Table 4. Identity for Complex Mixtures as Evaluated by the FEMA Expert Panel

FEMA No.	FEMA Primary Name	The Identification Description as Reviewed by the FEMA Expert Panel
4987	*Shorea stenoptera* seed butter	>95% of Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters including 35-50% stearic acid, 25-45% oleic acid and 10-25% palmitic acid, and minor amounts of Unsaturated linear and branched-chain aliphatic, nonconjugated aldehydes, related primary alcohols, carboxylic acids and esters
4988	Nootkatone 50%	>95% inclusive of 48-54% nootkatone, 13-22% *α*-nootkatol and 20-23% of related alicyclic ketones, secondary alcohols and related esters
4989	Cocoa bean shell extract	Derived from the shells of Cocoa beans (*Theobroma cacao*); 16% Volatile constituents including approximately: 10% Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters, <2% caffeine and <1% theobromine; Not less than 80% nonvolatile components including carbohydrates, fat, protein and water
4990	Sichuan pepper extract (*Zanthoxylum armatum*)	>40% Aliphatic and aromatic hydrocarbons; 35-45% Aliphatic and aromatic tertiary alcohols and related esters; 5-10% Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters; and 0.1-3% *alpha*-, *beta*-hydroxy sanshools
4991	*Persea americana* oil hydrolyzed fraction	Polyhydroxylated fatty alcohols (PFAs) typically measured as 60-80% avocadene and avocadyne; 10-30% Polyhydroxylated fatty alcohols - acetates (PFA acetates); 10-15% Citrates
4992	Rubusosides enriched Glucosylated Steviol Glycosides	Total steviol glycosides inclusive of glucosylated steviol glycosides 70-85%, glucosylated rubusosides 55-65%, rubusoside 9-13%, rebaudioside A <4%, stevioside <0.5%, not further glycosylated steviol glycosides individually <3%; Maltodextrin 10-20%
4999	*Adenophora stenanthina* root extract	Up to 1% amino acids and their derivatives such as citrulline; Up to 1% phenol derivatives such as icariside F2 and caffeic acid; Up to 5% sugar acids such as galacturonic acid; Up to 80% carbohydrates; Up to 15% protein; Less than 5% fat and water
5000	Prepared mixture of chloride salts of potassium, magnesium and calcium	5:3:3 molar composition of the chloride salts of potassium, magnesium and calcium, as a liquid concentrate or a solid blend

5001	Oak chips extract (*Quercus robur*)	>90% ethanol or propylene glycol, no more than 5% water and less than 1% each of: *trans*-sinapyl aldehyde, 4-hydroxy- 3,5-dimethoxybenzaldehyde, vanillin and furfural derivatives
5008	Finger Lime distillate	Aqueous distillate of the whole fruits of *Microcitrus australasica* (F. Muell.) Swingle. On a water-removed, concentrate basis, the material is: 70-90% Aliphatic tertiary alcohols typically measured as 4- carvomenthenol with smaller amounts of other tertiary alcohols; Up to 20% of aliphatic and aromatic terpene hydrocarbons typically measured as *d*-limonene; and 5-10% Alicyclic ketones such as isomenthone
5009	Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 40%	Total steviol glycosides no less than 95% inclusive of: Rebaudioside A >40%; Stevioside 16-19%; Rebaudioside C 9-12%; Rebaudioside B 3-6%; Rebaudioside F 2-4%; Other steviol glycosides not further glucosylated present at <2% individually
5011	Heat-treated Glucosylated Steviol Glycosides 45% with Steviol Glycosides 20%	Produced from enzymatically modified and heated steviol glycosides; >95% of identified constituents inclusive of: Supraglucosylated steviol glycosides 38-45%; Rubusoside 6-9%; Steviol glycosides not further glucosylated 5-9% with each individually, less than 2%; Polysaccharides 17-20%; Monosaccharides 8-9%; Water 3-6%; Disaccharides 3-4%; and Other non-volatiles 7-8% including sugar alcohols, amino acids, proteins and ash
5014	Modified Patchouli oil	Enzymatically modified distillate of patchouli Oil (*Pogostemon cablin* Benth. and *P. heyneanus* Benth.) (FEMA 2838); Contains: 60-75% Aliphatic and aromatic hydrocarbons, typically measured as *alpha*-guaiene, *alpha*-patchoulene, seychellene and related compounds; 10-15% Oxygenated sesquiterpenes; 5-12% Epoxide derivatives, typically measured as *beta*-caryophyllene oxide; and 5-15% Alicyclic ketones, secondary alcohols and related esters, typically measured as rotundone and related compounds
5015	Heat-treated Glucosylated Steviol Glycosides 20% with Steviol Glycosides 8%	Produced from enzymatically modified and heated steviol glycosides; >95% of identified constituents inclusive of: Supraglucosylated steviol glycosides 13-15%; Steviol glycosides not further glucosylated 4-8% with each individually less than 3%; Dextrins 60-70%; Monosaccharides 5-7%; and Other non-volatiles 5-7% including sugar alcohols, amino acids, proteins and water
5016	Heat-treated Glucosylated Steviol Glycosides 40% with Steviol Glycosides 15%	Produced from enzymatically modified and heated steviol glycosides; >95% of identified constituents inclusive of: Supraglucosylated steviol glycosides 20-40%; Steviol glycosides not further glucosylated 5-15% with each individually, less than 3%; Dextrins 25-40%; Other non-volatile constituents 20-25% including carbohydrates, sugar alcohols, amino acids, protein, ash and water

5017	Celtuce distillate	An ethanolic solution of the distillate of juiced lettuce stems of *Lactuca sativa* var. *augustana*; Contains: 45-60% Ethanol; 40-55% Water; and <0.1% Volatiles inclusive of 30-80 ppm of 2-acetyl-1-pyrroline
5023	Reactive distillation product of threonine and coconut oil	Obtained by distillation of thermally treated threonine in coconut oil; Contains: 36-47% nitrogen containing heterocyclic and heteroaromatic substances, such as 5-ethyl-2-methylpyridine, 2,3-dimethyl-5-ethyl-pyridine and dihydrooxazine derivatives; 9-21% Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters; and 7-13% Pyrazine derivatives such as 2,5-dimethyl-3-ethyl-pyrazine
5024	Camu Camu distillate	Aqueous distillate of the whole fruits of *Myrciaria dubia*; Contains >99% water with <1% of volatile constituents, including aliphatic and aromatic tertiary alcohols and related esters such as *alpha*-terpineol, 4-carvomenthenol and cubenol isomers
5025	Enzymatically modified *Stevia rebaudiana* extract enriched with Rebaudiosides AM, M and N2	Produced from enzymatically modified steviol glycosides, constituents inclusive of total steviol glycosides >95% inclusive of Rebaudioside AM 25-33%; Rebaudioside M 13-24%; Rebaudioside N2 13-16%; Supraglucosylated steviol glycosides 16-22%; Rebaudioside O4 5-9%; Other steviol glycosides not further glucosylated 7-15% with each individually, less than 3%
5026	*Eucommia ulmoides* leaf extract	Water/propylene glycol dilution (80%) of an ethanolic extraction (20%) of the aerial parts of *Eucommia ulmoides*; Contains 30-50% water, 30-40% propylene glycol, 6-10% carbohydrates, less than 2% polyphenols and less than 10% volatile constituents
5027	Fennel oleoresin (*Foeniculum vulgare* Miller)	Fennel Oleoresin (FEMA 5027) is prepared by solvent extraction of the seeds of the plant (*Foeniculum vulgare* Mill.) with an organic solvent followed by the removal of the solvent. The oleoresin is standardized with food grade diluents, preservatives, antioxidants and other substances consistent with GMP (see Food Chemical Codex) and contains 3 - 20% volatile oil in conformance with applicable residual solvent regulations.
5028	Nutmeg oleoresin (*Myristica fragrans* Houtt.)	Nutmeg Oleoresin (FEMA 5028) is prepared by solvent extraction of the seeds of the plant (*Myristica fragrans* Houtt.) with an organic solvent followed by the removal of the solvent. The oleoresin is standardized with food grade diluents, preservatives, antioxidants and other substances consistent with GMP (see Food Chemical Codex) and typically contains 25 - 90% volatile oil in conformance with applicable residual solvent regulations.

5029	*Corynebacterium casei* fermentation product	27-36% Amino acids, including 20-30% glutamic acid; 4-7% Heat-killed bacterial cells; 5-7% Minerals; Not more than 4% moisture; Not more than 3% of other non-volatiles such as carbohydrates, nucleic acids and lipids; Not more than 50% maltodextrin
Corrections of FEMA GRAS Identity Descriptions		
4845	Glucosylated stevia extract	At least 80% total steviol glycosides inclusive of: supraglucosylated steviol glycosides 50–70%; Not more than 10% Rebaudioside A; Not more than 4% Rebaudioside C; Not more than 5% Stevioside; And no individual steviol glycosides further glucosylated ≤3%; Maltodextrin <20%

Supplementary Information. Key Findings of the Expert Panel Safety Evaluations for GRAS 31

Since its initial publication of GRAS determinations for flavor ingredients (Hall and Oser, 1965), the Expert Panel has made available information on its determinations, including conditions of intended use for individual flavor ingredients, and the scientific basis and information supporting these determinations. Included herein are the key findings for each of the new GRAS determinations included within GRAS 31. Comprehensive monographs of the information relevant to the evaluations are also published as part of the Expert Panel's ongoing GRAS re-evaluation program (see Hallagan and Hall, 2009; Hallagan et al., 2020). For more information on the FEMA GRAS program, please see "About the FEMA GRAS Program" on femaflavor.org.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 8-methyl-4-methylenenon-7-en-2-one (CAS 6820-02-6) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4981) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and related esters (JECFA, 1999, 2003, 2017; SLR, A1). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 8-methyl-4-methylenenon-7-en-2-one from use as a flavor ingredient to be 7 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class II (540 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 8-methyl-4-methylenenon-7-en-2-one was reviewed by the Panel from a GLP- and OECD 407 guideline-compliant 28-day oral toxicity study (JECFA, 2017; Dhokale, 2008). In that study Sprague-Dawley rats (5/sex/dose) were administered the structural relative 9-decen-2-one (FEMA 4706) by gavage at 0, 250, 500 and 1000 mg/kg bw/day. No treatment-related mortalities or adverse effects were observed. The study authors established a no observed effect level (NOEL) of 1000 mg/kg bw/day for the structural relative 9-decen-2-one (FEMA 4706) (JECFA, 2017; Dhokale, 2008). This NOEL is 10,000,000 times the anticipated daily *per capita* intake of 8-methyl-4-methylenenon-7-en-2-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. It is presumed that 8-methyl-4-methylenenon-7-en-2-one will be reduced to the corresponding secondary alcohol, followed by conjugation and excretion. Epoxidation across the methylenenonene double bond group may also occur followed by glutathione conjugation and subsequent formation of the mercapturic acid derivative and excretion. Alternatively, P450 oxidation of the carbon chain and subsequent glucuronidation of the hydroxylation product(s) and excretion would likely occur (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of 8-methyl-4-methylenenon-7-en-2-one (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a two-strain screening bacterial reverse mutation assay, where 8-methyl-4-methylenenon-7-en-2-one was not mutagenic at concentrations up to 500 µg/plate in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 metabolic activation (Kino, 2020a). Similarly, corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay for the structurally related substance 9-decen-2-one (FEMA 4706), which was not mutagenic at concentrations up to 5 µl/plate (approximately 4 µg/plate) using both the plate incorporation and preincubation methodologies in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 strain pKM101 in the presence and absence of S9 metabolic activation (Garai, 2008).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 4-(4-methylpent-3-en-1-yl)-5,6-dihydro-2*H*-pyran-2-one (CAS 2492342-84-2) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4982) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones (Adams et al., 1998; JECFA, 1998, 2011; SLR, B1C). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 4-(4-methylpent-3-en-1-yl)-5,6-dihydro-2*H*-pyran-2-one from use as a flavor ingredient to be 0.7 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). The substance occurs naturally in beer (Natural Occurrence Analysis, 2021a). Based on the quantitative data, a consumption ratio of 50 could be calculated (Stofberg and Grundschober, 1987). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. 4-(4-Methylpent-3-en-1-yl)-5,6-dihydro-2*H*-pyran-2-one is expected to be hydrolyzed to the corresponding hydroxycarboxylic acid derivative followed by *β*-oxidation to short-chain metabolites that are excreted unchanged or in conjugated form (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of 4-(4-methylpent-3-en-1-yl)-5,6-dihydro-2*H*-pyran-2-one (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay for 4-(4-methylpent-3-en-1-yl)-5,6-dihydro-2*H*-pyran-2-one, which was not mutagenic at concentrations up to 1500 µg/plate using the plate incorporation method in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A in the presence and absence of S9 metabolic activation (Kino, 2020b). Corroborative evidence for the lack of genotoxic potential for 4-(4-methylpent-3-en-1-yl)-5,6-dihydro-2*H*-pyran-2-one was evaluated by the Expert Panel from a number of studies for the structurally related substances 4-hydroxy-2,3-dimethyl-2,4-nonadienoic acid

gamma-lactone (FEMA 4050) and 4-hydroxy-2-butenoic acid *gamma*-lactone (FEMA 4138). FEMA 4050 and FEMA 4138 were not mutagenic in GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in the presence or absence of S9 at concentrations up to 5000 µg/plate (Bowen, 2011a,b; Gooderham et al., 2020). In a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay, significant induction of micronuclei was observed in human lymphocytes treated with concentrations up to 140 µg/mL of FEMA 4050 for 3 hours with a 21-hour recovery period in the presence of S9, but not when tested for 24 hours in the absence of S9 at concentrations up to 15 µg/mL. In the 3-hour treatment in the absence of S9 at concentrations up to 90 µg/mL for FEMA 4050, increases in the micronuclei frequency at 70 and 90 µg/mL were not considered biologically relevant as they were within the historical control range (Whitwell, 2012a; Gooderham et al., 2020). In another GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay for the same structurally related substance (FEMA 4050) in human lymphocytes, significant induction of micronuclei was observed at 75 and 140 µg/mL in the presence of S9 in 3-hour treatments with 21-hour recovery periods when tested at concentrations of 40-140 µg/mL, but negative results were observed when tested in the absence of S9 for 3-hour treatments with a 21-hour recovery period at concentrations of 90-140 µg/mL, as well as in the continuous 24-hour treatment at concentrations of µg/mL (Watters, 2013a; Gooderham et al., 2020). Significant induction of micronuclei was observed at the top three concentrations in a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay when human lymphocytes were treated with FEMA 4138 at concentrations of 100-475 µg/mL for 3 hours with a 21-hour recovery period in the presence of S9. Increases in the top two tested concentrations in the 3-hour and 24-hour treatments in the absence of S9 were associated with high cytotoxicity (Whitwell, 2012b; Gooderham et al., 2020). In another GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay, FEMA 4138 induced a significant increase in micronuclei at the top three tested concentrations in lymphocytes treated for 3 hours with a 21-hour recovery period in the presence of S9 at concentrations of 100-400 µg/mL, but not when tested at 100-350 µg/mL or 10-65 µg/mL for 3 hours or 24 hours in the absence of S9, respectively (Watters, 2013b; Gooderham et al., 2020). In another GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay, no significant induction of micronuclei was observed for FEMA 4138 when it was tested in human TK6 cells at concentrations of 25-150 µg/mL for 4 hours in the presence and absence of S9 as well as at concentrations of 5-55 µg/mL for 27 hours in the absence of S9 (Dutta, 2018; Gooderham et al., 2020). In GLP- and OECD 474/489 guideline-compliant combination *in vivo* micronucleus/comet assays, no induction of micronuclei or DNA damage was observed in the bone marrow or liver of male Han Wistar rats (6/group) administered 63, 125 or 250 mg/kg bw/day of FEMA 4138 (Beevers, 2014a; Gooderham et al., 2020) or 125, 250 or 500 mg/kg bw/day of FEMA 4050 (Beevers, 2014b; Gooderham et al., 2020) via oral gavage for 0, 24 and 45 hours. Slight, 2-fold increases in tail intensity and tail moment observed in high-dose animals administered FEMA 4138 were within the historical control range and were therefore not considered to be biologically relevant (Beevers, 2014a). A dose-dependent decrease in bodyweight gain was observed in animals treated with the same structurally related substance (Beevers, 2014a). A slight decrease in mean aspartate aminotransferase activity was not indicative of treatment-related toxicity for FEMA 4138 (Beevers, 2014a). However, glycogen vacuolation in the liver and villous tip necrosis in the duodenum of high-dose animals treated with FEMA 4138 was suggestive of liver toxicity (Beevers, 2014a). A dose-dependent increase in bodyweight gain was observed in animals treated with FEMA 4050 (Beevers, 2014b). For this same structurally related substance, early evidence of liver injury was observed with dose-dependent increases in alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase (Beevers, 2014b). However, both the liver and duodenum showed no evidence of macroscopic findings while the liver in high-dose animals exhibited a reduced glycogen hepatocellular vacuolation and hepatocyte vacuolation when administered FEMA 4050 (Beevers, 2014b).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 4-mercapto-1-octanol (CAS 2491702-14-6) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4983) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic and aromatic sulfides and thiols (JECFA, 2000, 2004, 2008, 2011; SLR, B5B). The Expert Panel calculated the anticipated *per capita* intake (“eaters only”) of 4-mercapto-1-octanol from use as a flavor ingredient to be 0.01 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 4-mercapto-1-octanol was evaluated by the Expert Panel from a 90-day dietary toxicity study in Wistar rats (15/sex) that were provided approximately 0.7 mg/kg bw/day of the structural relative 2-mercapto-3-butanol (FEMA 3502) (Morgareidge, 1974a). No treatment-related effects or mortalities were observed. One death was observed at 7 weeks, but the cause of death could not be determined. A NOEL of 0.7 mg/kg bw/day was established, which is 3,500,000 times the anticipated daily *per capita* intake of 4-mercapto-1-octanol from use as a flavor ingredient. Though this substance occurs naturally in trace amounts in chicken fat, quantitative information was not available, and a consumption ratio could not be calculated (Natural Occurrence Analysis, 2021b). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. 4-Mercapto-1-octanol is expected to undergo metabolism by oxidation to the corresponding sulfoxide and then sulfone, followed by excretion. Alternatively, the alcohol moiety could be conjugated to the corresponding glucuronic acid or sulfonic acid derivative, followed by excretion (Smith et al., 2018). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of 4-mercapto-1-octanol (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential of 4-mercapto-1-octanol was evaluated by the Expert Panel from a two-strain screening bacterial reverse mutation assay for the substance, which was not mutagenic at concentrations up to 500 µg/plate

in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 metabolic activation (Kino, 2020c).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 2,11-tetradecadienal (CAS 2099712-94-2) and concluded that the use of the substance is GRAS (FEMA 4984) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. The substance was evaluated individually within the context of the chemical group of aliphatic linear and branched-chain *alpha*, *beta*-unsaturated aldehydes and related alcohols, acids and esters (Adams et al., 2008; JECFA, 2009, 2012; SLR, M1). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 2,11-tetradecadienal from use as a flavor ingredient to be 7 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 2,11-tetradecadienal was evaluated by the Expert Panel from 90-day National Toxicology Program (NTP) gavage studies with rats and mice, where administration of the structural relative *trans*,*trans*-2,4-decadienal (FEMA 3135) resulted in a no observed adverse effect level (NOAEL) of 100 mg/kg bw/day, which is 1,000,000 times the anticipated daily *per capita* intake of 2,11-tetradecadienal from use as a flavor ingredient (NTP, 2011). Additional corroborative evidence for the low toxicity potential of 2,11-tetradecadienal was evaluated by the Expert Panel from a series of toxicity studies for the structural relative *trans*,*trans*-2,4-hexadienal (FEMA 3429). In evaluating carcinogenicity studies for FEMA 3429 the Expert Panel noted clear effects, including increased incidences of forestomach epithelial hyperplasia (NTP, 2003). However, the Expert Panel determined that the effects were due to the high bolus doses administered in the studies and the strong irritating nature of the 2,4-hexadienal. In a subchronic toxicity study of FEMA 3429 administered to F344/N rats at doses of 7.5, 15, 30, 60, or 120 mg/kg bw/day by gavage 5 days per week for a total of 70 doses over 14 weeks, no mortalities were observed in this study (NTP, 2003). Significant reductions in final mean bodyweights and bodyweight gains were observed in male rats at doses of 30 mg/kg bw/day and above. No other signs of clinical toxicity were observed in treated animals at any dose, with the exception of increased salivation in males and females at 30 or 120 mg/kg bw/day during week 4 and only in 120 mg/kg bw/day groups at later times. Increased incidences of mild-to-moderate forestomach epithelial hyperplasia were reported in both males and females at 120 mg/kg bw/day, accompanied by forestomach-localized tissue degeneration and active chronic inflammation. Increased incidences of olfactory epithelial atrophy, osteofibrosis, and excessive exudate of the nose were also reported in males at 120 mg/kg bw/day. There were no biologically significant changes in organ weights at any dose level. Statistically significant but sporadic and non-dose dependent variations in hematological and clinical chemistry parameters were reported but were not considered related to treatment. Based on these findings, the NOEL was determined to be 15 and 60 mg/kg bw/day for male and female rats, respectively. This NOEL of 15 mg/kg bw/day is 150,000 times the anticipated daily *per capita* intake of 2,11-tetradecadienal from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. 2,11-Tetradecadienal is expected to undergo oxidation to the corresponding acid followed by *β*-oxidation to CO_2 and water. Alternatively, the resulting acid could be conjugated with glutathione followed by excretion as the mercapturic acid derivative (Adams et al., 2008; Smith et al., 2018). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 2,11-tetradecadienal (Gooderham et al., 2020). Corroborative evidence of the lack of genotoxic potential for 2,11-tetradecadienal was evaluated by the Expert Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, where the substance was not mutagenic when tested at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvr*A in the presence and absence of S9 metabolic activation using the preincubation and plate incorporation methods (Rao, 2020a). Similarly, corroborative evidence of the lack of genotoxic potential for 2,11-tetradecadienal was evaluated by the Expert Panel from an Ames assay conducted in *S. typhimurium* strains TA98 and TA100 for the structural relative (*Z*)-9-dodecenoic acid (FEMA 4917), which did not increase the frequency of revertant colonies in the absence and presence of S9 metabolic activation at concentrations up to 500 µg/plate (Kino, 2017a). Additionally, corroborative evidence for the lack of genotoxic potential for 2,11-tetradecadienal was evaluated by the Expert Panel available for 2,4-alkadienals, including assays conducted by the NTP during its investigations of the structural relatives *trans*,*trans*-2,4-decadienal (FEMA 3135) and *trans*,*trans*-2,4-hexadienal (FEMA 3429), and new genotoxicity studies for the structural relative 2-methyl-2-pentenal (FEMA 3194) (Adams et al., 2008; Bowen, 2011c; EFSA, 2019a; Keig-Shevlin, 2016a, b; Bastaki et al., 2019; Kilford, 2016; Lloyd, 2014; McKeon and Ciubotaru, 2016; ECHA, 2012a; NTP, 2011; Whitwell, 2011, 2016a,b).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 4,9-dodecadienal (CAS 1801275-27-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4985) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters (JECFA, 1999, 2012, 2020; SLR, M1). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 4,9-dodecadienal from use as a flavor ingredient to be 7 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 4,9-dodecadienal was evaluated by the Expert Panel from a GLP- and OECD 407 guideline-compliant 28-day toxicity study, where the administration of the structural relative 10-undecenal (FEMA 3095) administered to Wistar rats at concentrations up to 1000 mg/kg bw/day resulted in a NOAEL of 1000 mg/kg bw/day (ECHA, 2015). Similarly, corroborative evidence for the low toxicity potential of 4,9-dodecadienal was evaluated

by the Expert Panel from a GLP- and OECD 408 guideline-compliant 90-day dietary toxicity study, where the administration of the structural relative 10-undecenal (FEMA 3095) to Sprague-Dawley Crl:CD® (SD) IGS BR rats in the diet resulted in a NOAEL of 2000 ppm, or approximately 139 mg/kg bw/day (Api et al., 2022a), which is greater than 1,390,000 times the anticipated daily *per capita* intake of 4,9-dodecadienal from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. 4,9-Dodecadienal is anticipated to undergo oxidation to the corresponding acid followed by *β*-oxidation to CO_2 and water. Alternatively, the resulting acid could be conjugated with glutathione followed by excretion as the mercapturic acid derivative (Smith et al., 2018). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of 4,9-dodecadienal (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential for 4,9-dodecadienal was evaluated by the Expert Panel from GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays with the structurally related substances 10-undecenal (FEMA 3095) (Api et al., 2022a) and 4,7-decadienal (FEMA 4927) (Sokolowski, 2009; Api et al., 2022b) , where there were no increases in the frequency of revertant colonies in *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, and/or *E. coli* WP2*uvr*A in the absence or presence of S9 metabolic activation. Similarly, corroborative evidence for the lack of genotoxic potential for 4,9-dodecadienal was evaluated by the Expert Panel from a GLP-compliant *in vitro* chromosome aberration assay in Chinese hamster lung cells (CHL/IU) where no significant induction of chromosome aberrations was observed when the structural relative 10-undecenal (FEMA 3095) was tested for 6 hours with an 18-hour recovery period at concentrations of approximately 9-20 µg/mL in the absence of S9, at 24-50 µg/mL in the presence of S9 as well as for a continuous 24-hour treatment period in the absence of S9 at concentrations of approximately 9-20 µg/mL (JMHLW, 2018). Additional corroborative evidence of the lack of genotoxic potential for 4,9-dodecadienal was evaluated by the Expert Panel from an *in vivo* micronucleus assay in male and female NMRI mice (Api et al., 2022a), where gavage administration of the structural relative 10-undecenal (FEMA 3095) at doses up to 2000 mg/kg bw produced no statistically significant increases in the frequency of micronuclei (Api et al., 2022a). Additional corroborative evidence of the lack of genotoxic potential for 4,9-dodecadienal was evaluated by the Panel from a GLP- and OECD 474 guideline-compliant *in vivo* micronucleus assay, where oral administration of the structural relative 4,7-decadienal (FEMA 4927) to male Albino Hsd:ICR (CD-1) mice at doses up to 2000 mg/kg bw did not increase the frequency of micronucleated polychromatic or normochromatic erythrocytes (Flanders, 2017).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding hyaluronic acid, sodium salt (CAS 9067-32-7) and concluded that the substance is GRAS (FEMA 4986) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic polyhydroxy compounds and derivatives (SLR, B1F). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of hyaluronic acid, sodium salt from use as a flavor ingredient to be 46 µg/person/day. A decision tree structural class for the threshold of toxicological concern (TTC) could not be assigned for hyaluronic acid, sodium salt, as polymers are excluded from the Cramer/Ford/Hall Decision Tree classification (Cramer et al., 1978). Corroborative evidence for the low toxicity potential of hyaluronic acid, sodium salt was evaluated by the Expert Panel from a 90-day dietary toxicity study, in which Wistar rats (10/sex/group) were provided the equivalent of 330, 670 or 1000 mg/kg bw/day sodium hyaluronate (purity 96%) (INS-CCDCP, 2007). No treatment-related adverse effects or histopathological findings were observed, and a NOAEL of 1000 mg/kg bw/day was established. In a corroborative GLP-compliant 90-day repeated dose oral toxicity study, Sprague-Dawley rats (10/sex/group) were administered 30, 300 or 1000 mg/kg bw/day of the structural relative hydrolyzed chicken sternal cartilage known as BioCell Collagen II® (food-grade powder containing 60% collagen type II, 20% chondroitin sulfate, 10% hyaluronic acid and 1% other proteoglycans) by gavage (Schauss et al., 2007). A NOAEL of 1000 mg/kg bw/day at the top dose was established (Schauss et al., 2007). In a corroborative 13-week repeated dose oral toxicity study, rats were administered 2275 mg/kg bw/day of hyaluronic acid, sodium salt (MT7250000, 1996). Details of this study are limited but evidence of weight loss and changes in sodium and chlorine were observed. In another corroborative GLP-compliant 90-day repeated dose oral toxicity study in Sprague-Dawley rats (10/sex/group) administered 5, 55 or 600 mg/kg bw/day of rooster comb extract (includes 60-80% sodium hyaluronate along with other glycosaminoglycans and partially hydrolyzed proteins) by oral gavage, the study authors established the NOAEL at the top dose level of 600 mg/kg bw/day (Canut et al., 2012). This NOAEL is 750,000 times the anticipated daily *per capita* intake of hyaluronic acid, sodium salt from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of hyaluronic acid, sodium salt was evaluated by the Expert Panel from a sperm malformation test in which no statistically significant increases in sperm malformation rate were observed in male Kunming mice (10/group) administered 440, 880 or 1760 mg/kg bw/day sodium hyaluronate (purity 95.97%) by oral gavage for 5 days (INS-CCDCP, 2007). The substance occurs naturally in Tu-Chung tea leaves and bark, as well as tissues and organs of cows, rabbits, roosters, fish and Mediterranean mussels (Cowman et al., 2015; Nakano et al., 1994; Kim et al., 1992; Murado et al., 2012; Volpi and Maccari, 2003). However, quantitative information was not available and a consumption ratio could not be calculated. The Expert Panel noted the assay of the material was ≥91-94% of the named material with water as the secondary component (6-9%) and considered the food grade specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Hyaluronic

acid has a half-life of 2-6 minutes in circulation, with a total normal turnover of 10-100 mg/day, and 2-3 days in tissues (Lebel, 1991; Fraser et al., 1997). In skin and joints, the metabolism of hyaluronic acid occurs locally, and the remainder is eliminated through the lymphatic system. Elimination from circulation occurs in the liver via receptor-facilitated endocytosis and catabolism in the hepatic sinusoidal endothelial cells to CO_2 and water. Minor amounts (1-2%) are excreted in the urine (Laurent and Fraser, 1992; Kobayashi et al., 2020). Over 90% of ^{14}C-hyaluronic acid orally administered to male Sprague Dawley rats was excreted in the urine or exhaled after absorption in the digestive tract and used as an energy source or structural constituent of tissues (Oe et al., 2014). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of hyaluronic acid, sodium salt (Gooderham et al., 2020). Corroborative evidence of the lack of genotoxic potential for hyaluronic acid, sodium salt was evaluated by the Expert Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, where the test material, natural rooster comb extract (containing 60-80% sodium hyaluronate along with other glycosaminoglycans and partially hydrolyzed proteins), was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli uvr*A pKM101 in the presence and absence of metabolic activation using the plate incorporation method (Canut et al., 2012). Similarly, corroborative evidence of the lack of genotoxic potential for hyaluronic acid, sodium salt was evaluated by the Expert Panel from other bacterial reverse mutation assays for sodium hyaluronate materials, in which no statistically significant increases in the number of revertants relative to controls were observed at concentrations up to 5000 µg/plate in *S. typhimurium* TA97, TA98, TA100, TA102 and *E. coli uvr*A in both the presence and absence of metabolic activation using the plate incorporation and preincubation methodologies (INS-CCDCP, 2007; Aruga et al., 1994; Onishi et al., 1992 as described in Becker et al., 2009; Sugiyama and Yagame, 1991 as described in Becker et al., 2009). Additionally, corroborative evidence for the lack of genotoxic potential for hyaluronic acid, sodium salt was evaluated by the Expert Panel from *in vitro* chromosomal aberration assays in which no induction of chromosome aberrations was observed in Chinese hamster lung fibroblasts incubated with sodium hyaluronate (purity unknown) and 1% sodium hyaluronate at concentrations up to 1000 µg/mL for 24 and 48h with and without metabolic activation (Aruga et al., 1994; Onishi et al., 1992). Corroborative evidence for the lack of genotoxic potential for hyaluronic acid, sodium salt was evaluated by the Expert Panel from experimental studies of Orthovisc® (viscoelastic solution of sodium hyaluronate in saline at 15 mg/mL) which showed that high molecular weight sodium hyaluronate (1-2.9 MDa) was not mutagenic in an Ames assay and did not induce chromosomal damage in Chinese Hamster Ovary cells and a sister chromatid exchange assay (FDA, 2004). Additional corroborative evidence for the lack of genotoxic potential for hyaluronic acid, sodium salt was evaluated by the Expert Panel from *in vivo* micronucleus assays where no induction of micronuclei was observed in Kunming mice (5/sex/group) administered 440, 880 or 1760 mg/kg bw sodium hyaluronate (purity 95.97%) by oral gavage twice within 24 hours (INS-CCDCP, 2007); in CD-1 (ICR) male mice intraperitoneally injected twice 24 hours apart with 0, 75, 150, or 300 mg/kg bw of sodium hyaluronate solution (purity unknown) (Aruga et al., 1992); and in CD-1 (ICR) male treated with 90, 180 or 360 mg/kg bw of sodium hyaluronate for 1 or 4 consecutive days (information on the route of administration not provided in reference - Aruga et al., 1994).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *Shorea stenoptera* seed butter (CAS 91770-65-9) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4987) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *Shorea stenoptera* seed butter from use as a flavor ingredient to be 69 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). Corroborative evidence for the low toxicity potential of *Shorea stenoptera* seed butter is from a chronic drinking water study, where the administration of the constituent sodium oleate (FEMA 2815) to male and female F344 rats resulted in a NOAEL of 2.5% (equivalent to 2500 mg/kg bw/day) (Hiasa et al., 1985). This NOAEL is 2,500,000 times the anticipated daily *per capita* intake of *Shorea stenoptera* seed butter from use as a flavor ingredient. The material is produced from the nuts of the *Shorea stenoptera* tree. Though this material is consumed as food, no quantitative data are available, and a consumption ratio could not be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for representative members of the principal congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be expected to be metabolized primarily by well-established metabolic pathways to innocuous products (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of *Shorea stenoptera* seed butter (Gooderham et al., 2020; Cohen et al., 2018). Additional corroborative evidence for the lack of genotoxic potential for *Shorea stenoptera* seed butter was from bacterial reverse mutation assays, where the constituent oleic acid (FEMA 2815) was not mutagenic in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2uvrA at concentrations up to 5000 µg/plate using the preincubation methodology (Shimizu et al., 1985; Mortelmans et al., 1986). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, where the constituent palmitic acid (FEMA 2832) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA using the plate incorporation method (Api et al., 2019a). Corroborative evidence for the lack of genotoxic potential was also evaluated by the Panel from GLP- and OECD 487 guideline-compliant *in vitro*

micronucleus assays in human lymphocytes, where the constituents oleic acid (FEMA 2815) and palmitic acid (FEMA 2832) did not produce significant increases in the induction of micronuclei relative to controls in any treatment condition either in the presence or absence of S9 metabolic activation (Api et al., 2019a; 2021).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding Nootkatone 50% (CAS 4674-50-4) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4988) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of Nootkatone 50% from use as a flavor ingredient to be 280 µg/person/day. Corroborative evidence for the low toxicity potential of Nootkatone 50% was evaluated by the Expert Panel from a GLP- and OECD 407 guideline-compliant single-dose 28-day study in male and female Sprague-Dawley Crl:CD (SD) IGS BR rats, where oral gavage administration of the constituent, nootkatone (FEMA 3166), resulted in a NOEL of 10 mg/kg bw (Jones, 2004). Additional corroborative evidence for the low toxicity potential of Nootkatone 50% was evaluated by the Panel from an OCSPP guideline 870.3100-compliant 90-day oral toxicity study, where no adverse effects relevant to humans were observed when rats were administered 0, 100, 300 and 1000 mg/kg bw/day of the constituent, nootkatone (FEMA 3166) and a NOAEL of 1000 mg/kg bw/day was established. Corroborative evidence for the lack of developmental toxicity was evaluated by the Panel from an OCSPP guideline 870.3700-compliant developmental toxicity study, where rats administered 0, 50, 250 and 1000 mg/kg bw/day of the constituent nootkatone (FEMA 3166) showed no evidence of maternal and developmental toxicity, and NOAELs of 1000 mg/kg bw/day were established for both endpoints (EPA, 2020). The NOAELs of 1000 mg/kg bw/day are 200,000 times the anticipated daily *per capita* intake of Nootkatone 50% as a flavor ingredient. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for a representative member of the principal congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be expected to be metabolized primarily by well-established metabolic pathways to innocuous products (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of Nootkatone 50% (Gooderham et al., 2020; Cohen et al., 2018). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP-compliant liquid microplate bacterial reverse mutation assay, where up to 160 µg/ml of the structural relative nootkatone complex (FEMA 4941) did not increase the frequency of revertant colonies when tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* combined strains WP2(pKMN101) and WP2uvrA (Ziemianska, 2017). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from an *in vivo* micronucleus assay, where the structural relative nootkatone complex (FEMA 4941) did not increase the frequency of micronucleated bone marrow cells at up to 1000 mg/kg bw/day when intraperitoneally injected in male NMRI mice (Donath, 2017). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a bacterial reverse mutation assay for the constituent nootkatone (FEMA 3166), where no increases in the frequency of revertant colonies was observed in either the presence or absence of S9 in *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537, no (Marzin, 1998). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from an *in vitro* micronucleus assay for the constituent nootkatone (FEMA 3166), where no statistically significant effects on micronuclei frequency in the presence and absence of S9 were observed (Stone, 2011). Corroborative evidence for the lack of genotoxic potential for Nootkatone 50% was evaluated by the Panel from an OCSPP (EPA Office of Chemical Safety and Pollution Prevention) guideline 870.5100-compliant bacterial reverse mutation assay and an OCSPP guideline 870.5375-compliant *in vitro* chromosome aberration assay in human lymphocytes where the constituent nootkatone (FEMA 3166) in the presence and absence of S9 was not clastogenic or mutagenic (EPA, 2020). The same major marker constituent (FEMA 3166) was not clastogenic and not aneugenic when tested at concentrations of 1.38-353.4 µM (0.3-77 µg/mL) in human p53-competent TK6 lymphoblastoid cells (TK6 cells) for 4 hours in the presence and absence of S9 as well as for 24 hours in the absence of S9 (Hung et al., 2020).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding cocoa bean shell extract (CAS 84649-99-0) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4989) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of cocoa bean shell extract from use as a flavor ingredient to be 3 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). Corroborative evidence for the low toxicity potential of cocoa bean shell extract is available from a 13-week repeated dose oral toxicity study in which calcium lactate (related to the constituent, lactic acid (FEMA 2611)) was administered to F344 rats (5/sex/group) at 0.3, 0.6, 1.25, 2.5 or 5% (corresponding to doses of 300, 600, 1250, 2500 and 5000 mg/kg bw/day) in drinking water (ECHA, 1989; Matsushima et al., 1989). No adverse effects were observed, and a NOAEL was established at the top dose of 5000 mg/kg bw/day. Corroborative evidence from a chronic toxicity and carcinogenicity study in which the same test material was administered orally to F344 rats (50/sex/group) at 0, 2.5 and 5% for 2 years (approximately 0, 4510 or 8570 mg/kg bw/day and 0, 3260 or 5650 mg/kg bw/day in males and females, respectively) (Maekawa et al., 1991), resulted in a NOAEL of 8570 and 5650 mg/kg bw/day in males and

females, respectively. Additional corroborative evidence for the low toxicity potential of cocoa bean shell extract is from a chronic drinking water study, where the administration of the constituent sodium oleate (FEMA 2815) to male and female F344 rats resulted in a NOAEL of 2.5% (equivalent to 2500 mg/kg bw/day) (Hiasa et al., 1985). This NOAEL is 50,000,000 times the anticipated daily *per capita* intake of cocoa bean shell extract from use as a flavor ingredient. The material is produced from the shells of Cocoa beans (*Theobroma cacao*). Though this source material is consumed as food, quantitative data was not available, and a consumption ratio could not be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for representative members of the principal congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be expected to be metabolized primarily by well-established metabolic pathways to innocuous products (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of cocoa bean shell extract (Gooderham et al., 2020; Cohen et al., 2018). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, where cocoa bean shell extract was not mutagenic in *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of S9 (Shukla, 2020). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from bacterial reverse mutation assays, where the constituent oleic acid (FEMA 2815) was not mutagenic in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2uvrA at concentrations up to 5000 µg/plate using the preincubation methodology (Shimizu et al., 1985; Mortelmans et al., 1986). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, where the constituent palmitic acid (FEMA 2832) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA using the plate incorporation method (Api et al., 2019a). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assays, where FEMA 2815 and FEMA 2832 did not produce significant increases in the induction of micronuclei relative to controls in any treatment condition either in the presence or absence of S9 metabolic activation (Api et al., 2019a; 2021). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays, where the constituent lactic acid (FEMA 2611) was not mutagenic in *S. typhimurium* TA97, TA98, TA100, TA104, TA1535, TA1537, TA1538, *E. coli* WP2uvrA and *Saccharomyces cerevisiae* D4 in the presence and absence of S9 (ECHA, 2014a; NTP, 2018a; Al-Ani and Al-Lami, 1988; Brusick, 1976). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from an *in vitro* chromosomal aberration assay, where the same constituent (FEMA 2611) was not clastogenic in CHO-K1 cells with and without metabolic activation (D,L-isomer) at concentrations up to 1441 µg/ml (Morita et al., 1990) and in a GLP- and OECD 473 guideline-compliant *in vitro* chromosomal aberration assay in human lymphocytes at concentrations up to 901 µg/mL in the presence and absence of S9 (L-isomer) (ECHA, 2014b). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 476 guideline-compliant *in vitro* gene mutation assay, where no induction of mutant frequencies was observed for the same constituent (L-isomer) (FEMA 2611) when tested at concentrations up to 901 µg/mL for 3 hours in the presence and absence of S9 and for 24 hours in the absence of S9 (ECHA, 2014c).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding Sichuan pepper extract (*Zanthoxylum armatum*) (CAS 91770-90-0) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4990) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of Sichuan pepper extract (*Zanthoxylum armatum*) from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). Corroborative evidence for the low toxicity potential of Sichuan pepper extract (*Zanthoxylum armatum*) was evaluated by the Expert Panel from a GLP- and OECD 422 guideline-compliant combined repeat dose toxicity study for the constituent methyl cinnamate (FEMA 2698), Han Wistar rats (12/sex/group) were provided 0, 100, 300 and a high-dose of 1000 mg/kg bw/day (Week 1 only) or 600 mg/kg bw/day (Week 2 onwards) for at least 28 days in male rats and in female rats for 14 days prior to pairing, through pairing (14 days) and gestation (21 days) periods until Post-Partum Day 4 (ECHA, 2013a). Significant decreases in high-dose animals in mean absolute bodyweights during the pre-pairing and pairing periods were considered adverse since they did not fully recover even though bodyweight gains were relatively increased during the pairing period. Statistically significant decreases in gestation index in high-dose females were considered treatment-related due to other toxicologically relevant histopathological, hematological and clinical chemistry effects. No other adverse effects in the parental or offspring were observed. A NOAEL of 300 mg/kg bw/day was established for general toxicity due to absolute bodyweight changes and a reproductive and developmental toxicity NOAEL was established at the top dose. Further corroborative evidence is available from a GLP- and OECD 408 guideline-compliant 90-day repeat dose dietary toxicity study, Sprague Dawley rats (10/sex/group) were provided 0, 80, 250 and 750 mg/kg bw/day of the constituent linalool (FEMA 2635) (corresponding to mean overall daily intakes of approximately 78, 344 and 731 mg/kg bw/day and 79, 246 and 740 mg/kg bw/day in males and females, respectively) (Bauter, 2020). Stability studies at the highest dietary concentration showed that over 7 days, males and females received approximately 68-78% of the nominal dose (corresponding to approximately 498 and 532 mg/kg bw/day, respectively). No toxicologically relevant findings were observed and a NOAEL of 498 and 532 mg/kg bw/day was

established at the highest tested dietary concentration in male and female Sprague Dawley rats, respectively. Additional corroborative evidence is available, as described in the Expert Panel's prior assessment of the subchronic and chronic toxicity of the constituent d-limonene (FEMA 2633) in its review of *citrus-derived natural flavor complexes* (Cohen et al., 2019). A NOAEL of 215 mg/kg bw/day was determined for FEMA 2633 in female rats from a 103-week NTP study with F344/N rats (NTP, 1990). This NOAEL is greater than 1,000,000 times the anticipated daily *per capita* intake of Sichuan pepper extract (*Zanthoxylum armatum*) from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of Sichuan pepper extract (*Zanthoxylum armatum*) was evaluated by the Expert Panel from a reproductive toxicity study for the constituent linalool (FEMA 2635) as described in the Expert Panel's prior assessment of *Lavender, Guaiac Coriander-derived and related flavor ingredients*. A maternal toxicity NOAEL of 500 mg/kg bw/day and a developmental toxicity NOAEL of 1000 mg/kg bw/day was determined from an 11-day reproductive and developmental toxicity study in Sprague Dawley rats administered FEMA 2635 at doses of 0, 250, 500 or 1000 mg/kg bw/day (Fukami and Yokoi, 2012; Politano et al., 2008). Additional corroborative evidence is described in the Expert Panel's prior assessment of *citrus-derived natural flavor complexes* (Cohen et al., 2019), in which the reproductive and/or developmental toxicity of the constituent limonene (FEMA 2633) was reviewed (Kodama et al., 1974, 1977; Tsuji et al., 1975). Maternal and fetal toxicity NOAELs of 250 mg/kg bw/day were determined in pregnant Japanese white rabbits (Kodama et al., 1976). Corroborative evidence is available from a GLP- and OECD 422 guideline-compliant combined repeat dose toxicity study with reproduction/developmental toxicity screening in which Han Wistar rats (12/sex/group) were administered the constituent methyl cinnamate (FEMA 2698) for at least 28 days. Reproductive and developmental toxicity NOAELs of 1000 and 600 mg/kg bw/day were established, respectively (ECHA, 2013a). The Expert Panel determined that the most conservative maternal and developmental NOAELs of 250 mg/kg bw/day for the constituent d-limonene (FEMA 2633) and 600 mg/kg bw/day for the constituent methyl cinnamate (FEMA 2698) is greater than 1,000,000 and 2,500,000 times the anticipated daily per capita intake of Sichuan pepper extract (*Zanthoxylum armatum*), respectively, from use as a flavor ingredient. The material is produced from the berries of the Sichuan pepper (*Zanthoxylum armatum*) plant. Though the dried berries of this material are consumed as spice, quantitative information was not available, and a consumption ratio could not be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel noted that the constituent *beta*-phellandrene occurs naturally in many foods, including some vegetables (up to 154,600 ppm), fruits (up to 72,700 ppm), spices (up to 631,500 ppm), and herbs (up to 265,000 ppm) (Van Dongen et al., 2024). The Expert Panel concluded that metabolic data exist for representative members of the principal congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be expected to be metabolized primarily by well-established metabolic pathways to innocuous products (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of Sichuan pepper extract (*Zanthoxylum armatum*) (Gooderham et al., 2020; Cohen et al., 2018). The Expert Panel reviewed corroborative evidence from their prior assessment of the genotoxicity data for the constituent linalool (FEMA 2635) (Fukushima et al., 2020) and the constituent limonene (Cohen et al., 2019) and determined that the data were sufficient to indicate a lack of genotoxic concern for these two constituents of Sichuan pepper extract (*Zanthoxylum armatum*). Corroborative evidence for the constituent methyl cinnamate (FEMA 2698) indicates it is not mutagenic in an GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA in the presence and absence of S9 using the plate incorporation and preincubation methodologies (ECHA, 2012b). Additional corroborative evidence showed no significant increase in mutant frequency was observed when the same constituent (FEMA 2698) was tested in a GLP- and OECD 476 guideline-compliant *in vitro* mammalian cell gene mutation assay in Chinese Hamster V79 cells at concentrations up to 800 µg/ml for 4 hours in the absence of S9, at concentrations up to 1200 µg/ml for 4 hours in the presence of S9, at concentrations up to 600 µg/ml for 24 hours in the absence of S9 (ECHA, 2013b). Corroborative evidence is also available from non-guideline compliant assays for the same constituent (FEMA 2698), which was negative in the rec assay and sister chromatid exchange assay (Oda et al., 1979; Sasaki et al., 1989). However, the Expert Panel noted the limited value of the rec assay and sister chromatid exchange assay for the safety evaluation of Sichuan pepper extract (*Zanthoxylum armatum*). When tested in a corroborative OECD 471 guideline-compliant Ames assay with *S. typhimurium* TA98 and TA100 in the presence and absence of S9 using the plate incorporation method, the constituent *beta*-phellandrene was not mutagenic at concentrations of 0.5-50 µg/mL and 10-1000 µg/mL (Barhdadi et al., 2021). In another corroborative reverse mutation assay, the same constituent was tested at concentrations of 0.16%, 0.8%, 4%, 20% and 100% in corn oil in *S. typhimurium* TA97, TA98, TA100 and TA102 with and without S9 using the preincubation method and was mutagenic at 20-100% in TA98 and TA100, as well as at 100% in TA97 and TA102, in the absence of S9. In the presence of S9, mutagenicity was reported at 4% in TA97 and TA100 as well as at 4% and 20% in TA98. However, the Expert Panel noted several limitations of this study including lack of clarity if the test concentrations are done according to OECD guideline 471 (OECD, 2020). Under the assumption that the tested concentrations were 0, 8, 40, 200, 1000 and 5000 µg/plate, these represent a wide range of test concentrations more appropriate for a range finder assay than a mutagenicity assay. Additionally, the study does not indicate what substances were used as the positive, negative and vehicle controls, and thus the validity of the results of this assay is questionable. Corroborative evidence from an OECD 487 guideline-compliant *in vitro* micronucleus assay indicated the same constituent induced a statistically significant increase in micronuclei only at the highest tested concentration in CHO-K1 cells incubated with 27-2724 µg/mL for 4 hours in the presence of S9, but not for 24 hours in the absence of S9 at concentrations 14-681 µg/mL. The authors considered the test substance to be weakly positive (Barhdadi et al.,

2021). Additional corroborative evidence for the same constituent, *beta*-phellandrene showed no induction of chromosomal aberrations in Chinese hamster lung cells at concentrations of 16-164 µg/mL in the absence of S9 for 24 or 48 hours, and at concentrations of 41-409 µg/mL for 6 hours with a 24- or 48-hour recovery period (Cheng et al., 2017). Additionally, corroborative evidence is also available for the same constituent in a combined *in vivo* micronucleus and comet assay in female ICR mice at approximately 713, 1425 or 2825 mg/kg bw by gavage, in which no significant micronuclei induction was reported (Cheng et al., 2017). However significant increases in %tail DNA, tail moment and olive tail moment ([tail mean-head mean] x % of DNA in the tail) were observed at the mid-dose and a significant increase in Comet rate was observed at the mid- and high-doses. The Expert Panel noted that in this study, standard deviations reported for all metrics (% tail DNA, tail moment, olive tail moment) are extremely large and often larger than the reported value. Additionally, the authors do not clearly define or explain what the "Comet rate" is. Based on the limitations noted with the Ames and combined *in vivo* micronucleus and comet assay, the Expert Panel considered these studies for the constituent *beta*-phellandrene to be of limited relevance to the safety evaluation of Sichuan pepper extract (*Zanthoxylum armatum*) as a flavor ingredient. Corroborative evidence is available from the related preparation *Zanthoxylum piperitum* essential oil (18% *d*-limonene, 15.3% geranyl acetate, 8.5% cryptone, 7.1% citronellal, 6.2% cuminal and 5.2% phellandral) which was not mutagenic in an Ames assay with *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA in the presence and absence of S9, and did not induce chromosomal damage when tested in Chinese hamster lung (CHL) for 6 hours in the presence and absence of S9 as well as for 22 hours in the absence of S9 (Hwang and Kim, 2011). No induction of micronuclei was observed in a corroborative *in vivo* micronucleus assay in ICR mice (6/sex/group) orally administered the same related preparation at 250, 500 or 1000 mg/kg bw/day for 2 days (Hwang and Kim, 2011). The Expert Panel noted that while there are positive Ames and *in vivo* Comet assay data, as well as weakly positive *in vitro* for the constituent *beta*-phellandrene, due to questionable study methodology, other negative *in vitro* and *in vivo* genotoxicity data as well as the extensive natural occurrence of the constituent indicating greater exposure of the constituent from food than as a constituent of Sichuan pepper extract (*Zanthoxylum armatum*) used as a flavor ingredient, the Expert Panel did not identify a concern for the genotoxicity of this constituent or for Sichuan pepper extract (*Zanthoxylum armatum*).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *Persea americana* oil hydrolyzed fraction (CAS 1039550-44-1) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4991) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *Persea americana* oil hydrolyzed fraction from use as a flavor ingredient to be 208 µg/person/day. Corroborative evidence for the low toxicity potential of *Persea americana* oil hydrolyzed fraction was evaluated by the Expert Panel from a randomized, double-blind, placebo-controlled clinical trial of the related preparation Avocatin B (a 1:1 mixture of the constituents avocadene:avocadyne) provided to humans (n=6-10/group) at 0, 50 or 200 mg/day for 60 days (equivalent to approximately ¼ or 1 whole Hass avocado, respectively). Only minor symptoms were reported in the treatment groups and no significant differences were observed between the treatment groups, and the authors concluded that there was no "dose-limiting toxicity" (Ahmed et al., 2019). Corroborative evidence is also available from Balb/c mice administered the same related preparation at 0 or 100 mg/kg by gavage twice a week for 5 weeks after consumption of a high fat diet (60% lard) for 8 weeks showed improved insulin sensitivity and protection against lipotoxicity in pancreatic β-cells (Ahmed et al., 2019). Additional corroborative evidence is available from a 28-day repeated dose toxicity study in which rats of an unspecified strain (4/group) were orally administered up to 10,000 mg/kg of a related preparation of an aqueous extract of the avocado seeds (aqueous filtrate of powdered seeds soaked for 24 hours upon being chopped and dried for 5 days) (Ozolua et al., 2009). A significant increase in plasma protein concentration was observed in treated animals compared to the controls. A full battery of hematological and clinical chemistry variables was not provided. The control group had a 4.7% increase in bodyweight and a slight, but insignificant decrease in bodyweight was observed in the treatment group. The Expert Panel reviewed the key constituents of *Persea americana* oil hydrolyzed fraction and noted that the congeneric group intakes were below the respective TTC thresholds. The material is produced from the whole fruits and seeds of *Persea americana* Mill (Lauraceae). Some preparations of the source material are consumed as food, and based on the quantitative data, a consumption ratio of greater than 757,000 could be calculated (Stofberg and Grundschober, 1987). The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). The Expert Panel concluded that metabolic data exist for a representative member of the principal congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be expected to be metabolized primarily by well-established metabolic pathways to innocuous products (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of *Persea americana* oil hydrolyzed fraction (Gooderham et al., 2020; Cohen et al., 2018). Corroborative evidence from an OECD 471 guideline and GLP- compliant bacterial reverse mutation assay indicates that *Persea americana* oil hydrolyzed fraction was not mutagenic at concentrations up to 5000 µg/plate in a plate incorporation assay in *S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvrA pKM101 as well as at concentrations up to 1600 µg/plate in the same *S. typhimurium* strains and at concentrations up to 5000 µg/plate in the same *E. coli* strain in a preincubation assay, in the presence and absence of S9 (Mee, 2020). Corroborative evidence also indicates *Persea*

americana oil hydrolyzed fraction was non-genotoxic in a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay when tested in human lymphocytes at concentrations up to 2000 µg/mL for three hours in the presence and absence of S9 and for 24 hours in the absence of S9 (Clare, 2020). Additional corroborative evidence is available from a bacterial reverse mutation assay in which the related preparation, Avosafe (approximately 50% each of the constituents avocadene and avocadyne) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA (pKM101) in the presence and absence of S9 (Rodriguez-Sanchez et al., 2019). Corroborative evidence is also available from an *in vivo* micronucleus assay in Balb/c mice provided 0 or 250 mg/kg of the same related preparation where no significant induction of micronuclei was observed (Padilla-Camberos et al., 2013).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding rubusosides enriched glucosylated steviol glycosides (CAS 1225018-62-1) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4992) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake of rubusosides enriched glucosylated steviol glycosides from use as a flavor ingredient to be 6918 µg/person/day. Corroborative evidence for the low toxicity potential of rubusosides enriched glucosylated steviol glycosides was evaluated by the Expert Panel from a 108-week carcinogenicity study for stevioside in which no carcinogenic effects were observed (Toyoda et al., 1997). Corroborative evidence is also available from a 2-year feeding study in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 4,000 times the anticipated daily *per capita* intake of rubusosides enriched glucosylated steviol glycosides from use as a flavor ingredient. Additional corroborative evidence is available from a 52-week chronic toxicity study in which Beagle dogs (4/sex/group) were provided 0, 6200, 12500 or 50000 ppm of *beta*-cyclodextrin (Bellringer et al., 1995). The dietary concentrations correspond to actual intakes of 229, 456 or 1831 mg/kg bw/day and 224, 476 or 1967 mg/kg bw/day in male and female dogs, respectively (Bellringer et al., 1995). There were no toxicologically significant findings, and a NOAEL was established at the top dose (1831 and 1967 mg/kg bw/day for male and female dogs, respectively). This NOAEL is greater than 15,000 times the anticipated daily per capita intake of rubusosides enriched glucosylated steviol glycosides from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana*. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al., 2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997; Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; BeMiller, 2003; JECFA, 1982). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of studies. Based on the corroborative evidence noted below for the various steviol glycosides, the Expert Panel did not identify specific concerns related to the genotoxicity of rubusosides enriched glucosylated steviol glycosides (Gooderham et al., 2020). Corroborative evidence indicates that while some positive results are reported in *in vitro* mutagenicity assays, *in vivo* studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding methyl 3-methyl-2-buten-1-yl disulfide (CAS 34776-60-8) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4993) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic and aromatic sulfides and thiols (JECFA, 2000, 2004, 2008, 2011; SLR, B4). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of methyl 3-methyl-2-buten-1-yl disulfide from use as a flavor ingredient to be 0.1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of methyl 3-methyl-2-buten-1-yl disulfide was evaluated by the Expert Panel from a GLP- and OECD 408 guideline-compliant 90-day oral toxicity study for the structural relative methyl propyl trisulfide (FEMA 3308) (Purity: 57% methyl propyl trisulfide, 32% dipropyl trisulfide, 6.4% dipropyl disulfide, and 4.3% methyl isopropyl tetrasulfide) which was administered to Sprague-Dawley rats (10/sex/dose) by gavage at 0, 0.5, 2 and 6 mg/kg bw/day. No toxicologically significant findings were observed, and a NOAEL of 6 mg/kg bw/day was established (Bastaki et al., 2018). This NOAEL is 3,000,000 times the anticipated daily *per capita* intake of methyl 3-methyl-2-buten-1-yl disulfide from use as a flavor ingredient. The substance occurs naturally in beer (Natural Occurrence Analysis, 2021c). Based on the quantitative data, a consumption ratio of 155 could be calculated (Stofberg and Grundschober, 1987). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. It is presumed that methyl 3-methyl-2-buten-1-yl disulfide will be reduced either enzymatically by GSH reductase or thioltransferases, or reduced chemically by exchange with GSH, thioredoxin, cysteine or other endogenous thiols to yield 3-methyl-2-butenyl thiol (Waring, 1996; Wells et al., 1993). This simple thiol could undergo oxidation to yield the corresponding sulfenic acid, sulfinic acid and ultimately, sulfonic acid. The sulfinic and sulfonic acids are water soluble and easily excreted. Alternatively, the thiol that is formed may react with

glutathione and cysteine to form mixed disulfides that can then undergo reduction and oxidative desulfuration, or oxidation to sulfonic acid via the intermediate thiosulfinate and sulfinic acid (McBain and Menn, 1969; Dutton & Illing, 1972; Maiorino et al., 1989; Richardson et al., 1991). There are also several possible thiol-disulfide exchange reactions that may occur (Cotgreave et al., 1989). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of methyl 3-methyl-2-buten-1-yl disulfide (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a two-strain screening bacterial reverse mutation assay, where methyl 3-methyl-2-buten-1-yl disulfide was not mutagenic at concentrations up to 150 µg/plate in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 metabolic activation (Kino, 2020d). Similarly, corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from the structural relatives *bis*(3-methyl-2-butenyl) disulfide (FEMA 4914), allyl disulfide (FEMA 2028) and dimethyl disulfide (FEMA 3536) which were not mutagenic in bacterial reverse mutation assays in *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, TA1538 and/or *E. coli* strain WP2 uvrA in the presence or absence of S9 metabolic activation (Kino, 2017b; Eder et al., 1980; Aeschbacher et al., 1989; ECHA, 1985a,b, 2007). Additionally, corroborative evidence for the lack of genotoxic potential for methyl 3-methyl-2-buten-1-yl disulfide was evaluated by the Expert Panel in an *in vitro* chromosome aberration assay and an *in vitro* sister chromatid exchange assay for the structural relative allyl disulfide (FEMA 2028) at concentrations of 2-25 µg/mL and 2-10 µg/mL, respectively, in Chinese Hamster Ovary (CHO) cells (Musk et al., 1997). Significant induction of chromosome aberrations at concentrations of 10 µg/mL and above in the absence of S9 and in a non-dose responsive manner at concentrations of 4-25 µg/mL in the presence of S9, as well as a significant induction of sister chromatid exchanges at all tested concentrations in the presence and absence of S9, were observed (Musk et al., 1997). However, these effects were observed at concentrations that induced greater than 50% cytotoxicity in CHO cells and thus appear related to toxicity rather than truly positive results. The *in vitro* sister chromatid exchange assay has been removed from the OECD reference library due to a lack of evidence that this assay is predictive of a heritable mutagenic event (OECD, 2017). Corroborative evidence for the lack of genotoxic potential for methyl 3-methyl-2-buten-1-yl disulfide was evaluated by the Expert Panel in the following *in vitro* and *in vivo* assays. Corroborative evidence from a mouse micronucleus assay indicated gavage administration of a mixture containing the structurally related flavor ingredients allyl sulfide (FEMA 2042), allyl disulfide (FEMA 2028) and diallyl trisulfide (FEMA 3265) (in a ratio of 68:20:12) did not increase the frequency of micronucleated polychromatic erythrocytes in bone marrow cells. The test mixture provided estimated doses of the structural relative allyl disulfide (FEMA 2028) of 48 and 98 mg/kg bw/day at the two concentrations tested (Marks et al., 1992). Corroborative evidence from a GLP- and OECD 473 guideline-compliant *in vitro* chromosome aberration assay in human lymphocytes incubated with the structural relative dimethyl disulfide (FEMA 3536) at concentrations up to 300 µg/mL either for 24h in the absence of S9 or for 2h in the presence of S9, significant induction of chromosomal aberrations was observed only at the highest concentration tested, which was cytotoxic (ECHA, 1990a). Corroborative evidence from a GLP- and OECD 476 guideline-compliant *in vitro* HPRT mammalian cell gene mutation assay in Chinese Hamster Ovary (CHO) cells incubated with the structural relative FEMA 3536 for 3 hours in the presence and absence of S9 at concentrations up to 1000 µg/mL showed no conclusive evidence of genotoxicity was found, as slight increases in the mutation frequency in the presence and absence of S9 were not concentration dependent (ECHA, 1990b). Corroborative evidence also showed no induction of unscheduled DNA synthesis was observed in rat hepatocytes treated with the structural relative FEMA 3536 at concentrations up to 200 µg/mL for 18-20h in a GLP- and OECD 482 guideline-compliant assay (ECHA, 1990c).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding gallic acid (CAS 149-91-7) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4994) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of gallic acid from use as a flavor ingredient to be 277 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low reproductive and developmental toxicity potential of gallic acid was evaluated by the Expert Panel from the following reproductive toxicity studies. In a corroborative FDA GLP-compliant gavage toxicity study from gestation days (GD) 6-20, pregnant rats (10/group) were provided deionized water placebo, or 21.6, 215, 430 or 860 mg/kg bw/day of a related preparation of a mixture of 80% gallic acid and 20% of a herbal supplement known as "NT" (40% rhubarb root and stem (*radix et rhizoma rhei*), 13.3% astragalus root (*radix astragali*), 13.3% red sage root (*radix rhizoma miltiorrhizae*), 26-27% turmeric (*rhizoma curcumae longae*) and 6-7% dried ginger (*rhizome zingiberis officinalis*)) (Booth et al., 2010). Significant decreases in bodyweight and relative gravid uterine weights were observed at the high-dose group on GD21. In corroborative studies, no other treatment-related adverse effects were observed. A reproductive NOAEL of 430 mg/kg bw/day was established for the related preparation. No reproductive or developmental effects were observed in fetuses when the structural relative propyl gallate (FEMA 2947) was administered to pregnant rats and mice (3-300 mg/kg bw), hamsters (2.5-250 mg/kg bw) and rabbits (2.5-250 mg/kg bw) by oral gavage on GD15, GD6-10 and for 13 consecutive days, respectively (FDRL, 1972, 1973a). In a corroborative study in which female Wistar rats (18-20/group) were provided the structural relative propyl gallate (FEMA 2947) in the diet at 0, 0.4, 1 and 2.5% (approximately 0, 350, 880 and 2040 mg/kg bw/day) through pregnancy, no teratogenic effects attributed to the administration of the structural relative propyl gallate (FEMA 2947) were observed (Tanaka et al., 1979). Increased fetal resorption rates (18.3%) were observed in a corroborative study in which Walter Reed-Carworth Farms rats were fed the structural

relative FEMA 2947 at 0.5g (approximately 100 mg/kg bw/day) during pregnancy relative to control animals (10.6%) (Telford et al., 1962). This low observed adverse effect level (LOAEL) of 100 mg/kg bw/day is greater than 20,000 times the anticipated daily *per capita* intake of gallic acid from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of gallic acid was evaluated by the Expert Panel from the following toxicity studies for the structural relative propyl gallate (FEMA 2947). In corroborative 13-week NTP subchronic dietary toxicity studies 0, 1500, 3000, 6000, 12500 or 25000 ppm of the structural relative propyl gallate (FEMA 2947) were provided to F344/N rats (10/sex/group) (approximately 0, 75, 150, 300, 625 or 1250 mg/kg bw/day) and to B6C3F1 mice (10/sex/group) (approximately 0, 225, 450, 900, 1875 or 3750 mg/kg bw/day) (NTP, 1982; FDA, 1993). All rats except for 1 female rat at the second-highest treatment level and all mice survived. Dose-dependent increases in feed consumption in rats, weight gain depressions of 10% or more in males at the top two treatment levels and in females at the top treatment level were recorded. Reddish duodenal mucosa (8/10 males; 6/10 females), thickened stomach wall (4/10 males; 2/10 females), necrosis and ulceration of the mucosal surface of the stomach, accompanied with moderate to severe granulomatous inflammatory responses in the submucosa and muscular wall (4/10 males; 1/10 females) were observed in rats at the highest treatment level. No treatment-related effects treated mice were observed. The top two dietary concentrations were selected for a follow-up NTP carcinogenicity study in rats and mice. In follow-up corroborative 103-week NTP dietary carcinogenicity studies of the structural relative propyl gallate (FEMA 2947) at 6000 or 12000 ppm in F344 rats (50/sex/group) (approximately 300 or 600 mg/kg bw/day) and in B6C3F1 mice (50/sex/group) (approximately 900 or 1800 mg/kg bw/day), statistically significant increases in tumors of the preputial gland, pancreatic islet cells and adrenal gland in rats at the lowest concentration, increased thyroid follicular cell tumors in high-dose male rats, brain tumors in two female rats at the lowest test concentration and significant increases in liver adenomas in female mice at the highest test concentration were within historical controls as well (NTP, 1982; Abdo et al., 1983, 1986). The Expert Panel agreed with the NTP conclusion that the structural relative propyl gallate (FEMA 2947) was not carcinogenic to rats or mice. In a review of corroborative repeat-dose toxicity studies of the structural relative propyl gallate (FEMA 2947) conducted by BIBRA, mortalities and adverse effects were observed in the stomach, kidneys, forestomach and prostate of rats provided 1-5% in the diet (approximately 500-2500 mg/kg bw/day) (BIBRA, 1989; EFSA, 2014; FDA, 1993). No adverse effects on growth, liver and adrenal glands were reported in rats given 100-250 mg/kg bw/day of the structural relative propyl gallate (FEMA 2947) for 6 or 26 weeks (BIBRA, 1989; EFSA, 2014; FDA, 1993). Various studies of different durations in rats administered 100 mg/kg bw/day of the structural relative propyl gallate (FEMA 2947) resulted in changes of enzymatic activity in the lungs, intestines and livers along with increased organ weight and fatty changes of the liver (BIBRA, 1989; EFSA, 2014; FDA, 1993). No effects on growth, survival or microscopic treatment-related effects in major tissues were observed in rats (approximately 30/sex/group) administered 1-2 mg/kg bw/day of the structural relative propyl gallate (FEMA 2947), alone or as a constituent of an antioxidant mixture (den Tonkelaar et al., 1968). In a corroborative study, after gavage administration of 50, 100, 200 or 500 mg/kg bw/day of the structural relative propyl gallate (FEMA 2947) in SPF Carworth rats (4-12/sex/group) for 7 days, extensive fatty changes in the liver observed at the high-dose at 24h were reduced after a 14-day recovery period and returned to normal after 28 days in all treated rats except one treated female (Feuer et al., 1965). Increased abnormal mitotic figures in hepatocytes observed in all treated doses were still present after 14 days but not after 28 days. SPF Wistar RIVM:Tox rats (10/sex/group) were provided 0, 35, 135 or 527 mg/kg bw/day of the structural relative propyl gallate (FEMA 2947) in the diet for 13 weeks in a corroborative study (Speijers et al., 1993; EFSA, 2014). Decreases in hematological parameters, decreased extramedullary hematopoiesis in the spleen and decreased incidence of nephrocalcinosis and liver microsomal activity of ethoxy-resorufin-*O*-deethylase (EROD) were observed at the highest tested concentration. Increased glucuronyl-transferase and glutathione-*S*-transferase activities were observed at the top two concentrations. A NOAEL of 135 mg/kg bw/day was established for the structural relative propyl gallate (FEMA 2947). In another corroborative study, albino mice (25/sex/group) received the structural relative propyl gallate (FEMA 2947) at 0, 0.5 and 1.0% in the diet for 21 months (approximately 750 or 1500 mg/kg bw/day) and additional mice (25/sex/group) were maintained for the control and 1.0% groups (Dacre, 1974; FDA, 1993). No treatment-related adverse effects were observed, and a NOEL of 1500 mg/kg bw/day was established for the structural relative propyl gallate (FEMA 2947). In two 28-day studies conducted in Swiss albino mice, no mortalities or adverse effects were observed in 6- to 8-week old mice (6/sex/dose) orally administered 0 or 1000 mg/kg bw/day of gallic acid, or in 3-month old mice (5/sex/dose) at 0, 100, 300 or 900 mg/kg bw/day of gallic acid administered by gavage (Rajalakshmi et al., 2001; Variya et al., 2019). In a 13-week dietary toxicity study, F344 rats (15/sex) were provided with 0, 0.2, 0.6, 1.7 and 5% of gallic acid (approximately 0, 100, 300, 850 and 2500 mg/kg bw/day, respectively) (Niho et al., 2001; FDA, 1993). Significantly lower bodyweights and/or bodyweight gains at the top two treatment levels were observed. Hematological changes in males treated with 0.6% or more of gallic acid and 1.7% and above in treated females were observed. Significant changes in blood urea nitrogen and creatinine levels at the top two treatment levels, non-sex and non-dose dependent changes in blood biochemistry parameters in all treated rats, significantly increased potassium levels in males of the top three treatment groups and females of the top two treatment groups, extramedullary hematopoiesis, hemosiderin deposition and congestion in the spleen were observed. These were accompanied with increased absolute and relative spleen weights as well as fine, granular brown pigment in the proximal tubular cells in the kidney accompanied with berlin blue-negative brown pigment deposition in the proximal tubular epithelium of rats at the highest tested concentration. Dose-dependent centrilobular liver cell hypertrophy in the livers of rats at the top two treatment levels accompanied by increased relative liver weights at the 1.7% treatment group were observed. The authors considered the hematological and biochemical findings to be indicative of anemia and attributed the liver findings to the induction of *O*-methyltransferase and other enzymes. A NOAEL at 0.2%, calculated by the authors to be

approximately 119 and 128 mg/kg bw/day for male and female rats, respectively, was established. The NOAEL of 119 mg/kg bw/day for gallic acid was greater than 23,000 times the anticipated daily *per capita* intake of gallic acid from use as a flavor ingredient. This material occurs naturally in apple brandy, beer, armagnac (French brandy), bourbon whiskey, Canadian whiskey, cognac (French brandy), grape brandy, Irish whiskey, malt whiskey, mulberry (*Morus* spp.), red and white wines, rum, Scotch blended whisky, sour cherry (*Prunus cerasus* L.) and sweet cherry (*Prunus avium* L.) (Van Dongen et al., 2024). Based on the quantitative data, a consumption ratio of 12 could be calculated (Stofberg and Grundschober, 1987). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. Based on dietary, oral or i.p. administrations of up to 500 mg/kg bw of gallic acid in rats and up to 150 mg/kg bw of gallic acid in rabbits (FDA, 1993; Booth et al., 1959; Scheline, 1966; Zong et al., 1999), as well as oral administrations of approximately 250 mg/kg bw of the structural relative propyl gallate (FEMA 2947) in rats, gallic acid is expected to undergo rapid absorption and metabolism in the GI tract followed by excretion in the urine as the parent compound or as its main metabolite, 4-*O*-methylgallic acid (4-OmeGA) (Yang et al., 2020; Shahrzad and Bitsch, 1998; Abd et al., 2002; Ferruzzi et al., 2009). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of gallic acid (Gooderham et al., 2020). Gallic acid was not mutagenic in Ames assays at concentrations up to 3000 µg/plate in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 (Rashid et al., 1985; Wang and Klemencic, 1979; Chen and Chung, 2000), and at 15 µmol/plate (approximately 2552 µg/plate) in *S. typhimurium* TA1527 in the presence and absence of S9 (Wang and Klemencic, 1979). In a NTP Ames assay, gallic acid was not mutagenic at concentrations of 100-10,000 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537 in the presence and absence of S9, with the exception of equivocal or weakly positive results reported in one or more cultures of TA100 in the presence and absence of S9, in TA98 in the presence of S9, in TA1537 in the presence of S9 and in TA1535 in the absence of S9 (NTP, 2018b; Haworth et al., 1983). In a second NTP assay, gallic acid was not mutagenic at concentrations of 100-6666 µg/plate in the same tester strains in the presence and absence of S9 with the exception of one equivocal response in one culture of TA100 in the absence of S9 (NTP, 2018b; Haworth et al., 1983). No significant induction of micronuclei was observed in WIL2-NS cells treated with 1 or 10 µM of gallic acid (approximately 2 and 20 µg/mL, respectively) for 60 minutes (Sugisawa et al., 2004). Significant DNA damage was observed at the highest tested concentration in a corroborative *in vitro* comet assay of human lymphocytes treated with concentrations up to 100 µg/mL of gallic acid, but not when treated with the structural relative propyl gallate (FEMA 2947) at concentrations up to 100 µg/mL (Wu et al., 2004). No significant induction of micronuclei, DNA damage or %Comet was observed in Swiss albino mice (3 females + 2 males/group) orally administered 100, 200 and 400 mg/kg bw/day of gallic acid for 5 consecutive days in a bone marrow micronucleus assay, peripheral blood micronucleus assay and Comet assay (Shruthi and Shenoy, 2020). Additional evidence for the lack of genotoxic potential for gallic acid was evaluated by the Panel from corroborative *in vitro* and *in vivo* genotoxicity assays for the structural relative propyl gallate (FEMA 2947). The structural relative propyl gallate (FEMA 2947) was not mutagenic in *S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2 (uvrA) in several corroborative bacterial reverse mutation assays in the presence and absence of S9, including in an NTP reverse mutation assay (Ishidate et al., 1984; Shelef and Chin, 1980; Mortelmans et al., 1986; Chen and Chung, 2000; Rosin and Stich, 1981; Kawachi et al., 1980). In another corroborative Ames assay, the structural relative FEMA 2947 was not mutagenic in *S. typhimurium* TA97 at concentrations up to 100 µg/plate in the presence and absence of S9 but was weakly mutagenic at 100 µg/plate in TA102 in the presence and absence of S9 (Fujita, et al., 1988). The structural relative FEMA 2947 was non-clastogenic in corroborative *in vitro* chromosome aberration and sister chromatid exchange assays in human embryo fibroblasts as well as a corroborative *in vivo* rat bone marrow chromosome aberration assay (Kawachi et al., 1980) but was clastogenic in a corroborative *in vitro* chromosome aberration assay in hamster lung fibroblast (CHL) cells in the absence of S9 (Ishidate et al., 1984). In corroborative assays for the structural relative FEMA 2947, significant induction of chromosome aberrations was reported at 20-40 µg/mL in CHL cells incubated for 24h and 48h in the presence and absence of S9 (Ishidate et al., 1984), at 5-50 µg/mL in CHO cells in the absence of metabolic activation in an NTP assay (Gulati et al., 1989) and at 531-3183 µg/mL in CHO-K1 cells treated for 3 hours in the presence of S9 and approximately 1061-1592 µg/mL and above in the absence of S9 (Tayama and Nakagawa, 2001), but not in diploid human embryo fibroblast HE 2144 cells treated at concentrations of 2.1 and 21.2 µg/mL (Sasaki et al., 1980). These corroborative assays determined the frequency of chromosome aberrations with 100-200 metaphases per test concentration. The OECD guideline for this assay recommends scoring at least 300 well-spread metaphases for appropriate statistical analyses, though note that the number of metaphases may be reduced if the test substance is clearly positive (OECD, 2016). In corroborative assays for the structural relative FEMA 2947, significant induction of sister chromatid exchanges was observed in CHO cells treated at concentrations of 5-50 µg/mL and in CHO-K1 cells treated for 3h at concentrations of 531-3183 µg/mL in the presence and absence of S9 (Tayama and Nakagawa, 2001), but not at 2.1 and 21.2 µg/mL in diploid human embryo fibroblast HE 2144 cells (Sasaki et al., 1980). However, the sister chromatid exchange assay is no longer considered by the OECD to be a relevant assay for the detection of heritable mutagenic events (OECD, 2017). In the corroborative assay conducted using CHO-K1 cells, dose-dependent increases of endoreduplicated cells were observed (Tayama and Nakagawa, 2001). In at least one experiment of a corroborative assay, the structural relative FEMA 2947 significantly induced micronuclei at 5 and 7 µg/mL in V79 cells, at 23.5 and 48 µg/mL in CHL cells, 5.6-13.3 µg/mL in CHO cells, 4.2-31.6 µg/mL in TK6 cells, but not at 40-225 µg/mL in human lymphocytes, treated for 3h with a 21h recovery period in the absence of S9 (Fowler et al., 2012). Significant induction was also observed at 1000 µg/mL in HepG2 cells treated for 3h with a 21h recovery period in the absence of S9, however less than 200 cells were scored

(Fowler et al., 2012). The high levels of cytotoxicity in all cell lines (as much as 50% toxicity at concentrations below 50 µg/mL) noted by the study authors could have resulted in false positives (Fowler et al., 2012). In a corroborative NTP mouse lymphoma assay in cultures of L5178Y cells treated with the structural relative propyl gallate (FEMA 2947) at concentrations up to 1000 µg/mL for 4h in the absence of S9, significant mutagenicity was observed at all tested concentrations which were highly cytotoxic (McGregor et al., 1988).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding rebaudioside N 95% (CAS 1220616-46-5) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4995) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of rebaudioside N 95% from use as a flavor ingredient to be 55 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of rebaudioside N 95% was evaluated by the Expert Panel from a 108- week carcinogenicity study for stevioside, which showed no carcinogenic effects were observed (Toyoda et al., 1997). Additional corroborative evidence for the low toxicity potential of rebaudioside N 95% was evaluated by the Expert Panel from a 2-year feeding study, in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 600,000 times the anticipated daily *per capita* intake of rebaudioside N 95% from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana* leaves. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al., 2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997; Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; JECFA, 1982). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of rebaudioside N 95% (Gooderham et al., 2020). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of studies. While some positive results are reported in *in vitro* mutagenicity assays, *in vivo* studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding (2*S*)-7-(*beta*-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one (CAS 14982-11-7) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4996) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of (2*S*)-7-(*beta*-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of (2S)-7-(beta-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-1-benzopyran-4-one was evaluated by the Expert Panel from toxicity studies for structural relatives, neohesperidin dihydrochalcone (FEMA 3811) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715), summarized below. Weanling rats (5/sex) were provided 0-0.128% of the structural relative neohesperidin dihydrochalcone (FEMA 3811) (approximately 0-128 mg/kg bw/day) in the diet for 90 days (Booth et al., 1965; FDA, 1993). High mortality of the pups was attributed to an inadequate diet rather than treatment-related conditions. No adverse effects on reproductive performance were observed. No adverse effects or treatment-related mortalities were observed in a follow-up study of the structural relative FEMA 3811 using the same protocol at 0.5% in the diet of weanling rats (approximately 500 mg/kg bw/day) for 70 days before mating (Booth et al., 1965; FDA, 1993). SPF Fischer rats were provided 0, 0.5, 2.5 and 5.0% of the structural relative FEMA 3811 (0, 500, 2500 and 5000 mg/kg bw/day, respectively) in the diet in a three-generation reproduction and teratogenicity study (Booth, 1974; Gumbmann et al., 1978; FDA, 1993). Slight decreases in fetal survival in the mid- and high-intake levels were observed in the third generation. Apart from a few abnormalities, no evidence for the teratogenicity of the test substance was observed in the study. In a GLP- and OECD 414 guideline-compliant prenatal developmental toxicity study, mated female Wistar Crl:(WI)WU BR rats (28/group) were provided 0, 1.25%, 2.5% or 5% of the structural relative FEMA 3811 (approximately 0, 800-900, 1600-1700 or 3100-3400 mg/kg bw/day, respectively) in the diet until GD 21 (Waalkens-Berendsen et al., 2004). The NOAEL for maternal toxicity, fetotoxicity, embryotoxicity or teratogenicity according to the authors corresponded to the top dose level of 3100 mg/kg bw/day. This NOAEL is greater than 13,000,000 times the anticipated daily *per capita* intake of (2*S*)-7-(*beta*-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one from use as a

flavor ingredient. In an OECD 408 guideline-compliant 90-day dietary administration study of the structural relative 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715) in Sprague-Dawley rats (10/sex/group) that provided mean dietary daily intakes of 241, 487 and 968 mg/kg bw/day and 244, 491 and 983 mg/kg bw/day for males and females, the NOAEL was determined to be 968 and 983 mg/kg bw/day for males and females, respectively (Bauter, 2018). In a two-year dietary toxicity study in SPF Fischer rats (24/sex/group) that were provided the structural relative FEMA 3811 at 0, 0.5, 2.5 and 5.0% of the diet (approximately 0, 250, 1250 and 2500 mg/kg bw/day) (Gumbmann et al., 1978; FDA, 1993), lower body weight gains in high-intake males in the first ten weeks and high-intake females by the 60th week were recovered at 100 weeks after supplementation with 3% USP salts XIV (fortified with 40 ppm zinc and 3 ppm cobalt) and 3% additional brewers dried yeast at the expense of cornmeal in half of the high-intake group starting on Day 430. High mortalities in the high-intake group (50% survival) and control group (66% survival) were observed at the 100th week. Significantly increased plasma ornithine-carbamyl transferase (OCT) levels in treated rats were not reproduced in a separate 11-month feeding study of the structural relative FEMA 3811 at 10% of the diet. Decreased plasma cholesterol levels in high-intake rats were similar to the effects observed in the 11-month study. The authors attributed this to the intestinal fermentation of the test substance. Still, they noted that a different mode of action could not be excluded, based on the similar effects observed from other fermentable food constituents and bioflavonoids. Higher relative male kidney and liver weights and all organ weights in females were observed in the high-intake group with a non-supplemented diet. The organs of high-intake rats provided the supplemented diet had relative weights comparable to the controls and lower intake treatments, except thyroid weights which were lower than both control and other treatment groups but still within the expected normal range. Except for the supplemented high-intake group, diffuse thyroid follicular hyperplasia and hypertrophy were observed in all other control and treated rats. Increased focal cortical kidney atrophy incidence was observed in low-intake females, mid-intake rats, and non-supplemented high-intake rats compared to the controls and supplemented high-intake rats. The incidence of tumors in rats over 18 months of age did not reveal any treatment-related differences between the different experimental groups. Young beagle dogs (3/sex/group) were provided the structural relative FEMA 3811 in the diet for two years at levels of 0, 200, 1000 and 2000 mg/kg bw/day (Gumbmann et al., 1978). Slightly increased plasma alkaline phosphatase levels in high-intake males at 12, 18 and 24 months were not observed in high-intake females and were not accompanied with changes in other plasma enzymes or histology. Decreased plasma thyroxine concentrations in high-intake females were observed from 6 months onwards. Non-significant increased absolute and relative liver weights were observed in high-intake males and females. Decreased relative testes weights observed in one dog in the mid- and high-intake groups were accompanied by testicular atrophy and degeneration. Increased absolute and relative thyroid weights in high-intake males and females were accompanied by mild thyroid hypertrophy and hyperplasia (2/3 per sex) and increased thyroid follicular epithelium diameter with little alterations to thyroid architecture. The most severely affected high-intake dog exhibited increased cell diameter and architectural changes, including mild follicular epithelium folding and reduced follicular size. However, the authors acknowledge that the number of animals per tested intake level was too low to draw firm conclusions about the degree these results represent reversible, adaptive metabolic responses to high intakes of the test substance. Additionally, in the absence of historical data, the Expert Panel noted that the authors were unclear on the relevance of testicular atrophy in 1 of 3 mid- and high-intake dogs each. In a 90-day dietary toxicity study followed by a reproductive toxicity study for a total study period of 148 days, weanling rats of an unspecified strain (5/sex/group) were provided 0, 0.00064, 0.0064, 0.064 or 0.128% of the structural relative FEMA 3811 (approximately 0, 0.64, 6.4, 64 and 128 mg/kg bw/day) (Booth et al., 1965; FDA, 1993). Decreased mean body weights in high-intake males and all treated females were comparable at all doses by the end of the reproductive phase except for high-intake males. Decreased red and white blood cell counts and hemoglobin levels in treated females were not considered toxicologically significant. In a follow-up 90-day dietary toxicity study, six female weanling rats were provided 0.128% of the structural relative FEMA 3811 (approximately 128 mg/kg bw/day) in the diet at a different basal ration (using Purina laboratory chow) than the previous study to avoid liver lipidosis (Booth et al., 1965). No other effects were observed apart from decreased mean body weight gains in treated rats. In a third study, no significant effects were observed in 5 male and 20 female weanling rats provided 0.5% of the structural relative FEMA 3811 (approximately 500 mg/kg bw/day) in the diet for 70 days followed by mating, and until Day 92 and Day 113-140 in males and females, respectively, after mating. These studies included parallel arms of treated animals with naringin dihydrochalcone, naringin and hesperidin (Booth et al., 1965). Marginal effects on body weight and food consumption, cecal enlargement and some clinical chemistry variables of high-dose animals observed in a 91-day dietary toxicity study of Wistar rats (20/sex/group) fed the structural relative neohesperidin dihydrochalcone (FEMA 3811) at 0, 0.2, 1.0, and 5.0% (approximately 0, 150, 757, and 4011 mg/kg bw/day and 0, 166, 848 and 4334 mg/kg bw/day in male and female rats, respectively) were not considered to be toxicologically significant. The study authors determined the NOAEL to be 1.0% or 750 mg/kg bw/day (Lina et al., 1990). This NOAEL is greater than 3,700,000 times the anticipated daily *per capita* intake of (2*S*)-7-(*beta*-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). It is presumed that (2*S*)-7-(*beta*-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one is expected to be metabolized similarly to other dietary flavonoids (Day et al., 1998; Hollman and Katan, 1997, 1999; Walle et al., 2005). Before entering the bloodstream, the substance is expected to undergo metabolism forming sulfates, glucuronide conjugates and/or methylated metabolites. Upon entry into the bloodstream the metabolites are expected to be further transformed in

metabolic processes in the liver. Metabolites not absorbed in the small intestine are expected to undergo further metabolism by the microflora in the large intestine. The microflora is expected to cleave the conjugates and the resulting aglycones are expected to undergo ring fission leading to phenolic acid and cinnamic acid derivatives. These metabolites are expected to be absorbed and ultimately excreted in the urine (Smith et al., 2018). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of (2*S*)-7-(*beta*-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one (Gooderham et al., 2020). The structural relatives neohesperidin dihydrochalcone (FEMA 3811) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715) were not mutagenic in several Ames assays in *S. typhimurium* TA98, TA100, TA1535, TA1536, TA1537 or TA1538 in the presence and absence of S9 (Batzinger and Bueding, 1977; MacGregor & Jurd, 1978; Brown et al., 1977; MacGregor, 1979; Brown & Dietrich, 1979; Nagao et al., 1981; Zeiger et al., 1987). No treatment-related induction of micronuclei was observed in the bone marrow of Swiss-Webster mice (6/dose) administered doses of 200, 500, 1000 and 5000 mg/kg bw of the structural relative neohesperidin dihydrochalcone (FEMA 3811) by oral gavage in 2% acacia (gum arabic) in water at 30 hours and 6 hours before sacrifice (MacGregor, 1979; MacGregor et al., 1983). Significant induction of micronuclei observed at 500 mg/kg bw due to a single mouse was not observed in a repeat experiment at the same dose in 6 additional mice.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one (CAS 2864335-29-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4997) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one was evaluated by the Expert Panel from toxicity studies for structural relatives, neohesperidin dihydrochalcone (FEMA 3811) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715), summarized below. Weanling rats (5/sex) were provided 0-0.128% of the structural relative neohesperidin dihydrochalcone (FEMA 3811) (approximately 0-128 mg/kg bw/day) in the diet for 90 days (Booth et al., 1965; FDA, 1993). High mortality of the pups was attributed to an inadequate diet rather than treatment-related conditions. No adverse effects on reproductive performance were observed. No adverse effects or treatment-related mortalities were observed in a follow-up study of the structural relative FEMA 3811 using the same protocol at 0.5% in the diet of weanling rats (approximately 500 mg/kg bw/day) for 70 days before mating (Booth et al., 1965; FDA, 1993). SPF Fischer rats were provided 0, 0.5, 2.5 and 5.0% of the structural relative FEMA 3811 (0, 500, 2500 and 5000 mg/kg bw/day, respectively) in the diet in a three-generation reproduction and teratogenicity study (Booth, 1974; Gumbmann et al., 1978; FDA, 1993). Slight decreases in fetal survival in the mid- and high-intake levels were observed in the third generation. Apart from a few abnormalities, no evidence for the teratogenicity of the test substance was observed in the study. In a GLP- and OECD 414 guideline-compliant prenatal developmental toxicity study, mated female Wistar Crl:(WI)WU BR rats (28/group) were provided 0, 1.25%, 2.5% or 5% of the structural relative FEMA 3811 (approximately 0, 800-900, 1600-1700 or 3100-3400 mg/kg bw/day, respectively) in the diet until GD 21 (Waalkens-Berendsen et al., 2004). The NOAEL for maternal toxicity, fetotoxicity, embryotoxicity or teratogenicity according to the authors corresponded to the top dose level of 3100 mg/kg bw/day. This NOAEL is greater than 13,000,000 times the anticipated daily *per capita* intake of 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one from use as a flavor ingredient. In an OECD 408 guideline-compliant 90-day dietary administration study of the structural relative 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715) in Sprague-Dawley rats (10/sex/group) that provided mean dietary daily intakes of 241, 487 and 968 mg/kg bw/day and 244, 491 and 983 mg/kg bw/day for males and females, the NOAEL was determined to be 968 and 983 mg/kg bw/day for males and females, respectively (Bauter, 2018). In a two-year dietary toxicity study in SPF Fischer rats (24/sex/group) that were provided the structural relative FEMA 3811 at 0, 0.5, 2.5 and 5.0% of the diet (approximately 0, 250, 1250 and 2500 mg/kg bw/day) (Gumbmann et al., 1978; FDA, 1993), lower body weight gains in high-intake males in the first ten weeks and high-intake females by the 60th week were recovered at 100 weeks after supplementation with 3% USP salts XIV (fortified with 40 ppm zinc and 3 ppm cobalt) and 3% additional brewers dried yeast at the expense of cornmeal in half of the high-intake group starting on Day 430. High mortalities in the high-intake group (50% survival) and control group (66% survival) were observed at the 100th week. Significantly increased plasma ornithine-carbamyl transferase (OCT) levels in treated rats were not reproduced in a separate 11-month feeding study of the structural relative FEMA 3811 at 10% of the diet. Decreased plasma cholesterol levels in high-intake rats were similar to the effects observed in the 11-month study. The authors attributed this to the intestinal fermentation of the test substance. Still, they noted that a different mode of action could not be excluded, based on the similar effects observed from other fermentable food constituents and bioflavonoids. Higher relative male kidney and liver weights and all organ weights in females were observed in the high-intake group with a non-supplemented diet. The organs of high-intake rats provided the supplemented diet had relative weights comparable to the controls and lower intake treatments, except thyroid weights which were lower than both control and other treatment groups but still within the expected normal range. Except for the supplemented high-intake group, diffuse thyroid follicular hyperplasia and hypertrophy

were observed in all other control and treated rats. Increased focal cortical kidney atrophy incidence was observed in low-intake females, mid-intake rats, and non-supplemented high-intake rats compared to the controls and supplemented high-intake rats. The incidence of tumors in rats over 18 months of age did not reveal any treatment-related differences between the different experimental groups. Young beagle dogs (3/sex/group) were provided the structural relative FEMA 3811 in the diet for two years at levels of 0, 200, 1000 and 2000 mg/kg bw/day (Gumbmann et al., 1978). Slightly increased plasma alkaline phosphatase levels in high-intake males at 12, 18 and 24 months were not observed in high-intake females and were not accompanied with changes in other plasma enzymes or histology. Decreased plasma thyroxine concentrations in high-intake females were observed from 6 months onwards. Non-significant increased absolute and relative liver weights were observed in high-intake males and females. Decreased relative testes weights observed in one dog in the mid- and high-intake groups were accompanied by testicular atrophy and degeneration. Increased absolute and relative thyroid weights in high-intake males and females were accompanied by mild thyroid hypertrophy and hyperplasia (2/3 per sex) and increased thyroid follicular epithelium diameter with little alterations to thyroid architecture. The most severely affected high-intake dog exhibited increased cell diameter and architectural changes, including mild follicular epithelium folding and reduced follicular size. However, the authors acknowledge that the number of animals per tested intake level was too low to draw firm conclusions about the degree these results represent reversible, adaptive metabolic responses to high intakes of the test substance. Additionally, in the absence of historical data, the Expert Panel noted that the authors were unclear on the relevance of testicular atrophy in 1 of 3 mid- and high-intake dogs each. In a 90-day dietary toxicity study followed by a reproductive toxicity study for a total study period of 148 days, weanling rats of an unspecified strain (5/sex/group) were provided 0, 0.00064, 0.0064, 0.064 or 0.128% of the structural relative FEMA 3811 (approximately 0, 0.64, 6.4, 64 and 128 mg/kg bw/day) (Booth et al., 1965; FDA, 1993). Decreased mean body weights in high-intake males and all treated females were comparable at all doses by the end of the reproductive phase except for high-intake males. Decreased red and white blood cell counts and hemoglobin levels in treated females were not considered toxicologically significant. In a follow-up 90-day dietary toxicity study, six female weanling rats were provided 0.128% of the structural relative FEMA 3811 (approximately 128 mg/kg bw/day) in the diet at a different basal ration (using Purina laboratory chow) than the previous study to avoid liver lipidosis (Booth et al., 1965). No other effects were observed apart from decreased mean body weight gains in treated rats. In a third study, no significant effects were observed in 5 male and 20 female weanling rats provided 0.5% of the structural relative FEMA 3811 (approximately 500 mg/kg bw/day) in the diet for 70 days followed by mating, and until Day 92 and Day 113-140 in males and females, respectively, after mating. These studies included parallel arms of treated animals with naringin dihydrochalcone, naringin and hesperidin (Booth et al., 1965). Marginal effects on body weight and food consumption, cecal enlargement and some clinical chemistry variables of high-dose animals observed in a 91-day dietary toxicity study of Wistar rats (20/sex/group) fed the structural relative neohesperidin dihydrochalcone (FEMA 3811) at 0, 0.2, 1.0, and 5.0% (approximately 0, 150, 757, and 4011 mg/kg bw/day and 0, 166, 848 and 4334 mg/kg bw/day in male and female rats, respectively) were not considered to be toxicologically significant. The study authors determined the NOAEL to be 1.0% or 750 mg/kg bw/day (Lina et al., 1990). This NOAEL is greater than 3,700,000 times the anticipated daily *per capita* intake of 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). It is presumed that 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one is expected to be metabolized similarly to other dietary flavonoids (Day et al., 1998; Hollman and Katan, 1997; Hollman et al., 1999; Walle et al., 2005). Before entering the bloodstream, the substance is expected to undergo metabolism forming sulfates, glucuronide conjugates and/or methylated metabolites. Upon entry into the bloodstream the metabolites are expected to be further transformed in metabolic processes in the liver. Metabolites not absorbed in the small intestine are expected to undergo further metabolism by the microflora in the large intestine. The microflora is expected to cleave the conjugates and the resulting aglycones are expected to undergo ring fission leading to phenolic acid and cinnamic acid derivatives. These metabolites are expected to be absorbed and ultimately excreted in the urine (Smith et al., 2018). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of 2-[4-(D-glucopyranosyloxy)-3-hydroxyphenyl]-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one (Gooderham et al., 2020). The structural relatives neohesperidin dihydrochalcone (FEMA 3811) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715) were not mutagenic in several Ames assays in *S. typhimurium* TA98, TA100, TA1535, TA1536, TA1537 or TA1538 in the presence and absence of S9 (Batzinger and Bueding, 1977; MacGregor & Jurd, 1978; Brown et al., 1977; MacGregor, 1979; Brown & Dietrich, 1979; Nagao et al., 1981; Zeiger et al., 1987). No treatment-related induction of micronuclei was observed in the bone marrow of Swiss-Webster mice (6/dose) administered doses of 200, 500, 1000 and 5000 mg/kg bw of the structural relative neohesperidin dihydrochalcone (FEMA 3811) by oral gavage in 2% acacia (gum arabic) in water at 30 hours and 6 hours before sacrifice (MacGregor, 1979; MacGregor et al., 1983). Significant induction of micronuclei observed at 500 mg/kg bw was due to increases in a single mouse, but this was not observed in a repeat experiment at the same dose in 6 additional mice.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one (CAS 153733-96-1) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4998) (Smith et al.,

2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one was evaluated by the Expert Panel from toxicity studies for structural relatives, neohesperidin dihydrochalcone (FEMA 3811) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715), summarized below. Weanling rats (5/sex) were provided 0-0.128% of the structural relative neohesperidin dihydrochalcone (FEMA 3811) (approximately 0-128 mg/kg bw/day) in the diet for 90 days (Booth et al., 1965; FDA, 1993). High mortality of the pups was attributed to an inadequate diet rather than treatment-related conditions. No adverse effects on reproductive performance were observed. No adverse effects or treatment-related mortalities were observed in a follow-up study of the structural relative FEMA 3811 using the same protocol at 0.5% in the diet of weanling rats (approximately 500 mg/kg bw/day) for 70 days before mating (Booth et al., 1965; FDA, 1993). SPF Fischer rats were provided 0, 0.5, 2.5 and 5.0% of the structural relative FEMA 3811 (0, 500, 2500 and 5000 mg/kg bw/day, respectively) in the diet in a three-generation reproduction and teratogenicity study SPF Fischer rats were provided 0, 0.5, 2.5 and 5.0% of the structural relative FEMA 3811 (0, 500, 2500 and 5000 mg/kg bw/day, respectively) in the diet in a three-generation reproduction and teratogenicity study (Booth, 1974; Gumbmann et al., 1978; FDA, 1993). Slight decreases in fetal survival in the mid- and high-intake levels were observed in the third generation. Apart from a few abnormalities, no evidence for the teratogenicity of the test substance was observed in the study. In a GLP- and OECD 414 guideline-compliant prenatal developmental toxicity study, mated female Wistar Crl:(WI)WU BR rats (28/group) were provided 0, 1.25%, 2.5% or 5% of the structural relative FEMA 3811 (approximately 0, 800-900, 1600-1700 or 3100-3400 mg/kg bw/day, respectively) in the diet until GD 21 (Waalkens-Berendsen et al., 2004). The NOAEL for maternal toxicity, fetotoxicity, embryotoxicity or teratogenicity according to the authors corresponded to the top dose level of 3100 mg/kg bw/day. This NOAEL is greater than 13,000,000 times the anticipated daily per capita intake of (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one from use as a flavor ingredient. In an OECD 408 guideline-compliant 90-day dietary administration study of the structural relative 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715) in Sprague-Dawley rats (10/sex/group) that provided mean dietary daily intakes of 241, 487 and 968 mg/kg bw/day and 244, 491 and 983 mg/kg bw/day for males and females, the NOAEL was determined to be 968 and 983 mg/kg bw/day for males and females, respectively (Bauter, 2018). In a two-year dietary toxicity study in SPF Fischer rats (24/sex/group) that were provided the structural relative FEMA 3811 at 0, 0.5, 2.5 and 5.0% of the diet (approximately 0, 250, 1250 and 2500 mg/kg bw/day) (Gumbmann et al., 1978; FDA, 1993), lower body weight gains in high-intake males in the first ten weeks and high-intake females by the 60th week were recovered at 100 weeks after supplementation with 3% USP salts XIV (fortified with 40 ppm zinc and 3 ppm cobalt) and 3% additional brewers dried yeast at the expense of cornmeal in half of the high-intake group starting on Day 430. High mortalities in the high-intake group (50% survival) and control group (66% survival) were observed at the 100th week. Significantly increased plasma ornithine-carbamyl transferase (OCT) levels in treated rats were not reproduced in a separate 11-month feeding study of the structural relative FEMA 3811 at 10% of the diet. Decreased plasma cholesterol levels in high-intake rats were similar to the effects observed in the 11-month study. The authors attributed this to the intestinal fermentation of the test substance. Still, they noted that a different mode of action could not be excluded, based on the similar effects observed from other fermentable food constituents and bioflavonoids. Higher relative male kidney and liver weights and all organ weights in females were observed in the high-intake group with a non-supplemented diet. The organs of high-intake rats provided the supplemented diet had relative weights comparable to the controls and lower intake treatments, except thyroid weights which were lower than both control and other treatment groups but still within the expected normal range. Except for the supplemented high-intake group, diffuse thyroid follicular hyperplasia and hypertrophy were observed in all other control and treated rats. Increased focal cortical kidney atrophy incidence was observed in low-intake females, mid-intake rats, and non-supplemented high-intake rats compared to the controls and supplemented high-intake rats. The incidence of tumors in rats over 18 months of age did not reveal any treatment-related differences between the different experimental groups. Young beagle dogs (3/sex/group) were provided the structural relative FEMA 3811 in the diet for two years at levels of 0, 200, 1000 and 2000 mg/kg bw/day (Gumbmann et al., 1978). Slightly increased plasma alkaline phosphatase levels in high-intake males at 12, 18 and 24 months were not observed in high-intake females and were not accompanied with changes in other plasma enzymes or histology. Decreased plasma thyroxine concentrations in high-intake females were observed from 6 months onwards. Non-significant increased absolute and relative liver weights were observed in high-intake males and females. Decreased relative testes weights observed in one dog in the mid- and high-intake groups were accompanied by testicular atrophy and degeneration. Increased absolute and relative thyroid weights in high-intake males and females were accompanied by mild thyroid hypertrophy and hyperplasia (2/3 per sex) and increased thyroid follicular epithelium diameter with little alterations to thyroid architecture. The most severely affected high-intake dog exhibited increased cell diameter and architectural changes, including mild follicular epithelium folding and reduced follicular size. However, the authors acknowledge that the number of animals per tested intake level was too low to draw firm conclusions about the degree these results represent reversible, adaptive metabolic responses to high intakes of the test substance. Additionally, in the absence of historical data, the Expert Panel noted that the authors were unclear on the relevance of testicular atrophy in 1 of 3 mid-

and high-intake dogs each. In a 90-day dietary toxicity study followed by a reproductive toxicity study for a total study period of 148 days, weanling rats of an unspecified strain (5/sex/group) were provided 0, 0.00064, 0.0064, 0.064 or 0.128% of the structural relative FEMA 3811 (approximately 0, 0.64, 6.4, 64 and 128 mg/kg bw/day) (Booth et al., 1965; FDA, 1993). Decreased mean body weights in high-intake males and all treated females were comparable at all doses by the end of the reproductive phase except for high-intake males. Decreased red and white blood cell counts and hemoglobin levels in treated females were not considered toxicologically significant. In a follow-up 90-day dietary toxicity study, six female weanling rats were provided 0.128% of the structural relative FEMA 3811 (approximately 128 mg/kg bw/day) in the diet at a different basal ration (using Purina laboratory chow) than the previous study to avoid liver lipidosis (Booth et al., 1965). No other effects were observed apart from decreased mean body weight gains in treated rats. In a third study, no significant effects were observed in 5 male and 20 female weanling rats provided 0.5% of the structural relative FEMA 3811 (approximately 500 mg/kg bw/day) in the diet for 70 days followed by mating, and until Day 92 and Day 113-140 in males and females, respectively, after mating. These studies included parallel arms of treated animals with naringin dihydrochalcone, naringin and hesperidin (Booth et al., 1965). Marginal effects on body weight and food consumption, cecal enlargement and some clinical chemistry variables of high-dose animals observed in a 91-day dietary toxicity study of Wistar rats (20/sex/group) fed the structural relative neohesperidin dihydrochalcone (FEMA 3811) at 0, 0.2, 1.0, and 5.0% (approximately 0, 150, 757, and 4011 mg/kg bw/day and 0, 166, 848 and 4334 mg/kg bw/day in male and female rats, respectively) were not considered to be toxicologically significant. The study authors determined the NOAEL to be 1.0% or 750 mg/kg bw/day (Lina et al., 1990). This NOAEL is greater than 3,700,000 times the anticipated daily *per capita* intake of (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). It is presumed that (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one is expected to be metabolized similarly to other dietary flavonoids (Day et al., 1998; Hollman and Katan, 1997; Hollman et al., 1999; Walle et al., 2005). Before entering the bloodstream, the substance is expected to undergo metabolism forming sulfates, glucuronide conjugates and/or methylated metabolites. Upon entry into the bloodstream the metabolites are expected to be further transformed in metabolic processes in the liver. Metabolites not absorbed in the small intestine are expected to undergo further metabolism by the microflora in the large intestine. The microflora is expected to cleave the conjugates and the resulting aglycones are expected to undergo ring fission leading to phenolic acid and cinnamic acid derivatives. These metabolites are expected to be absorbed and ultimately excreted in the urine (Smith et al., 2018). Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of (2*S*)-2-(3,4-dihydroxyphenyl)-8-*beta*-D-glucopyranosyl-2,3-dihydro-5,7-dihydroxy-4*H*-1-benzopyran-4-one (Gooderham et al., 2020). The structural relatives neohesperidin dihydrochalcone (FEMA 3811) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanon (FEMA 4715) were not mutagenic in several Ames assays in *S. typhimurium* TA98, TA100, TA1535, TA1536, TA1537 or TA1538 in the presence and absence of S9 (Batzinger and Bueding, 1977; MacGregor & Jurd, 1978; Brown et al., 1977; MacGregor, 1979; Brown & Dietrich, 1979; Nagao et al., 1981; Zeiger et al., 1987). No treatment-related induction of micronuclei was observed in the bone marrow of Swiss-Webster mice (6/dose) administered doses of 200, 500, 1000 and 5000 mg/kg bw of the structural relative neohesperidin dihydrochalcone (FEMA 3811) by oral gavage in 2% acacia (gum arabic) in water at 30 hours and 6 hours before sacrifice (MacGregor, 1979; MacGregor et al., 1983). Significant induction of micronuclei observed at 500 mg/kg bw due to a single mouse was not observed in a repeat experiment at the same dose in 6 additional mice.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *Adenophora stenanthina* root extract (CAS 2622180-83-8) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 4999) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *Adenophora stenanthina* root extract from use as a flavor ingredient to be 7 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). Corroborative evidence for the low toxicity potential of *Adenophora stenanthina* root extract was evaluated by the Expert Panel from a series of 5- to 10-day reproductive toxicity and teratology studies in mammals (25-30/group) for the constituent citric acid (FEMA 2306) administered by gavage which resulted in no effects on fetal or maternal survival, the number of pregnancies, live litters, resorptions, fetal weight or fetal skeletal abnormalities were observed. NOAELs of >241 mg/kg bw/day, >295 mg/kg bw/day, >425 mg/kg bw/day and >272 mg/kg bw/day were established for albino mice, rats, rabbits and hamsters, respectively (FDRL, 1973b). The NOAEL of >241 mg/kg bw/day is greater than 2,000,000 times the anticipated daily *per capita* intake of *Adenophora stenanthina* root extract as a flavor ingredient. No subchronic toxicity data on the material of structural relatives were available for consideration. The material is produced from the roots of the *Adenophora stenanthina* plant. Though some preparations of this material are consumed as traditional medicine, quantitative information was not available, and a consumption ratio could not be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns

related to the genotoxicity of *Adenophora stenanthina* root extract (Gooderham et al., 2020; Cohen et al., 2018). Corroborative evidence showed that *Adenophora stenanthina* root extract was not mutagenic at concentrations up to 5000 µg/plate in a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay in *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of S9 (Patel, 2021). Corroborative evidence from a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay showed no significant induction of micronuclei was observed in human peripheral blood lymphocytes incubated with the same candidate substance at concentrations of 1250-5000 µg/plate for 4h with a 20h recovery period in the presence and absence of S9 as well as for 24h in the absence of S9 (Desai, 2021). Corroborative evidence available for the constituent citric acid (FEMA 2306) showed FEMA 2306 was not mutagenic in bacterial reverse mutation assays in *Salmonella typhimurium* TA92, TA94, TA97, TA98, TA100, TA104, TA1535 and TA1537 in the presence and absence of metabolic activation (Al-Ani and Al-Lami et al., 1988; Ishidate et al., 1984). Corroborative evidence also showed no induction of chromosome aberrations was observed when the same constituent (FEMA 2306) was tested *in vitro* in CHO cells (Ishidate et al., 1984) or human embryonic lung WI-38 cells, or *in vivo* in rats orally administered 1.2, 12 or 120 mg/kg bw for 5 days, at 500 or 3500 mg/kg bw in a single dose and at 300 and 3000 mg/kg bw for 5 days (Fiume et al., 2014). Corroborative evidence from an *in vivo* dominant lethal assay in rats at the same dose levels and treatment periods showed that the constituent citric acid (FEMA 2306) was not mutagenic in rats (Fiume et al., 2014). Corroborative evidence from a host-mediated assay showed that mitotic recombination was observed in *Saccharomyces* D3 inoculated mice that were provided the constituent citric acid (FEMA 2306) at 1.2, 12 and 120 mg/kg bw for 5 days but not when treated with a single, acute dose of 3500 mg/kg bw (Fiume et al., 2014). Additional corroborative evidence is available for the same constituent (FEMA 2306) which was not mutagenic at the same dose levels in *S. typhimurium* TA1530 and G46 mice in a host-mediated assay (Fiume et al., 2014).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding the material, Prepared mixture of chloride salts of potassium, magnesium and calcium (CAS 7447-40-7; 7786-30-3; 10035-04-8) and concluded that the mixture is GRAS (FEMA 5000) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of this mixture from use as a flavor ingredient to be 1384 µg/person/day. A decision tree structural class for the threshold of toxicological concern (TTC) could not be assigned for this mixture as inorganic substances are excluded from the Cramer/Ford/Hall Decision Tree classification (Cramer et al., 1978). Corroborative evidence for the low reproductive and developmental toxicity potential of Prepared mixture of chloride salts of potassium, magnesium and calcium was evaluated by the Expert Panel from toxicity studies for the constituents, KCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, and calcium salts summarized below. In a corroborative developmental dietary toxicity study of the constituent KCl provided to pregnant ICR mice (5-10/group) at 0 and 5% (10,000 mg/kg bw/day, approximately 13x greater than the nutritional requirement of potassium in mice) (Murai et al., 2013; EFSA, 2019b), significantly higher water intake and urine volume were observed in mice treated from GD 6.5 to 1 day after birth, and decreased body weight gains in pregnant mice and offspring as well as significantly increased relative kidney weights and serum potassium were observed in mice treated from GD 6.5 to 14 days after birth. No other developmental effects were observed. In prenatal developmental toxicity studies, no treatment-related effects were observed when virgin adult female albino CD-1 outbred mice (25/group) and virgin female albino Wistar rats (21-28/group) were administered the constituent KCl in a water solution by oral gavage at doses of 2, 11, 50 or 235 mg/kg bw/day and at doses of 3, 14, 67 or 310 mg/kg bw/day from GD 6-15, respectively (FDRL, 1975; EFSA, 2019b). In corroborative prenatal developmental toxicity studies, no treatment-related adverse effects were observed when female Dutch rabbits (16-22 total; 11-14 pregnant/group), virgin adult female albino CD-1 outbred mice (25/group) and virgin female albino Wistar rats (25/group) were administered the constituent $CaCl_2$ in a water solution by oral gavage at doses of 2, 8, 36 or 169 mg/kg bw/day from GD 6-18, at doses of 2, 9, 41 or 189 mg/kg bw/day from GD 6-15 and at doses of 2, 8, 38 or 176 mg/kg bw/day from GD 6-15, respectively (FDRL, 1974; EFSA, 2019b). In a corroborative prenatal developmental toxicity study in pregnant Wistar rats administered the constituent $MgCl_2{\cdot}6H_2O$ by gavage at 200, 400 or 800 mg/kg bw/day from GD 6-15, no treatment-related maternal or fetal mortalities, signs of toxicity or adverse effects were observed, and a NOAEL of 800 mg/kg bw/day was established (Usami et al., 1996). In a corroborative GLP- and OECD 422 guideline-compliant combined repeated dose toxicity with reproductive/developmental screening test, no treatment-related mortalities or adverse effects were observed in Wistar rats (10-15/sex/group) treated with 0, 250, 500 or 1000 mg/kg bw/day of the constituent $MgCl_2{\cdot}6H_2O$ in a water solution by oral gavage for 28 (males) or 54 (females) days (ECHA, 2010b). A NOAEL of 1000 mg/kg bw/day was established for both sexes for repeated dose toxicity and reproductive/developmental toxicity. The NOAEL of 1000 mg/kg bw/day for the constituent $MgCl_2{\cdot}6H_2O$ was greater than 43,000 times the anticipated daily *per capita* intake of Prepared mixture of chloride salts of potassium, magnesium and calcium as a flavor ingredient. Corroborative evidence for the low toxicity potential of Prepared mixture of chloride salts of potassium, magnesium and calcium was evaluated by the Expert Panel from toxicity studies for the constituents, KCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, and calcium salts summarized below. In a corroborative 13-week repeat dose dietary toxicity study, B6C3F1 mice (10/sex/group) were administered the constituent $MgCl_2{\cdot}6H_2O$ in a corn oil solution by oral gavage at 0, 0.3, 0.6, 1.25, 2.5 or 5% (equivalent to 0, 610, 1220, 2690, 5410 or 11,400 mg/kg bw/day and 0, 770, 1580, 3260, 6810 or 13,830 mg/kg bw/day for males and females, respectively) (Tanaka et al., 1994). Significant body weight and relative organ weight changes at the top two doses were incidental. Renal cell vacuolation was found in the proximal tubules of high-dose males. In B6C3F1 mice, NOAELs of 5% in females (equivalent to 13,830 mg/kg bw/day) and 2.5% in males (equivalent to 5410 mg/kg bw/day) were established. In another corroborative 90-day dietary toxicity study, Fischer 344/DuCrj rats (10/sex/group) were provided the constituent $MgCl_2{\cdot}6H_2O$ at 0, 0.1, 0.5 or 2.5% in feed, or

approximately 0, 100, 500 or 2500 mg/kg bw/day (Takizawa et al., 2000; JECFA, 2012). Significantly lower male body weights, soft stool and increased water consumption were observed in the high-dose group. No other significant differences in hematological, clinical biochemistry, organ weights or gross pathological findings were observed. A NOAEL of 0.5% or 500 mg/kg bw/day was established for male and female rats. In a corroborative chronic dietary toxicity study, no evidence of carcinogenicity was observed in B6C3F1 mice provided 0, 0.5 or 2% of the constituent $MgCl_2 \cdot 6H_2O$ (equivalent to 0, 570 or 2810 mg/kg bw/day or 0, 730 or 3930 mg/kg bw/day for males and females, respectively) for 96 weeks and a control diet for 8 weeks after the end of treatment prior to necropsy (Kurata et al., 1989). Significantly decreased bodyweights, increased feed consumption, significantly increased serum albumin and significant increases in absolute and relative brain weights and relative kidney and heart weights as well as significantly decreased absolute liver weights were observed in high-dose females. However, the authors noted that there were no differences in survival rates between the treatment and control groups and that there were no other significant changes in clinical chemistry, hematological or urinalysis parameters. The authors also attributed the changes in organ weights in high-dose females to significantly decreased bodyweights without further explanation. Tumors in organs of both sexes were observed; however, hepatocellular carcinomas in high-dose males were significantly decreased compared to controls. The Expert Panel concluded that non-significant, dose-dependent increases in malignant lymphoma/leukemia in treated females were not considered relevant based on their high spontaneous occurrences in B6C3F1 mice. A NOAEL of 0.5%, equivalent to 730 mg/kg bw/day, was established in females based on decreased body weight gain, and a NOAEL of 2%, or 3930 mg/kg bw/day, was established in males. In corroborative toxicity studies, Wistar rats were provided the constituent KCl at 0 or 3% (equivalent to 0 or 1500 mg/kg bw/day) in the diet for 4 weeks (10/sex/group), 13 weeks (10/sex/group), 18 months (15/sex/group) and 30 months (50/sex/group) (Lina and Kuijpers, 2004). Decreased mean body weights observed in treated rats in the 30-month study and in treated males in the 18-month study were correlated with reduced feed intake as well as increased water intake, urinary volume and urinary potassium. Statistically significant increases in relative kidney weights were observed in treated males in the 18-month study, and significantly increased incidences of hypertrophy of the adrenal zone glomerulosa were observed in treated animals in the 30-month study. The Expert Panel noted that these effects are expected given the administration of high doses of KCl. No other significant hematological, clinical chemistry parameters or tumor incidences were observed at the end of the 30-month study. In a corroborative 2-year chronic dietary toxicity study, male F344/Slc rats (50/group) were provided 0, 110, 450 and 1820 mg/kg bw/day of the constituent KCl (Imai et al., 1961). Survival of treated animals was higher than in the control group. Higher incidences of gastritis in the mid- and high-dose groups compared to the control group indicated an irritant effect. Chronic progressive nephropathy (CPN) was observed in all treated rats and the control rats. Although the biological relevance of this effect could not be determined, the Expert Panel concluded that incidences of CPN in control and treated rats were not biologically relevant to human risk assessment. No carcinogenic effects were observed. In a corroborative 60-day dietary toxicity study, 3-month-old male Wistar rats (8/group) were provided the constituent $CaCl_2$ at 0, 0.5, 1.0, and 1.5 gm/100gm diet (approximately 250, 500 and 750 mg/kg bw/day, respectively) (Chandra et al., 2012). Statistically significant increases in absolute and relative thyroid weights as well as hypertrophy of thyroid follicular epithelial cells without inflammatory changes were observed at the mid- and high-dose groups. Morphometric and histomorphometric analysis of the thyroid follicular cells supported mild follicular cell hypertrophy observed in the histological studies. The changes observed were interpreted as treatment-dependent cytological changes as a result of subchronic exposure to calcium. At all treatment levels, thyroid peroxidase activity was decreased, but this was not associated with the reduction of serum total or free T4. The thyroid 5'-deoidinase I activity levels were significantly lower compared to controls, which was associated with reduced serum total and free T3. Thyroid Na+-K+-ATPase activity was significantly increased. The serum total T4 measurements at the highest treatment level and the free T4 at the highest two treatment levels were significantly increased. At those same two high dose levels, total and free T3 levels were significantly decreased compared to controls. Statistically significant decreases in T3/T4 ratios were observed in all treated rats in a dose-dependent manner. Serum thyroid stimulating hormone (TSH) was increased in the two highest treatment groups. The authors determined that chronic exposure to high concentrations of calcium chloride led to mild thyroid hypertrophy with secondary adaptive changes in serum T4, T3, and TSH. A LOAEL of 250 mg/kg bw/day was established for thyroid toxicity. In a corroborative study in which female albino Sprague-Dawley rats (7/group) were administered calcium salts (calcium carbonate, calcium chloride and calcium aspartate hydrochloride) by gavage at 3.5, 7 or 14 mmol/kg bw/day (equivalent to 388, 777 and 1554 mg/kg bw/day) (Classen et al., 1995), statistically significant decreases in urinary pH and significant increases in urinary magnesium, calcium and chloride were observed at the mid- and high-doses. Significant increases in hyperchloremia and hypomagnesemia along with metabolic acidosis were also observed at the high dose. The Expert Panel determined that the NOAEL of 500 mg/kg bw/day for the constituent $MgCl_2 \cdot 6H_2O$ (Takizawa et al., 2000; JECFA, 2012) was greater than 21,000 times the anticipated daily *per capita* intake of Prepared mixture of chloride salts of potassium, magnesium and calcium as a flavor ingredient. Based on the Expert Panel's review of thyroid toxicity from high doses of $CaCl_2$ administration in male Wistar rats in the diet for 60 days, the Expert Panel determined the LOAEL of 250 mg/kg bw/day for thyroid toxicity for the constituent $CaCl_2$ (Chandra et al., 2012) was greater than 10,000 times the anticipated daily per capita intake of Prepared mixture of chloride salts of potassium, magnesium and calcium as a flavor ingredient. Based on the Expert Panel's review, it was determined that the LOAEL of 6 mg/kg bw/day for magnesium-induced diarrhea in adults (IOM, 1997) described below was greater than 200 times the anticipated daily *per capita* intake of prepared mixture of chloride salts of potassium, magnesium and calcium as a flavor ingredient. The constituents of the mixture are essential ingredients for humans; however, quantitative information was not available, and a consumption ratio could not be calculated. The Expert Panel noted the risk of hyperkalemia in individuals with pre-existing medical conditions upon

excessive potassium consumption, adverse gastrointestinal effects in patients given doses above 100 mg of potassium chloride (KCl) generally by bolus administration, and the LOAEL of 360 mg/day (equivalent to 6 mg/kg bw/day) for magnesium-induced diarrhea in adults (with the US Institute of Medicine (IOM) using an uncertainty factor of 1.0) (IOM, 1997). The Expert Panel concluded that these data indicate that the consumption of these ions from food or at high concentrations exceed the consumption of these ions from use of prepared mixture of chloride salts of potassium, magnesium and calcium as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). The constituents of the candidate substance are essential dietary substances that are expected to dissociate into their respective ions in biological fluids and be absorbed in the GI tract followed by distribution mainly to soft tissues, bones and muscle (OECD, 2002, 2003, 2011a; EFSA, 2015a,b, 2016, 2019b; Swaminathan, 2003; IOM 1997, 2005, 2011). These ions participate in various essential cellular functions and modulate membrane permeability and potential (Elin, 1987; IOM 1997, 2005, 2011). Calcium is essential for skeletal formation, neuronal transmission, muscle contraction and blood coagulation. Upon filtration in the kidney, most of the ions are reabsorbed. Minor amounts of these ions are excreted in the urine, and in sweat, through the skin, in breast milk and in the feces (Lakshamanan et al., 1984; Weiner et al., 2010; Mickelsen et al., 1977; Pietinen, 1982; Holbrook et al., 1984; Tasevska et al., 2006; Yoshida et al., 2012; OECD, 2003; Kiela and Ghishan, 2016; Greger, 2000). Based on the structure of the constituents, the arrangement and identity of the functional groups therein, and the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of Prepared mixture of chloride salts of potassium, magnesium and calcium (Gooderham et al., 2020). The constituents KCl, $CaCl_2$ and $MgCl_2$ were not mutagenic in corroborative bacterial reverse mutation assays in *S. typhimurium* TA92, TA1535, TA100, TA1537, TA94 and TA98 in the absence and/or presence of S9 (Ishidate et al., 1984; Mortelmans et al., 1986). In a corroborative GLP- and OECD 473 guideline-compliant *in vitro* chromosome aberration assay, no induction of chromosome aberrations was observed in human lymphocytes incubated with the constituent $MgCl_2 \cdot 6H_2O$ at concentrations of 508-2033 µg/mL for 4 hours in the presence and absence of S9 as well as for 24 hours in the absence of S9 (ECHA, 2010a). Corroborative evidence showed no induction of chromosomal aberrations was observed in Chinese hamster fibroblasts incubated with the constituents $CaCl_2$ at concentrations up to 4000 µg/mL and $MgCl_2$ at concentrations up to 2000 µg/mL in the absence of metabolic activation (Ishidate et al., 1984). Significant increases in chromosome aberrations and a slight increase in sister chromatid exchange frequencies were observed in CHO cells tested with up to 180 mM of the constituent KCl, however, these results were attributed to cytotoxicity and cell cycle delay. No significant increases in single-strand DNA breaks were observed (Galloway et al., 1987). In another corroborative assay of the constituent KCl tested in Chinese hamster lung fibroblast V79 cells at concentrations of 2000-12,000 µg/mL without metabolic activation, chromosome aberrations observed only at the highest tested concentration were attributed to high osmotic pressure of the medium compared to the control medium (Hasegawa et al., 1984). In this same corroborative study, no significant increases of sister chromatid exchanges were observed at any tested concentration (Hasegawa et al., 1984). However, the OECD guideline for the sister chromatid exchange assay has been deleted due to a lack of understanding of the mechanism(s) of action detected by the test (OECD, 2017). No significant changes in mutant frequency were observed in L5178Y mouse lymphoma cells treated with concentrations of 22-36 mg/mL of the constituent $MgCl_2$ in a corroborative *in vitro* mammalian cell gene mutation assay in the absence and presence of S9 (Oberly et al., 1982). In a corroborative *in vitro* mammalian cell gene mutation assay of concentrations up to 9000 µg/mL of the constituent KCl (well above OECD recommendations) in L5178Y mouse lymphoma cells in the presence and absence of S9 metabolic activation, significant increases in mutant frequencies were observed at cytotoxic concentrations of 5000 µg/mL and above in the absence of S9 (Myhr and Caspary, 1988). In another corroborative *in vitro* mammalian cell gene mutation assay of the constituent KCl at concentrations up to 5000 µg/mL in the presence and absence of S9 metabolic activation, weak increases in mutant frequencies at concentrations of 4000 µg/mL and above were attributed to changes in osmotic pressure of the test medium due to high salt concentrations (Mitchell et al., 1988).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding oak chips extract (*Quercus robur*) (CAS 71011-28-4) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5001) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of oak chips extract (*Quercus robur*) from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day). Corroborative evidence for the low toxicity potential of oak chips extract (*Quercus robur*) was evaluated by the Expert Panel from a drinking water toxicity study in which Sprague-Dawley rats (50/sex/group) were provided 1% or 3% of the constituent ethanol (FEMA 2419) or glucose (control group) in a synthetic diet calorically equal to the ethanol group for 120 weeks (equivalent to 1000 or 3000 mg/kg bw/day of the constituent ethanol) (Holmberg and Eckstom, 1995). Statistically significant increases in the occurrence of pituitary tumors (not further described by the authors) were observed in the high-dose ethanol-treated females compared to the glucose control group. Increased incidences of mammary gland fibromas, fibroadenomas and adenomas were observed in the low-dose ethanol-treated females compared to the glucose control group. No increase in tumor incidence was observed in ethanol-treated males compared to the glucose controls. The overall information described by the authors indicate an absence of carcinogenic activity. Corroborative evidence is also available from another chronic drinking water toxicity study in

which male Sprague Dawley rats were provided 5% (v/v) of the constituent ethanol for 130 weeks (equivalent to 5000 mg/kg bw/day) (Radike et al., 1981; FDA, 1993). Hyperplastic nodules of the liver were observed, and significantly increased adenomas of the pancreas, adrenal gland and pituitary gland were observed compared to the controls. The Expert Panel noted that no further information was provided on the types of adrenal and pancreatic tumors observed in treated rats compared to the controls. Pancreatic acinar cell tumors and adrenal pheochromocytomas are the most common tumors in this strain of rats and are considered not relevant to human cancer risk. For both studies (Holmberg and Eckstom, 1995; Radike et al., 1981), the Expert Panel noted that pituitary tumors are common in Sprague Dawley rats and are not relevant to humans (Son and Gopinath, 2004; Edler et al., 2014). Corroborative evidence is also available from a chronic exposure and reproductive toxicity study in which Sprague Dawley rats (30-55/sex/group) were provided 10% of the constituent ethanol (FEMA 2419) in drinking water for 104 weeks (equivalent to 10,000 mg/kg bw/day) and were necropsied after deaths from natural causes (Soffritti et al., 2002; FDA, 1993). Increased benign and malignant tumor formation including increased incidences of carcinomas of the oral cavity, lips and tongue in male and female breeding rats and offspring were observed. The majority of the increase in total tumors was due to oral carcinomas. Minimal increases in tumor incidences were present in additional tissues; however, the data were not consistent between males and females or rat groups and there was a lack of comparisons to historical data. Since only a single dose was used, there was no evaluation of a dose-response relationship. Benign and malignant tumors of the forestomach were observed in breeding males and increased incidence of lymphomas and leukemias were observed in dams. Increased interstitial-cell adenomas of the testes and osteosarcomas of several sites were observed in parental males. Limited statistical analyses were conducted in this study and the IARC Working Group noted some unconventional approaches in their review (IARC, 2018). The Expert Panel concurs with the IARC conclusions. The Expert Panel reviewed the key constituents of oak chips extract (*Quercus robur*) and noted that the congeneric group intakes were below the respective TTC thresholds. The Expert Panel also noted that the intake of ethanol (FEMA 2419) from the use of oak chips extract (*Quercus robur*) as a flavor ingredient is expected to be lower than the intake resulting from the levels at which it is present as an endogenous substance and in food and beverages. Corroborative evidence for the constituent ethanol (FEMA 2419) has been reviewed by the IARC in published monographs of the human health effects of the consumption of alcoholic beverages (IARC, 1988, 2010, 2012, 2018). The IARC has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of ethanol and to consider ethanol in alcoholic beverages as a Group 1 carcinogen (IARC, 1988, 2010, 2012, 2018). The material is produced from the chips of the *Quercus robur* tree and is not consumed as food. Therefore, a consumption ratio could not be calculated. The Expert Panel noted that in adult humans, exposure to the constituent ethanol (FEMA 2419) occurs mainly from consumption of alcohol, and up to 90% of ingested alcohol is metabolized to acetaldehyde (FEMA 2003) in the liver and, to a lesser extent in extrahepatic tissues. In children, exposure to ethanol (FEMA 2419) is expected to be up to 12.5-23 mg/kg bw/day via consumption of bananas, fruit juices as well as bread and bakery products. Additionally, the Expert Panel noted the estimated endogenous levels of ethanol in non-alcohol consuming adults to be 0.39 ± 0.45 µg/mL (Jones et al., 1983) The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of oak chips extract (*Quercus robur*) (Gooderham et al., 2020; Cohen et al. 2018). The Expert Panel noted the equivocal results reported by the International Agency for Research on Cancer (IARC) for the constituent ethanol (FEMA 2419), as well as ability of acetaldehyde (FEMA 2003), a metabolite of ethanol (FEMA 2419) to bind to proteins, DNA and other macromolecules (IARC, 1988, 2010, 2012, 2018; Nakao et al., 2000). Despite the results from the mutagenicity and chromosomal damage testing for the constituent ethanol (FEMA 2419) and its metabolite acetaldehyde (FEMA 2003), the Expert Panel concluded that the use of oak chips extract (*Quercus robur*) as a flavor ingredient would not raise an additional concern for genotoxicity relative to the consumption of ethanol and acetaldehyde from food. Additionally, the exposure to acetaldehyde and ethanol from use of oak chips extract (*Quercus robur*) as a flavor ingredient is expected to be negligible relative to the endogenous levels of exposure. Corroborative evidence for the constituent ethanol (FEMA 2419) was also evaluated by JECFA in 1970 as a solvent with an ADI limited by good manufacturing processes (GMP) (JECFA, 1970). In its 46th meeting, based on corroborative evidence, JECFA concluded that ethanol (FEMA 2419) does not pose a safety concern at the intake levels from the use of ethyl esters as flavor ingredients (JECFA, 1997). In its evaluation, the Expert Panel considered the high consumption ratio of ethanol (FEMA 2419) from food excluding alcoholic beverages and subsequent high endogenous exposure to ethanol and its metabolite, acetaldehyde (FEMA 2003), compared to their intake from the use of oak chips extract (*Quercus robur*) as a flavor ingredient, and concluded, based on these considerations, that the anticipated intake of ethanol (FEMA 2419) and its metabolite acetaldehyde (FEMA 2003) from consumption of oak chips extract (*Quercus robur*) as a flavor ingredient is not expected to be of concern with respect to carcinogenicity. The Expert Panel noted that approximately 97% of oak chips extract (*Quercus robur*) could be propylene glycol (FEMA 2940). Based on the anticipated annual volume of use of propylene glycol as a flavor ingredient (97 kg) from its presence in oak chips extract (*Quercus robur*), the *per capita* intake was calculated to be 14 µg/person/day (0.2 µg/kg bw/day). Both JECFA (JECFA, 1974, 2002) and EFSA (EFSA, 2018) have set an Acceptable Daily Intake (ADI) for propylene glycol at 25 mg/kg bw/day, which is greater than 107,000 times the anticipated daily per capita intake of propylene glycol from use of oak chips extract (*Quercus robur*) as a flavor ingredient.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-

prop-2-enamide (CAS 2294887-29-7) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5002) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. The substance was evaluated individually within the context of the chemical group of aliphatic and aromatic amines and related amides (JECFA, 2006a, 2008, 2011, 2012, 2017; SLR, A7, C21). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide from use as a flavor ingredient to be 69 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low reproductive and developmental toxicity potential of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide was evaluated by the Expert Panel from toxicity studies for the structural relatives, (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) and 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809). Corroborative evidence is available from a GLP- and OECD 414 guideline-compliant prenatal developmental toxicity study in pregnant Sprague Dawley rats administered 0, 125, 250, 500 and 1000 mg/kg bw/day of the structural relative (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) by oral gavage from implantation until the day before delivery, which resulted in maternal and fetal NOAELs of 1000 mg/kg bw/day due to a lack of treatment-related adverse effects (Bauter, 2017a). After doses of 0, 125, 300 and 1000 mg/kg bw/day of the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) were administered by gavage to pregnant female Sprague Dawley rats (25/group) from GD 6-20 in a corroborative GLP- and OECD 414 guideline-compliant prenatal developmental toxicity study (Karanewsky et al., 2015), no adverse effects were observed. Maternal and fetal NOAELs of 1000 mg/kg bw/day were established for the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) based on a lack of treatment-related adverse effects. The maternal and fetal NOAELs of 1000 mg/kg bw/day for the structural relatives (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) and 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) were greater than 800,000 times the anticipated daily *per capita* intake of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide was evaluated by the Expert Panel from a GLP- and OECD 408 guideline-compliant 90-day dietary toxicity study in which Sprague Dawley rats were provided 0, 30, 100 or 500 mg/kg bw/day of the structural relative (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) (corresponding to average daily intakes of 0, 29, 98 or 490 mg/kg bw/day and 0, 29, 99 or 492 mg/kg bw/day for male and female rats, respectively). NOAELs of 490 and 492 mg/kg bw/day were established in males and females, respectively, based on a lack of treatment-related adverse effects (Koetzner, 2013; JECFA, 2017). Additionally, corroborative evidence is also available from a GLP-compliant combined 90-day toxicity and toxicokinetic study in which Sprague Dawley rats (20/sex/group) were administered 0, 10, 30 or 100 mg/kg bw/day of the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) by oral gavage. A NOAEL of 100 mg/kg bw/day was established based on a lack of treatment-related adverse effects (Karanewsky et al., 2015). The NOAEL of 100 mg/kg bw/day for the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) is greater than 86,000 times the anticipated daily *per capita* intake of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. Based on corroborative evidence for the absorption, distribution, metabolism, and/or excretion of the structural relatives 3-[(4-amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (FEMA 4701), *N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide (FEMA 4232), *N*-(2-methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide (FEMA 4678), piperine (FEMA 2909) and 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl) acetamide (FEMA 4809), the Expert Panel expected that (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide undergoes cytochrome P450-mediated oxidation to polar metabolites, followed by sulfation or glucuronidation and excretion in the urine (Arthur et al., 2015; Karanewsky et al., 2016; Foster, 2009; Bhat and Chandrasekhara, 1986,1987). Corroborative evidence is available from both rat and human microsomal incubations with a structurally similar substance, 3-[(4-amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (FEMA 4701), which resulted in ≥99% of the parent compound remained intact after one hour. Only trace amounts of amide hydrolysis products were detected after *in vivo* oral or i.v. administration of the same test substance to male and female Sprague-Dawley rats (Arthur et al., 2015). In a corroborative *in vitro* assay, polar and conjugated metabolites, including a catechol derivative, indicative of hydroxylation and demethylation, were detected when rat and human liver microsomes were incubated with another structurally similar substance, *N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide (FEMA 4232) (Karanewsky et al., 2016). Additionally, corroborative oral and i.v. administrations of this same substance to male and female Sprague-Dawley rats resulted in similar demethylated metabolites, including the catechol derivative that was quickly conjugated directly or after ensuing *O*-demethylation before further oxidation into additional metabolites (Karanewsky et al., 2016). No amide hydrolysis products of this substance were observed in either the *in vitro* or *in vivo* experiments. Corroborative evidence is also available when another structurally similar substance, *N*-(2-methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide (FEMA 4678), was incubated with rat microsomes. Small amounts of metabolites resulting from the hydroxylation of the methylcyclohexyl ring of the parent compound, as well as evidence of limited amide hydrolysis were observed (Foster, 2009; JECFA, 2012). Increased excretion of conjugated glucuronides, sulfates, and phenol metabolites, suggestive of demethylation, was observed from corroborative evidence after administration of another structurally similar substance, piperine (FEMA 2909), by gavage (170 mg/kg bw) or i.p. injection (85 mg/kg bw) to male Albino Wistar rats (Bhat and Chandrasekhara, 1986, 1987). Hydrolysis of the structural relative, 2-(4-methylphenoxy)-*N*-(1H-pyrazol-3-yl)-*N*-

(thiophen-2-ylmethyl) acetamide (FEMA 4809), to its carboxylic acid derivative and secondary amine was detected from corroborative *in vitro* in incubations with rat, dog, rabbit, Gottingen pig, and human microsomes (Karanewsky et al., 2015). However, corroborative evidence from oral administration of FEMA 4809 to either rat, mouse or dog, systemic exposure to the carboxylic acid derivative was significantly greater relative to the parent amide or secondary amine hydrolysis product. Based on the structure of the substance, the arrangement and identity of the functional groups therein, and supported by the corroborative evidence cited below, the Expert Panel did not identify specific concerns related to the genotoxicity of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential was evaluated by the Panel from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, in which (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide was not mutagenic at concentrations up to 3160 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA pKM101 in the presence and absence of S9 metabolic activation using the preincubation and plate incorporation methods (Spruth, 2020). Additional corroborative evidence for the lack of genotoxic potential of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*-phenyl-*N*-tetrahydrofuran-3-yl-prop-2-enamide was evaluated from the structural relatives (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) and 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809). Corroborative evidence is available from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay in which the structural relative (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) was not mutagenic at concentrations of 22-5500 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA pKM101 in the presence and absence of S9 metabolic activation using the preincubation and plate incorporation methods (Schulz and Landsiedel, 2009). Corroborative evidence is also available from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay in which the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) was not mutagenic when tested at concentrations of 63-1000 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA pKM101 in the presence and absence of S9 metabolic activation using the preincubation and plate incorporation methods (Karanewsky et al., 2015). Corroborative evidence is available from a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay in which no significant induction of micronuclei was observed in human peripheral blood lymphocytes treated with the structural relative (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (FEMA 4788) for 3 hours with a 21-hour recovery period in the presence and absence of S9 metabolic activation at concentrations of 10-60 µg/mL and for 24 hours in the absence of S9 metabolic activation at concentrations of 8-20 µg/mL (Watters, 2014; EFSA, 2022). Corroborative evidence from a GLP- and OECD 473 guideline-compliant *in vitro* chromosomal aberration assay showed no significant induction of structural or numerical chromosome aberrations were observed in human peripheral blood lymphocytes treated with the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) for 3 hours and a 20-hour recovery period in the presence of S9 at concentrations of 35-160 µg/mL, for 3 hours and a 20-hour recovery period in the absence of S9 at concentrations of 1.3-5 µg/mL and for 20 hours in the absence of S9 at concentrations of 23-65 µg/mL (Karanewsky et al., 2015). No significant increases in mononuclear polychromatic erythrocytes, changes in body weight, feed consumption, adverse effects or reduction in polychromatic erythrocytes/total erythrocytes were observed in CD-1 mice (3/sex/dose) administered doses up to 2000 mg/kg bw of the structural relative 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide (FEMA 4809) from a corroborative GLP- and OECD 474 guideline-compliant *in vivo* micronucleus assay (Karanewsky et al., 2015).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 2,6-octadienal (CAS 149231-57-2) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5003) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters (JECFA, 1999, 2012, 2020; SLR, M1). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 2,6-octadienal from use as a flavor ingredient to be 7 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 2,6-octadienal was evaluated by the Expert Panel from a 4-week dietary toxicity study in which rats were provided the structural relative 2-*trans*-6-*cis*-dodecadienal (FEMA 3377) (0.2, 0.4, 1.0, 2.0, 4.0, 10.0 or 20.0 ppm) and 2-*trans*-4-*cis*-7-*cis*-tridecatrienal (FEMA 3638) (3.2, 6.4, 16, 32, 64, 160 or 320 ppm) in the diet (Edwards, 1973; Adams et al., 2008). Based on feed consumption measurements, the highest concentrations were equivalent to intakes of 1.93 and 2.06 mg/kg bw/day of FEMA 3377 for males and females, respectively, and 30.9 and 33 mg/kg bw/day of the structural relative FEMA 3638 for males and females, respectively. No significant treatment-related adverse effects were observed, and the NOAEL was reported to be the highest dose tested (1.93 and 2.06 mg/kg bw/day of the structural relative FEMA 3377 for males and females, respectively, and 30.9 and 33 mg/kg bw/day of FEMA 3638 for males and females, respectively). The NOAEL of 2 mg/kg bw/day is greater than 17,000 times the anticipated daily *per capita* intake of 2,6-octadienal from use as a flavor ingredient. This material occurs naturally in hops essential oil (*Humulus lupulus* L.), sweet basil oil (*Ocimum basilicum* L.), navel orange juice (*C. sinensis* (L.) Osbeck.), *Syzygium polyanthum*, and *Satureja cuneifolia* (Bocquet et al., 2018; Fattahi et al., 2019; Sun et al. 2021; Hamad et al., 2017; Oke et al., 2009). Based on the quantitative data, a consumption ratio of 1 could be calculated (Stofberg and Grundschober, 1987). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. 2,6-Octadienal is expected to undergo oxidation to the corresponding acid followed by *β*-oxidation to CO_2 and water. Alternatively, the compound could be conjugated with glutathione followed by excretion in the urine as the

mercapturic acid derivative (Adams et al., 2008; Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 2,6-octadienal (Gooderham et al., 2020). The Expert Panel reviewed their prior assessment of the genotoxicity data for the structural relative nona-2-*trans*-6-*cis*-dienal (FEMA 3377) and determined it sufficient to indicate a lack of genotoxic concern for 2,6-octadienal (Adams et al., 2008). Since the publication of Adams et al., corroborative evidence from new genotoxicity data available for the structural relative, FEMA 3377, are described below. In a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay, the structural relative FEMA 3377 was tested in human lymphocytes at concentrations up to 60 µg/mL for 4 hours in the absence and presence of S9, as well as for 24 hours in the absence of S9 (Api et al., 2022c). Significant induction of micronuclei was observed at concentrations of 15 and 20 µg/mL in the 4 hours treatment period in the absence of S9, at concentration of 40 µg/mL in the 4 hours treatment period in the presence of S9 and at concentrations of 20 and 30 µg/mL in the 24 hours treatment period in the absence of S9. In a GLP- and OECD 474 and 489 guidelines-compliant combined *in vivo* micronucleus and comet assay in male Han Wistar rats administered the structural relative FEMA 3377 by oral gavage at doses of 175, 350 or 700 mg/kg bw/day, at 1h, 24h and 45h after dosing, no significant induction of micronucleated polychromatic erythrocytes in the bone marrow was observed in the tested rats compared to the controls (Beevers, 2015; Api et al., 2022c). No significant increases in tail intensity or hedgehogs in the liver were observed at any tested dose compared to the controls. No treatment-related macroscopic findings were observed. Dose-dependent decreases in glycogen vacuolation were observed in the liver of all test groups. Soft/loose brown feces and reduced body weight gain in all animals and brown stained fur in one animal were observed in the high-dose group. Clinical chemistry findings at the high dose included a slight decrease in calcium levels, a slight increase in phosphate animals in all animals, and an increase in glucose levels in one animal. A slight increase in blood urea was observed in the mid and high doses.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 2-methyloctan-4-olide (CAS 40556-69-2) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5004) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones (Adams et al., 1998; JECFA, 1998, 2011; SLR, B1C). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 2-methyloctan-4-olide from use as a flavor ingredient to be 0.1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 2-methyloctan-4-olide was reviewed by the Expert Panel from a 90-day single-dose toxicity study in which male and female rats were fed diets containing a mixture of four flavor materials: structural relative undecalactone (isomer not specified), nonalactone, allyl hexanoate, and dihydrocoumarin (Oser, 1957). The total consumption of flavor material was 109 mg/kg bw/day, and the achieved level of undecalactone intake was calculated to be approximately 47 mg/kg bw/day. A noted but statistically non-significant depression in growth was observed in both sexes. Feed consumption was slightly higher in test animals, leading to a significant depression in the efficiency of food utilization for both sexes. Trace amounts of albumin were detected in the urine of one male rat, but the authors did not consider this to be significant. Due to the decrease in growth and feed efficiency and the use of a mixture in the test diet, the authors did not derive a NOAEL for this study. Corroborative evidence is also available from 2-year feeding studies in which male and female rats were administered a diet containing the structural relative γ-undecalactone (FEMA 3091) or the structural relative γ-nonalactone (FEMA 2781), at doses of 0, 50 or 250 mg/kg bw/day (Bär and Griepentrog, 1967). Animals were observed throughout the study period and no adverse effects were reported. The FEMA Expert Panel agreed with JECFA's evaluation of this study and their selected NOAELs of 250 mg/kg bw/day for FEMA 2781 and FEMA 3091 (JECFA, 1998). Additional corroborative evidence is also available from single-dose 90-day toxicity studies in which FDRL (derived from Wistar) rats (15/sex) were provided diets containing the structural relative γ-nonalactone (FEMA 2781) or 2% of the structural relative γ-undecalactone (FEMA 3091) diluted in cottonseed oil. The concentration in the feed corresponded to oral doses of approximately 15 and 17 mg/kg bw/day of FEMA 3091 or 63 and 73 mg/kg bw/day of FEMA 2781 in males and females, respectively (Oser et al., 1965). No significant changes in body weight, food consumption, food efficiency, or significant gross pathological abnormalities were observed in either study. A NOAEL of 15 mg/kg bw/day for the structural relative γ-undecalactone (FEMA 3091) and a NOAEL of 63 mg/kg bw/day for the structural relative γ-nonalactone (FEMA 2781) was established for male rats. The NOAEL of 15 mg/kg bw/day for FEMA 3091 is greater than 9,000,000 times the anticipated daily *per capita* intake of 2-methyloctan-4-olide from use as a flavor ingredient. The substance occurs naturally in beer and hops pellets (Natural Occurrence Analysis, 2021d). Based on the quantitative data, a consumption ratio of 1,861,200 could be calculated (Stofberg and Grundschober, 1987). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. It is predicted that the lactone ring of 2-methyloctan-4-olide will undergo hydrolysis followed by conjugation and excretion of the resulting hydroxycarboxylic acid derivative or *β*-oxidation of the hydroxy acid to yield short-chain polar metabolites that are excreted either unchanged or in conjugated form (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 2-methyloctan-4-olide (Gooderham et al., 2020). Corroborative evidence from a two-strain screening bacterial reverse mutation assay showed 2-methyloctan-4-olide was not mutagenic at concentrations up to 1500 µg/plate in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 metabolic activation (Kino, 2021a). Corroborative evidence from an Ames assay in *S. typhimurium* strains TA92, TA1535,

TA100, TA1537, TA94 and TA98 showed that the structural relative γ-undecalactone (FEMA 3091) did not increase the frequency of revertant colonies in the absence or presence of S9 metabolic activation at concentrations up to 5000 µg/plate (Ishidate et al., 1984). Corroborative evidence from another Ames assay in *S. typhimurium* strains TA97 and TA102 showed that the structural relative γ-undecalactone (FEMA 3091) did not increase the frequency of revertant colonies in the absence or presence of S9 metabolic activation at concentrations up to 100 µg/plate (Fujita and Sasaki, 1987). Corroborative evidence from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay indicated that the structural relative γ-nonalactone (FEMA 2781) was not mutagenic at concentrations up to 5000 µg/plate in the presence and absence of S9 using the plate incorporation and preincubation assays in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA (Rao, 2020b). Corroborative evidence from another Ames assay using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 for the structural relative γ-nonalactone (FEMA 2781) did not show an increase in the frequency of revertant colonies in either the absence or presence of S9 metabolic activation using the plate incorporation methodology at concentrations up to 36 µg/plate (Heck et al., 1989; Jagannath, 1982). Corroborative evidence showed that no induction of chromosome aberrations was observed when the structural relative γ-nonalactone (FEMA 2781) was tested in Chinese hamster ovary (CHO) cells for 20h in the absence of S9 at concentrations of 251-754 µg/mL and for 10 h in the presence of S9 at concentrations of 495-3710 µg/mL (Murli, 1989). Corroborative evidence for the structural relative γ-undecalactone (FEMA 3091) gave negative results in a chromosomal aberration assay in Chinese hamster fibroblast cells at concentrations up to 500 µg/mL (Ishidate et al., 1984). Corroborative evidence from a GLP- and OECD 476 guideline-compliant *in vitro* mammalian cell gene mutation assay in L5178Y mouse lymphoma cells showed no increases in mutant frequencies when the structural relative γ-nonalactone (FEMA 2781) was tested at concentrations of 200-550 µg/mL for 3 h in the absence of S9, 100-550 µg/mL for 24 hours in the absence of S9 and 200-1050 µg/mL for 3 h in the presence of S9 (ECHA, 2012c). Corroborative evidence for the structural relative γ-nonalactone (FEMA 2781) showed negative results in the absence of S9 metabolic activation in a mouse lymphoma forward mutation assay in L5178Y TK +/- mouse lymphoma cells at concentrations up to 1000 nl/ml (approximately 1 µg/mL based on a specific gravity of 0.96). Increases in mutagenicity were reported at the top two concentrations of 400-600 nl/ml (approximately 0.4 and 0.6 µg/mL2) in the presence of S9 (Heck et al., 1989; Cifone, 1982). Corroborative evidence for the structural relative γ-nonalactone (FEMA 2781) was negative in an unscheduled DNA synthesis assay in rat hepatocytes from adult male Fischer or Sprague-Dawley rats at concentrations up to 500 µg/ml (Heck et al., 1989; Cifone, 1988). Corroborative evidence from an *in vivo* micronucleus assay in which groups of 6 male ddY mice were administered the structural relative γ-undecalactone (FEMA 3091) intraperitoneally at doses of 250, 500, 1000 or 2000 mg/kg bw in olive oil showed no increases in the frequency of micronuclei in the polychromatic erythrocytes of the bone marrow were observed (Hayashi et al., 1988). Additional corroborative evidence is from a GLP- and OECD 474 guideline-compliant *in vivo* mouse micronucleus assay for NMRI mice (6/sex/dose) administered oral doses of the structural relative γ-nonalactone (FEMA 2781) at 500, 1000 and 2000 mg/kg bw (Api et al., 2019b). No significant induction of micronuclei was observed at all tested doses.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 3-hydroxyhexanoic acid (CAS 10191-24-9) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5005) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic primary alcohols, aldehydes, carboxylic acids, acetals, and esters containing additional oxygenated functional groups (JECFA, 2000, 2011; SLR, B1B). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 3-hydroxyhexanoic acid from use as a flavor ingredient to be 0.1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low reproductive and developmental toxicity potential of 3-hydroxyhexanoic acid was reviewed by the Expert Panel from a developmental toxicity study in female rats (20-24/group) in which the structural relative adipic acid (FEMA 2011) was administered by gavage once daily from gestation days 6-15 at dosage levels of 2.9, 13, 62 and 288 mg/kg bw/day (JECFA, 2000; Morgareidge, 1973). On Gestation Day 20, all female rats were subjected to cesarean section and all recorded observations were comparable to the control group. The reported number of abnormalities was comparable to the occurrence in the control group (JECFA, 2000; Morgareidge, 1973). Based on corroborative evidence, no teratogenic or embryotoxic effects were observed when the structural relative adipic acid (FEMA 2011) was administered once daily by gavage to CD-1 mice (21-24/group) from gestation days 6-15 at doses of 2.6, 12, 56 and 263 mg/kg bw/day, to hamsters (20-24/group) from gestation days 6-10 at doses of 2, 9.5, 44 and 205 mg/kg bw/day (JECFA, 2000; Morgareidge, 1973) and rabbits (10-14/group) from gestation days 6-18 at doses of 2.5, 12, 54 and 250 mg/kg bw/day (JECFA, 2000; Morgareidge, 1974b). Evaluation protocols were similar to the developmental toxicity study in rats for the structural relative adipic acid (FEMA 2011), except that dams were subject to cesarean sections on gestation day 17 for mice, gestation day 14 for hamsters and gestation day 29 for rabbits. Corroborative evidence is available from a GLP-compliant reproductive and developmental toxicity screening in which female Charles River Cr1:CD®(SD)BR rats (10/group) were orally administered the structural relative hexanoic acid (FEMA 2559) in corn oil by gavage at doses of 36, 182 or 364 mg/kg bw/day for up to one week before cohabitation, through gestation and parturition and four days postpartum (Hoberman, 1990). No mortalities or gross lesions were observed in adults and pups of the treatment groups. Rales were observed in two mid-dose and five high-dose adults during the premating and/or gestation periods. Decreases in body weight and body weight gains in mid- and high-dose rats were not significant and were transient at the mid-dose. Non-significant decreases in absolute and relative feed consumption were observed in high-dose rats in the premating period and the first week of gestation relative to controls. A slight reduction in weight gain of litters over the

four-day lactation period was not significant. No other significant effects were observed. Based on the clinical signs of toxicity and slightly decreased feed consumption and body weights at the top dose level, the study authors considered the maternal toxicity NOAEL to be 182 mg/kg bw/day. The developmental toxicity NOAEL was considered to be the top dose level of 364 mg/kg bw/day (Hoberman, 1990). The maternal toxicity NOAEL of 182 mg/kg bw/day is greater than 109,000,000 times the anticipated daily *per capita* intake of 3-hydroxyhexanoic acid from use as a flavor ingredient, and the developmental toxicity NOAEL of 364 mg/kg bw/day is greater than 218,000,000 times the anticipated daily *per capita* intake of 3-hydroxyhexanoic acid from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of 3-hydroxyhexanoic acid was reviewed by the Expert Panel from a 2-year dietary study in which male and female albino rats were administered the structural relative adipic acid (FEMA 2011) at doses of approximately 75, 750, 2250 and 3750 mg/kg bw/day (Horn et al., 1957). Female rats were only included at the control and 750 mg/kg bw/day levels. Survival rates were comparable between treatment and control groups. Bodyweight gains in the two highest dose groups were significantly less during the rapid growth period. No significant body weight changes were recorded in the latter half of the experiment, but the authors noted that the 3750 mg/kg bw/day dose group showed consistently lower body weights. The only dose group with a reduction in food consumption, when compared to controls, was the 3750 mg/kg bw/day dose group. Incidences of tumors and/or lung pathology were comparable between treatment and control groups. Gross and microscopic histopathological examination showed no notable changes in any treatment group. The examined organ weights were similar between treatment and control groups. The authors of this study did not select a NOAEL, but the Expert Panel determined a NOAEL of 750 mg/kg bw/day based on the slight body weight reductions in the 2250 and 3750 mg/kg bw/day treatment groups (Horn et al., 1957). Corroborative evidence is also available from a 28-day repeat-dose oral toxicity study in which Fischer 344 rats (10/sex/group) were administered the structural relative hexanoic acid (FEMA 2559) at doses of 250, 1250 or 2500 mg/kg bw/day (Wenk, 1990). Early deaths of seven high-dose males (six were moribund sacrificed due to respiratory problems and one found dead) and two mid-dose males (one due to gavage error) were observed. Additional early deaths were observed in five high-dose females (two were moribund sacrificed due to respiratory problems and three found dead) and one mid-dose female (gavage error). Clinical signs of toxicity were observed in both sexes for most animals at the top two dose levels including respiratory problems which were displayed as rales along with shallow and rapid breathing by the second day of the study. Other clinical signs of toxicity observed in individual animals at the top two levels include gasping and red nasal secretions. No signs of clinical toxicity were observed at the lowest dose level with either sex. At both the mid- and high-dose levels for both sexes, statistically significant and dose-dependent decreases in bodyweight and food consumption were observed throughout the study period and during the first three weeks, respectively. While statistically significant changes were observed for several clinical chemistry and hematological parameters at the mid- and high-dose levels, only sodium and/or potassium reductions were considered toxicologically relevant. The study authors opined that acid-base balances were considered a possible reason for the respiratory effects and the electrolyte changes observed with sodium and potassium reduction. High-dose males exhibited test article-related cytoplasmic changes in the liver along with degenerative changes in the testes. Renal tubular regeneration observed in high-dose females were considered equivocal. Based on premature deaths and clinical signs of toxicity observed at the top two dose levels, the NOAEL for this study was 250 mg/kg bw/day (Wenk, 1990). The NOAEL of 250 mg/kw bw/day for the structural relative hexanoic acid (FEMA 2559) is 150,000,000 times the anticipated daily *per capita* intake of 3-hydroxyhexanoic acid from use as a flavor ingredient. The substance occurs naturally in strawberries and milk; however, quantitative data are unavailable and therefore a consumption ratio cannot be calculated (Mussinan and Walradt, 1975; Parks, 1977). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. 3-Hydroxyhexanoic acid is anticipated to undergo fatty acid metabolism resulting in oxidation to acetoacetic acid, an endogenous compound which is then released into the blood and peripheral tissues. it is then converted to acetyl CoA, which is available for metabolism (Saito et al., 2017; Smith et al., 2018; Voet and Voet, 1990). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 3-hydroxyhexanoic acid (Gooderham et al., 2020). Corroborative evidence is available from a two-strain screening bacterial reverse mutation assay in which 3-hydroxyhexanoic acid was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 metabolic activation (Kino, 2021b). Corroborative evidence is also available for 3-hydroxyhexanoic acid, which was also not mutagenic at concentrations up to 5000 µg/plate in an OECD 471 guideline-compliant bacterial reverse mutation assay conducted in *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *E. coli* strain WP2 uvrA in the absence or presence of S9 metabolic activation (EFSA, 2019c). Negative results were reported in corroborative evidence from GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays for the structural relatives *trans*-2-hexenoic acid (FEMA 3169) (Api et al., 2018) and hexanoic acid (FEMA 2559) (Rao, 2020c) when tested in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA in the absence or presence of S9 at concentrations up to 5000 µg/plate. Corroborative evidence from Ames assays in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 TA1538 and *E. coli* strain WP2uvrA showed that the structural relative adipic acid (FEMA 2011) did not increase the frequency of revertant colonies in either the absence or presence of S9 metabolic activation at concentrations up to 10,000 µg/plate (ECHA, 1996; Kubo, 2002; NTP, 2018c; Prival et al., 1991; Shimizu et al., 1985). The structural relative hexanoic acid (FEMA 2559) was non-genotoxic based on corroborative evidence from an unscheduled DNA synthesis assay at concentrations up to 927 µg/mL (Heck et al., 1989). Corroborative evidence available from a mouse lymphoma assay in L5178Y cells at reported concentrations up to 1000 nl/mL in the absence of S9 and at concentrations up to 800 nl/mL in the presence of

S9 indicated the structural relative hexanoic acid (FEMA 2559) induced significant increases in mutant frequencies at 700-800 nl/mL (corresponding to 644-736 µg/mL based on a specific gravity of 0.922) in the absence of S9 (Heck et al.,1989). However, the study author noted that culture conditions of low pH and high osmolality, which may occur upon incubation with acidic substances, have been shown to produce artefactual results (Heck et al.,1989). In reviewing these results, JECFA noted that these results should be interpreted with caution (JECFA, 1998). Furthermore, negative results were observed in the presence of S9 (Heck et al.,1989). Corroborative evidence from a GLP- and OECD 476 guideline-compliant *in vitro* mammalian cell gene mutation assay in V79 Chinese hamster cells showed that the structural relative adipic acid (FEMA 2011) at concentrations up to 10 mM (or approximately 1461 µg/mL) gave no significant increases in mutant frequency at the HPRT locus in the presence and absence of S9 metabolic activation (ECHA, 2009). No chromosomal aberrations were observed based on corroborative evidence from an *in vitro* chromosome aberration assay in human embryonic lung fibroblast cells (WI-38) treated with the structural relative adipic acid (FEMA 2011) at concentrations up to 200 µg/mL without metabolic activation (ECHA, 1974a). Corroborative evidence from an *in vivo* chromosome aberration assay showed no statistically significant increases in chromosome aberrations were observed in Sprague-Dawley rats (5/sex/group) administered the structural relative hexanoic acid (FEMA 2559) by oral gavage in corn oil at doses of 500, 1667 or 5000 mg/kg bw (Murli, 1991). High-dose males sacrificed at 6h and 30h appeared prostrate immediately after administration, while others appeared lethargic. High-dose animals in the 18h group appeared languid after administration. By 3.7 h, one high-dose male and female in the 6 hours group had died, while other animals at this dose from the six h and 30 hours groups were normal (Murli, 1991). Based on corroborative evidence, no significant increases in chromosome aberrations were observed in a series of studies in which male rats (5/group) were administered the structural relative adipic acid (FEMA 2011) at doses of 3.75, 37.5 and 375 mg/kg bw/day for one or five days, a single dose of 5000 mg/kg bw or doses of 2500 mg/kg bw/day for five days (ECHA, 1974b).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 3-methyl-3-butene-1-thiol (CAS 58156-49-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5006) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of simple aliphatic and aromatic sulfides and thiols (JECFA, 2000, 2004, 2008, 2011; SLR, A8). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 3-methyl-3-butene-1-thiol from use as a flavor ingredient to be 0.01 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 3-methyl-3-butene-1-thiol was reviewed by the Expert Panel from an OECD 408 guideline-compliant 90-day study in male and female Sprague-Dawley rats in which gavage administration of the structural relative 4-mercapto-4-methyl-2-pentanone (FEMA 3997) at doses of 13, 20 and 26 mg/kg/day resulted in a NOAEL of 26 mg/kg bw/day (Bauter, 2017b). Additional corroborative evidence from a 90-day study in albino weanling rats showed that dietary addition of the structural relative 2,3-butanedithiol (FEMA 3477) at a dose of 0.7 mg/kg bw/day resulted in no hematological, biochemical and urinary deviations from normal ranges of tested parameter or any unusual microscopic pathological observations (Morgareidge, 1974c). The NOAEL of 0.7 mg/kg bw/day is greater than 3,500,000 times the anticipated daily *per capita* intake of 3-methyl-3-butene-1-thiol from use as a flavor ingredient. The substance occurs naturally in roasted coffee beans; however, quantitative information was not available, and a consumption ratio could not be calculated (Natural Occurrence Analysis, 2021e). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. It is predicted that 3-methyl-3-butene-1-thiol will undergo oxidation of the thiol group resulting in the corresponding sulfenic acid, sulfinic acid, and sulfonic acid (McBain & Menn, 1969; Dutton & Illing, 1972; Maiorino et al., 1988; Richardson et al., 1991; Renwick, 1996). Sulfenic acids are unstable and readily undergo further oxidation to sulfinic and sulfonic acids or combine with nucleophiles (Klancnik, et al., 1992). The sulfinic and sulfonic acids are water-soluble and easily excreted. Alternatively, the thiol may react with glutathione and cysteine to form mixed disulfides that can then undergo reduction and oxidative desulfuration, or oxidation to sulfonic acid via the intermediate thiosulfinate and sulfinic acids. 3-Methyl-3-butene-1-thiol is also anticipated to undergo *S*-methylation on mammals to produce the corresponding methyl thioether that can be successively oxidized to the corresponding sulfoxide and sulfone (Tateishi et al., 1978; Shaw & Blagbrough, 1989; Tateishi & Tomisawa, 1989). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 3-methyl-3-butene-1-thiol (Gooderham et al., 2020). Corroborative evidence available for 3-methyl-3-butene-1-thiol indicated that the substance was not mutagenic in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA at concentrations of 39.1-1250 µg/plate in the presence of S9 and at concentrations of 9.8-313 µg/plate in the absence of S9 (Sato, 2021). Further corroborative evidence from an Ames assay for the structural relative 2-methyl-3-butene-2-thiol (FEMA 4916) did not increase the frequency of revertant colonies in the absence and presence of S9 metabolic activation in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 at concentrations of 9.8-313 µg/plate, and *E. coli* strain WP2 uvrA at concentrations of 39.1-1250 µg/plate (Sato, 2017). Additional corroborative evidence from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay showed that the structural relative 4-mercapto-4-methyl-2-pentanone (FEMA 3997) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102 in the presence and absence of S9 using the plate incorporation and preincubation methods (McGarry, 2012).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding myoglobin (CAS 9008-45-1) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5007) (Smith et al., 2005a) in the food categories and at the use levels

specified in Table 2. This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake of myoglobin from use as a flavor ingredient to be 3,238 µg/person/day. A decision tree structural class for the threshold of toxicological concern (TTC) could not be assigned for myoglobin as polymers are excluded from the Cramer/Ford/Hall Decision Tree classification (Cramer et al., 1978). Corroborative evidence for the low toxicity potential of myoglobin was reviewed by the Expert Panel from a soy leghemoglobin preparation (containing 6-9% of soy leghemoglobin protein) provided to Sprague-Dawley rats (10/sex/group) at dietary levels of 512, 1024 or 1536 mg/kg bw/day (corresponding to 250, 500 or 750 mg/kg bw/day of soy leghemoglobin) in a GLP- and OECD 407 guideline-compliant 28-day dietary toxicity study (mean overall daily intakes of soy leghemoglobin protein were 0, 234, 466 and 702 mg/kg bw/day in male rats, as well as 0, 243, 480 and 718 mg/kg bw/day in female rats) (Fraser et al., 2018). No adverse treatment-related effects were observed, and a NOAEL was established at the top dose of 702 mg/kg bw/day and 718 mg/kg bw/day in male and female rats, respectively. Additionally, corroborative evidence is also available from a follow-up OECD-compliant 28-day dietary toxicity study, the same related material, soy leghemoglobin preparation, was provided to female Sprague Dawley rats (15/group) at dietary levels of 512, 1024 or 1536 mg/kg bw/day, which correspond to soy leghemoglobin protein levels of 250, 500 or 750 mg/kg bw/day (mean overall daily intakes of soy leghemoglobin protein were 0, 250, 496 and 738 mg/kg bw/day) (Fraser et al., 2018). No adverse treatment-related effects were observed, and the authors established a NOAEL at the top dose. The NOAEL of 702 mg/kg bw/day is greater than 13,000 times the anticipated daily *per capita* intake of myoglobin from use as a flavor ingredient. The substance occurs naturally in muscles and tissues of beef, pork and chicken; however, quantitative information was not available, and a consumption ratio could not be calculated (Yip and Dallman, 1996). The Expert Panel considered the specification of ≥95% the material, including ≥3% bovine myoglobin as a secondary component, to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. Proteins, such as myoglobin, are expected to be denatured in the acidic environment of the stomach accompanied by hydrolysis of the polypeptide by pepsins in the gut to free amino acids and oligopeptides, which are absorbed in the proximal jejunum unchanged or hydrolyzed by brush border peptidases (Nelson and Cox, 2008). Once absorbed into the intestinal mucosal cells the oligopeptides are further hydrolyzed by cytoplasmic amino oligopeptidases to free amino acids. Corroborative evidence is available from the related preparation soy leghemoglobin which was found to be rapidly digested at 1 ug in 10-unit activity of pepsin, and 1 ug in 1 unit activity of pepsin in human stimulated gastric fluid of pH 2 (Jin et al., 2018; Reyes et al., 2018). No pepsin-stable fragments were identified. Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of myoglobin (Gooderham et al., 2020). Corroborative evidence is available from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay in which a related material, soy leghemoglobin preparation (containing 6-9% of soy leghemoglobin protein) was not mutagenic at concentrations up to 74,000 µg/plate (corresponding to 5000 µg/plate of soy leghemoglobin protein) in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA in the presence and absence of S9 using the plate incorporation and preincubation methods (Fraser et al., 2018). Corroborative evidence is also available from a GLP- and OECD 473 guideline-compliant *in vitro* mammalian chromosome aberration assay in which a soy leghemoglobin preparation (containing 6-9% of soy leghemoglobin protein) was not clastogenic when tested in human peripheral blood lymphocytes at concentrations up to 7,400-74,000 µg/mL for 4 hours in the absence of S9, concentrations of 14,800-74,000 µg/mL for 4 hours in the presence of S9 and at concentrations of 1,480-14,800 µg/mL for 24 hours in the absence of S9 (Fraser et al., 2018). These levels corresponded to test concentrations of soy leghemoglobin protein at 500-5000 µg/ml for 4 hours in the absence of S9, 1000-5000 µg/mL for 4 hours in the presence of S9 and 100-1000 µg/mL for 24 hours in the absence of S9. The FEMA Expert Panel noted that myoglobin does not contain protein or ingredients derived from one of nine foods or food groups defined as major allergens by the Food Allergen Labeling and Consumer Protection Act of 2004 (P.L. 108-282) (FALCPA) and the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act of 2021 (P.L. 117-11).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding finger lime distillate (CAS 1174331-57-7) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5008) (Smith et al., 2005a) for use as a flavor ingredient in the food categories and at the use levels specified in the GRAS application (see Table 2). This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005a). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of finger lime distillate from use as a flavor ingredient to be 6 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). On a water-removed basis, the per capita intake for the concentrate from use as a flavor ingredient was calculated to be 0.006 µg/person/day. The material is produced from the fruits of *Microcitrus australasica* (F. Muell.) Swingle (synonym: *Citrus australasica* F. Muell.). Though the source material is consumed as food, quantitative information was not available, and a consumption ratio could not be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for a representative members of the principal identified congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be predicted to be metabolized primarily by well-established detoxication pathways to innocuous products or to be excreted as such (Smith et al., 2018). Corroborative evidence for the low reproductive and developmental toxicity potential of finger lime distillate was reviewed by the Expert Panel from data available for its constituents. As described in the Expert Panel's prior assessment of citrus-derived natural flavor complexes (Cohen et al., 2019), corroborative evidence of the reproductive and/or developmental toxicity of the constituent limonene (FEMA 2633) was reviewed (Kodama et al., 1974, 1977; Tsuji et al., 1975), and maternal and fetal toxicity NOAELs of 250 mg/kg bw/day was determined in pregnant Japanese white rabbits (Kodama et al., 1976). Corroborative evidence is available from a GLP- and OECD 421 guideline-

compliant one-generation reproductive toxicity study in male and female Albino rats (10/sex/group) administered the constituent 2-methyl-3-buten-2-ol at doses of 12.5, 50 or 200 mg/kg bw/day for two weeks before mating. High-dose male body weights were reduced. A slight test substance-related decrease in feed consumption was observed in high-dose females. In the F1 generation, the pup viability index decreased by 23% in the high-dose group. No other treatment-related effects were observed in the parental and first filial generations, and NOAELs of 50 mg/kg bw/day was established for both the parental and first filial generations (OECD, 1995). The NOAELs of 250 mg/kg bw/day for FEMA 2633 are greater than 2,700,000,000 times the anticipated daily per capita intake of finger lime distillate on a water-removed, concentrate basis from use of finger lime distillate as a flavor ingredient. The NOAELs of 50 mg/kg bw/day for the constituent 2-methyl-3-buten-2-ol are 500,000,000 times the anticipated daily *per capita* intake of finger lime distillate on a water-removed, concentrate basis from use of finger lime distillate as a flavor ingredient. Further corroborative evidence was evaluated from swine spermatozoa incubated with both tea tree oil and the constituent 4-carvomenthenol FEMA 2248 at concentrations of 0.2-1 mg/mL and 0.08-0.83 mg/mL, respectively, for 3 hours. Evaluations were conducted for motility, pH, acrosome status and viability. The first toxic effect was observed at 0.67 mg/mL of FEMA 2248, which was the third-highest test concentration. The most sensitive reproductive parameters (i.e., the most easily impacted by the test article) for FEMA 2248 were acrosome reaction and viability while the motility was significantly altered at the top tested concentration only. No synergistic effects between tea tree oil and the constituent FEMA 2248 were observed (Elmi et al., 2019). Corroborative evidence for the low toxicity potential of finger lime distillate was reviewed by the Expert Panel from data available for its constituents. Corroborative evidence is available from a GLP- and OECD 407 guideline-compliant repeat dose 28-day oral toxicity study in which Wistar rats (5/sex/dose) were administered the constituent 2-methyl-3-buten-2-ol at doses of 30, 150 or 750 mg/kg bw/day for four weeks by gavage (ECHA, 1994a). Significantly decreased chloride and cholesterol levels were observed in high-dose males and females. Significantly decreased triglyceride levels and significantly increased magnesium levels were observed in high-dose males. Significantly decreased potassium levels were observed in high-dose females. The authors attributed the significant changes in triglyceride and cholesterol levels in high-dose males to slight changes in lipid metabolism. The study authors considered the changes in chloride, potassium and magnesium levels to be treatment-related but could not assign a specific toxic effect. No other significant treatment related effects were observed, and a NOAEL of 150 mg/kg bw/day was established (ECHA, 1994a). Additional corroborative evidence is available from another GLP- and OECD 407 guideline-compliant repeat dose 28-day oral toxicity study in which Wistar rats (10-14/sex/dose) were administered the constituent 2-methyl-3-buten-2-ol at doses of 50, 200 or 600 mg/kg bw/day for four weeks by gavage (OECD, 1995). One male and one female died at the top dose level spontaneously with no apparent cause of death determined, so treatment-related effects were considered a possibility. Minimal hypertrophy of hepatocytes and increased liver weights were observed in mid-dose females. Minimal increases in kidney weights and accumulation of renal hyaline droplets were observed in mid-dose males. The statistical significance of these findings was not reported. Marginally increased liver weights and periacinar hypertrophy of liver cells were observed in both sexes at the top dose level. No functional changes in the kidney were observed and the only pathological findings were confined to the liver in both sexes and male kidneys only. The kidney findings were evident of α2u-globulin nephropathy which is male rat-specific and not relevant to humans. Clinical signs of toxicity including ataxia, sedation and uncoordinated gait were observed at the highest dose level upon first dose administration. Salivation occurred after repeated administration of the test article. Small increases in transaminases were noted at unspecified dose levels Based on the increased liver weights and hypertrophy of hepatocytes observed at the mid-dose, the NOAEL is 50 mg/kg bw/day (OECD, 1995). As described in the Expert Panel's prior assessment of corroborative evidence from the subchronic and chronic toxicity of the constituent d-limonene (FEMA 2633) in its review of citrus-derived natural flavor complexes (Cohen et al., 2019), a NOAEL of 215 mg/kg bw/day was determined for female rats from a 103-week NTP study with F344/N rats (NTP, 1990). The NOAEL of 215 mg/kg bw/day for the constituent d-limonene (FEMA 2633) is 2,150,000,000 times the anticipated daily *per capita* intake of finger lime distillate on a water-removed, concentrate basis from use of finger lime distillate as a flavor ingredient. The NOAEL of 50 mg/kg bw/day for the constituent 2-methyl-3-buten-2-ol is 500,000,000 times the anticipated daily *per capita* intake of finger lime distillate on a water-removed, concentrate basis from use of finger lime distillate as a flavor ingredient. The Expert Panel reviewed the key constituents of finger lime distillate and noted that the congeneric group intakes were below the respective TTC thresholds. Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of finger lime distillate (Gooderham et al., 2020; Cohen et al., 2018).The Expert Panel reviewed their prior assessment of the corroborative genotoxicity data for the constituent limonene (Cohen et al., 2019) and determined it sufficient to indicate a lack of genotoxic concern for finger lime distillate. Corroborative evidence is available from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay in which the constituent 4-carvomenthenol (FEMA 2248) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA97a, TA98, TA100, TA102 and TA1535 in the presence and absence of S9 using the plate incorporation method (ECHA, 2017). Negative results were obtained from a corroborative GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay for the same constituent, FEMA 2248 (ECHA, 2017). Corroborative evidence is available for the constituent 2-methyl-3-buten-2-ol, which was not mutagenic in GLP- and/or OECD 471 guideline-compliant bacterial reverse mutation assays at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537 in the presence and absence of S9 (Api et al., 2015). Additional corroborative evidence is available from a GLP- and OECD 474 guideline-compliant *in vivo* micronucleus assay, NMRI mice (5/sex/group) were administered single oral doses of 500, 1000 or 1500 mg/kg bw of the constituent 2-methyl-3-buten-2-ol. Clinical signs of toxicity, including irregular respiration and piloerection, were observed at all dose levels 1 to 2 days after treatment. Bone marrow was collected from all doses 24 hours after administration, as well as after 16 hours and 48 hours after administration from high-dose animals only. No significant increases in the frequency of micronucleated erythrocytes were observed relative to the vehicle controls (Api et al., 2015).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding steviol glycoside extract, *Stevia rebaudiana*, rebaudioside A

40% (CAS 91722-21-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5009) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake of steviol glycoside extract, *Stevia rebaudiana*, rebaudioside A 40% from use as a flavor ingredient to be 415 µg/person/day. Corroborative evidence for the low toxicity potential of steviol glycoside extract, *Stevia rebaudiana*, rebaudioside A 40% was evaluated by the Expert Panel from a 108-week carcinogenicity study for stevioside showed no carcinogenic effects were observed (Toyoda et al., 1997). Corroborative evidence is also available from a 2-year feeding study, in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 79,000 times the anticipated daily *per capita* intake of steviol glycoside extract, *Stevia rebaudiana*, rebaudioside A 40% from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana*. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to the intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al., 2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997; Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; JECFA, 1982). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of steviol glycoside extract, *Stevia rebaudiana*, rebaudioside A 40% (Gooderham et al., 2020). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of corroborative studies. While some positive results are reported in corroborative *in vitro* mutagenicity assays, corroborative *in vivo* studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding thaumatin II (CAS 83271-81-2) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5010) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of thaumatin II from use as a flavor ingredient to be 2,906 µg/person/day. A decision tree structural class for the threshold of toxicological concern (TTC) could not be assigned for thaumatin II as polymers are excluded from the Cramer/Ford/Hall Decision Tree classification (Cramer et al., 1978). Corroborative evidence for the low reproductive and developmental toxicity potential of thaumatin II was reviewed by the Expert Panel in a study in which no effects on body weight, number of litters, fetal weight or implantation viability were observed in pregnant female rats (20/group) administered 0, 200, 600 or 2000 mg/kg bw/day of the protein thaumatin (FEMA 3732) from GD 6-15 (Higginbotham et al., 1983). The FEMA Expert Panel determined the NOAEL of 2000 mg/kg bw/day is greater than 40,000 times the anticipated daily *per capita* intake of thaumatin II from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of thaumatin II was reviewed by the Expert panel from a subchronic toxicity study in which CD rats (5/sex/group) were provided 0, 30,000 or 80,000 ppm of the structural relative thaumatin in the diet for 3 weeks, followed by administration via drinking water for 2 weeks (equivalent to 0, 1500 or 4000 mg/kg bw/day or 0, 3000 or 8000 mg/kg bw/day in feed and water, respectively) (Ben-Dyke et al., 1976a). The highest tested intake level exceeds the FDA test substance intake limit of 5%, and any findings in this level could be due to nutritive differences and a dietary imbalance rather than overt toxicity of the test substance, as noted in the FDA Redbook 2000 guidance. Diarrhea was observed in three high dose males during the first week of the water administration study. Lower feed consumption and feed intakes were observed in all test females and high dose males compared to the controls in the dietary study. In the drinking water study, decreased feed intake was observed in high dose males and increased feed intake was observed in high dose females. Reduced fluid intake in all treatment groups during the first 2 days of the drinking water study returned to normal by the second week. Reduced absolute spleen weights were observed in high dose males. Significant dose-dependent increases in relative kidney weights were observed in males but were not accompanied by any correlating histopathology and no biochemical parameters were measured in the study. No serum antibody to the structural relative thaumatin was detected in test rats at the end of five weeks in the Ouchterlony test. The FEMA Expert Panel established a NOAEL of 1500 mg/kg bw/day in the diet or 3000 mg/kg bw/day in drinking water provided to CD rats. Corroborative evidence is also available from a 13-week study in which CD rats (10/sex/group) were provided the protein thaumatin (FEMA 3732, described using the alternative name Talin) at 0 (supplemented with 8% casein), 1, 4 and 8% in feed (equivalent to 0, 500, 2000 or 4000 mg/kg bw/day). No adverse treatment-related mortalities or effects were observed (Ben-Dyke et al., 1976b). The FEMA Expert Panel established a NOAEL at the highest tested level of 4000 mg/kg bw/day in CD rats. Corroborative evidence is also available from a GLP-compliant 90-day toxicity study in which COBS-CD rats (20/sex/group) were provided 0, 0.3, 1 or 3% of the protein thaumatin (FEMA 3732) (equivalent to 0, 256, 861 or 2418 mg/kg bw/day and 0, 293, 998 or 2822 mg/kg bw/day for males and females, respectively). No adverse, treatment-related mortalities or effects were observed (Higginbotham et al., 1983). A NOAEL was established at the top dose of 2418 mg/kg bw/day and 2822 mg/kg bw/day in

males and females, respectively. Additional corroborative evidence is available from a 90-day repeated dose oral toxicity study in which 4-week-old Crj:CD (SD) IGS rats (10/sex/group) were provided 0%, 0.3%, 1% or 3% thaumatin sterilized by electron beam irradiation (5.0 kGy) or non-irradiated 3% thaumatin (Hagiwara et al., 2005). The non-irradiated dietary levels correspond to 2394 and 2925 mg/kg bw/day for males and females, respectively. The test material was not further described. No treatment-related mortalities or adverse effects. Corroborative evidence is also available from a GLP-compliant 90-day toxicity study, four male and female beagle dogs were provided 0, 0.3, 1 or 3% of the protein thaumatin (FEMA 3732) for 90 days (equivalent to average daily intakes of 0, 133, 435 or 1298 mg/kg bw/day for males and 139, 469 or 1476 mg/kg bw/day for females, respectively). No adverse treatment-related mortalities or effects were observed (Higginbotham et al., 1983). A NOAEL was established at the top dose of 1298 mg/kg bw/day and 1476 mg/kg bw/day in males and females, respectively, for the structural relative thaumatin. This NOAEL of 1298 mg/kg bw/day is greater than 26,000 times the anticipated daily *per capita* intake of thaumatin II from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of thaumatin II (Gooderham et al., 2020). Corroborative evidence is available from bacterial reverse mutation assays conducted in *S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1530, TA1531, TA1532, TA1535, TA1537, TA1538 and TA1964 for thaumatin (FEMA 3732) (Higginbotham et al., 1983) as well as the structural relatives glycine (FEMA 3287) (Fujita et al., 1994; Haworth et al., 1983), L-glutamic acid (FEMA 3285) (Fujita et al., 1994; Zeiger et al., 1992), monosodium glutamate (FEMA 2756) (De Flora et al., 1984; Fujita et al., 1994; Zeiger et al., 1992), L-proline (FEMA 3319) (Green and Savage, 1978), L-cysteine (FEMA 3263) (Calle and Sullivan, 1982) and L-methionine (De Serres and Ashby, 1981; Sugimura et al., 1976; Hubbard et al., 1981), which were not mutagenic in the absence and presence of S9. Corroborative evidence is available from a standard Ames assay, a microsomal Ames assay and a host-mediated assay in mice in which proline was not mutagenic (Green and Savage, 1978). Based on corroborative evidence in *E. coli* Q13, WP2, WP2uvrA, JC9238, JC8471, JC5519, JC7689, JC7623, 58-161envA, C600, WP67uvrApolA, CM871 uvrArecAlexA, 343/113/uvrB, 343/113/uvrB/leu8, P3478 polA- and P3310 polA+ incubated with the protein thaumatin (FEMA 3732) (Higginbotham et al., 1983) as well as with the structural relatives glycine (FEMA 3287) (Kubinski et al., 1981), DL-valine (FEMA 3444) (Fluck et al., 1976; Kubinski et al., 1981), L-leucine (FEMA 3297) (Kubinski et al., 1981), L-isoleucine (FEMA 4675) (Kubinski et al., 1981), tyrosine (Martinez et al., 2000), monosodium glutamate (FEMA 2756) (De Flora et al., 1984), L-lysine (FEMA 3847) (Kubinski et al., 1981), L-arginine (FEMA 3819) (Kubinski et al., 1981), L-proline (FEMA 3319) (Kubinski et al., 1981), L-histidine (FEMA 3694) (Kubinski et al., 1981; Martinez et al., 2000) and L-cysteine (FEMA 3263) (Kubinski et al., 1981), no mutagenicity was observed. Available corroborative evidence also showed no induction of lethal mutations in germ cells of CD-1 male mice (15/group) administered 0, 200 or 2000 mg/kg bw/day of the protein thaumatin (FEMA 3732) for five consecutive days (Higginbotham et al., 1983). The FEMA Expert Panel noted that thaumatin II does not contain protein or ingredients derived from one of nine foods or food groups defined as major allergens by the Food Allergen Labeling and Consumer Protection Act of 2004 (P.L. 108-282) (FALCPA) and the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act of 2021 (P.L. 117-11).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding heat-treated glucosylated steviol glycosides 45% with steviol glycosides 20% (CAS 2649239-34-7) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5011) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake of heat-treated glucosylated steviol glycosides 45% with steviol glycosides 20% from use as a flavor ingredient to be 692 µg/person/day. Corroborative evidence for the low toxicity potential of heat-treated glucosylated steviol glycosides 45% with steviol glycosides 20% was evaluated by the Expert Panel from a 108-week carcinogenicity study for stevioside showed no carcinogenic effects were observed (Toyoda et al., 1997). Corroborative evidence is also available from a 2-year feeding study, in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 45,000 times the anticipated daily *per capita* intake of heat-treated glucosylated steviol glycosides 45% with steviol glycosides 20% from use as a flavor ingredient. Additional corroborative evidence is available from a 52-week chronic toxicity study in which Beagle dogs (4/sex/group) were provided 0, 6200, 12500 or 50000 ppm of *beta*-cyclodextrin (Bellringer et al., 1995). The dietary concentrations correspond to actual intakes of 229, 456 or 1831 mg/kg bw/day and 224, 476 or 1967 mg/kg bw/day in male and female dogs, respectively (Bellringer et al., 1995). The dietary concentrations correspond to actual intakes of 229, 456 or 1831 mg/kg bw/day and 224, 476 or 1967 mg/kg bw/day in male and female dogs, respectively (Bellringer et al., 1995). There were no toxicologically significant findings, and a NOAEL was established at the top dose (1831 and 1967 mg/kg bw/day for male and female dogs, respectively). This NOAEL is greater than 152,000 times the anticipated daily *per capita* intake of heat-treated glucosylated steviol glycosides 45% with steviol glycosides 20% from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana*. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al., 2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997;

Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; BeMiller, 2003; JECFA, 1982). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of heat-treated glucosylated steviol glycosides 45% with steviol glycosides 20% (Gooderham et al., 2020). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of corroborative studies. While some positive results are reported in corroborative in vitro mutagenicity assays, corroborative in vivo studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding ethyl 5-acetoxyoctadecanoate (CAS 2762033-61-2) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5012) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters (JECFA, 1999, 2012, 2020; SLR, M1). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of ethyl 5-acetoxyoctadecanoate from use as a flavor ingredient to be 1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class I (1800 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of ethyl 5-acetoxyoctadecanoate was reviewed by the Expert Panel from a GLP-compliant combined repeat dose and reproductive/developmental toxicity study in which Sprague Dawley rats (12/sex/group) were administered 0 (corn oil), 100, 300 and 1000 mg/kg bw/day of the structural relative and minor constituent ethyl octadecanoate (FEMA 3490) by oral gavage for 42 days (JMHLW, 2017c). Significantly decreased body weights (at necropsy) and hindlimb grip strength were observed in high-dose males of the main study period. Significantly higher white blood cell counts, proportions of large unstained cells and lymphocyte counts were observed in unmated high-dose females in the main study period. No other significant, treatment-related adverse effects were observed. Based on the hindlimb grip strength findings, a NOAEL of 300 mg/kg bw/day was established for the repeat dose toxicity and a NOAEL of 1000 mg/kg bw/day for the reproductive/developmental toxicity of the structural relative and minor constituent ethyl octadecanoate (FEMA 3490). The NOAEL of 1000 mg/kg bw/day is 50,000,000 times the anticipated daily *per capita* intake of ethyl 5-acetoxyoctadecanoate from use as a flavor ingredient. The NOAEL of 300 mg/kg bw/day is 15,000,000 times the anticipated daily *per capita* intake of ethyl 5-acetoxyoctadecanoate from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. Ethyl 5-acetoxyoctadecanoate is expected to be rapidly hydrolyzed to ethanol, acetic acid, and 5-hydroxyoctadecanoic acid. Ethanol and acetic acid would enter the citric acid cycle, while 5-hydroxydecanoate would undergo metabolism via *beta*-oxidation in fatty acid pathways (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of ethyl 5-acetoxyoctadecanoate (Gooderham et al., 2020). Ethyl 5-acetoxyoctadecanoate was not mutagenic at concentrations up to 5000 µg/plate when tested in a corroborative GLP-compliant bacterial reverse mutation assay in the presence and absence of S9 using *S. typhimurium* TA98, TA100, TA1535 and TA1537, as well as *E. coli* WP2 uvrA (Hosoya, 2022). Corroborative evidence is also available from GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays in which the structural relative ethyl octadecanoate (FEMA 3490) and the structural relative ethyl 2-acetyloctanoate (FEMA 4459) were not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537, as well as *E. coli* WP2uvrA in the presence and absence of S9 metabolic activation using the preincubation and plate incorporation methodologies, respectively (JMHLW, 2017a; Api et al., 2019c). Corroborative evidence from a bacterial reverse mutation assay indicates the structural relative ethyl 5-formyloxydecanoate (FEMA 4765) (with secondary components *delta*-decalactone (FEMA 2361) and ethyl-5-acetoxydecanoate) was not mutagenic at concentrations up to 500 µg/plate when tested in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 using the preincubation method (Kino, 2011). Additional corroborative evidence is available from a GLP- and OECD 473 guideline-compliant *in vitro* chromosome aberration assay in which no significant induction of chromosomal aberrations was observed in Chinese hamster lung-derived fibroblasts (CHL/IU) treated with concentrations of 500-2000 µg/mL of the structural relative ethyl octadecenoate (FEMA 3490) for 6 hours with an 18-hour recovery period in the presence and absence of S9 and for 24 hours in the absence of S9 (JMHLW, 2017b). Corroborative evidence from a GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay showed no induction of micronuclei was observed in human peripheral blood lymphocytes treated with the structural relative ethyl 2-acetyloctanoate (FEMA 4459) at concentrations up to 2145 µg/mL for 3 hours in the presence and absence of S9, as well as for 24 hours in the absence of S9 (Api et al., 2019c).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding the Prepared mixture of potassium chloride, magnesium sulfate and calcium lactate (CAS 7440-09-07; 10034-99-8; 5743-47-5) and concluded that the mixture is GRAS (FEMA 5013) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of this mixture from use as a flavor ingredient to be 2076 µg/person/day. A decision tree structural class for the threshold of toxicological concern (TTC) could not be assigned for this Mixture as inorganic substances are excluded from the Cramer/Ford/Hall Decision Tree classification (Cramer et al., 1978). Corroborative evidence for the low reproductive and developmental toxicity potential of Prepared mixture of

potassium chloride, magnesium sulfate and calcium lactate was evaluated by the Expert Panel from toxicity studies for the constituent ions summarized below. In a corroborative developmental dietary toxicity study of the constituent KCl provided to pregnant ICR mice (5-10/group) at 0 and 5% (10,000 mg/kg bw/day, approximately 13x greater than the nutritional requirement of potassium in mice) (Murai et al., 2013; EFSA, 2019b), significantly higher water intake and urine volume were observed in mice treated from GD 6.5 to 1 day after birth, and decreased body weight gains in pregnant mice and offspring as well as significantly increased relative kidney weights and serum potassium were observed in mice treated from GD 6.5 to 14 days after birth. No other developmental effects were observed. In corroborative prenatal developmental toxicity studies, no treatment-related effects were observed when virgin adult female albino CD-1 outbred mice (25/group) and virgin female albino Wistar rats (21-28/group) were administered the constituent KCl in a water solution by oral gavage at doses of 2, 11, 50 or 235 mg/kg bw/day and at doses of 3, 14, 67 or 310 mg/kg bw/day from GD 6-15, respectively (FDRL, 1975; EFSA, 2019b). A NOAEL of 570 mg/kg bw/day for maternal and developmental toxicity was established for CD-1 mice administered the related compound lactic acid at doses of 0 or 570 mg/kg bw/day by gavage (OECD, 2011b). In the reproductive/developmental toxicity arm of the corroborative OECD 422 guideline and GLP- compliant combined repeat dose and reproductive/developmental toxicity study for the constituent magnesium sulfate (0, 50, 150 or 450 mg/kg bw/day) reported in the previous section, no statistically significant changes in gestation index, post-implantation loss rates, live birth indices or viability indices were observed in treated Sprague Dawley rats. There was a statistically significant decrease in the body weight of male pups in the 450 mg/kg bw/day group (not further described). Based on the effects in male pups at the highest dose level, the NOAEL for reproductive and developmental toxicity was considered to be 150 mg/kg bw/day (OECD, 2010). The NOAEL of 150 mg/kg bw/day was greater than 4,000 times the anticipated daily *per capita* intake of this mixture as a flavor ingredient. A NOAEL of 0.5%, equivalent to 730 mg/kg bw/day, was established in females based on decreased body weight gain, and a NOAEL of 2%, or 3930 mg/kg bw/day, was established in males. Corroborative evidence for the low toxicity potential of Prepared mixture of potassium chloride, magnesium sulfate and calcium lactate was evaluated by the Expert Panel from toxicity studies for the constituent ions summarized below. In corroborative studies, Wistar rats were provided the constituent KCl at 0 or 3% (equivalent to 0 or 1500 mg/kg bw/day) in the diet for 4 weeks (10/sex/group), 13 weeks (10/sex/group), 18 months (15/sex/group) and 30 months (50/sex/group) (Lina and Kuijpers, 2004). Decreased mean body weights observed in treated rats in the 30-month study and in treated males in the 18-month study were correlated with reduced feed intake as well as increased water intake, urinary volume and urinary potassium. Statistically significant increases in relative kidney weights were observed in treated males in the 18-month study, and significantly increased incidences of hypertrophy of the adrenal zone glomerulosa were observed in treated animals in the 30-month study. The Expert Panel noted that these effects are expected given the administration of high doses of KCl. No other significant hematological, clinical chemistry parameters or tumor incidences were observed at the end of the 30-month study. In a corroborative 2-year chronic dietary toxicity study, male F344/Slc rats (50/group) were provided 0, 110, 450 and 1820 mg/kg bw/day of the constituent KCl (Imai et al., 1961). Survival of treated animals was higher than in the control group. Higher incidences of gastritis in the mid- and high-dose groups compared to the control group indicated an irritant effect. Chronic progressive nephropathy (CPN) was observed in all treated rats and the control rats. Although the biological relevance of this effect could not be determined, the Expert Panel concluded that incidences of CPN in control and treated rats were not biologically relevant to human risk assessment. No carcinogenic effects were observed. In a corroborative chronic toxicity and carcinogenicity study, calcium lactate (related to the constituent, lactic acid (FEMA 2611)) was administered orally to F344 rats (50/sex/group) at 0, 2.5 and 5% for 2 years (approximately 0, 4510 or 8570 mg/kg bw/day and 0, 3260 or 5650 mg/kg bw/day in males and females, respectively) (Maekawa et al., 1991). A NOAEL of 8570 and 5650 mg/kg bw/day was established for males and females, respectively. Calcium lactate was considered neither toxic nor carcinogenic in F344 rats by the authors. In a corroborative 13-week repeated dose oral toxicity study, calcium lactate was administered to F344 rats (5/sex/group) at 0.3, 0.6, 1.25, 2.5 or 5% (corresponding to doses of 300, 600, 1250, 2500 and 5000 mg/kg bw/day) in drinking water (ECHA, 1989; Matsushima et al., 1989). No adverse effects were observed, and a NOAEL was established at the top dose of 5000 mg/kg bw/day. In a corroborative GLP- and OECD 422 guideline-compliant combined repeat dose and reproductive/developmental toxicity study, Sprague Dawley rats (13/sex/group) were administered the constituent magnesium sulfate by gavage at doses of 0, 50, 150 or 450 mg/kg bw/day (OECD, 2010). Sporadic or frequent stool observed at 450 mg/kg bw/day in the main study period was recovered during the recovery period. Treatment related histopathological effects, including squamous cell hyperplasia of forestomach, edema of submucosa, inflammation of submucosa, focal erosion of mucosa, focal ulcer of mucosa into forestomach and hyperplasia of mucosa into cecum, were observed in one high-dose female. No treatment-related mortalities, significant changes in body weight gain, feed consumption, clinical biochemistry, hematology or organ weights were observed. Based on the soft stool observed in several animals and the histopathological effects observed in one female at the top dose level, the authors considered the NOAEL to be 150 mg/kg bw/day for repeated dose toxicity (OECD, 2010). The NOAEL of 150 mg/kg bw/day for the constituent magnesium sulfate was greater than 4,000 times the anticipated daily *per capita* intake of the Prepared mixture of potassium chloride, magnesium sulfate and calcium lactate as a flavor ingredient. The LOAEL of 6 mg/kg bw/day for magnesium-induced diarrhea (IOM, 1997) was greater than 170 times the anticipated daily *per capita* intake of the Prepared mixture of potassium chloride, magnesium sulfate and calcium lactate as a flavor ingredient. The constituents of the mixture are essential ingredients for humans; however, quantitative information was not available, and no consumption ratio could be calculated. The Expert Panel noted the risk of hyperkalemia in individuals with pre-existing medical conditions upon excessive potassium consumption, adverse gastrointestinal effects in patients given doses above 100 mg of potassium chloride (KCl) generally by bolus administration, and the LOAEL of 360 mg/day (equivalent to 6 mg/kg bw/day) for magnesium-induced diarrhea in adults (with the US Institute of Medicine (IOM) using an uncertainty factor of 1.0) (IOM, 1997). The Expert Panel concluded that

these data indicate that the consumption of these ions from food or at high concentrations exceed the consumption of these ions from use of Prepared mixture of potassium chloride, magnesium sulfate and calcium lactate as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). The constituents of the candidate substance are essential dietary substances that are expected to dissociate into their respective ions in biological fluids and be absorbed in the GI tract followed by distribution mainly to soft tissues, bones and muscle (OECD, 2002, 2003, 2011; EFSA, 2015a,b, 2016, 2019b; Swaminathan, 2003; IOM 1997, 2005, 2011). These ions participate in various essential cellular functions and modulate membrane permeability and potential. Calcium is essential for skeletal formation, neuronal transmission, muscle contraction and blood coagulation (Elin, 1987; IOM 1997, 2005, 2011). Upon filtration in the kidney, most of the ions are reabsorbed. Ions are excreted in the urine, and in sweat through the skin, in breast milk and in the feces (Lakshamanan et al., 1984; Weiner et al., 2010; Mickelsen et al., 1977; Pietinen, 1982; Holbrook et al., 1984; Tasevska et al., 2006; Yoshida et al., 2012; OECD, 2003; Kiela and Ghishan, 2016; Greger, 2000). Based on the structure of the constituents, the arrangement and identity of the functional groups therein, and the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of Prepared mixture of potassium chloride, magnesium sulfate and calcium lactate (Gooderham et al., 2020). The constituents potassium chloride and magnesium sulfate were not mutagenic in corroborative bacterial reverse mutation assays in *S. typhimurium* TA92, TA94, TA98, TA100, TA1535 or TA1537, or *E. coli* WP2uvrA, in the absence and presence of S9 (Ishidate et al., 1984; Oguma et al., 1998; Mortelmans et al., 1986). The related compound lactic acid was not mutagenic in corroborative Ames assays and corroborative GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays in *S. typhimurium* TA97, TA98, TA100, TA104, TA1535, TA1537, TA1538, *E. coli* WP2uvrA and Saccharomyces cerevisiae D4 in the presence and absence of S9 (ECHA, 2014a; NTP, 2018a; Al-Ani and Al-Lami, 1988; Brusick, 1976). The constituent magnesium sulfate was non-clastogenic in a corroborative GLP- and OECD 473 guideline-compliant *in vitro* chromosomal aberration assay in Chinese hamster lung fibroblasts (V79) cells in the absence and presence of S9 at 1000, 2000 or 5000 µg/mL for 6h in the presence and absence of S9 or for 24 hours in the absence of S9 (OECD, 2010). No significant increases in chromosome aberrations were observed at concentrations up to 4000 µg/mL in another chromosome aberration assay for the constituent magnesium sulfate (Ishidate et al., 1984). In a corroborative study, significant increases in chromosome aberrations and a slight increase in sister chromatid exchange frequencies were observed in CHO cells tested with up to 180 mM of the constituent KCl, however, these results were attributed to cytotoxicity and cell cycle delay. No significant increases in single-strand DNA breaks were observed (Galloway et al., 1987). In another corroborative assay of the constituent KCl tested in Chinese hamster lung fibroblast V79 cells at concentrations of 2000-12,000 µg/mL without metabolic activation, chromosome aberrations observed only at the highest tested concentration were attributed to high osmotic pressure of the medium compared to the control medium (Hasegawa et al., 1984). In this same study, no significant increases of sister chromatid exchanges were observed at any tested concentration (Hasegawa et al., 1984). In a corroborative study, no significant increases in sister chromatid exchanges were observed in human peripheral blood lymphocytes treated with the constituent magnesium sulfate (Debova, 1982). However, the OECD guideline for the sister chromatid exchange assay has been deleted due to a lack of understanding of the mechanism(s) of action detected by the test (OECD, 2017). The related compound L(+)-lactic acid (FEMA 2611) was not clastogenic in a corroborative *in vitro* chromosomal aberration assay in CHO-K1 cells with and without metabolic activation (D,L-isomer) at concentrations up to 1441 µg/ml (Morita et al., 1990) and in a corroborative GLP- and OECD 473 guideline-compliant *in vitro* chromosomal aberration assay in human lymphocytes at concentrations up to 901 µg/mL in the presence and absence of S9 (L-isomer) (ECHA, 2014b). In a corroborative *in vitro* mammalian cell gene mutation assay of concentrations up to 9000 µg/mL of the constituent KCl (well above OECD recommendations) in L5178Y mouse lymphoma cells in the presence and absence of S9 metabolic activation, significant increases in mutant frequencies were observed at cytotoxic concentrations of 5000 µg/mL and above in the absence of S9 (Myhr and Caspary, 1988). In another corroborative *in vitro* mammalian cell gene mutation assay of the constituent KCl at concentrations up to 5000 µg/mL in the presence and absence of S9 metabolic activation, weak increases in mutant frequencies at concentrations of 4000 µg/mL and above were attributed to changes in osmotic pressure of the test medium due to high salt concentrations (Mitchell et al., 1988). In a corroborative GLP- and OECD 476 guideline-compliant *in vitro* mammalian cell gene mutation assay for the constituent magnesium sulfate, no significant increases in mutant frequencies were observed when L5178 mouse lymphoma cells were treated with concentrations of 2 µg/mL for 3 hours without S9, concentrations up to 17 µg/mL for 3 hours with S9 and concentrations up to 1.8 µg/mL for 24 hours without S9 (ECHA, 2010c). In a corroborative GLP- and OECD 476 guideline-compliant *in vitro* gene mutation assay in mouse lymphoma L5178Y cells, no induction of mutant frequencies was observed for the same constituent (L-isomer) (FEMA 2611) when tested at concentrations up to 901 µg/mL for 3 hours in the presence and absence of S9 and for 24 hours in the absence of S9 (ECHA, 2014c). In a corroborative *in vivo* micronucleus assay conducted in male Swiss mice (5/group) administered the constituent magnesium sulfate at doses of 125, 250 and 500 mg/kg bw/day by oral gavage for 7 days, no signs of clinical toxicity, significant changes in the proportion of polychromatic to normochromatic erythrocytes (PCE/NCE ratio) or significant increases in the frequency of micronuclei in the bone marrow were observed (OECD, 2010).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding modified patchouli oil and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5014) (Smith et al., 2005a) for use as a flavor ingredient in the food categories and at the use levels specified in the GRAS application (see Table 2). This material was evaluated within the context of the procedure for the safety evaluation of

natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of Modified Patchouli Oil from use as a flavor ingredient to be 3 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day). The reproductive and developmental toxicity of the source material patchouli oil (FEMA 2838) was recently reviewed in the FEMA Expert Panel evaluation of lavender, guaiac coriander-derived natural flavor complexes (Fukushima et al., 2020). Corroborative evidence for the low reproductive and developmental toxicity potential of modified patchouli oil was evaluated by the Expert Panel from a corroborative GLP- and OECD 407 guideline-compliant 28-day combined dietary and reproductive/developmental toxicity study in which Wistar Han rats (10/sex/group) were provided the related preparation of the source material, patchouli oil, at concentrations of 0, 1300, 4000 and 13,000 ppm (equivalent to 0, 91, 277 and 810 mg/kg bw/day) for up to 8 weeks (Fukushima et al., 2020). As described in Fukushima et al., the NOAEL was determined to be 277 mg/kg bw/day for systemic toxicity in the adult rats and for reproduction and offspring development (Fukushima et al., 2020). This NOAEL is greater than 5,500,000 times the anticipated daily *per capita* intake of modified patchouli oil from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of modified patchouli oil was evaluated by the Expert Panel from a corroborative 90-day dietary toxicity study in which a related preparation of the source material, patchouli oil, was diluted in cottonseed oil and provided to FDRL rats at a concentration of 2% of the diluted material in the diet (equivalent to patchouli oil doses of 12 mg/kg bw/day and 15 mg/kg bw/day in males and females, respectively). No adverse effects, changes in body weights, feed consumption, hematological, blood biochemistry and histopathological analyses were observed (Oser et al., 1965). In a corroborative GLP- and OECD 408 guideline-compliant 90-day dietary toxicity study, Sprague Dawley rats (10/sex/group) were provided the constituent β-caryophyllene epoxide (FEMA 4085) at 0, 1750, 10,500 or 21,000 ppm (equivalent to mean daily dietary intakes of 0, 109, 672 and 1398 mg/kg bw/day in males and 0, 137, 800 and 1,660 mg/kg bw/day in females) (Bauter, 2013). Increased relative kidney weights in mid- and high-dose females were not accompanied by changes in kidney weights relative to brain weights or other clinical and histopathological findings. The authors considered these findings to be due to slight, non-statistically significant reductions in bodyweight and considered these findings to be non-adverse. In all treated males, fine granular casts were found upon examination of the urine. Tubular cytoplasmic droplets were observed with dose-dependent intensity in the kidneys of treated males. These renal findings are consistent with α2u-globulin nephropathy, an effect that is not biologically relevant to humans. Increased absolute and relative liver weights in mid- and high-dose males and females were correlated with hepatocyte hypertrophy. Based on the reported hepatocyte hypertrophy in the mid- and high-intake males and females, as well as increased liver weights in mid- and high-intake females, the authors established a NOAEL of 109 mg/kg bw/day (Bauter, 2013). In a corroborative GLP- and OECD 407 guideline-compliant 28-day combined dietary and reproductive/developmental toxicity study in Wistar Han rats (10/sex/group) at concentrations of 0 (control), 500, 4000 and 13,000 ppm (equivalent to 0, 41, 323 and 977 mg/kg bw/day) (Fukushima et al., 2020). As described in Fukushima et al., the Expert Panel assigned a NOAEL of 41 mg/kg bw/day (Fukushima et al., 2020). This NOAEL is 820,000 times the anticipated daily *per capita* intake of modified patchouli oil as a flavor ingredient. The Expert Panel reviewed the key constituents of modified patchouli oil and noted that the congeneric group intakes were below the respective TTC thresholds. The material is produced from the distillate of patchouli oil, derived from *Pogostemon cablin*; however, quantitative data are unavailable and therefore a consumption ratio cannot be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for a representative members of the principal identified congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be predicted to be metabolized primarily by well-established detoxication pathways to innocuous products or to be excreted as such (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of modified patchouli oil (Gooderham et al., 2020; Cohen et al., 2018). In a corroborative GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, modified patchouli oil was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537, as well as *E. coli* WP2uvrA, in the presence and absence of S9 using the plate incorporation and preincubation methodologies (Kovács, 2022a). In a corroborative GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay for modified patchouli oil, no significant induction of micronuclei formation was observed in mouse lymphoma L5178Y TK+/- 3.7.2 C cells treated with 3-20 µg/mL for 3 hours in the presence of S9, 3-30 µg/mL for 3 hours in the absence of S9 and 3-25 µg/mL for 24 hours in the absence of S9 (Kovács, 2022b). The related preparation of the source material, patchouli oil was not mutagenic when tested in a corroborative GLP-compliant bacterial reverse mutation assay at concentrations up to 50 µg/plate in the presence and absence of S9 in *S. typhimurium* TA98, TA100, TA1535 and TA1537 (Fukushima et al., 2020). Negative results were reported in a corroborative GLP- and OECD 473 guideline-compliant *in vitro* chromosome aberration assays in Chinese hamster ovary cells treated with the related preparation of the source material, patchouli oil, at concentrations up to 90 µg/mL in the presence and absence of S9 (Fukushima et al., 2020). Another preparation of the source material, patchouli oil, was not mutagenic in a corroborative GLP- and OECD 476 guideline-compliant forward mutation assay with mouse lymphoma cells treated at concentrations up to 50 µg/mL for 4 h in the absence of S9, at concentrations up to 275 µg/mL for 4 h in the presence of S9 and at concentrations up to 36 µg/mL for 24 hours in the absence of S9 (Fukushima et al., 2020). The constituent β-caryophyllene epoxide (FEMA 4085) was not mutagenic in several corroborative Ames assays at concentrations up to 10,000 µg/plate tested in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and/or *E. coli* WP2uvrA in the presence and absence of S9 (Api et al., 2020; DiSotto et al., 2013; Leuschner, 2018). In a corroborative GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay, no significant induction of micronuclei was observed in human peripheral blood lymphocytes treated with the constituent β-caryophyllene epoxide (FEMA 4085) at concentrations up to 90 µg/mL for 4 h in the absence and

presence of S9, as well as for 24 hours in the absence of S9 (Api et al., 2020). The same constituent (FEMA 4085) was negative in another corroborative *in vitro* micronucleus assay conducted in human lymphocytes at concentrations up to 50 µg/mL (Di Sotto, 2013).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding heat-treated glucosylated steviol glycosides 20% with steviol glycosides 8% (CAS 2766152-47-8) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5016) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of heat-treated glucosylated steviol glycosides 20% with steviol glycosides 8% from use as a flavor ingredient to be 692 µg/person/day. Corroborative evidence for the low toxicity potential of heat-treated glucosylated steviol glycosides 20% with steviol glycosides 8% was evaluated by the Expert Panel from a 108-week carcinogenicity study for stevioside showed no carcinogenic effects were observed (Toyoda et al., 1997). Corroborative evidence is also available from a 2-year feeding study, in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 45,000 times the anticipated daily *per capita* intake of heat-treated glucosylated steviol glycosides 20% with steviol glycosides 8% from use as a flavor ingredient. Additional corroborative evidence is available from a 52-week chronic toxicity study in which Beagle dogs (4/sex/group) were provided 0, 6200, 12500 or 50000 ppm of *beta*-cyclodextrin (Bellringer et al., 1995). The dietary concentrations correspond to actual intakes of 229, 456 or 1831 mg/kg bw/day and 224, 476 or 1967 mg/kg bw/day in male and female dogs, respectively (Bellringer et al., 1995). There were no toxicologically significant findings, and a NOAEL was established at the top dose (1831 and 1967 mg/kg bw/day for male and female dogs, respectively). This NOAEL is greater than 152,000 times the anticipated daily *per capita* intake of heat-treated glucosylated steviol glycosides 20% with steviol glycosides 8% from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana*. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to the intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al., 2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997; Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; JECFA, 1982). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of heat-treated glucosylated steviol glycosides 20% with steviol glycosides 8% (Gooderham et al., 2020). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of corroborative studies. While some positive results are reported in corroborative *in vitro* mutagenicity assays, corroborative *in vivo* studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding heat-treated glucosylated steviol glycosides 40% with steviol glycosides 15% (CAS 2766151-56-6) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5016) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of heat-treated glucosylated steviol glycosides 40% with steviol glycosides 15% from use as a flavor ingredient to be 692 µg/person/day. Corroborative evidence for the low toxicity potential of heat-treated glucosylated steviol glycosides 40% with steviol glycosides 15% was evaluated by the Expert Panel from a 108-week carcinogenicity study for stevioside showed no carcinogenic effects were observed (Toyoda et al., 1997). Corroborative evidence is also available from a 2-year feeding study, in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 45,000 times the anticipated daily *per capita* intake of heat-treated glucosylated steviol glycosides 40% with steviol glycosides 15% from use as a flavor ingredient. Additional corroborative evidence is available from a 52-week chronic toxicity study in which Beagle dogs (4/sex/group) were provided 0, 6200, 12500 or 50000 ppm of *beta*-cyclodextrin (Bellringer et al., 1995). The dietary concentrations correspond to actual intakes of 229, 456 or 1831 mg/kg bw/day and 224, 476 or 1967 mg/kg bw/day in male and female dogs, respectively (Bellringer et al., 1995). There were no toxicologically significant findings, and a NOAEL was established at the top dose (1831 and 1967 mg/kg bw/day for male and female dogs, respectively). This NOAEL is greater than 152,000 times the anticipated daily *per capita* intake of heat-treated glucosylated steviol glycosides 40% with steviol glycosides 15% from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana*. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to the intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al.,

2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997; Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; JECFA, 1982). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of heat-treated glucosylated steviol glycosides 40% with steviol glycosides 15% (Gooderham et al., 2020). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of corroborative studies. While some positive results are reported in corroborative *in vitro* mutagenicity assays, corroborative *in vivo* studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding celtuce distillate (CAS 84696-33-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5017) (Smith et al., 2005a) for use as a flavor ingredient in the food categories and at the use levels specified in the GRAS application (see Table 2). This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of celtuce distillate from use as a flavor ingredient to be 28 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day). Corroborative evidence for the low toxicity potential of celtuce distillate evaluated by the Expert Panel from a drinking water toxicity study in which Sprague-Dawley rats (50/sex/group) were provided 1% or 3% of the constituent ethanol (FEMA 2419) or glucose (control group) in a synthetic diet calorically equal to the ethanol group for 120 weeks (equivalent to 1000 or 3000 mg/kg bw/day of the constituent ethanol) (Holmberg and Eckstom, 1995). Statistically significant increases in the occurrence of pituitary tumors (not further described by the authors) were observed in the high-dose ethanol-treated females compared to the glucose control group. Increased incidences of mammary gland fibromas, fibroadenomas and adenomas were observed in the low-dose ethanol-treated females compared to the glucose control group. No increase in tumor incidence was observed in ethanol-treated males compared to the glucose controls. The overall information described by the authors indicate an absence of carcinogenic activity. Corroborative evidence is also available from another chronic drinking water toxicity study in which male Sprague Dawley rats were provided 5% (v/v) of the constituent ethanol for 130 weeks (equivalent to 5000 mg/kg bw/day) (Radike et al., 1981; FDA, 1993). Hyperplastic nodules of the liver were observed, and significantly increased adenomas of the pancreas, adrenal gland and pituitary gland were observed compared to the controls. The Expert Panel noted that no further information was provided on the types of adrenal and pancreatic tumors observed in treated rats compared to the controls. Pancreatic acinar cell tumors and adrenal pheochromocytomas are the most common tumors in this strain of rats and are considered not relevant to human cancer risk (Son and Gopinath, 2004; Edler et al., 2014). For both studies (Holmberg and Eckstom, 1995; Radike et al., 1981), the Expert Panel noted that pituitary tumors are common in Sprague Dawley rats and are not relevant to humans. Corroborative evidence is also available from a chronic exposure and reproductive toxicity study in which Sprague Dawley rats (30-55/sex/group) were provided 10% of the constituent ethanol (FEMA 2419) in drinking water for 104 weeks (equivalent to 10,000 mg/kg bw/day) and were necropsied after deaths from natural causes (Soffritti et al., 2002; FDA, 1993). Increased benign and malignant tumor formation including increased incidences of carcinomas of the oral cavity, lips and tongue in male and female breeding rats and offspring were observed. The majority of the increase in total tumors was due to oral carcinomas. Minimal increases in tumor incidences were present in additional tissues; however, the data were not consistent between males and females or rat groups and there was a lack of comparisons to historical data. Since only a single dose was used, there was no evaluation of a dose-response relationship. Benign and malignant tumors of the forestomach were observed in breeding males and increased incidence of lymphomas and leukemias were observed in dams. Increased interstitial-cell adenomas of the testes and osteosarcomas of several sites were observed in parental males. Limited statistical analyses were conducted in this study and the IARC Working Group noted some unconventional approaches in their review (IARC, 2018). The Expert Panel concurs with the IARC conclusions. The Expert Panel also noted that the intake of ethanol (FEMA 2419) from the use of celtuce distillate as a flavor ingredient is expected to be lower than the intake resulting from the levels at which it is present as an endogenous substance and in food and beverages. Corroborative evidence for the constituent ethanol (FEMA 2419) has been reviewed by the IARC in published monographs of the human health effects of the consumption of alcoholic beverages (IARC, 1988, 2010, 2012, 2018). The IARC has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of ethanol and to consider ethanol in alcoholic beverages as a Group 1 carcinogen (IARC, 1988, 2010, 2012, 2018). The material is produced from the lettuce of *Lactuca sativa* var. augustana. Based on quantitative data, a consumption ratio of 127 could be calculated (Stofberg and Grundschober, 1987). The IARC has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of ethanol and to consider ethanol in alcoholic beverages as a Group 1 carcinogen (IARC, 1988, 2010, 2012, 2018). Ethanol (FEMA 2419) was also evaluated by JECFA in 1970 as a solvent with an ADI limited by good manufacturing processes (GMP) (JECFA, 1970). In its 46th meeting, JECFA concluded that ethanol (FEMA 2419) does not pose a safety concern at the intake levels from the use of ethyl esters as flavor ingredients (JECFA, 1997). In its evaluation, the Expert Panel considered the high consumption ratio of ethanol (FEMA 2419) from food excluding alcoholic beverages and subsequent high endogenous exposure to ethanol and its metabolite, acetaldehyde (FEMA 2003), compared to their intake from the use of celtuce distillate as a flavor ingredient, and concluded, based on these considerations, that the anticipated intake of ethanol (FEMA 2419) and its metabolite acetaldehyde (FEMA 2003) from consumption of celtuce distillate as a flavor ingredient is not expected to be of concern with respect to carcinogenicity. The Expert Panel noted that

in adult humans, exposure to the constituent ethanol (FEMA 2419) occurs mainly from consumption of alcohol, and up to 90% of ingested alcohol is metabolized to acetaldehyde (FEMA 2003) in the liver and, to a lesser extent in extrahepatic tissues. In children, exposure to ethanol (FEMA 2419) is expected to be up to 12.5-23 mg/kg bw/day via consumption of bananas, fruit juices as well as bread and bakery products. Additionally, the Expert Panel noted the estimated endogenous levels of ethanol in non-alcohol consuming adults to be 0.39 ± 0.45 µg/mL (Jones et al., 1983). The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for a representative members of the principal identified congeneric groups that indicate, in the context of anticipated levels of intake, that substance would be predicted to be metabolized primarily by well-established detoxication pathways to innocuous products or to be excreted as such (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of Celtuce distillate (Gooderham et al., 2020; Cohen et al., 2018). The Expert Panel noted the equivocal results reported by the International Agency for Research on Cancer (IARC) for the constituent ethanol (FEMA 2419), as well as ability of acetaldehyde (FEMA 2003), a metabolite of ethanol (FEMA 2419) to bind to proteins, DNA and other macromolecules (IARC, 1988, 2010, 2012, 2018; Nakao et al., 2000). Despite the results from the mutagenicity and chromosomal damage testing for the constituent ethanol (FEMA 2419) and its metabolite acetaldehyde (FEMA 2003), the Expert Panel concluded that the use of Celtuce distillate as a flavor ingredient would not raise an additional concern for genotoxicity relative to the consumption of ethanol and acetaldehyde from food. Additionally, the exposure to acetaldehyde and ethanol from use of Celtuce distillate as a flavor ingredient is expected to be negligible relative to the endogenous levels of exposure.

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-2*H*-1-benzopyran-2-one (CAS 350982-20-6) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5018) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-2*H*-1-benzopyran-2-one from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of regarding 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-2*H*-1-benzopyran-2-one was reviewed by the Panel based on a corroborative OECD 407 guideline-compliant 28-day oral toxicity study in Sprague Dawley rats (5/sex/dose) administered the structural relative mixture of 5-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2- one and 7-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-5-methylchroman-2-one (FEMA 4888) by gavage at doses of 250, 500 and 1000 mg/kg bw/day, high-dose males had decreased absolute and relative thymus and spleen weights that were not accompanied with corresponding histopathological findings. Based on the absolute and relative thymus and spleen weights changes, the authors determined a NOAEL of 500 mg/kg bw/day (Chen, 2017). This NOAEL is 2,500,000 times the anticipated daily *per capita* intake of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-2*H*-1-benzopyran-2-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). 3,4-Dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-2*H*-1-benzopyran-2-one is anticipated to be partially absorbed and conjugated with glucuronic acid and/or sulphate. It may also undergo hydroxylation and/or *O*-demethylation before urinary or biliary excretion (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-2*H*-1-benzopyran-2-one (Gooderham et al., 2020). No increases in the number of reverse mutations were observed in corroborative GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays for the structural relatives (R)-5-hydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-7-methylchroman-2-one (FEMA 4834) (Soltesova, 2015) and the mixture of 5-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2- one and 7-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-5-methylchroman-2-one (FEMA 4888) (Wisher, 2016) in S. typhimurium strains TA98, TA100, TA1535, and TA1537 and E. coli WP2 uvrA (pKM101) in either the absence or presence of S9 metabolic activation. Based on corroborative evidence, no increases in micronuclei were observed in vitro when the same structural relatives were incubated with CHO-K1 cells in the absence or presence of metabolic activation (Zhao, 2015, 2016; JECA, 2022).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2*H*-1-benzopyran-2-one (CAS 2803352-58-9) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5019) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2*H*-1-benzopyran-2-one from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day)

(Munro et al., 1996). Corroborative evidence for the low toxicity potential of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2*H*-1-benzopyran-2-one was reviewed by the Panel based on a corroborative OECD 407 guideline-compliant 28-day oral toxicity study in Sprague Dawley rats (5/sex/dose) administered the structural relative mixture of 5-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2- one and 7-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-5-methylchroman-2-one (FEMA 4888) by gavage at doses of 250, 500 and 1000 mg/kg bw/day, high-dose males had decreased absolute and relative thymus and spleen weights that were not accompanied with corresponding histopathological findings. Based on the absolute and relative thymus and spleen weights changes, the authors determined a NOAEL of 500 mg/kg bw/day (Chen, 2017). This NOAEL is 2,500,000 times the anticipated daily *per capita* intake of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2*H*-1-benzopyran-2-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2*H*-1-benzopyran-2-one is anticipated to be partially absorbed and conjugated with glucuronic acid and/or sulphate. It may also undergo hydroxylation and/or *O*-demethylation before urinary or biliary excretion (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2*H*-1-benzopyran-2-one (Gooderham et al., 2020). No increases in the number of reverse mutations were observed in corroborative GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays for the structural relatives (R)-5-hydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-7-methylchroman-2-one (FEMA 4834) (Soltesova, 2015) and the mixture of 5-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2- one and 7-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-5-methylchroman-2-one (FEMA 4888) (Wisher, 2016) in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* WP2 uvrA (pKM101) in either the absence or presence of S9 metabolic activation. Based on corroborative evidence, no increases in micronuclei were observed *in vitro* when the same structural relatives were incubated with CHO-K1 cells in the absence or presence of metabolic activation (Zhao, 2015, 2016; JECA, 2022).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding 4-(3,4-dihydroxyphenyl)-3,4-dihydro-7-hydroxy-5-methyl-2*H*-1-benzopyran-2-one (CAS 2803352-59-0) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5020) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of phenol and phenol derivatives (JECFA, 2001, 2011, 2012, 2022; SLR, C12). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of 4-(3,4-dihydroxyphenyl)-3,4-dihydro-7-hydroxy-5-methyl-2*H*-1-benzopyran-2-one from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Corroborative evidence for the low toxicity potential of 4-(3,4-dihydroxyphenyl)-3,4-dihydro-7-hydroxy-5-methyl-2*H*-1-benzopyran-2-one was reviewed by the Panel based on a corroborative OECD 407 guideline-compliant 28-day oral toxicity study in Sprague Dawley rats (5/sex/dose) administered the structural relative mixture of 5-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2- one and 7-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-5-methylchroman-2-one (FEMA 4888) by gavage at doses of 250, 500 and 1000 mg/kg bw/day, high-dose males had decreased absolute and relative thymus and spleen weights that were not accompanied with corresponding histopathological findings. Based on the absolute and relative thymus and spleen weights changes, the authors determined a NOAEL of 500 mg/kg bw/day (Chen, 2017). This NOAEL is 2,500,000 times the anticipated daily *per capita* intake of 4-(3,4-dihydroxyphenyl)-3,4-dihydro-7-hydroxy-5-methyl-2*H*-1-benzopyran-2-one from use as a flavor ingredient. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). 4-(3,4-Dihydroxyphenyl)-3,4-dihydro-7-hydroxy-5-methyl-2*H*-1-benzopyran-2-one is anticipated to be partially absorbed and conjugated with glucuronic acid and/or sulphate. It may also undergo hydroxylation and/or *O*-demethylation before urinary or biliary excretion (Smith et al., 2018). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of 4-(3,4-dihydroxyphenyl)-3,4-dihydro-7-hydroxy-5-methyl-2*H*-1-benzopyran-2-one (Gooderham et al., 2020). No increases in the number of reverse mutations were observed in corroborative GLP- and OECD 471 guideline-compliant bacterial reverse mutation assays for the structural relatives (R)-5-hydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-7-methylchroman-2-one (FEMA 4834) (Soltesova, 2015) and the mixture of 5-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2- one and 7-hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-5-methylchroman-2-one (FEMA 4888) (Wisher, 2016) in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* WP2 uvrA (pKM101) in either the absence or presence of S9 metabolic activation. Based on corroborative evidence, no increases in micronuclei were observed *in vitro* when the same structural relatives were incubated with CHO-K1 cells in the absence or presence of metabolic activation (Zhao, 2015, 2016; JECA, 2022).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *S*-(3-methylbut-3-en-1-yl) 4-(formyloxy)butanethioate (CAS 2775350-66-6) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5021) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within

the context of the chemical group of aliphatic and aromatic sulfides and thiols (JECFA, 2000, 2004, 2008, 2011; SLR, C5). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *S*-(3-methylbut-3-en-1-yl) 4-(formyloxy)butanethioate from use as a flavor ingredient to be 0.1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). No toxicity data on the material or structural relatives were available for consideration. The Expert Panel noted that the intake was below the TTC for Structural Class III. The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. *S*-Thioesters such as *S*-(3-methylbut-3-en-1-yl) 4-(formyloxy)butanethioate are anticipated to undergo rapid hydrolysis by lipases and esterases (Kurooka et al., 1976). The rate of hydrolysis increases as the carbon chain length of the carboxylic acid fragment increases and decreases as oxygenation of the carbon chain in the thiol moiety increases (Jencks and Greenzaid, 1971). Thiols may oxidize to form unstable sulfenic acids, which can be further oxidized to the corresponding sulfinic and sulfonic acids. Simple aliphatic and aromatic thiols may also undergo methylation to yield methyl sulfides, which then form sulfoxides and sulfones. Thiols may react with physiological thiols to form mixed disulfides or undergo conjugation with glucuronic acid followed by elimination in the urine (Anders, 1989; Graffner-Nordberg et al., 1998; Heymann, 1980). Based on the structure of the substance and the arrangement and identity of the functional groups therein, and supported by the corroborative evidence noted below, the Expert Panel did not identify specific concerns related to the genotoxicity of *S*-(3-methylbut-3-en-1-yl) 4-(formyloxy)butanethioate (Gooderham et al., 2020). *S*-(3-Methylbut-3-en-1-yl) 4-(formyloxy)butanethioate was not mutagenic in a corroborative bacterial reverse mutation assay conducted in *S. typhimurium* TA98 and TA100 at concentrations up to 5000 µg/plate in the presence and absence of S9 (Kino, 2022a). Neither the structural relatives, methylthio-2-(acetyloxy)propionate (FEMA 3788) or methylthio-2-(propionyloxy)propionate (FEMA 3790), were mutagenic when tested at concentrations up to 5000 µg/plate in corroborative bacterial reverse mutation assays conducted in *S. typhimurium* TA98, TA100, TA1535 and TA1537, as well as in *E. coli* WP2uvrA, in the presence or absence of S9 (Watanabe and Morimoto, 1989a,b).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *S*-butan-2-yl 4-(formyloxy)butanethioate (CAS 2775350-67-7) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5022) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This substance was evaluated individually within the context of the chemical group of aliphatic and aromatic sulfides and thiols (JECFA, 2000, 2004, 2008, 2011; SLR, C5). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *S*-butan-2-yl 4-(formyloxy)butanethioate from use as a flavor ingredient to be 0.1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day) (Munro et al., 1996). Based on the mutagenicity testing results for the candidate substance and the structural relatives FEMA 3788 and 3790, as well as the structure of the substance and the identity and arrangement of functional groups therein, the Expert Panel did not identify a concern for the genotoxic potential of *S*-butan-2-yl 4-(formyloxy)butanethioate (Gooderham et al., 2020). Corroborative evidence for the lack of genotoxic potential for *S*-butan-2-yl 4-(formyloxy)butanethioate was evaluated by the Expert Panel from a bacterial reverse mutation assay conducted in *S. typhimurium* TA98 and TA100 at concentrations up to 5000 µg/plate in the presence and absence of S9, where no evidence of mutagenicity was observed (Kino, 2022b). Corroborative evidence for the lack of genotoxic potential was evaluated by the Expert Panel from bacterial reverse mutation assays for the structural relatives, methylthio-2-(acetyloxy)propionate (FEMA 3788) or methylthio-2-(propionyloxy)propionate (FEMA 3790), which were not mutagenic when tested at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535 and TA1537, as well as in *E. coli* WP2uvrA, in the presence or absence of S9 (Watanabe and Morimoto, 1989a,b). The Expert Panel considered the specification of the material to be adequately characterized by the purity assay and supporting spectral data provided for the FEMA GRAS evaluation. *S*-Thioesters such as *S*-butan-2-yl 4-(formyloxy)butanethioate are anticipated to undergo rapid hydrolysis by lipases and esterases (Kurooka et al., 1976). The rate of hydrolysis increases as the carbon chain length of the carboxylic acid fragment increases and decreases as oxygenation of the carbon chain in the thiol moiety increases (Jencks and Greenzaid, 1971). Thiols may oxidize to form unstable sulfenic acids, which can be further oxidized to the corresponding sulfinic and sulfonic acids. Simple aliphatic and aromatic thiols may also undergo methylation to yield methyl sulfides, which then form sulfoxides and sulfones. Thiols may react with physiological thiols to form mixed disulfides or undergo conjugation with glucuronic acid followed by elimination in the urine (Anders, 1989; Graffner-Nordberg et al., 1998; Heymann, 1980).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding reactive distillation product of threonine and coconut oil and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5023) (Smith et al., 2005a) for use as a flavor ingredient in the food categories and at the use levels specified in the GRAS application (see Table 2). This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of reactive distillation product of threonine and coconut oil from use as a flavor ingredient to be 1 µg/person/day, which is below the threshold of toxicological concern (TTC) for structural class III (90 µg/person/day). Corroborative evidence for the low reproductive and developmental toxicity potential of reactive distillation product of threonine and coconut oil was reviewed by the Panel based on a corroborative GLP- and OECD 421 guideline-compliant combined reproductive and developmental toxicity study in which Sprague-Dawley rats (10/sex/group) were administered 30, 95 or 300 mg/kg bw/day of the constituent 5-ethyl-2-methylpyridine (FEMA 3546) for 15 days before pairing until post-partum Day 4 for females, and for males after successful littering. Clinical signs of toxicity were observed in parental animals (ECHA, 1994b). Two treatment-related mortalities were observed in high-dose

males. These two males exhibited clinical signs of ataxia, partially closed eyes, prostrate posture and underactivity. Pathological examination of these rats demonstrated accentuated lobular liver patterns, reduced and dehydrated gastrointestinal contents. In each of these two animals, a small mass was observed on one epididymis. Examination of the epididymal masses showed spermatozoa granulomas. Significantly reduced absolute weights of the epididymides and seminal vesicles in high-dose males were considered by the authors to be due to reduced body weight and not related to treatment. Increased, dose-dependent incidences of salivation were observed in all high-dose animals, most mid-dose animals and a few low-dose animals. Reduced body temperature and abnormal respiration were observed in the high-dose rats in weeks 2-4 of treatment. Body weight gain was reduced throughout the treatment period in high-dose males but not top dose females. Statistically significant reductions in body weight change were also observed in mid- and high-dose females during gestation. Body weights were reduced in high-dose females during lactation. Relative liver weights (both sexes) and male kidney weights of the high-dose rats were increased relative to controls, but statistical significance was not described. Feed consumption was unaffected in parental animals. Reproductive indices including length of estrous cycles, mating performance and fertility were unaffected by treatment. No gross or histopathological changes were observed in any of the organs examined from the high-dose groups compared to controls. Since no adverse effects were observed on reproductive performance of parental animals, the authors considered the reproductive NOAEL to be 300 mg/kg bw/day. Based on signs of clinical toxicity and morbidity at the high-dose level, the authors considered the NOAEL for parental males to be 95 mg/kg bw/day. For females, signs of clinical toxicity were observed at the high-dose level along with significantly reduced body weight changes at the two highest dose levels, so the authors considered the NOAEL for parental females to be 30 mg/kg bw/day. Offspring viability was unaffected at the low dose levels but at the high dose, poor weight gain was observed, and viability was reduced. Mean body weights of both sexes at the high dose level were significantly lower than controls at days 1 and 4 of age. Based on the reduction in viability and body weight at the high dose level, the authors considered the offspring NOAEL to be 95 mg/kg bw/day (ECHA, 1994b). The NOAEL of 30 mg/kg bw/day for parental female Sprague-Dawley rats is 1,800,000 times the anticipated daily *per capita* intake of the reactive distillation product of threonine and coconut oil from use as a flavor ingredient. The developmental toxicity NOAEL of 95 mg/kg bw/day is 5,700,000 times the anticipated daily *per capita* intake of the reactive distillation product of threonine and coconut oil from use as a flavor ingredient. The reproductive toxicity NOAEL of 300 mg/kg bw/day is 18,000,000 times the anticipated daily *per capita* intake of the reactive distillation product of threonine and coconut oil from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of reactive distillation product of threonine and coconut oil was reviewed by the Panel based on a corroborative GLP- and OECD 407 guideline-compliant 28-day oral toxicity study for the constituent 5-ethyl-2-methylpyridine (FEMA 3546) which was administered by gavage to Sprague-Dawley rats (6/sex/group) at 30, 95 or 300 mg/kg bw/day (ECHA, 1988). Clinical signs of toxicity were observed at the top two doses in both sexes from Week 2 onwards. High-dose males had reduced feed efficiency as well as statistically significant reductions in body weight gain and feed consumption. High-dose females had statistically significant decreases in erythrocytes and hematocrit levels as well as statistically significant increases in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). High-dose males exhibited statistically significant increases in blood urea and aspartate aminotransferase levels. Significant increases in creatinine levels were observed in mid-dose males. Significantly decreased chloride levels were observed in high-dose females. Relative brain, testes and kidney weights were significantly higher than controls. Absolute heart weights in low-dose females were significantly lower than controls. Relative liver weights were significantly higher in mid- and high-dose males compared to controls, and absolute liver weights were significantly higher in mid-dose males compared to controls. Absolute spleen weights were significantly lower in high-dose males compared to controls. Significant changes in brain, kidney and testes weights were observed in high-dose males. Significant increases in relative liver weights were observed in high-dose males and females as well as mid-dose males. Mottled kidneys were observed in 5/6 high-dose males. Nephropathy was observed in mid-dose males and high-dose males, consistent with α2u-globulin accumulation. The authors considered the blood urea and creatinine effects to be related to α2u-globulin nephropathy. No significant differences in mortalities were observed in treated rats compared to control rats. Based on the clinical effects and renal lesions at the top two dose levels and the lack of clinical effects and histopathological lesions at the low-dose level, the authors established a NOAEL of 30 mg/kg bw/day for the constituent FEMA 3546 (ECHA, 1988). Corroborative evidence is also available from a single dose oral toxicity study in which the constituent 3-ethyl-2,6-dimethylpyrazine (FEMA 3150) was provided to Sprague Dawley rats (16/sex/group) in the diet at mean intake levels of 13 and 12 mg/kg bw/day for 13 weeks for males and females, respectively. No treatment related effects were observed (Posternak et al., 1975). Additional corroborative evidence is available from a 90-day toxicity study in which Sprague-Dawley rats (10/sex/group) were administered the constituent 5-ethyl-2-methylpyridine (FEMA 3546) by gavage at doses of 0.03, 0.3 or 3 mg/kg bw/day. No dose-dependent effects were observed, and a NOAEL of 3 mg/kg bw/day was established (JMHLW, 2017d). This NOAEL is 180,000 times the anticipated daily *per capita* intake of the reactive distillation product of threonine and coconut oil mixture from use as a flavor ingredient. The Expert Panel reviewed the key constituents of the Reactive distillation product of threonine and coconut oil and noted that the congeneric group intakes were below the respective TTC thresholds. The Expert Panel also noted that the intake of Reactive distillation product of threonine and coconut oil at the anticipated annual volume of use was below the TTC for structural class III (90 µg/person/day). The material is produced from distillation of thermally treated threonine in coconut oil. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The material is not known to occur in nature and thus no consumption ratio can be calculated. The Expert Panel concluded that metabolic data exist for a representative members of the principal identified congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be predicted to be metabolized primarily by well-established detoxication pathways to innocuous products or to be

excreted as such (Smith et al., 2018). The constituent 5-ethyl-2-methylpyridine (FEMA 3546) is expected to undergo alkyl side-chain oxidation followed by glucuronic acid conjugation and excretion or oxidation to nicotinic acid (JECFA, 2006b). Pyridine derivatives that are constituents of the reactive distillation product of threonine and coconut oil are weak tertiary bases that are expected to undergo rapid absorption in the gastrointestinal tract (Hogben et al., 1959). Pyrazine derivatives that are constituents of the reactive distillation product of threonine and coconut oil are expected to undergo oxidation of side chain alkyl substituents to yield the corresponding acids that are excreted either unchanged or in conjugated form. If pyrazine rings are activated by alkoxy substituents, ring hydroxylation may occur to yield polar metabolites (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of reactive distillation product of threonine and coconut oil (Gooderham et al., 2020). Corroborative evidence from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay showed that the reactive distillation product of threonine and coconut oil was not mutagenic in the presence and absence of S9 when tested at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, and TA1537, as well as *E. coli* WP2 uvrA using the plate incorporation method, at concentrations up to 2500 µg/plate in *S. typhimurium* TA100 using the preincubation method and at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA1535, TA1537 and *E. coli* WP2 uvrA using the preincubation method in triplicate (Chang, 2022). The reactive distillation product of threonine and coconut oil did not induce the formation of micronuclei in a corroborative GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay in human peripheral blood lymphocytes for 3 h in the presence of S9 at concentrations of 39, 87 and 130 µg/mL, for 3 h in the absence of S9 at concentrations of 133, 200, 226 and 248 µg/mL and for 24 h in the absence of S9 at concentrations of 26, 39 and 58 µg/mL (Clare, 2022). Corroborative evidence from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay indicated the constituent 5-ethyl-2-methylpyridine (FEMA 3546) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 in both the absence and presence of S9 using the plate incorporation method in triplicate (ECHA, 1986). Corroborative evidence from a GLP- and OECD 476 guideline-compliant *in vitro* mammalian cell gene mutation assay showed no significant increases in mutant frequencies relative to the control were observed when the constituent 5-ethyl-2-methylpyridine (FEMA 3546) was tested in Chinese hamster ovary cells either for 5 hours or 20 hours in the presence and absence of S9 (ECHA, 2012d). Additionally, corroborative evidence is available from a GLP- and OECD 474 guideline-compliant *in vivo* micronucleus assay in which CD-1 mice (5/sex/group) were administered the constituent 5-ethyl-2-methylpyridine (FEMA 3546) via oral gavage at doses of 0, 156, 313 or 625 mg/kg bw. Doses were established based on a preliminary dose-range finding assay which identified evidence of bone marrow toxicity at 1000 mg/kg bw. In the main corroborative micronucleus assay, upon dosing, prone/hunched posture and unstable gait were observed in two high-dose males that recovered after 4 hours. No mortalities or significant induction of micronuclei were observed under all treatment conditions of the micronucleus assay compared to the controls (ECHA, 1990d).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding Camu Camu distillate (CAS 363158-42-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5024) (Smith et al., 2005a) for use as a flavor ingredient in the food categories and at the use levels specified in the GRAS application (see Table 2). This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of Camu Camu distillate from use as a flavor ingredient to be 31 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). On a water-removed basis, the *per capita* intake for the concentrate from use as a flavor ingredient was calculated to be 0.03 µg/person/day. Corroborative evidence for the low toxicity potential of Camu Camu distillate was reviewed by the Panel based on a corroborative 28-day toxicity study for the constituent 4-carvomenthenol (FEMA 2248) which was administered to male Sprague-Dawley rats by oral gavage at 400 mg/kg bw/day to investigate its nephrotoxic potential. The study authors concluded that 4-carvomenthenol did not induce any treatment-related renal changes (Fukushima et al., 2020; Schilcher and Leuschner, 1997). Additional corroborative evidence is available from a 28-day repeated dose oral toxicity study in which adult CF-1 mice (5/sex/group) were administered the juice of Camu Camu (*Myrciaria dubia*) by gavage at dose levels of 0 (water), 25%, 50% or 100% juice concentrations (approximately 0; 2500; 5000 or 10,000 mg/kg bw/day in males and females). No mortalities were observed in the treated mice. Statistically significant increases in high-dose body weights were observed in males but not females. No body weight changes were found in other dose groups. No other findings were reported (da Silva et al., 2012). Corroborative evidence is available from a 56-day repeated-dose oral toxicity study in which, adult CF-1 mice (5/sex/group) were administered the juice of Camu Camu (*Myrciaria dubia*) by gavage at dose levels of 0 (water), 25%, 50% or 100% juice concentrations (approximately 0; 2500; 5000 or 10,000 mg/kg bw/day in males and females). No mortalities were observed in treated mice and no significant differences in body weight gain were observed in treated mice compared to controls. No other findings were reported (da Silva et al., 2012). Corroborative evidence is also available from a GLP- and OECD 408 guideline-compliant 90-day repeated-dose oral toxicity study in which the constituent *alpha*-terpineol (FEMA 3045) was provided to Sprague-Dawley rats (10/sex/group) in the diet at targeted intake levels of 50, 150 or 500 mg/kg bw/day. Average intake levels were calculated to be 49, 146 and 487 mg/kg/ bw/day for males, and 50, 148 and 496 mg/kg bw/day, for females, respectively (Bauter, 2021). There were no deaths observed throughout the treatment period. Treated animals showed no signs of clinical toxicity or changes in food consumption, body weight, ophthalmologic findings, or food efficiency throughout the treatment period. Additionally, no statistically significant changes in treated animals were observed in hematological, clinical chemistry or urinalysis parameters. There were also no microscopic or macroscopic findings in the tissues examined. Statistically significant increases in relative

thyroid/parathyroid weights in top dose males and relative liver weights in top dose females were observed but these changes were not considered related to test substance administration. Overall, there were no organ weight changes related to the test article administration. Three males at the top dose level had non-motile sperm noted at evaluation. One other male at the top dose exhibiting reduced motile sperm compared to other animals in the same treatment group. The authors noted the four animals with reduced sperm motility exhibited excessive fragmentation with sperm heads separated from the tails, which were correlated to reduced sperm motility. However, there were no microscopic or macroscopic changes observed for these four animals, so it was unclear if these were treatment-related effects. The NOAEL was assigned to 146 mg/kg bw/day for males due to the reduced sperm motility observed at the top dose level that could be attributed to the constituent *alpha*-terpineol (FEMA 3045). The dietary stability of 83-96% results in the adjusted dietary intake of 122 mg/kg bw/day for this NOAEL. For females, the NOAEL, initially considered to be at the top dose level of 496 mg/kg bw/day, was adjusted to 413 mg/kg bw/day based on dietary stability analysis. The Expert Panel reviewed the key constituents of Camu Camu distillate and noted that the congeneric group intakes were below the respective TTC thresholds. This NOAEL is greater than 240,000,000 times the anticipated daily per capita intake of the entire NFC, Camu Camu distillate, on a water-removed, concentrate basis from use of Camu Camu distillate as a flavor ingredient. Based on corroborative evidence, swine spermatozoa were incubated with both tea tree oil and the constituent 4-carvomenthenol FEMA 2248 at concentrations of 0.2-1 mg/mL and 0.08-0.83 mg/mL, respectively, for 3 hours. Evaluations were conducted for motility, pH, acrosome status and viability. The first toxic effect was observed at 0.67 mg/mL of FEMA 2248, which was the third-highest test concentration. The most sensitive reproductive parameters (i.e., the most easily impacted by the test article) for FEMA 2248 were acrosome reaction and viability while the motility was significantly altered at the top tested concentration only. No synergistic effects between tea tree oil and the constituent FEMA 2248 were observed (Elmi et al., 2019). The material is produced from the whole fruits of *Myrciaria dubia*. Though the source material is consumed as food, quantitative information was not available, and a consumption ratio could not be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel concluded that metabolic data exist for a representative members of the principal identified congeneric groups that indicate, in the context of anticipated levels of intake, that the substance would be predicted to be metabolized primarily by well-established detoxication pathways to innocuous products or to be excreted as such (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of Camu Camu distillate (Gooderham et al., 2020; Cohen et al. 2018). Corroborative evidence from a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay indicated the constituent 4-carvomenthenol (FEMA 2248) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA97a, TA98, TA100, TA102 and TA1535 in the presence and absence of S9 using the plate incorporation method (ECHA, 2017). Negative results were obtained from a corroborative GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay for the same constituent, FEMA 2248 (ECHA, 2017). In a corroborative GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay, the constituent *alpha*-terpineol (FEMA 3045) was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA97a, TA98, TA100, TA102 and TA1535 in the presence and absence of S9 using the plate incorporation and preincubation methods (Rao, 2020d). The same constituent (FEMA 3045) was also not mutagenic in other corroborative Ames assays in *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 at concentrations up to 1000 µg/plate in the presence and absence of S9 (Florin et al., 1980; Heck, 1989; Seifried et al., 2006). Corroborative evidence from an OECD 487 guideline-compliant *in vitro* micronucleus assay in human peripheral blood lymphocytes treated with the same constituent (FEMA 3045) for a 20-hour treatment period at concentrations of 0, 25, 50 or 100 ug/mL showed no significant induction of micronuclei was observed relative to the controls (Drosopoulou et al., 2018). The same constituent (FEMA 3045) was not mutagenic in a corroborative mouse lymphoma L5178Y forward mutation assay at concentrations of 0.1-0.4 µg/mL or 0.2-0.6 µg/mL in the absence and presence of S9, respectively (Seifried et al., 2006). In corroborative *in vivo* Comet assays, the DNA damage frequency (number of cells with tail vs. those with no tail) was evaluated in blood samples from adult CF-1 mice (5/sex/group) administered the juice of Camu Camu (*Myrciaria dubia*) by gavage at dose levels of 0 (water), 25%, 50% or 100% juice concentrations (approximately 0; 2500; 5000 or 10,000 mg/kg bw/day in males and females) after both the 28- and 56-day repeated dose oral toxicity studies, and at single doses of 0 (water), 25%, 50% or 100% juice concentrations (approximately 0; 2500; 5000 or 10,000 mg/kg bw in males and females). No statistically significant increases in damage frequency were observed for any treated animals relative to controls (da Silva et al., 2012).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding enzymatically modified *Stevia rebaudiana* extract enriched with rebaudiosides AM, M and N2 (CAS 91722-21-3) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5025) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake of enzymatically modified *Stevia rebaudiana* extract enriched with rebaudiosides AM, M and N2 from use as a flavor ingredient to be 415 µg/person/day. Corroborative evidence for the low toxicity potential of enzymatically modified *Stevia rebaudiana* extract enriched with rebaudiosides AM, M and N2 was evaluated by the Expert Panel from a 108-week carcinogenicity study for stevioside, no carcinogenic effects were observed (Toyoda et al., 1997). Corroborative evidence is also available from a 2-year feeding study in which male and female rats were administered the equivalent of 0, 50, 150, or 550 mg/kg bw/day of a stevia extract comprised of 74% stevioside and 16% rebaudioside A. The authors considered the NOAEL from this 2-year rat feeding study of a stevia extract to be equal to 550 mg/kg bw/day (Yamada et al., 1985), which is greater than 79,000 times the anticipated daily *per capita*

intake of enzymatically modified *Stevia rebaudiana* extract enriched with rebaudiosides AM, M and N2 from use as a flavor ingredient. This material is derived from the leaves of *Stevia rebaudiana*. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. The Expert Panel evaluated sensory data included within the application and found it satisfactory with regard to intended conditions of use for the flavor ingredient (Harman and Hallagan, 2013). Metabolic data exist that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Cardoso et al., 1996; Gardana et al., 2003; Geuns et al., 2003a,b; Geuns and Pietta, 2004; Geuns et al., 2007; Hutapea et al., 1997; Koyama et al., 2003a,b; Nakayama et al., 1986; Purkayastha et al., 2014, 2015, 2016; Purkayastha and Kwok, 2020; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Roberts et al., 2016; Wheeler et al., 2008; Wingard et al., 1980; JECFA, 1982). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of enzymatically modified *Stevia rebaudiana* extract enriched with rebaudiosides AM, M and N2 (Gooderham et al., 2020). The genotoxicity of the major marker constituents (steviol glycosides) has been thoroughly examined in a wide range of corroborative studies. While some positive results are reported in corroborative in vitro mutagenicity assays, corroborative in vivo studies do not provide evidence of genotoxic effects (Nakajima, 2000a,b; Pezzuto et al., 1985, 1986; Rumelhard et al., 2016; Suttajit et al., 1993; Terai et al, 2002; Williams and Burdock, 2009).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *Eucommia ulmoides* leaf extract (CAS 223749-00-6) and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5026) (Smith et al., 2005a) in the food categories and at the use levels specified in the GRAS application (see Table 2). This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *Eucommia ulmoides* leaf extract from use as a flavor ingredient to be 14 µg/person/day, which is below the threshold of toxicological concern for Structural Class III materials (90 µg/person/day). Corroborative evidence for the low toxicity potential of *Eucommia ulmoides* leaf extract was reviewed by the Expert Panel from a 30-day repeated dose oral toxicity study in which Sprague Dawley rats (10/sex/group) were administered a related preparation consisting of an aqueous extract of *E. ulmoides* leaves at doses of 0, 0.83, 1.67 and 3.30 mL/kg bw/day (equivalent to 0, 83, 167 or 330 mg/kg bw/day). No adverse effects were reported, and the study authors considered the NOAEL to be 330 mg/kg bw/day (Zhu et al., 2017). Additional corroborative evidence is available from another 28-day repeated dose oral toxicity study in which a NOAEL was established at the top dose level of 330 mg/kg bw/day in Sprague Dawley rats (10/sex/group) that were administered a related preparation consisting of an aqueous extract of *E. ulmoides* leaves at doses of 0, 0.83, 1.67 and 3.30 mL/kg bw/day (equivalent to 0, 83, 167 or 330 mg/kg bw/day) (Yuefeng et al., 2009 as cited in Hong-xin et al., 2020). Corroborative evidence is available from another related preparation consisting of a stock solution of *E. folium* (no further details available) was administered to Sprague-Dawley rats at 0, 0.83, 1.67 and 3.30 mL/kg bw/day (equivalent to 0, 83, 167 or 330 mg/kg bw/day) in a 30-day repeated dose oral toxicity study. No adverse effects were observed and the NOAEL for the study was once again considered the highest dose level of 330 mg/kg bw/day (Huang et al., 2000 as cited in Hong-xin et al., 2020). Corroborative evidence was reviewed from a toxicity study in which Kunming mice were administered the related preparation consisting of the extract of *E. ulmoides* (no further details available) at doses of 0, 690, 2060 and 6170 mg/kg bw/day via oral gavage for 8 weeks for 5 days per week. Significant weight gain, as well as increases in red blood cell count, total protein, hemoglobin, blood urea nitrogen and albumin levels, were observed at the two lowest treatment levels (Jianqin et al., 1990 as cited in Hong-xin et al., 2020). However, no histopathological findings were observed in treated mice relative to controls, and a LOAEL of 690 mg/kg bw/day was established by the study authors. Based on its review of the above studies, the Expert Panel concluded that the toxicity data provided for an aqueous extract of the leaves of *E. ulmoides* provided only limited value for read across to the candidate substance. The Expert Panel noted that the estimated intake of the substance, at 14 µg/person/day, was significantly below the TTC for Structural Class III. Additionally, corroborative evidence is available from a mouse sperm deformity test in which no statistically significant increases in the number, rate or type of sperm malformations were observed in ICR male mice (5/group) administered the related preparation of the aqueous extract from the leaves of *E. ulmoides* via oral gavage at doses of 0, 2500, 5000 or 10,000 mg/kg bw/day for 5 days (Zhu et al., 2017). The leaves of *Eucommia ulmoides* are edible, but no qualitative data are available and thus no consumption ratio can be calculated. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of *Eucommia ulmoides* leaf extract (Gooderham et al., 2020; Cohen et al., 2018). Based on corroborative evidence, *Eucommia ulmoides* leaf extract was not mutagenic in a GLP- and OECD 471 guideline-compliant bacterial reverse mutation assay conducted in *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of S9 at concentrations up to 5000 µg/plate (Thakor, 2022).Corroborative evidence is also available from a bacterial reverse mutation assay conducted with *S. typhimurium* TA97, TA98, TA100 and TA102 in the presence and absence of S9, in which a related preparation of an aqueous leaf extract of *E. ulmoides* was not mutagenic at concentrations up to 5,000 µg/plate (Cai et al., 2016; Zhu et al., 2017). No significant increases in micronuclei induction or the ratio of polychromatic erythrocytes and normochromic erythrocytes were observed in a corroborative *in vivo* micronucleus assay conducted with ICR mice (5/sex/group) that were administered a related preparation of an aqueous leaf extract of *E. ulmoides* via oral gavage at doses of 0, 2500, 5000 or 10,000 mg/kg bw twice in 24 hours (Zhu et al., 2017).

Using scientific procedures, the Expert Panel reviewed the GRAS applications and supporting information regarding fennel oleoresin (*Foeniculum vulgare* Miller) and nutmeg oleoresin (*Myristica fragrans* Houtt.) and concluded that the uses of the substances as flavor ingredients are GRAS (FEMA 5027 and 5028) (Smith et al., 2005a) for use as flavor ingredients in the food categories and at the use levels specified in the GRAS applications (see Table 2). These materials were evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). These natural flavor complexes are derived from commonly consumed spices. The Expert Panel considered the identity descriptions of each material to be adequate for the FEMA GRAS evaluation. These natural flavor complexes were evaluated using a rigorous procedure that considers the chemical composition, anticipated *per capita* intake, as well as principal and corroborative evidence regarding the metabolic fate and toxicity of the identified constituents and potential toxicity and genotoxicity of unidentified constituents (Rietjens et al., 2023; Davidsen et al., 2023).

Using scientific procedures, the Expert Panel reviewed the GRAS application and supporting information regarding *Corynebacterium casei* fermentation product and concluded that the use of the substance as a flavor ingredient is GRAS (FEMA 5029) (Smith et al., 2005a) in the food categories and at the use levels specified in Table 2. This material was evaluated within the context of the procedure for the safety evaluation of natural flavor complexes (Cohen et al., 2018; Smith et al., 2005b). This material was evaluated within the context of the procedure for the FEMA GRAS evaluation of flavor ingredients produced through biotechnology processes (Cohen et al., 2015). The Expert Panel calculated the anticipated *per capita* intake ("eaters only") of *Corynebacterium casei* fermentation product from use as a flavor ingredient to be 415 µg/person/day. Corroborative evidence for the low reproductive and developmental toxicity potential of *Corynebacterium casei* fermentation product was reviewed by the Expert Panel from pregnant rhesus monkeys that received the structural relative monosodium glutamate at 4000 mg/kg bw/day during the last trimester and did not exhibit any treatment-related effects (Newman et al., 1973). Corroborative evidence is also available from CD-1 COBS (Caesarean Originated Barrier Sustained) mice provided the structural relative monosodium glutamate (FEMA 3285; 2756) in feed at concentrations of 0, 1 or 4% in a three-generation reproductive toxicity study (equivalent to 0, 1500 or 6000 mg/kg bw/day for males and 0, 1800 or 7200 mg/kg bw/day for females) (Anantharaman, 1979). The highest tested concentration of 4% (equivalent to 6000 and 7200 mg/kg bw/day for males and females, respectively) was considered to be the NOAEL for parental, reproductive and developmental toxicity. Corroborative evidence from a study in which three-month-old female Wistar rats were paired with mature male rats at a 4:1 ratio. Following the onset of pregnancy, pregnant female rats (10/group) were placed in either a control group or experimental group that was administered 200 mg/kg bw/day of the structural relative, monosodium glutamate (1% solution) throughout pregnancy and lactation (Gusev et al., 2021). Offspring from the treatment group had increased relative weights of the cerebral hemispheres (19.1%) and kidneys (7.8%) as well as decreased absolute and relative weights of the thymus and spleen. Motor activity was decreased and horizontal hanging time on a wire was increased in the offspring of treated females. Histological examinations of the 25-day-old offspring from the treated group showed an increased number of nucleoli in the neurons (statistically significant compared to controls), decreased indicators of the nucleolar apparatus of cardiomyocytes, and an increased mitotic index of the anterior corneal epithelium (statistically significant compared to controls). Decreased resistance of the erythrocyte membranes to acid hemolysis was also reported in the offspring of treated females. The LOAEL of 200 mg/kg bw/day is greater than 20,000 times the anticipated daily *per capita* intake of *Corynebacterium casei* fermentation product from use as a flavor ingredient. Corroborative evidence for the low toxicity potential of *Corynebacterium casei* fermentation product was reviewed by the Expert Panel from a number of safety studies available for glutamate and monosodium glutamate as structural relatives of the constituent, L-glutamic acid (FEMA 3285), including subacute toxicity studies in rats and mice (Onaolapo et al., 2016; Egbuonu et al., 2010a,b; Harper et al., 2009 as cited in JECFA, 2022; CIT, 1997a,b as cited in EFSA, 2017), and subchronic toxicity studies in rats (Reddy et al., 2021; Biosafety Research Center, 2007a; del Carmen Contini et al., 2017; TNO, 2014 as cited in JECFA, 2022). Corroborative evidence is available from an OECD 409 guideline-compliant 90-day feeding study in which groups of beagle dogs (4/sex/group) were fed diets containing monosodium glutamate monohydrate (purity unknown) at concentrations calculated to provide doses of 0 (basal diet), 150, 500 or 1500 mg/kg bw per day. No adverse, treatment-related findings were observed, and the authors established a NOAEL of 1500 mg/kg bw/day for both sexes (Biosafety Research Center, 2007b as cited in JECFA, 2022). Additional corroborative evidence is available from a chronic toxicity study of the structural relative monosodium glutamate (FEMA 2756) at dietary levels up to 4000 mg/kg bw/day in Charles River rats where increased incidence and earlier onset of spontaneous subepithelial basophilic deposits in the renal pelvis of treated rats were observed. In female rats, there was an increase in the incidence of focal mineralization beneath the epithelium of the renal pelvis at all intake levels for the duration of the study. However, this incidence was also higher in control female rats compared to control males by 104 weeks and it was considered a spontaneous occurrence within the historical control incidence rates and unrelated to the administration of the test material (Owen et al., 1978). Corroborative evidence is also available from another chronic toxicity study in which no evidence of carcinogenicity was observed when Fisher 344 rats were administered the structural relative monosodium glutamate (FEMA 2756) in the diet at concentrations up to 1982 mg/kg bw/day in males and 2311 mg/kg bw/day in females for 104 weeks (Shibata et al., 1995).The NOAEL of 1500 mg/kg bw/day for the structural relative monosodium glutamate (FEMA 2756) was greater than 200,000 times the anticipated daily *per capita* intake of *Corynebacterium casei* fermentation product from use as a flavor ingredient. Corroborative evidence is available from a 52-week chronic toxicity study in which Beagle dogs (4/sex/group) were provided 0, 6200, 12500 or 50000 ppm of *beta*-cyclodextrin (Bellringer et al., 1995). The dietary concentrations correspond to actual intakes of 229, 456 or 1831 mg/kg bw/day and 224, 476 or 1967 mg/kg bw/day in male and female dogs, respectively (Bellringer et al., 1995). There were no toxicologically significant findings, and a NOAEL was established at the top dose (1831 and 1967

mg/kg bw/day for male and female dogs, respectively). This NOAEL is greater than 260,000 times the anticipated daily *per capita* intake of *Corynebacterium casei* fermentation product from use as a flavor ingredient. The Expert Panel considered the identity description of the material to be adequate for the FEMA GRAS evaluation. Metabolic data exist for representative members of the principal identified congeneric groups that would predict, at the intake levels proposed, metabolism by well-established detoxication pathways to innocuous products (Smith et al., 2018). Based on the corroborative evidence noted below, the structures of the constituents and the arrangement and identity of the functional groups therein, and the Expert Panel's consideration of the unidentified constituents, the Expert Panel did not have specific concerns related to the genotoxicity of *Corynebacterium casei* fermentation product (Gooderham et al., 2020). Corroborative evidence is available from a GLP- and OECD 471 guideline-compliant Ames assay in which *Corynebacterium casei* fermentation product was not mutagenic at concentrations up to 5000 µg/plate in *S. typhimurium* TA98 and TA100 in the presence and absence of S9 using the preincubation methodology (Shimada, 2020). Based on corroborative evidence, no evidence of genotoxicity was observed when the structural relative monosodium L-glutamate monohydrate (FEMA 2756) was tested in a GLP- and OECD 471 guideline-compliant Ames assay, GLP-compliant *in vitro* chromosomal aberration assay with CHL/IU cells, GLP- and OECD 490 guideline-compliant *in vitro* mouse lymphoma assay conducted in TK +/- L5178Y cells, GLP- and OECD 487 guideline-compliant *in vitro* micronucleus assay in human peripheral blood lymphocytes, or a GLP- and OECD 474 guideline-compliant *in vivo* micronucleus assay (Takumi et al., 2019).

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