence of a dose response and data on whether the response observed was related to general toxicity considering the high doses administered. In addition, % tail DNA, tail lengths and tail moments were not reported, as required by the OECD guideline for the *in vivo* comet assay (OECD, 2014a).

In an *in vivo* CA assay, male (SD) rats (5/group) were administered sodium benzoate by gavage at dose levels 0, 50, 500 or 5000 mg/kg bw in a single dose or once a day for 5 days (Fabrizio, 1974). In this study the animals were terminated at 6, 24 or 48 h following treatment in the acute study and 6 h after treatment in the 5-day study. Bone marrow metaphase chromosomes were evaluated for aberrations, but no evidence of toxicity or evidence of exposure was provided in the study report. No significant increase in DNA damage was observed upon administration of benzoic acid or sodium benzoate under the conditions tested.

7.1.3. Summary of genotoxicity of benzyl derivative constituents

The genotoxicity studies of benzaldehyde (FEMA 2127), benzoic acid (FEMA 2131), benzyl acetate (FEMA 2135), benzyl alcohol (FEMA 2137), benzyl benzoate (FEMA 2138) and cuminaldehyde (FEMA 2341) reviewed by the FEMA Expert Panel are summarized in tabular form in Appendix B. The overview in Appendix B includes studies that were previously reviewed (non-shaded) for the safety assessment of these benzyl derivative flavoring ingredients (Adams et al., 2005a) as well as studies that have become available since its publication in 2005 (shaded).

In its previous safety assessment, based on the studies reviewed, the Panel concluded that the group of benzyl derivatives is not genotoxic *in vivo* (Adams et al., 2005a). For benzaldehyde, several Ames test results showed no evidence of mutagenicity, including a study conducted by the National Toxicology Program (NTP, 1990b). However, there are mixed results for benzaldehyde reported in several non-guideline *in vitro* studies. Benzaldehyde was negative for genotoxicity when tested up to 251 μg/mL in an unscheduled DNA synthesis (UDS) assay in rat hepatocytes (Heck et al., 1989), an assay no longer included in the OECD guidelines (OECD, 2015, 2017), but positive in two *in vitro* mouse lymphoma assays at concentrations of 600–800 μg/mL (Adams et al., 2005a; Heck et al., 1989; McGregor et al., 1991). Both positive and negative results were reported for CA studies in Chinese hamster cells (Galloway et al., 1987; Kasamaki et al., 1982; Sofuni et al., 1985) and in sister chromatid exchange (SCE) assays, the latter of which also no longer included in the OECD guidelines (Galloway et al., 1987; OECD, 2015, 2017; Sofuni et al., 1985). For benzoic acid, benzyl alcohol, benzyl acetate and cuminaldehyde, the table in Appendix B summarizes several Ames test and mutation study results showing no evidence of mutagenicity (Adams et al., 2005a). For benzoic acid, no genotoxicity was reported in indirect DNA repair and SCE assays (Glosnicka and Dziadziuszko, 1986; Jansson et al., 1988), which are no longer included in the OECD guidelines, but weakly positive results were reported in a CA assay and a rec assay (Ishidate et al., 1984; Nonaka, 1989). However, the concentrations of benzoic acid (mM) used in these tests that might be expected to appreciably lower the pH of the medium (1 mM benzoic acid ~ pH 3.6); low pH is well known to induce artifactual chromosomal damage in eukaryote cells (Scott et al., 1991) and OECD guidelines specify the need to control for such pH changes (OECD, 2014b).

In more recent studies, benzaldehyde tested negative in both OECD guideline-compliant *in vitro* and *in vivo* micronucleus assays (ECHA, 2019b; Honarvar, 2009) and the *in vitro* gene mutation assay (ECHA, 2019a). Studies reported by Demir and co-workers and Yilmaz and co-workers indicated positive results for benzaldehyde, benzoic acid, sodium and potassium salts of benzoic acid, benzyl acetate and benzyl alcohol in the *in vitro* alkaline comet assay and for benzoic acid in the *in vitro* comet, CA, SCE and micronucleus assays (Demir et al., 2010;

Yilmaz et al., 2009, 2014; Zengin et al., 2011). As described above, concentrations of benzoic acid (mM) were used in these tests which might be expected to appreciably lower the pH of the medium (1 mM benzoic acid ~ pH 3.6) and OECD guidelines specify the need to control for such pH changes (OECD, 2014b). Although some of these studies report that the pH of the medium was not altered with treatment, confirmatory data are not reported. Furthermore, these assays appear to have been performed under non-standard experimental conditions, as noted in their summaries above, and their results are superseded by the results of *in vivo* studies. In addition to the negative results for *in vivo* assays previously evaluated (Foureman et al., 1994; Hayashi et al., 1988; Longnecker et al., 1990; Mirsalis et al., 1989; NTP, 1993b; Shelby et al., 1993; Steinmetz and Mirsalis, 1984), there are four additional *in vivo* studies that have become available and are summarized above: a micronucleus induction assay that was negative for benzaldehyde (Honarvar, 2009), two *in vivo* comet assays that were negative for benzoic acid and its sodium salt (Sasaki et al., 2002), and a CA assay that was negative for sodium benzoate (Fabrizio, 1974).

For benzyl alcohol, mixed results were reported among three rec assays and three CA assays (Anderson et al., 1990; Ishidate et al., 1984; Kuroda et al., 1984; NTP, 1989; Oda et al., 1979; Yoo, 1986). Negative results were reported for benzyl acetate in the CA, sister chromatic exchange (SCE) and UDS assays with positive results reported in the mouse lymphoma assay (Caspary et al., 1988; Galloway et al., 1987; Matsuoka et al., 1996; McGregor et al., 1988; Mirsalis et al., 1983; Rudd et al., 1983). No genotoxicity was reported for cuminaldehyde in the SCE assay or in the Ames and GLP compliant *in vitro* CA assay in CHL cells summarized above (Rockwell and Raw, 1979; Sasaki et al., 1989). Since the initial reporting of these results, the OECD has removed the SCE and UDS assays from the OECD library of standardized assays due to a lack of understanding of the underlying mechanism(s) of action and the OECD has noted that indicator tests such as the rec assay should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015, 2017).

In reviewing the *in vivo* assay results in the previous evaluation, negative results were reported for *in vivo* assays of benzyl alcohol and benzyl acetate in the micronucleus study on bone marrow in mice as well as in other *in vivo* assays (Foureman et al., 1994; Hayashi et al., 1988; Longnecker et al., 1990; Mirsalis et al., 1989; NTP, 1993b; Shelby et al., 1993; Steinmetz and Mirsalis, 1984). However, positive *in vivo* results were reported for benzyl alcohol and benzyl acetate in comet assays (Sasaki et al., 2000). These assays were performed at a single dose of 400 mg/kg for benzyl alcohol and 1600 mg/kg for benzyl acetate in ddY mice with tissues collected for testing 3, 8 and 24 h following dose administration by oral gavage using olive oil as a vehicle (Sasaki et al., 2000). In a later publication by the same group (Sasaki et al., 2002), the related substances, benzoic acid and sodium benzoate were negative for genotoxicity when tested at a single dose level of 2000 mg/kg in ddY mice using the comet assay. Investigative studies into the interpretation of comet assay results have determined several issues that can increase the variability of results (Vasquez, 2012; Vasquez and Dewhurst, 2024). These single dose studies are not considered helpful to the safety evaluation of benzyl acetate or benzyl alcohol due to the lack of data at lower doses that would indicate the presence or absence of a dose response and data on whether the response observed was related to general toxicity considering the high doses administered. In addition, % tail DNA, tail lengths and tail moments were not reported, as required by the OECD guideline for the *in vivo* comet assay (OECD, 2014a).

Two-year carcinogenicity studies conducted by the NTP are available for both benzyl alcohol and benzyl acetate. For benzyl alcohol, a two-year oral gavage study showed no evidence of carcinogenicity in male and female F344/N rats and B6C3F1 mice administered doses of 0, 200 or 400 mg/kg bw (NTP, 1989). For benzyl acetate, a 2-yr oral gavage study conducted by NTP reported no evidence of carcinogenicity in female F344/N rats at doses up to 500 mg/kg bw/day. Male F344/N rats administered doses up to 500 mg/kg bw and male and female B6C3F1 mice administered doses up to 1000 mg/kg bw developed pancreatic acinar cell neoplasms and hepatocellular neoplasms, respectively (NTP, 1986). A subsequent, 2-yr dietary study on benzyl acetate conducted in male and female F344/N rats at targeted doses up to 575 mg/kg bw/day and in male or female B6C3F1 mice at targeted doses up to 375 mg/kg bw/day showed no evidence of carcinogenicity, consistent with the determination that the effects observed in the oral gavage study were correlated with the route of administration, diet versus oral gavage, of the test substance (Adams et al., 2005a; NTP, 1993b). The NTP stated:

In the feeding study, no increased incidences of neoplasms or nonneoplastic lesions could be associated with benzyl acetate administration in male or female rats. By comparison, male rats administered benzyl acetate in corn oil by gavage at doses of 250 or 500 mg/kg had significantly increased incidences of pancreatic acinar cell adenoma and hyperplasia. This effect did not occur in females. These contrasting results were probably due to the use of corn oil as a vehicle in the latter study. The administration of high levels of fat to experimental animals has been shown to enhance the development of spontaneous and chemical-induced neoplasms.

Furthermore, induction of pancreatic acinar neoplasms in rats is cholecystokinin-dependent, a mode of action not relevant to human cancer risk (Obourn et al., 1997). As shown in Fig. 8 and discussed in the "Metabolic fate of benzyl derivatives" section of this manuscript, benzyl alcohol, benzyl acetate, sodium benzoate and benzoic acid are all metabolized to hippuric acid, in humans. Hippuric acid is a non-toxic and non-carcinogenic compound that is rapidly excreted in the urine.

Based on this review of the available genotoxicity studies for benzaldehyde, benzoic acid, benzyl acetate, benzyl alcohol, benzyl benzoate, cuminaldehyde and other related benzyl derivative flavoring ingredients (see Appendix B for summary table) the FEMA Expert Panel affirms the conclusion that benzyl derivative flavoring ingredients are not of concern with respect to genotoxicity based on the weight of evidence approach (Gooderham et al., 2020a).

7.1.4. Short-term toxicity of benzyl derivatives

7.1.4.1. Benzyl benzoate (FEMA 2138). In a 90-day study, male Sprague-Dawley rats (8/group) were administered 0, 25 or 100 mg/kg bw/day benzyl benzoate by oral gavage (Kılıç Süloğlu et al., 2022). The control rats were administered a saline solution and the treatment groups were administered benzyl benzoate without dissolving it in solvent. Animals were checked twice a day for signs of clinical toxicity. Body weights were measured weekly. At the end of the study, all animals were euthanized and the kidney, liver, thymus, cauda epididymis, prostate and testis were collected. Blood samples were collected from the heart for clinical chemistry analyses.

During the study, there were no mortalities. There were no significant differences in final body weights, food consumption or relative organ weights between the treatment groups and the control group. Water consumption was significantly increased in the low dose group but not in the high dose group. Analysis of blood samples showed significant but non-dose-related increases in % monocytes and aspartate aminotransferase (AST) in the low dose group and a dose-dependent decrease in % neutrophils that was statistically significant in the high dose group compared to the control group.

Histopathology of the liver revealed enlargement in sinusoids, mononuclear cell infiltration, local lysis, and cytoplasmic degeneration in the treatment groups. Congestion, tubular degeneration, mononuclear cell infiltration and tissue lysis were observed in the kidney tissues of high dose rats. Rats in the high dose treatment group also showed increases in the number of Hassall's bodies, increased lipids, degeneration, congestion and fibrosis, a decrease in lymphocytes in the thymus, vacuolization and irregular secretion in the prostate and increased connective tissue in the epididymis with the presence of cells in the lumen. The study authors, however, did not provide tabulated incidences of these effects. There were no significant differences in

sperm count, daily sperm production or sperm anomalies between the treatment and control groups. Immunohistochemical analyses were performed to detect fibronectin (FN), type IV collagen, transforming growth factor β (TGF-β), matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in the liver, kidney and thymus with 16 tissue preparations analyzed for each group/tissue. FN, TGF-β, MMPs and TIMPs are each involved in tissue repair processes. Dose-related increases in FN staining were observed in the liver, kidney and thymus. Increases in staining intensities were observed for type IV collagen in the kidney and for TGF-β in the thymus for the high dose animals compared to controls. In addition, MMP-2 staining was increased in the treatment groups compared to the control group in a dose-dependent manner in all tissues. In the thymus, staining of TIMP-2 showed dose-related decreases in intensity. The study authors concluded that benzyl benzoate caused significant changes in the liver, kidney and thymus of treated rats but did not determine a no-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) for this study (Kılıç Süloğlu et al., 2022). The Expert Panel concluded a LOAEL of 25 mg/kg, the lowest dose tested, but considering the limitations of this study, including the lack of tabular data and administration of the test substance without dissolving in solvent, it is not relevant to the safety evaluation of benzyl benzoate as a constituent of the NFCs under evaluation.

7.1.4.2. Cuminaldehyde (FEMA 2341). In an OECD and GLP guideline-compliant subchronic study, cuminaldehyde was administered to male and female CRL: SD CD® IGS rats (10/sex/group) in the diet to provide target doses of 0, 30, 100 or 300 mg/kg/day for 90 days (Bauter, 2021). At the end of the study, the actual doses, based on food consumption and stability of cuminaldehyde in the feed, were determined to be 0, 18, 60 and 178 mg/kg bw/day for males and 0, 18, 60 and 180 mg/kg bw/day for females. There were no treatment-related deaths during the study. However, two animals were euthanized: one mid-dose male due to suspected incidental trauma to the nose and snout and one emaciated high dose female on day 42 of the study. Statistically significant decreases in body weights and body weight gains occurred in high dose male and female groups over the course of the study. In mid-dose males, there was a significant decrease in body weights on days 56–91 and decreases in body weight gains over the course of the study. These decreases in body weight and body weight gains were concurrent with decreased food consumption in the high dose male and female groups and the mid-dose male group. Mean body weight gains for low-dose male and female groups and the mid-dose female group were comparable to the control groups.

Clinical chemistry showed decreased triglyceride levels in all treated groups, increased blood urea nitrogen and creatinine levels in high dose males and females, decreased total protein and globulin in mid- and high dose males and decreased albumin in all female treatment groups. Urinalysis showed ketonuria in all male and female treated groups and decreased total protein in all the female treatment groups. These treatment-related changes were within historical control ranges and considered as non-adverse.

At the end of the study, there were statistically significant decreases in the absolute weights of the adrenal glands, brain, epididymides, heart, testes, thyroid/parathyroid and the combined weight of the prostate, seminal vesicles and coagulation gland and in the relative weights of the epididymides and testes in the high dose male group. Histopathological analysis found degeneration/atrophy of the testicular germinal epithelium, and small epididymides which were correlated with decreased sperm content in the epididymal tubules secondary to decreased sperm production in the testes.

A significant, dose-related increase in relative kidney weights was observed in mid- and high dose males and high dose females. Histopathological analysis of the kidney found degeneration of tubular epithelial cells in 10/10 high dose males, 9/10 high dose females, and 1/10 mid-dose female. This finding was characterized by cytoplasmic vacuolation of collecting ducts and distal convoluted tubules in both the cortex and medulla. The cells were enlarged by the vacuoles and often projected into tubular lumens. Renal papillary necrosis was also present in 8/10 high dose males, 8/10 high dose females, and 1/10 mid-dose female. The collecting duct and distal tubule degeneration and papillary necrosis were both considered to be adverse findings. Gray/green granular pigment was noted sporadically in the kidneys of some males for all treatment groups and in two high dose females. This pigment was present within the cytoplasm of proximal convoluted tubules in the renal cortex and did not show an apparent association with the degenerative changes noted in the collecting ducts and distal tubules or the papillary necrosis. The specific identity of the pigment could not be determined based on the tissue sections examined.

There were significant dose-related increases in relative liver weights in all female treatment groups and significant, but non-dose dependent increases in relative liver weights in the mid- and high dose male treatment groups. Histopathological analysis of the liver showed hepatocellular hypertrophy characterized by an increase in hepatocyte cytoplasm in all male and female treatment groups. In the high dose animals, the findings were diffuse, while in lower doses, the effect was mainly present in centrilobular hepatocytes. The severity of this finding was dose dependent. The study authors considered these effects to be the result of an adaptive response by the rats to the administration of an exogenous substance. Also, small numbers of Kupffer cells in the hepatic sinusoids, in or adjacent to portal regions and central veins, were observed in the livers of the male and female treatment groups. The Kupffer cells contained gray/green pigment within their cytoplasm, similar to the pigment in the cytoplasm of renal proximal convoluted tubules. The pigment was not associated with cell injury and was not considered to be adverse.

Based on clinical chemistry observations, decreases in body weight and food consumption, histopathologic changes in the kidneys and decreased reproductive organ weights that were correlated with microscopic findings, the FEMA Expert Panel determined a NOAEL for this study to be the low dose, targeted to be 30 mg/kg bw/day. The actual intake of the low dose was calculated to be 18 mg/kg/day for male and female rats based on the dietary stability, refreshment and intake data (Bauter, 2021). Based on this NOAEL, a reassuring MOE of greater than 5700 was calculated for the estimated intake of cuminaldehyde in Cumin Oil (FEMA 2343), indicating a low concern for toxicity when used as a flavoring ingredient.

7.1.5. Reproductive and developmental toxicity of benzyl derivatives

In a preliminary study, pregnant female Wistar rats were administered benzaldehyde at doses of 0 (0.5 % methylcellulose with 0.5 % Tween-80 in purified water), 100, 300 or 600 mg/kg bw/day by oral gavage. There were no mortalities or treatment-related clinical signs during this preliminary study. At the high dose, transient signs of decreased activity, irregular breathing and salivation were observed. Also at the high dose, mean maternal body weight gain, mean maternal body weight adjusted for the contribution of the gravid uterine weight, mean gravid uterine weight, mean total litter weight and fetal weights were reduced compared to controls. There were no findings by macroscopic examination of the adult females and no treatment related findings by external examination of the fetuses at any dose level investigated. Based on these findings in the preliminary study, a high dose of 600 mg/kg bw/day was selected for the main study.

In an OECD 414 guideline and GLP guideline-compliant prenatal developmental toxicity study, benzaldehyde was administered to pregnant female rats Wistar rats (22/dose) at 0 (0.5 % methylcellulose with 0.5 % Tween-80 in purified water), 100, 300 or 600 mg/kg bw/day by oral gavage on days 6–20 following mating (ECHA, 2022). Animals were terminated on day 21, blood samples were taken for thyroid hormone analyses and the gravid uterus and thyroid weights were recorded.

One female in the high dose group was euthanized due to poor

clinical condition. Macroscopic examination revealed a distended stomach with abnormal, pale contents. This female was also pregnant with 12 live fetuses. Because there were no other premature deaths or similar observations in other treatment group females, this premature death was not considered treatment related. There were no significant differences in maternal body weight gains, food consumption or mean body weight gains adjusted for the weight of the gravid uterus between the treatment and control groups. The mean gravid uterine weight in the high dose group was slightly lower than the control group but this was not statistically significant. There were no significant differences in absolute or relative weights of the gravid uterus, including the cervix and ovaries or the thyroid between the treatment and control groups. Gross necropsy and histopathological analyses of the thyroid did not reveal any treatment-related findings.

Significantly lowered mean serum triiodothyronine (T3) and thyroxine (T4) concentrations were observed in the high dose group compared to controls, an effect which was not observed in the low or mid-dose groups. The mean serum concentration of thyroid stimulating hormone (TSH) was significantly higher in the mid-dose group compared to controls, an effect not observed in the low or high dose maternal females. This result is due to a very high TSH level in one female in the mid-dose group and was not considered to be treatment related. There were no treatment related histopathological findings in the thyroid for any of the treatment groups.

There were no differences in the number of abortions, pre- and post-implantation loss, total litter losses by resorptions, early or late resorptions, dead fetuses, changes in pregnancy duration or mean placental weights between the maternal treatment and control groups. Three rats in the control group, two in the low dose group, one in the mid-dose group, and one high-dose group were found to be not pregnant at macroscopic examination but these incidences were not considered treatment related.

There were no differences in the number of live offspring, changes in sex ratio, effects on anogenital distance or offspring survival between the fetuses of the treatment and control groups. At the high dose, male, female and overall fetal weights were statistically lower than controls. Mean total litter weights of high dose females were lower than controls but this did not reach statistical significance. Eight fetuses from four high dose group litters were observed to have major eye abnormalities; anophthalmia and microphthalmia, exceeding the historical control data (HCD) range and were considered to be adverse, treatment-related effects. Minor skeletal changes that exceeded the HCD were observed in high dose litters. In the low and high dose litters, there were 5 fetuses with apparent malrotation of the hindlimb(s) noted at necropsy. In subsequent skeletal examinations, although the limbs still appeared slightly malrotated, both the bones and cartilage of the hindlimbs were normal. This finding was therefore considered to be incidental and not treatment related.

Based on a lack of adverse findings in maternal female rats, a NOAEL for maternal toxicity of 600 mg/kg bw/day benzaldehyde was determined. Based on the abnormalities in fetuses of high dose females, a NOAEL for developmental toxicity of 300 mg/kg bw/day benzaldehyde was determined in this study.

In an OECD 443 guideline and GLP guideline-compliant extended one-generation reproductive toxicity study, benzoic acid was administered to male and female Crl:CD(SD) rats at concentrations of 0, 7,500, 11,500 and 15,000 ppm (equivalent to 0, 500, 750 and 1000 mg/kg bw/day) in the feed (Turnbull et al., 2021). Thirty F_0 males were administered benzoic acid for 2 weeks before mating until termination at 10–11 weeks. Thirty F_0 female rats were administered benzoic acid for 2 weeks before mating, throughout mating, gestation, and lactation until termination at 10–11 weeks. Similar methods of administration were followed for F_1 and F_2 offspring, beginning at weaning until termination. Dietary concentrations of benzoic acid were adjusted weekly for the F_0 and F_1 females during gestation and lactation and for F_1 and F_2 pups following weaning (post-natal day (PND) 21–70) due to differing body weight and food consumption habits. At the end of the weaning period (PND 21), F1 pups were randomly assigned to Cohorts 1A (20/sex/group), 1B (20/sex/group), 2A (12/sex/group), 2B (12/sex/group), 3 (10/sex group) and 3A (10/sex) such that not more than 1 pup/sex/litter was assigned to each group. Cohort 1A animals were evaluated for reproductive toxicity, Cohort 1B animals were mated to produce an F_2 generation, Cohort 2A and 2B animals were given developmental neurotoxicity assessments and Cohort 3 animals were subjected to developmental immunotoxicity testing with Cohort 3A serving as a positive control for this evaluation.

All animals were observed daily for moribundity, mortality and clinical signs. Body weights and food consumption were recorded weekly, and twice weekly for mated females through gestation and lactation. Following administration of benzoic acid, female estrous cycles were monitored until confirmation of mating. Litters were scored for live and stillborn pups. Blood samples from parental males and females were collected to assess thyroid hormones and clinical pathology. At necropsy, organ weights were analyzed, and a histopathological evaluation was conducted. For all males, sperm parameters were evaluated, and terminal vaginal lavage samples were collected for females to determine estrous cycle stage.

In the F_1 generation, clinical signs and survival data were collected on PND 1, 4, 7, 14 and 21, and body weights were collected on PND 0, 4, 14 and 21. Additionally, animals were examined for anogenital distance and presence of nipples/areolae. F_1 animals in Cohort 1A were evaluated for reproductive parameters including onset of estrous and estrous cyclicity, splenic lymphocyte immunophenotyping at PND 91, as well as thyroid hormones, sperm parameters and clinical pathology, organ weights and histopathology at termination (PND 91). Cohort 1B animals were mated to produce F_2 offspring to evaluate the impact of benzoic acid on pup reproductive behavior, offspring survival, growth and development, parturition and litter parameters. To assess the potential neurotoxic effects of benzoic acid, F_1 animals in Cohort 2A were evaluated for auditory startle response (PND 24), functional observation battery (PND 65), locomotor activity (PND 65), and learning and memory. At termination (PND 78), brain measurements, including weight, length and width, were recorded. F_1 animals in Cohort 2B were terminated on PND 22 for measurements and neuropathological evaluation of the brain. F_1 animals in Cohort 3 were evaluated for immune function using the T-cell dependent antibody response assay (TDAR) at termination (PND 59), with Cohort 3A serving as a positive control.

No effects on survival, body weights, and food consumption were observed in F_0 male and female rats. No treatment-related effects were noted on reproductive performance, female estrous cyclicity or sperm parameters. Administration of benzoic acid did not affect thyroid levels, and no macroscopic or microscopic observations or effects were reported on clinical pathology or organ weights.

No effects on the mean number of pups, live litter size, offspring sex ratio or postnatal survival were noted for F_1 and F_2 generations following administration of benzoic acid. In the F_1 generation, lactation transfer of benzoic acid and its metabolite hippuric acid during preweaning were noted at the two highest dose concentrations in the dose range-finding study. No changes in clinical signs and food consumption were reported, and the body weights of treatment groups were comparable to those of the control groups (PND 1–14). Low pup body weight gain was reported in both sexes between PND 14–21, but the reduction was within the historical control data range. No effects were observed on developmental landmarks, thyroid hormone levels, reproductive performance, female estrous cyclicity, time to first estrus, female primordial follicle counts or sperm parameters. The administration of benzoic acid resulted in no effects on auditory startle responsiveness, functional observation battery, locomotor activity, or learning and memory in the treatment groups. No effects on the humoral immune system or splenic lymphocyte populations were observed. In F_2 offspring, no differences in body weights or body weight gains, weanling organ weight, food consumption or absolute/relative brain weights were observed between

treatment and control groups. In summary, there were no effects reported in any of the parameters evaluated in this study. A NOAEL of 1000 mg/kg bw/day, the highest dose tested, was determined based on the lack of adverse effects at all dose levels.

A GLP repeat-dose and reproductive/developmental toxicity study was conducted on cuminaldehyde in male and female SD rats (JMHLW, 2013). Male rats were administered doses of cuminaldehyde at levels of 0 (corn oil), 30, 100 or 300 mg/day by oral gavage for up to 42 days with a recovery period (14 days). Female rats received the substance at the same doses for 2 weeks before mating, throughout pregnancy and up to 4 days of nursing for a total of 42–45 days. Post-pregnancy examinations were performed only for the low dose female group in the absence of pregnancies in the mid- and high dose groups. There were additional satellite groups of non-mating control females and a recovery group of high dose females. Due to marked toxicity observed in females of the high dose group, the dose level was lowered to 150 mg/kg bw/day on Day 18. Four females of the main high dose group were found dead or were moribund and displayed decreased locomotor activity, decreased stool volume and thin appearance during clinical observations. Similar observations were noted in the other females of the high dose group that survived to scheduled necropsy. No deaths or adverse clinical observations were reported in any of the treated males. Lower terminal body weights were observed in high dose males (Control: 512.5 ± 34.6 g; low dose: 494 ± 33.9 g; mid-dose: 485.6 ± 36.7 g; high dose: 466.5 ± 28.8 g) of the main group and mid- and high dose recovery group males (Control: 552.8.5 ± 39.6 g; mid-dose: 490.7 ± 36.2 g; high dose: 489.7 ± 29.6 g). Lower body weights were also observed in high dose females belonging to the main group (Control: 263.7 ± 16.8 g; low dose: 260.9 ± 15.4 g; mid-dose: 260.7 ± 14.0 g; high dose: 255.6 ± 24.2 g), the satellite group (Control: 312 ± 26.3 g; high dose: 280.3 ± 37.5 g) and the recovery group (Control: 326 ± 29.8 g; high dose: 283.2 ± 36.5 g). Lower food consumption was observed in the middle and high dose males and in the high dose females during pregnancy and lactation periods. Urinalysis and hematology revealed increased urine volume, decreased specific gravity and electrolytes, decreased mean corpuscular hemoglobin and decreased platelet counts in high dose satellite females. These same changes were observed in recovery group females to a lesser extent but were not statistically significant. Serum chemistry revealed increased AST, urea nitrogen and inorganic phosphorus and decreased total protein, total cholesterol and calcium in high dose males, none of which were observed in recovery group males at the same dose level. In females, decreases of albumin and calcium in the high dose of the satellite group were observed. At necropsy, thymus weights were decreased in males at all dose levels. Since decreased thymus weights were not observed in the recovery groups and the thymus had normal histology, this effect was interpreted as a stress response. Increased liver weights were reported in males at 100 mg/kg bw/day and higher, in females at all dose levels, and in satellite females. Increased relative kidney weights were observed in high dose males, mid- and high dose females and high dose satellite females. In addition, the relative weight of the testis decreased in the high dose group, and the relative weight of the epididymis increased in the mid-dose group.

For surviving males at the end of the dosing period, histopathological findings that increased in incidence and severity in relation to dose level included diffuse hepatocyte hypertrophy, seminiferous tubule atrophy, decreases in elongated spermatids in the seminiferous tubules, Leydig cell hyperplasia, multinucleated giant cells in the seminiferous tubules, luminal cell debris in the epididymis, and decreases in sperm bilaterally in mid- and/or high dose groups. These findings were observed in rats during the recovery period. The findings in the testes and epididymis corresponded to infertility in males of the high dose group. The presence of microgranulomas and increased focal and single cell necrosis of the liver, as well as spermatic granulomas in the epididymis and lymphocyte infiltration in the prostate, were reported in main group males but appeared to be resolved in the two-week recovery period. The Panel noted that two weeks is too short for the recovery of such lesions, including sperm granulomas. In surviving females, diffuse hepatocyte hypertrophy, fatty change in periportal hepatocytes, and vacuolar degeneration of the distal collecting tubule in the kidneys were observed in the mid- and/or high dose groups.

There were no effects on the estrus cycle, gestation period, birth rate, corpora luteal count, number of implantations or implantation rate in the low-dose females with confirmed pregnancies. As noted previously, there were no confirmed pregnancies in the mid- and high dose groups. Mean body weights of female pups born to litters of the low-dose group were lower at birth (Control: 6.2 ± 0.5 g; low dose: 5.6 ± 0.6 g) but were comparable to the control group 4 days after birth (Control: 10.0 ± 1.5 g; low dose: 9.1 ± 1.6 g). Mean body weights of male pups born to litters of the low-dose group were comparable to the control both at birth (Control: 6.4 ± 0.5 g; low dose: 5.9 ± 0.7 g) and 4 days after birth (Control: 10.5 ± 1.7 g; low dose: 9.4 ± 1.8 g). External malformations were not observed for any of the live pups born in either the control or low-dose groups. Based on these findings, the no-observed-adverse-effect level (NOAEL) for the repeat dose toxicity study, as well as for reproductive/developmental toxicity study of cuminaldehyde was determined to be 30 mg/kg bw/day (JMHLW, 2013).

Pregnant adult female Wistar albino rats (gestational day (GD) 0–20) were given daily oral administrations of 0, 25 or 100 mg/kg bw/day of benzyl benzoate (Kockaya and Kilic, 2014). Body weights of each dam as well as maternal food and water consumption were recorded daily from GD 0 to 20. Maternal weights were recorded at the initiation and termination of the study. Dams were terminated at GD 20. Absolute and relative weights of maternal liver, kidney, heart, brain and placenta were recorded at caesarian section. Uterine horns were removed from the body and assessed for implantations as well as any living, dead or resorbed fetuses. Living fetuses were examined for physical abnormalities, and the fetal liver, kidneys, heart and brain were weighed. Maternal and fetal tissues were subjected to histopathology and maternal blood was collected for hematological analysis.

No statistically significant changes were observed in body weight gain as well as food and water consumption between the control and treatment groups. There were no significant changes in the relative or absolute weights of the female rats in the treatment groups compared to the control groups. In the fetuses, there were no significant differences in the absolute and relative weights of the liver, kidneys or brain between the treatment and control groups. A significant increase in the absolute weight of the fetal heart of the high dose group compared to the control group was reported. Hematological analysis revealed a statistically significant increase in % lymphocytes between the high dose group and the control groups. Other significant differences included: decrease in % monocytes in the high dose group, increase in % eosinophils in the low dose but not the high dose group and a decrease in red blood cell count and hematocrit values in the low dose but not the high dose group ($p \leq 0.05$).

The female rats in the low and high dose groups had non-dose dependent, statistically significant higher AST levels compared to the control group. The high dose group had a statistically significant decrease in creatinine levels compared to the control group ($p \leq 0.05$). Histological findings were reported in the liver, brain, heart and placenta of the maternal rats in both treatment groups, but the description of the effects was not standard and the FEMA Expert Panel found it difficult to interpret the implications for risk assessment. Furthermore, there were only 5 rats per group, limiting statistical comparisons.

An examination of the placenta and fetuses found that the placental weight and diameter, *trans*-umbilical length, and fetal length and weight and resorption ratio were significantly increased in the high dose group compared to the control group. The placental index values were lower for both the low and high dose treatment groups compared to the control group. There were no gross malformations found in the fetuses. Neither a maternal nor fetal NOAEL was identified by the study authors. The LOAEL was 25 mg/kg bw/day, the lowest dose tested (Kockaya and

Kilic, 2014). Although difficult to interpret, based on this LOAEL, very conservative MOEs for NFCs with benzyl benzoate as a constituent range from greater than 1.7×10^6 for Jasmin Absolute (FEMA 2598) to greater than 3.8×10^8 for Jasmine Concrete (FEMA 2599).

7.2. Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives)

7.2.1. Methyl salicylate (FEMA 2745)

7.2.1.1. Metabolic fate. In a radiolabel experiment, complete (100 %) absorption from the oral route was reported following a single gavage dose of 97 mg/kg of [^{14}C]-labelled methyl salicylate (0.76 μCi/μmol) in hairless HRS/J (hr) mice (Yamagata et al., 1976). Blood, tissues and excreta were sampled from 9 animals terminated at 0.25, 0.5, 1, 2, 4, 8, 12, 24 and 48 h after dosing. Maximum blood concentrations and maximum tissue concentrations were reached 30 min after dose administration followed by a rapid decline. Methyl salicylate was distributed primarily to the liver, kidneys and adrenals with lower levels detected in the lungs, heart, spleen and pancreas, and the lowest level in the brain (Yamagata et al., 1976).

In a review of the metabolic fate of methyl salicylate in humans (Graham et al., 2004), hydrolysis to salicylate and subsequent conjugation with glycine to yield salicyluric acid was reported to be the major metabolic pathway for its elimination (Fig. 9). The relative contribution of glycine conjugation declined with increasing dose of methyl salicylate indicating saturation of this pathway at high dose levels (from ~100 % to 80–85 % as the dose increases from 1 to 4 g per day). Conjugation with glucuronic acid was observed as a secondary pathway that yielded up to 20 % of the administered dose as salicylic acid phenolic glucuronide (SAPG) and/or 10 % as salicylic acid acyl glucuronide (SAAG). In a minor pathway, oxidation of the aromatic ring of methyl salicylate yielded up to 5 % of the administered dose as 2,5-dihydroxybenzoic acid (gentisic acid) and lower yields of 2,3-dihydroxybenzoic acid, with subsequent glucuronide conjugation.

7.2.1.2. Short term toxicity study. In a 30-day repeat dose study in which male *Rattus Norvegicus* albino rats received either 0, 80 or 250 mg/kg bw/day of methyl salicylate by oral gavage, no statistically significant differences in body weight gain, % growth rate, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), glucose, alanine transaminase (ALT), AST, alkaline phosphatase (ALP), total protein, albumin, urea or creatinine levels were found in treated rats compared to control rats. Hematological or histopathological analyses were not performed in this study (Amin and AlMuzafar, 2015).

7.2.1.3. Genotoxicity. In its assessment of hydroxy- and alkoxy-substituted benzyl derivative flavoring ingredients, the FEMA Expert

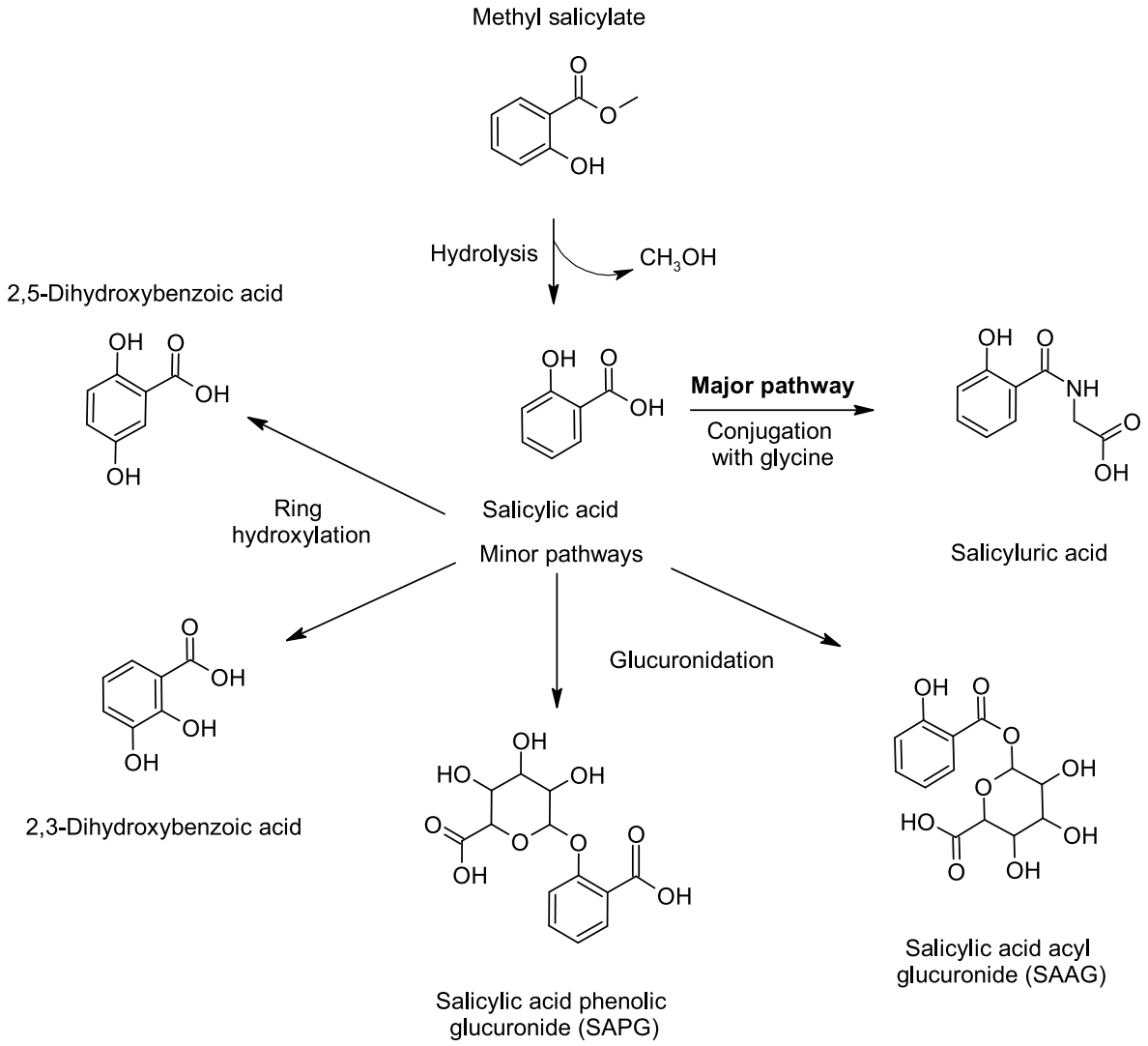

Fig. 9. Metabolic fate of methyl salicylate.

Table 7
Summary of results from benchmark dose modeling (Greene et al., 2017) of reproductive and developmental effects observed upon the administration of methyl salicylate to mice in an NTP study (Gulati et al., 1984).

Effect	BMD (mg/kg bw/day)	$BMDL_5$ (mg/kg bw/day)	Best Fitting Model(s)
Decreased numbers of litters per mating pair	428	220	Linear model Power model
Adjusted average pup weights	415	293	Exponential 3 model
Number of dead pups per individual breeding	2710	770	Logistic model

panel reviewed genotoxicity studies for methyl salicylate (Adams et al., 2005b). Methyl salicylate was negative for mutagenicity in the Ames and rec assays (Ishidate et al., 1984; Kawachi et al., 1980a; Mortelmans et al., 1986) as well as in the CA assays in Chinese hamster and human embryo fibroblast cells and an SCE assay in human embryo fibroblast cells (Ishidate et al., 1984; Kawachi et al., 1980a, 1980b). Since the initial reporting of these results, however, the OECD has removed the SCE assay from the OECD library of standardized assays due to a lack of understanding of the underlying mechanism(s) of action and the OECD has noted that indicator tests such as the rec assay should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015, 2017). Based on the weight of evidence approach for the evaluation of genotoxicity of flavoring ingredients (Gooderham et al., 2020a), methyl salicylate does not present a concern for genotoxicity.

7.2.1.4. Reproductive/developmental toxicity. In a review article examining the oral toxicity of methyl salicylate, the quality of the studies reviewed were ranked using Klimisch scores (Greene et al., 2017). In addition to reviewing the literature, the authors performed benchmark dose modeling of data reported by the NTP sponsored study on the reproductive and developmental effects of methyl salicylate in mice published in 1984 (Greene et al., 2017; Gulati et al., 1984; Morrissey et al., 1989). The FEMA Expert Panel, in their safety evaluation of hydroxy- and alkoxy-substituted benzyl derivatives used as flavoring ingredients, reviewed this study noting that there were no significant effects from the administration of methyl salicylate on mating behavior, fertility rate or reproductive performance and determined that at doses up to 100 mg/kg bw/day, methyl salicylate was not a reproductive toxicant in mice (Adams et al., 2005b). In their modeling of the study data, summarized in Table 7, a 95 % lower bound confidence limit (BMDL) was presented for the best fitting models (Greene et al., 2017). The benchmark response (BMR) of one standard deviation below the mean control value was used for the first two endpoints (numbers of litters per mating pair and adjusted average pup weights) and a BMR of 5 % was used for the third endpoint (number of dead pups per individual breeding). The most sensitive endpoint was the number of litters per mating pair with a $BMDL_5$ of 220 mg/kg bw/day. Based on this $BMDL_5$, the MOE for the estimated intakes of methyl salicylate from the consumption of Wintergreen Oil (FEMA 3113) and Tuberose Oil (FEMA 3084) are greater than 14,000 and greater than 2×10^9, respectively.

In the FEMA Expert Panel's safety assessment of hydroxy- and alkoxy-substituted benzyl derivatives used as flavoring ingredients (Adams et al., 2005b), a two-generation reproductive study was reported in which methyl salicylate was administered to Wistar rats (25/sex/group) at levels of 0, 0.25 or 0.5 % in the diet, corresponding to doses of 250 or 500 mg/kg bw/day (FDA, 1993), for 60 days (Abbott, 1978). In that study, no significant differences were observed in any reproductive parameters including mating performance, number of stillborn, viability, mean litter size, number born, number live-born and number alive at 5 days (Abbott, 1978). In a separate two-generation reproductive study not reported in Adams et al. (2005b), methyl salicylate was also administered to mice (25/sex/group) in the diet at levels of 0.25 % or 0.5 %, corresponding to doses of 375 or 750 mg/kg bw/day (FDA, 1993), for 30 days prior to mating. The F_0 animals were mated twice to produce F_{1a} and F_{1b} litters. The F_{1a} litter was sacrificed after weaning for analysis and the F_{1b} litter (30/sex) were mated to produce F_{2a} and F_{2b} litters. All litters were culled to 10 neonates on postnatal day (PND) 5 and results were reported for females only. There were no external abnormalities in any litter, and no differences in growth, appearance or behavior. Methyl salicylate did not induce any adverse reproductive effects in mice. In fact, a higher conception rate and a lower rate of unsuccessful mating were reported in the treated groups compared to controls. Litter size was slightly smaller in treated groups but pup survival was greater than in control pups. A higher rate of stillbirths was reported in the control group than in the treated groups. Viability, lactation and reproduction indices for the treatment groups were comparable or better than the controls (Abbott, 1978). The Panel noted that statistical analyses for the reported effects were not provided in the study report.

Methyl salicylate was orally administered to pregnant LVG hamsters at a dose of 1750 mg/kg bw on gestation day (GD) 7 (Overman and White, 1983). Most fetuses were recovered on GD 9 but some were allowed to continue to GD 12, although few survived. Plasma salicylate in dams reached a maximum concentration (125 mg/100 mL) 2 h after oral dosing and returned to background level within 8–10 h. A high incidence of 72 % of neural tube defects was reported among 35 litters. Failure of closure of the neural tube most commonly resulted in both cranium bifidum and spina bifida. No differences in the morphology of the malformation were observed between embryos receiving oral or topical treatment. Salicylate was also detected in whole fetal homogenates demonstrating that it reached the fetus and reached maximum concentrations later than in maternal blood. Several concerns were noted for this study: the use of hamsters as a test animal (uncommon for reproductive toxicity testing), the very high dose administered (1750 mg/kg orally), unclear description of the number of animals in the study and unclear description of whether the results are per animal or per litter. The value of this study for the safety assessment is therefore limited.

7.2.2. Vanillin (FEMA 3107)

In a GLP and OECD guideline-compliant Ames assay, vanillin was non-mutagenic when tested in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvr*A at concentrations of 2–5000 μg/plate, in the presence or absence of a phenobarbital/5,6-benzoflavone-induced male SD rat liver (S9) metabolic activation system using the plate incorporation and preincubation methods (Rao, 2019).

In a GLP guideline-compliant *in vitro* CA assay, vanillin was incubated in Chinese hamster ovary (CHO) cells at concentrations of 500–1500 μg/mL without S9 metabolic activation and at concentrations of 609–2440 μg/mL in the presence of S9. There were no statistically significant increases in the frequency of cells with aberrations except at the highest tested concentration in the presence of S9 which was highly cytotoxic. The study authors concluded that vanillin was non-clastogenic under the conditions tested, up to the limit of cytotoxicity (ECHA, 1991b).

Genotoxicity studies for vanillin previously reviewed (un-shaded) by the FEMA Expert Panel (Adams et al., 2005b) as well as the more recently reported studies reviewed above (shaded) are summarized in Appendix B. In addition to the Rao study reviewed above, there are seven additional Ames studies that found vanillin to be non-mutagenic.

Seven of eight CA studies for vanillin reported negative results with only a single positive reported at concentrations greater than 300 μg in the absence of metabolic activation (Tamai et al., 1992). In a non-standard *in vitro* micronucleus induction in human hepatoma (HepG2) cells, vanillin was reported to be positive at a concentration of 500 μg/mL in the absence of metabolic activation, but the increase observed was small and difficult to evaluate in the absence of a positive control and historical data (Sanyal et al., 1997). An *in vivo* micronucleus test, vanillin was negative at 500 mg/kg bw/day in male mice (Inouye et al., 1988). Based on the weight of evidence summarized here, vanillin does not present a concern for genotoxicity (Gooderham et al., 2020a).

7.2.3. Ethyl vanillin (FEMA 2464)

7.2.3.1. Genotoxicity. In an OECD and GLP guideline-compliant Ames assay, ethyl vanillin was tested in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvr*A at concentrations of 52–5000 μg/plate in the presence or absence of an exogenous mammalian S9 metabolic activation system, utilizing the direct plate method and the pre-incubation method (ECHA, 2017b). Ethyl vanillin was not mutagenic under the conditions tested.

In an OECD and GLP guideline-compliant mouse lymphoma assay, ethyl vanillin was tested in mouse lymphoma L5178Y cells at the thymidine kinase (*tk*) gene locus (ECHA, 2017c). Ethyl vanillin was tested at concentrations of 100–1200 μg/mL in the absence of S9 metabolic activation and at 100–1100 μg/mL in the presence of S9 metabolic activation in 3 h exposure experiments. The S9 metabolic activation system was prepared from phenobarbital/β-naphthoflavone-induced rat livers. At 1200 μg/mL, in the absence of S9 metabolic activation, a 3.2-fold dose related-increase in mutation frequency was observed (3.7-fold increase in small colonies, 3.5-fold increase in large colonies), with a relative total growth of 16 % compared to controls. At 1000 and 1100 μg/mL in the presence of S9 metabolic activation, a 4.6-fold dose-related increase in mutation frequency was observed (5.3-fold increase in small colonies, 2.8-fold increase in large colonies), with a relative total growth of 16 % and 11 %, respectively, compared to controls. The large colonies are attributed to mutants with mutations in the TK gene and the small colonies are considered the results of chromosomal damage to the TK and adjacent genes. The observed increases in mutation frequency surpassed the control data and there was a significant dose-related trend ($p < 0.001$); therefore, ethyl vanillin was reported to be positive for mutagenicity.

Ethyl vanillin was tested in a GLP-compliant *in vivo* CA study in the bone marrow, SD rats (5/sex/dose/post-dose time) were administered a single dose of ethyl vanillin, by oral gavage, at doses of 0 (corn oil) 500, 1667 or 5000 mg/kg bw (Murli, 1991). Groups were euthanized at either 6, 18, or 30 h post-dose and the bone marrow was harvested. Some males at all concentrations of the test substance appeared languid immediately after dosing, but this observation was not elaborated upon. All animals in the 6 and 30 h groups appeared normal 3 h post-administration, and all animals in the 18 h group appeared normal about 1.5 h post-administration. There were no mortalities reported throughout the study and no evidence of exposure or toxicity in the bone marrow was reported. There were no significant differences in the percentage of chromosomally aberrant cells between the treatment and control groups under the conditions tested.

Genotoxicity studies for ethyl vanillin previously reviewed (unshaded) by the FEMA Expert Panel (Adams et al., 2005b) as well as the more recently reported studies reviewed above (shaded) are summarized in Appendix B. In addition to the negative Ames assay reviewed above, the Panel previously reviewed 4 additional Ames assays that found ethyl vanillin to be non-mutagenic in both the presence and absence of metabolic activation systems. Ethyl vanillin was positive in the mouse lymphoma assay reviewed above. However, ethyl vanillin was negative for the induction of CA and micronucleus formation in rodents, *in vivo* (Murli, 1991; Wild et al., 1983). Based on this weight of evidence, ethyl vanillin does not present a concern for genotoxicity (Gooderham et al., 2020a).

7.2.3.2. Combined repeat-dose, reproductive and developmental toxicity study. In a GLP and OECD guideline combined repeated dose, reproductive and developmental toxicity study, Wistar (Crl:WI (Han)) rats (10 sex/group) were administered ethyl vanillin at doses of 0 (propylene glycol), 250, 500 or 1000 mg/kg bw/day by oral gavage for 14 days before mating, during the mating period and up to and including the day before necropsy (ECHA, 2017d). Males were treated for 31 days and females were treated during the gestation period, with the first day of gestation designated G0, and at least 13 days after giving birth. The dose levels for this study were selected based on a 10-day preliminary dose range finder study in which doses of 500 and 1000 mg/kg bw/day ethyl vanillin were not associated with any systemic effects.

No treatment-related mortalities occurred; however, there was one low dose female that died on day 18 of treatment and whose necropsy revealed dark lungs and moderate alveolar hemorrhage, likely due to the administration procedure rather than due to the test substance. During the pre-mating period, 5/10 high dose females and 4/10 high dose males briefly exhibited adverse clinical signs. For females, these consisted of decreased activity, abnormal breathing, partly closed eye(s), piloerection, cold body and/or recumbency, while males exhibited decreased activity. In addition, there were observations of occasional noisy and/or laboured breathing for three males in the low-dose group and two males in the high dose group, but because there were no comparable findings in the mid-dose group, these were regarded as incidental. One female receiving the low dose experienced bilateral exophthalmos as a result of mating, which was deemed as incidental. Other incidental clinical signs during the pre-mating period were localized hair loss, erythema, scabs and scratches. During gestation, two females in the high dose group showed occasional piloerection that was not considered treatment-related due to the lack of findings in other females. One female in the high dose group had a cold body and was pale on gestation day 22; this female also exhibited decreased activity, recumbency, partly closed eyes and piloerection, before having subsequent total litter death. These observations were attributed to the pregnancy status of the female and unrelated to the treatment. Both sexes in all treatment groups and the control group exhibited hypersalivation accompanied by abnormal foraging and/or pedalling, but because the treatment groups exhibited a higher incidence and a longer duration of these effects than that observed in the controls, the study authors suggested that these clinical signs may be related to the palatability of the test substance.

For males, throughout the pre-mating and mating periods, the low-dose group did not experience a treatment-related effect on body weight gain. During the dosing period, the mean body weight gain for males receiving the high dose was slightly lower than the control group (−6 %), but the final mean body weight was 1 % lower than the control group. When compared to the low-dose group, for the mid-dose males during the first week and the high dose males during the third week there was a slightly lower mean body weight. Additionally, when compared to the control group during the last week of dosing, males in all treatment groups had a higher mean body weight gain. Mean food consumption for males was comparable or higher than controls in all treated groups during the premating period.

For females, throughout the pre-mating period the mean body weight gain in all treatment groups was either similar or greater than the control group. The mean body weight gain was lower in all treatment groups during the first week, in which a few females lost weight (2/10 in the low-dose group, 6/10 in the mid-dose group and 5/10 in the high dose group). The high dose females had a 5 % lower mean body weight than the control group during the pre-mating period; this group also had a correlated slightly lower food consumption than other groups during the first week of the pre-mating period. When compared to the control

group, the mid- and high dose females had a transient slightly lower mean body weight gain from gestation days 6–12; however, the overall mean body weight gain was comparable for all groups from gestation days 0–20. There was a slight, minimal decrease in food consumption during the gestation period for the mid- and high dose females which was not considered adverse. For the high dose group, the mean body weight gain was 5–7 % lower during the gestation period and 5–8 % lower during the lactation period, but this was attributed to the lower initial mean body weight. Ultimately, there was no treatment-related effect on mean body weight gain during lactation. There was no treatment-related effect on food consumption for the low-dose group.

At necropsy, examination of organ weights and gross pathology in males and females showed no treatment-related effects. Histopathological analysis of the male treatment groups and the female control and high dose groups reported no treatment-related microscopic findings. In the mid-dose group, one male had unilateral small testis and epididymis that was histologically correlated with marked unilateral tubular hypoplasia/atrophy with epididymal aspermia, but this was deemed to be incidental because it was not observed in other treatment groups.

Total T4 levels in males of the mid- and high dose groups were statistically significantly lower than the control group, but this was considered to be slight, and there were no changes in the weight or histology of the thyroid for all treatment groups. The fertility, mating performance and histology were also not affected by the test substance. In females, there were no significant differences in the length or regularity of the estrous cycle between the treatment and control groups and mating performance was comparable in all groups. Nine of the ten females in the control, low- and high dose groups, as well as all ten females in the mid-dose group, delivered fetuses successfully. The three unsuccessful deliveries were not considered related to the test substance. Mean duration of gestation was similar for all groups.

There were no treatment-related effects on the number of implantations compared to the number of offspring born, as well as on the offspring viability and bodyweight. In all treatment groups, there was a lower percentage pre-birth loss compared to controls, and in the mid- and high dose groups there was a slightly lower number of mean offspring delivered compared to the low-dose group and the controls. These findings were considered within the historical control range and unrelated to the test substance. There were no treatment-related clinical signs or body weight changes reported for the offspring. The number of areola/nipples in the offspring and the total T4 levels (both examined on post-natal day 13), as well as the anogenital distance for both male and female offspring, were also unaffected.

Transient parental toxicity (adverse clinical signs, body weight and weight gain changes) observed at 1000 mg/kg bw per day resulted in the assignment of a NOAEL for parental toxicity at 500 mg/kg bw/day. Because there was no evidence of reproductive and developmental toxicity observed, the NOAEL for reproductive and developmental toxicity was the highest dose tested, 1000 mg/kg bw per day. Based on the NOAEL of 500 mg/kg bw/day for parental toxicity, an MOE of greater than 25,000 was calculated for the estimated intake of Group 15 constituents for Vanilla Extract (FEMA 3105) in Step 13 of the safety procedure.

In an OECD guideline 414 and GLP guideline-compliant prenatal developmental toxicity study, ethyl vanillin was administered to pregnant female Wistar (Crl:WI (Han)) rats (22/dose) at doses of 0 (propylene glycol), 250, 500 or 1000 mg/kg bw daily from gestation day 6 to gestation day 20, by oral gavage (ECHA, 2017a). Food consumption was measured once for the six-day period between gestation day 0 and the beginning of treatment (gestation day 6) and then measured every three days through gestation day 21.

No mortalities were reported during the study. Shortly after the first or second dose (gestation day 6 or 7), 10 high dose females briefly exhibited irregular and/or rapid breathing, partly closed eye(s), wheezing, decreased activity and/or dark eyes; these adverse findings were considered treatment related. Six of these animals exhibited behavior described as recumbent and/or subdued. Hypersalivation, along with abnormal foraging, pedaling and/or Straub tail, occurred in all 66 animals in the treatment groups except 1 animal in the low-dose group. These effects were attributed to the poor palatability of the test substance were not considered toxicologically significant.

There were no significant differences in final body weight gains between the treatment groups and the control group. There was a slightly lower mean body weight gain than in other groups in animals receiving the high dose from gestation day 6 to gestation day 9, but these slight decreases were considered non-adverse. Food consumption for the high dose group was slightly reduced between gestation days 6–12 compared to the other treatment groups and controls, but not significantly different after gestation day 12, and was not considered adverse.

There were no significant differences in the relative and absolute organ weights or the gravid uterus between the treatment and control groups. Gross pathology reported only incidental findings: a cyst on the left ovary for one mid-dose animal and a dark spot on the liver of one high dose animal. At termination, all females were pregnant with viable fetuses. There were no significant differences in the mean numbers of corpora lutea, implantation sites, embryo-fetal survival or mean live litter size between the treatment and control groups. For low- and mid-dose groups, there was a slightly higher pre-implantation loss, but this finding was considered to be incidental since it was within the historical range not observed in the high dose group.

Examination of fetuses found no significant differences between the treatment groups and the control group in fetal mean body weight, sex ratio, litter size or postnatal survival. In the high dose group, external malformations were reported for 3 fetuses from separate litters and skeletal malformations were reported for 5 fetuses from 5 separate litters but these incidences of external and skeletal malformations were within the limits of the historical control data and not attributed to treatment with ethyl vanillin. Visceral malformations were reported in 3 fetuses from 2 litters from the high dose group and were also within the historical control range and considered to not be treatment-related. While the incidences of external, skeletal and visceral malformations were within the historical control range and not directly attributable to a treatment related effect, the study authors noted that these malformations in the high dose group fetuses were more likely attributed to a maternal-mediated effect as evidenced by the clinical signs observed for the maternal dams of this group.

Based on the observation of adverse clinical findings in the high dose maternal dams, the study authors determined a NOAEL for maternal toxicity of 500 mg/kg bw/day for ethyl vanillin. The NOAEL for embryo-fetal development was also considered to be the mid-dose of 500 mg/kg/day; the authors hypothesized that the range of malformations for the high dose may indicate a maternal-fetal mediation effect even though their incidences were within the historical control data and were considered non-treatment-related. Based on this NOAEL, an MOE of greater than 25,000 was calculated for the estimated intake of Group 15 constituents for Vanilla Extract (FEMA 3105) in Step 13 of the safety procedure.

7.3. Natural flavor complexes

A summary of genotoxicity studies for the NFCs under consideration is listed in Table 8.

7.3.1. Almonds bitter oil

7.3.1.1. Genotoxicity. In an *in vitro* reverse mutation assay, *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were treated with almonds bitter oil at concentrations up to 1 μg/plate. Almonds bitter oil did not induce revertants in the presence or absence of S9 (Ishidate et al., 1988). The same authors tested almonds bitter oil in a rec assay using cultures of *Bacillus subtilis.* At concentrations up to 10

Table 8
Summary of genotoxicity studies for the NFCs under consideration.

Name of Substance Tested	Test Type (System)	Doses Tested	Results	Reference
Almond oil, bitter	Reverse mutation assay in *S. typhimurium*[a]	Up to 1 μg/plate	Negative[a]	Ishidate et al. (1988)
Almond oil, bitter	Rec assay in *B. subtilis*[a]	Up to 10 mg/disk	Positive[a]	Ishidate et al. (1988)
Almond oil, bitter	*In vitro* chromosomal aberration assay in CHL cells[a]	Up to 0.5 mg/mL Up to 2 mg/mL	Positive[b] Negative[c]	Ishidate et al. (1988)
Cumin extract (EtOH)	Reverse mutation assay in *S. typhimurium*	10 mg/plate	Negative[c]	Mahmoud et al. (1992)
Cumin oil	Reverse mutation assay in *S. typhimurium and E. coli*[a]	Up to 5000 μg/plate	Negative[a]	Dakoulas (2021)
Cumin oil	Reverse mutation assay in *S. typhimurium*[a]	550 to 34,000 μg/plate[d]	Negative[a]	Nirogi et al. (2014)
Cumin oil	Rec assay in *B. subtilis*[a]	15 mg/disk	Positive[b] Negative[c]	Ueno et al. (1984)
Cumin oil	*In vitro* chromosomal aberration assay in human lymphocytes	Up to 50 μg/mL	Negative	Ishidate et al. (1984)
Cumin oil	*In vitro* chromosomal aberration assay in human lymphocytes	46–180 μg/mL[d]	Positive	Yildiz et al. (2015)
Cumin oil	*In vitro* micronucleus assay in CHO cells[a]	0.005, 0.016, 0.05 μL/mL	Negative[a]	Goyal et al. (2019)
Cumin oil	*In vitro* micronucleus assay in HPBL	8–120 μg/mL[e] 8–77 μg/mL[f]	Negative[a]	Xie (2021)
Jasmine absolute	Reverse mutation assay in *S. typhimurium*[a]	0.0316–5 μL/plate	Negative[a]	ECHA (2018)
Oak Moss absolute	Reverse mutation assay in *S. typhimurium*[a]	1.5–5000 μg/plate	Negative[a]	ECHA (2017e)
Vanilla Extract	Rec assay in *B. subtilis*[a]	20 mg/disk	Negative[a]	Ueno et al. (1984)
Vanilla Extract (Methanolic)	Reverse mutation assay in *S. typhimurium*[a]	1.5–5000 μg/plate	Positive/Negative[g]	Araujo et al. (2024)
Vanilla Extract (Ethanolic)	Reverse mutation assay in *S. typhimurium*[a]	1.5–5000 μg/plate	Positive/Negative[h]	de Oliveira et al. (2024)
Vanilla Extract (Methanolic)	*In vitro* micronucleus assay in HepG2 cells	0.5–500 μM	Negative	Araujo et al. (2024)
Vanilla Oleoresin	Reverse mutation assay in *S. typhimurium*[a]	100 to 20,000 μg/plate	Negative[a]	Bonin and Baker (1980)
Wintergreen oil	*In vitro* comet assay in rat neurons and N2a neuroblastoma cells	10–400 μg/mL	Negative	Celik and Turkez (2016)

[a] With and without metabolic activation system, S9.
[b] With metabolic activation system, S9.
[c] Without metabolic activation system, S9.
[d] Based on median density of 0.915 g/mL (Source: Food Chemical Codex 12th Edition, United States Pharmacopeia (USP), Rockville, MD, USA).
[e] 4 h experiment with and without metabolic activation system, S9.
[f] 24 h experiment metabolic activation system, S9.
[g] For *V. planitfolia,* positive for strain TA98 in the absence of S9, negative in strains TA97a, TA100, TA102 and TA1535 with and without S9. For *V. chamissonis* and *V. bahiana*, negative with and without S9 in all strains tested.
[h] For *V. cribbiana,* positive for strain TA98 in the absence of S9, negative in strains TA97a, TA100, TA102 and TA1535 with and without S9. For *V. planitfolia, V. chamissonis* and *V. bahiana*, negative with and without S9 in all strains tested.

mg/disk, almonds bitter oil was positive for mutagenicity in the presence and absence of S9 in the rec assay (Ishidate et al., 1988). This assay, which lacks a standardized testing protocol, tested a concentration of almonds bitter oil that greatly exceeded the typical maximum concentration of 2 mg/mL utilized in various *in vitro* genotoxicity assays. Almonds bitter oil was positive for the induction of CA in CHL cells in treatments with S9 activation. Briefly, CHL cells were incubated with almonds bitter oil in the absence of S9 up to concentrations of 0.5 mg/mL for 24 and 48 h and in the presence of S9 at concentrations up to 2 mg/mL for 24 h only. The oil increased the frequency of aberrant cells, but a concentration-dependent trend could not be demonstrated (Ishidate et al., 1988). No statistical analyses were conducted on the observed increases, nor was the laboratory's historical negative control range referenced. Therefore, this study does not meet the criteria for a positive outcome based on OECD testing guidelines (OECD, 2016).

In summary, almonds bitter oil was negative for mutagenicity in an Ames assay but positive results for genotoxicity were reported in the rec assay and CA assay in CHL cells. However, these positive results were reported in non-guideline studies. The rec assay is only an indicator assay that should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015, 2017). The CA study was performed under non-standard conditions and lacked historical control data. The weight of evidence presented above, that included negative results reported for an OECD guideline *in vivo* micronucleus study (Honarvar, 2009), indicates that the primary component, benzaldehyde, is non-genotoxic. Similarly, Almonds Bitter Oil (FEMA, 2046), whose constituent profile is greater than 95 % (mean %) benzaldehyde (see Appendix A), is also not genotoxic.

7.3.2. Apricot kernel oil

7.3.2.1. Subchronic toxicity. In a 13-week toxicity study, there were no adverse effects observed when apricot kernel oil (95 % unsaturated fatty acids) was administered to albino Haffkine Wistar rats (6/sex/group) in the feed at levels of 0 or 10 %, corresponding to a dose of 10 mg/kg bw/day (FDA, 1993). The control diet contained 10 % refined groundnut (peanut) oil and the rats were given food and water *ad libitum*. Body weight was measured twice a week and food consumption was recorded daily. Following termination, hematological and biochemical analyses were conducted. Liver, heart, kidney, spleen, and testes weights were measured. No differences were observed in food consumption, body weight gain, locomotor activity, histopathology, or hematological or biochemical parameters between the control and test groups (Gandhi et al., 1997). Based on a NOAEL of 10 mg/kg bw/day, the only dose tested in this study, an MOE of greater than 800 was calculated for Apricot Kernel Oil (FEMA 2105).

7.3.3. Birch sweet oil

7.3.3.1. Chronic toxicity. In a chronic two-year dietary study, there were no adverse effects observed when birch sweet oil was administered to weanling albino rats (25/sex/group) at target concentrations in the diet of 0, 700 and 2100 ppm (Packman et al., 1961), equivalent to approximately 0, 70 and 210 mg/kg bw/day, respectively (FDA, 1993). Body weights and survival were unaffected, and no changes in hematological or urinalysis parameters were reported. Macroscopic and histopathological examinations did not report any adverse findings when compared to the control group, including no differences in incidences of neoplasms in any of the tissues. The NOAEL for the two-year study of birch sweet oil was 210 mg/kg bw/day, providing an adequate MOE of >260,000 based on the estimated daily intake of Birch Sweet Oil (FEMA 2154).

7.3.4. Cumin oil

7.3.4.1. Genotoxicity. In an OECD and GLP guideline-compliant reverse mutation assay, cumin oil, a composition similar to Cumin Oil (FEMA 2343) was tested in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2*uvrA*. In both the presence and absence of S9 metabolic activation derived from the livers of Aroclor 1254-induced male SD rats, cumin oil was non-mutagenic at concentrations up to 1500 μg/plate in strains TA100, TA1535, TA1537 and *E. coli* WP2*uvrA* and 5000 μg/plate in strain TA98 (Dakoulas, 2021). An ethanol extract of cumin was negative for mutagenicity in a reverse mutation assay using *S. typhimurium* strains TA98 and TA102 at a concentration of 10 mg/plate without metabolic activation (Mahmoud et al., 1992). In a five-strain Ames assay, cumin oil was evaluated for mutagenic potential in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of phenobarbital/β-naphthoflavone-induced rat liver S9 metabolic activation. The tested oil was reported to contain 15–40 % cuminaldehyde, 18–29 % terpinenes, 1 % α-pinene, 2 % β-pinene and 30 % menthadienal, a composition consistent with Cumin Oil (FEMA 2343). Based on a preliminary cytotoxicity experiment, concentrations ranging from 550 to 34,000 μg/plate in the absence of S9 or 1830 to 34,000 μg/plate[15] in the presence of S9 were tested. No increases in revertant frequency were observed in treatments with cumin oil using the pre-incubation method (Nirogi et al., 2014). In a rec assay in *B. subtilis,* cumin oil was positive for mutagenicity at a concentration of 15 mg/disk in the absence of S9 but negative in the presence of S9 (Ueno et al., 1984). As noted previously, the rec assay does not provide an appropriate primary line of evidence for mutagenic potential (OECD, 2015, 2017). The negative five-strain reverse mutation assay supports the weight of evidence that cumin oil is not expected to be mutagenic.

In an *in vitro* CA assay, the essential oil of *C. cyminum* (origin and preparation not reported) was incubated with human peripheral blood for 24 h at concentrations of 46, to 180 μL/mL[15] in the absence of metabolic activation (Yildiz et al., 2015). Cultures treated with cumin oil exhibited a significant, dose-dependent increase in the frequency of chromosomal aberrations compared to the negative control at all tested concentrations that also significantly reduced the mitotic index, indicating cytotoxicity at these levels. In another *in vitro* CA assay, cumin oil, supplied by the Japan Food Additives Association and verified for purity by the Ministry and Health and Welfare of Japan, was negative for genotoxicity when tested up to 50 μg/mL in CHL cells for 48 h (Ishidate et al., 1984).

In a GLP and OECD guideline-compliant *in vitro* micronucleus assay, HPBL were incubated with cumin oil in 4 h exposure experiments in the presence and absence of S9 metabolic activation at concentrations of 8–120 μL/mL and in a 24 h experiment at concentrations of 8–77 μL/mL in the absence of S9. The S9 metabolic activation system was prepared from the livers of Aroclor 1254-induced male SD rats. Cumin oil was negative for the induction of micronuclei in HPBL under the conditions tested (Xie, 2021). In an *in vitro* micronucleus assay conducted in Chinese hamster ovarian (CHO–K1) cells, cumin oil was tested at concentrations of 0.005, 0.016 and 0.05 μL/mL in both the presence and absence of a phenobarbital/β-naphthoflavone-induced rat liver S9 metabolic activation system. The test substance was composed of approximately 15–40 % cuminaldehyde, 18–29 % terpenes, 30 % menthadienal and minor amounts of other terpenoids, a composition consistent with Cumin Oil (FEMA 2343). In the shorter duration (5 h) treatments with and without S9, as well as in the continuous (21 h) treatment without S9, cumin oil did not increase the frequency of micronucleated CHO–K1 cells at any of the concentrations tested (Goyal et al., 2019).

In summary, the results of several Ames assays on an ethanolic extract of cumin and cumin oil were negative for mutagenicity. In non-standard CA assays, one positive and one negative result for genotoxicity were reported for cumin oil. A negative *in vitro* micronucleus assay in CHO–K1 cells was also reported for cumin oil. The constituent profile of Cumin Oil (FEMA 2343) (see Appendix A) contains 28 % cuminaldehyde, a constituent that is not genotoxic, and Group 19 constituents which are also non-genotoxic (Adams et al., 2011). Based on the weight of evidence (Gooderham et al., 2020a), it was concluded that Cumin Oil (FEMA 2343) is not genotoxic.

7.3.4.2. Subacute toxicity. In a 28-day repeat dose subacute toxicity study, groups of female mice (5/group) of an unspecified strain were administered doses of 0 (distilled water, control), 250, 500 or 1000 mg/kg bw/day *C. cyminum* L. seed essential oil once daily by oral gavage for 6 days a week for 2 weeks. An additional group received 25 mg/kg bw/day of *C. cyminum* L. seed essential oil for 28 days (Haddad et al., 2018). The essential oil was isolated by steam distillation of cumin seeds and was composed of 41 % 1-phenyl-1-butanol, 28 % cuminaldehyde, 10 % 1-isopropylidene-3-n-butyl-2-cyclobutene, 4 % *p*-mentha-1,4-diene and other minor constituents and although this test cumin oil sample contains similar amounts of cuminaldehyde as Cumin Oil (FEMA 2343) (reported in Appendix A), the test sample is reported to contain 41 % 1-phenyl-1-butanol and 10 % 1-isopropylidene-3-n-butyl-2-cyclobutene which are not reported constituents of Cumin Oil (FEMA 2343).

In the 2-week study, total body weights of all dose groups were significantly increased compared to the control group, but these increases were not dose-dependent. There were no significant differences in water or food consumption. There were no significant differences in the relative or absolute weights of the liver, lung, kidney or heart measured at day 14 between the control and treatment groups. A significant increase in the uterus weight was observed in the female mice of all treatment groups, which correlated with mild to moderate edema of the endometrium and perimetrium of the uterus. Incidental, non-dose related changes were observed in estradiol and progesterone concentrations in the blood. In the 250 and 500 mg/kg bw/day groups, focal infiltration of mononuclear cells and hydropic degeneration of hepatocytes were reported in the liver, in addition to hydropic degeneration of proximal tubules and multifocal mononuclear cell infiltration in the kidneys. In the 1000 mg/kg bw/day group, histopathological findings included necrosis of hepatocytes, accumulation of mononuclear cells in the alveolar ducts and mild congestion in the lungs, mild degeneration and epithelial necrosis of proximal tubules in the kidney and hyperplastic endometrial epithelium in the uterus. The severity of these findings was not reported.

In the 28-day study, female mice (strain not specified) administered cumin oil at 25 mg/kg bw/day for 28 days showed a significant increase in body weight compared to control group mice without significant differences in water or food consumption. This group showed no significant differences in the absolute or relative weights of the liver, lung, kidney or heart measured at day 28 compared to the control group. Similar to the 2-week study, a significant increase in the uterus weight was observed in the treatment group. Concentrations of progesterone and estradiol in the blood were lower in the treatment group compared to the control group. The study authors indicated that histopathological examinations were performed for the liver, kidneys, lungs and uterus, but these results were not discussed in the manuscript.

To evaluate the oral toxicity of cumin essential oil, a repeat dose toxicity study was conducted in accordance with GLP guidelines (Taghizadeh et al., 2017). The essential oil tested contained 39 % cuminaldehyde, 18 % *p*-mentha-1,4-diene, 15 % *p*-cymene, 12 % β-pinene, 5 % 1,3-*p*-menthadien-7-al and 3 % 1,4-*p*-menthadien-7-al, which is consistent with the constituent profile for Cumin Oil (FEMA 2343). Female Wistar rats (20/group) were administered cumin oil formulated in olive oil at dose levels of 0 (sterile water), 250, 500 or 1000 mg/kg bw/day by oral gavage. After 23 days of administration, 10

[15] Based on median density of 0.915 g/mL (Source: Food Chemical Codex, United States Pharmacopeia (USP), Rockville, MD, USA).

animals per dose level were sacrificed for an interim toxicological evaluation, while the remaining 10 animals continued treatment for an additional 22 days.

Throughout the study period, there were no reported clinical signs of toxicity or deaths in treatment or control groups. Mean group body weights were significantly lower ($p < 0.05$) in all treatment groups compared to the control group. No significant differences in food or water consumption were observed. Hematological analyses conducted on Day 23 for the first 10 rats and on Day 45 for the remaining 10 rats did not reveal any differences in the measured parameters. Clinical chemistry revealed non-dose-dependent decreases in cholesterol and triglycerides and non-dose-dependent increases in alanine aminotransferase and lactate dehydrogenases on Day 23. Similar non-linear changes were reported for these clinical chemistry parameters on Day 45.

The study authors stated that histopathological examination of animals sacrificed on Day 23 revealed mild infiltration of mononuclear cells and mild dilation of the sinusoids of the liver and mild edema and infiltration of mononuclear cells in the lungs at all dose levels. There were no pathological findings in the kidneys of the treated groups. Similar histopathological findings were reported in animals necropsied on Day 45. However, the study authors did not report the incidences or severity of these effects for each dose level and in summarizing their observations concluded that there were no significant changes in the liver, spleen, lungs or kidneys in the treated groups compared to the control group at either interval of the study. In the absence of significant adverse findings in this study, the FEMA Expert Panel derived a NOAEL of 1000 mg/kg bw/day, the highest dose level tested, from the repeat dose study, which provides an MOE of >160,000 for Cumin Oil (FEMA 2343).

In a non-standard study, cumin essential oil (29 % α-pinene, 21.5 % limonene, 17.9 % eucalyptol, 10.4 % linalool with other minor components, a composition that differs significantly from Cumin Oil (FEMA 2343) was administered to Wistar rats of both sexes (number of animals in the study was not specified) by oral gavage at a dose 100 μL/day for 30 days (Allahghadri et al., 2010). A set of control rats were administered 3 % (v/v) Tween 80 in saline. Blood samples for hematology and biochemical analyses were collected at days 0 and 30 of the study. Rats in the treatment group showed decreased white blood cell count and increased hemoglobin concentration, hematocrit and platelet counts at 30 days. In addition, the treated rats showed increased levels of cholesterol and triglycerides and decreased levels of aspartate aminotransferase and alanine aminotransferase activities. Necropsy and histopathological examinations were not performed. Because the constituent profile of the test sample significantly differs from Cumin Oil (FEMA 2343), lacking cuminaldehyde in its composition, and the limited scope and reporting of this study, it is not relevant to the safety evaluation.

7.3.5. Jasmine absolute

7.3.5.1. Genotoxicity. An OECD and GLP guideline-compliant Ames assay was conducted on jasmine absolute (*Jasminum grandiflorum*) obtained from ethanol extraction of the concrete. Using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102, the absolute was tested in two independent experiments at concentrations of 0.0316, 0.1, 0.316, 1, 2.5 and 5 μL/plate in the presence and absence of rat liver S9. When using both the plate incorporation and pre-incubation methods, jasmine absolute did not increase revertant counts in any of the tester strains and was concluded to be non-mutagenic (ECHA, 2018).

7.3.6. Oak moss absolute

7.3.6.1. Genotoxicity. Oak moss absolute was tested in an OECD and GLP guideline-compliant reverse mutation assay, using *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 at concentrations of 1.5–5000 μg/plate. In both the presence and absence of S9 liver homogenate, oak moss absolute did not induce any increases in the frequency of revertant mutant colonies under the conditions tested (ECHA, 2017e).

7.3.7. Vanilla extract, vanilla oleoresin

7.3.7.1. Genotoxicity. In an Ames assay, methanolic extracts of the fruits of three vanilla species, *V. planifolia, V. chamissonis* and *V. bahiana* were tested in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535 at concentrations of 0.5–5000 μg/plate in the presence and absence of S9 metabolic activation derived from the livers of Aroclor 1254-induced SD rats (Araujo et al., 2024). The test sample was prepared from the fruit of each species, each procured from a different public garden in Brazil, after which the fruit was dried, chopped, treated enzymatically in a citrate-phosphate buffer to accelerate the curing process, frozen and lyophilized to dryness. Each sample was extracted with methanol and concentrated to dryness. Analytical data on the composition of the final extracts, whose preparation differs significantly from the preparation of Vanilla Extract (FEMA 3105) and Vanilla Oleoresin (FEMA 3106), was not reported. The extracts of all three species were negative for mutagenicity in all strains tested with and without S9, with the exception of a positive result for the *V. planifolia* extract which showed a dose-dependent increase in revertants in strain TA98 in the absence of S9 that reached statistical significance at concentrations of 4000 and 5000 μg/plate. However, it is noted that the number of revertants observed for the 4-nitroquinoline 1-oxide positive control in strain TA98 in the absence of S9 reported with the results of *V. planifolia* methanolic extract was 3-times higher (~225) compared to the number of revertants observed for this strain (~70) in the absence of S9 reported with the results of *V. chamissonis* and *V. bahiana* methanolic extracts.

In an *in vitro* micronucleus induction assay, the same methanolic extracts of *V. planifolia, V. chamissonis* and *V. bahiana* described above did not induce formation of micronuclei in HepG2 cells in the absence of metabolic activation (Araujo et al., 2024). An experiment with S9 metabolic activation was not reported.

In another study from the same research group, ethanolic extracts of the fruits of the three vanilla species studied by Araujo et al., including *V. planifolia, V. chamissonis* and *V. bahiana,* as well as the fruit of a fourth species *V. cribbiana,* sourced from Brazilian national gardens, were tested in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535 at concentrations of 0.5–5000 μg/plate in the presence and absence of S9 metabolic activation derived from the livers of Aroclor 1254-induced SD rats (de Oliveira et al., 2024). In this study, the test samples were prepared as described by Araujo et al., except that following enzymatic treatment and lyophilization, each sample was extracted with ethanol, instead of methanol, prior to concentration to dryness. Extract samples were diluted in dimethyl sulfoxide for the Ames assay. The extracts of *V. planifolia, V. chamissonis* and *V. bahiana* were nonmutagenic in all strains in the presence and absence of S9. The study authors reported the extract of *V. cribbiana* as positive in strain TA98 in the absence of S9 at the highest concentration tested, 5000 μg/plate, but there was no dose response and there was only a ~2-fold increase in revertants compared to the control (control: 19.3 ± 1.2; 5000 μg/plate: 40.7 ± 2.3 revertants). These studies were not considered relevant to the safety of FEMA 3105 and FEMA 3106 given the large differences in the preparation of the test samples that would result in significant differences in the composition of the test substances compared to the NFCs under consideration. In addition, the vanilla samples were sourced from public gardens, not cultivated under controlled conditions, and may have been treated with various plant protection products.

In a rec assay using *B. subtilis,* vanilla extract (composition not reported) was non-mutagenic when tested at a concentration of 20 mg/

Table 9
NFCs affirmed FEMA GRAS.

FEMA No.	Name
2046	Almonds Bitter Oil (FFPA) (*Prunus amygdalus* Batsch var. *amara* (DC.) Focke *P. armeniaca* L., *P. persica* (L.) Batch))
2105	Apricot Kernel Oil (*Prunus armeniaca* L.)
2154	Birch Sweet Oil (*Betula lenta* L.)
2260	Cassie Absolute (*Acacia farnesiana* (L.) Willd)
2276	Cherry Bark Wild Extract (*Prunus serotina* Ehrh.)
5063	Cherry Bark Wild Distillate (*Prunus serotina* Ehrh.)
2277	Cherry Laurel Oil ((FFPA) *Prunus laurocerasus* L.)
2343	Cumin Oil (*Cuminum cyminum* L.)
5064	Cumin Oleoresin (*Cuminum cyminum* L.)
2598	Jasmine Absolute (*Jasminum grandiflorum* L.)
2599	Jasmine Concrete (*Jasminum grandiflorum* L.)
2600	Jasmine Oil (*Jasminum grandiflorum* L.)
2795	Oak Moss Absolute (*Evernia prunastri* (L.) Ach., *E. furfuracea* (L.) Mann, and other lichens)
3021	Sloe Berries Extract (*Prunus spinosa* L.)
3084	Tuberose Oil (*Polianthes tuberosa* L.)
3105	Vanilla Extract (*Vanilla planifolia Andrews, V. tahitensis J.W. Moore, V. pompona*)
3106	Vanilla Oleoresin (*Vanilla planifolia* Andrews, *V. tahitensis* J.W. Moore, *V. pompona*)
3113	Wintergreen Oil (*Gaultheria procumbens* L.)

disk in the presence and absence of S9 metabolic activation (Ueno et al., 1984). The rec assay has not been standardized in an OECD guideline for genotoxicity testing and OECD has noted that indicator tests such as the rec assay should be correlated to the results of other assays that measure DNA damage or mutagenicity that can be passed on to subsequent generations (OECD, 2015, 2017).

In an Ames assay, vanilla oleoresin, tested at concentrations of 100 to 20,000 μg/plate, was negative for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, TA 1537 and TA1538 in the presence and absence of S9 metabolic activation derived from the livers of Aroclor 1254-induced SD rats (Bonin and Baker, 1980).

7.3.8. Wintergreen oil

7.3.8.1. Genotoxicity. In an *in vitro* alkaline comet assay, wintergreen oil was evaluated at concentrations of 0, 10, 25, 50, 75, 100, 200 and 400 μg/mL in rat primary neurons and N2a neuroblastoma cells. In both cell lines, cell viabilities were >60 % for all concentrations of wintergreen oil tested. The frequency of neurons and neuroblastoma cells presenting evidence of DNA damage was comparable between treated and control groups. Under the test conditions, there was no evidence of DNA damage induced by wintergreen oil (Celik and Turkez, 2016). However, due to the lack of a standardized OECD guideline and procedure for the performance and evaluation of results of this *in vitro* comet assay, the relevance of the results cannot be assessed (Gooderham et al., 2020a).

7.3.9. Summary on genotoxicity of NFCs

The review of available genotoxicity studies of almond bitter oil, cumin oil, jasmine absolute, oakmoss absolute, vanilla extract, vanilla oleoresin and wintergreen oil indicate that there is no concern for genotoxicity of these substances. Furthermore, the weight of evidence supports the assessment that Group 14 (Benzyl derivatives) and Group 15 (Hydroxy- and alkoxy-substituted benzyl derivatives) constituents of these NFCs also are not genotoxic. The available studies of the NFCs and their respective constituents indicating no concern for genotoxicity provide a weight of evidence (Gooderham et al., 2020a) to conclude that the NFCs under consideration do not present a genotoxic concern.

8. Recognition of GRAS status

The safety evaluation procedure of the FEMA Expert Panel was applied to the NFCs listed in Table 9, which were concluded to not present a safety concern. For each constituent congeneric group of these NFCs, the estimated intakes are below the TTC for their respective structural classes, indicating no safety concern. In addition, the group of unidentified constituents was below the TTC for Structural Class III, 90 μg/person/day for each NFC. Available genotoxicity studies for both the NFCs and their Group 14 (Benzyl derivatives) and Group 15 ((Hydroxy- and alkoxy-substituted benzyl derivatives)) constituents were reviewed and support the conclusion that these flavoring ingredients do not raise a concern for genotoxicity. Based on the safety evaluation described in this manuscript, the FEMA Expert Panel has affirmed the GRAS status for the NFCs listed in Table 9.

CRediT authorship contribution statement

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

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 and Taylor are employed by Verto Solutions which provides scientific and management support services to FEMA. The members of the FEMA Expert Panel are solely responsible for GRAS determinations. 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2026.115969.

Abbreviations

ADI, Acceptable Daily Intake; ALP, Alkaline phosphatase; ALT, Alanine transaminase; ARfD, Acute Reference Dose; AST, Aspartate transferase; ASTA, American Spice Trade Association; $BMDL_{10}$, Lower confidence limit of the benchmark dose resulting in a 10 % extra cancer incidence; BMR, Benchmark response; CA, Chromosome Aberration; CAC, Codex Alimentarius Commission; CF, Correction factor; CFR, Code of Federal Regulations; CHL Chinese Hamster Lung (cells); CHO, Chinese hamster ovary (cells); DTC, Decision tree class; EFFA, European Flavour Association; EFSA, European Food Safety Authority; FAS GATS, Foreign Agricultural Service Global Agricultural Trade System; FCC, Food Chemicals Codex; FDA, Food and Drug Administration; FEMA, Flavor and Extract Manufacturers Association; FFPA, Free from Prussic Acid; FID, Flame ionization detector; FN, fibronectin; GC, Gas chromatography; GC-MS, Gas chromatography-mass spectrometry; GD, Gestational Day; GLP, Good laboratory practices; GMP, Good Manufacturing Practice; GRAS, Generally Recognized As Safe; GSFA, General Standard for Food Additives; HCD, Historical Control Data; HDL, High Density Lipoprotein; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; HPBL Human Peripheral Blood Lymphocytes; 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; JMHLW, Japan Ministry of Heath, Labor and Welfare; LC-MS, Liquid chromatography-mass spectrometry; LDL, Low density lipoprotein; LOAEL, Lowest Observed Adverse Effect Level; LOD, Limit of detection; MMP-2, matrix metalloproteinase-2; MOE, Margin of Exposure; MS, Mass spectrometry; NAS, National Academy of Sciences; NFC, Natural flavoring complex; NTP, National Toxicology Program; NOAEL, No-observed-adverse-effect-level; NOEL, No Observed Effect Level; NRC, National Research Council; OECD, Organization for Economic Co-Operation and Development; PCI, Per capita intake; PMTDI, Preliminary Tolerable Daily Intake; PND, Postnatal day; SAAG, salicylic acid acyl glucuronide; SAPG, salicylic acid phenolic glucuronide; SCE, Sister chromatid exchange; SD, Sprague-Dawley (rat); SPET, Single Portion Exposure Technique; TD_{50}, Dose giving a 50 % tumor incidence; TGF-β, transforming growth factor β; TIMP-2, tissue inhibitor of metalloproteinase-2; TK, Thymidine kinase; TSH, Thyroid-stimulating hormone; TTC, Threshold of toxicological concern; T3, triiodothyronine; T4, Thyroxine; UDS, Unscheduled DNA Synthesis; USDA, United States Department of Agriculture; WHO, World Health Organization.

Data availability

Data will be made available on request.

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