GRAS 27

Flavoring Substances

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The 27th publication by the Expert Panel of the Flavor and Extract Manufacturers Association provides an update on recent progress in the consideration of flavoring ingredients generally recognized as safe under the Food Additives Amendment.

More than 55 years have passed since the first Flavor and Extract Manufacturers Association (FEMA) Expert Panel began a program to assess the safety of flavor ingredients for their intended use in human food. Throughout that time, the primary objective of the FEMA GRAS™ program has been to evaluate whether materials nominated by the flavor industry can be considered "generally recognized as safe" (GRAS) for their intended use as flavor ingredients. Operating since 1960 (Hallagan and Hall 1995a, 2009), the FEMA GRAS program continues as the longest-running and most widely recognized industry-sponsored GRAS assessment program.

The FEMA GRAS program operates within the confines of the 1958 Food Additives Amendment, which defines a food additive as: "… any substance … which … may … [become] a component or … [affect] the characteristics of any food … if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures … to be safe under the conditions of its intended use." With the Food Additives Amendment, Congress for the first time established a premarket approval requirement for all substances meeting the definition of "food additive." In essence, a substance that is "generally recognized as safe" (GRAS) is excluded from the definition of food additive. Such substances are not subject to mandatory review by the Food and Drug Administration (FDA), but they are subject to the requirements established by the agency and the courts for GRAS assessments, and the rigor of a GRAS determination is not less than that for a food additive (Hallagan and Hall 1995a, 2009). The intention by Congress in excluding GRAS substances from the definition of "food additive" was to provide FDA with flexibility and discretion in allocating resources to food additive issues of potentially greater safety concern.

This GRAS 27 publication includes the results of the Expert Panel's review of 38 new FEMA GRAS flavoring substances (Tables 1 and 2). In addition, the Expert Panel determined that new use levels and/or use in new food categories for seven flavoring substances are consistent with their current FEMA GRAS status (Table 3) and concluded that the FEMA GRAS status of three ingredients should be changed. The Panel also describes its updated approach toward the evaluation of flavoring ingredients that are produced through biotechnology processes and the framework for describing the identity of natural flavor complexes. Finally, the Panel describes in brief key relevant studies in the recent FEMA GRAS determination for the flavor uses of palmitoylated green tea catechins. »»

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FEMA GRAS Evaluation of Flavor Ingredients Produced Through Biotechnology Processes—An Update

The safety evaluation of all flavoring substances includes review of all available studies for any evidence of toxicity in the context of their use in food. In essence, it is not only the specific flavoring substance that is subject to scrutiny but also as discussed in previous publications (Smith et al. 1996; Smith et al. 2005a), any potential impurities or contaminants that result from extraction of the flavoring substance from its source (if found in nature) or from substances used or formed during its manufacturing. The review and evaluation of methods of production and the associated hazards that may be involved are therefore an integral part of the GRAS evaluation process. Considering that technologies change and new technological advances may introduce substances not previously encountered, the FEMA Expert Panel makes a deliberate effort to monitor changes in the production methods of flavor materials. Because FEMA GRAS status is granted in the context of safety information of potential impurities from the manufacturing process, any significant change in the method of manufacturing a flavor substance is considered a reason for reevaluation of its safety and its FEMA GRAS status.

The introduction of biotechnology in producing highly purified flavoring substances took place in the 1990s and largely consisted of the use of production processes such as fermentation employing genetically engineered organisms designed to mimic the natural biosynthetic process for

naturally occurring substances. A review of flavor ingredient production using these processes addressed the safety questions that pertained to the technology available at the time and their incorporation in the FEMA GRAS evaluation process (Hallagan and Hall 1995b). Since then, new advances in biotechnology have been made that have brought about significant advantages to the scale and efficiency of flavor ingredient production along with improved batch-to-batch consistency of final product quality. The technological changes were significant enough to merit a review of their implications in flavor ingredient development, production, and safety evaluation and an update of the previously established procedure for the safety evaluation of flavors derived from biotechnology through the FEMA GRAS assessment program.

The safety evaluation of a biotechnology-produced flavor ingredient relies on the same core data for ingredients produced using more traditional methods (e.g., extraction from a natural source, chemical synthesis). Information about exposure, metabolism, toxicity, and specifications are required regardless of the origin of the flavor ingredient. However, in the case of biotechnology-derived materials, a second set of considerations is included in evaluating the safety of the final product, such as a) the nature of the host organism, b) the nature of the genetic sequence(s) cloned, c) the specification of the flavor ingredient relative to the nonbiotechnology substance, if already approved, and d) the purity of the final product and level of residual contaminants, if any.

In light of the changes in biotechnology, the following focus areas within the safety assessment of biotechnology-derived materials (Hallagan and Hall 1995b) were reconsidered.

a) Host Organisms

Previous Consideration. The safety concerns related to the host organism were primarily based on 1) whether any new host organism carries genes that code for enzymes that produce toxins and 2) whether there are products of endogenous processes that may present toxicological concern.

Current State of Technology. While a large number of possible host organisms exist that are appropriate and versatile in the context of research activities in the development of biotechnology products, only a selected list of wild-type hosts can be appropriate for the production of food ingredients. The wild-type hosts used in the current methods are among well-known and approved organisms for production of food ingredients, and therefore these hosts are fully characterized, with a large body of prior data and experience available for them. The European Commission and the European Food Safety Authority (EFSA) have reviewed the safety of host microbial

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organisms, including bacteria, yeast, fungi, and viruses, in the context of food applications. In light of the increasing use of biotechnological developments in food applications, the EFSA developed a system for the safety evaluation of organisms that are often used in the production of food and feed ingredients. This program leads to a determination of a host organism as to its "Qualified Presumption of Safety" (QPS) designation (EFSA 2007). The most recent list of QPS-recommended biological agents used in food ingredient production and biological control, including bacteria, yeast, and viruses, was reassessed in 2012 and is updated periodically (EFSA 2013). The classification of organisms as to their QPS status is based on the available body of safety data on their characterization and history of safe use. This eliminates the concern of 1) new or unknown toxins or 2) products of endogenous biochemical processes being introduced into the final product.

b) Introduced Sequences

Previous Consideration. Most biosynthetic gene clusters or individual genes that could be used for the production of biotechnology-derived flavor ingredients were introduced and maintained within the host organism in a vector when the previous guidance was published (Hallagan and Hall 1995b). The vector maintenance relied on the use of selection via

antibiotic resistance, which was also typically encoded within the vector. Briefly, when an external DNA sequence was introduced in a vector that remained separate from the host's genome, the continuous presence of the vector in the daughter cells after each cell division could not be guaranteed. While cell division results in a complete copy of the genome in each of the daughter cells, the same cannot be said for vector DNA (Summers 1998). The vector remained in the cytoplasm independent of the organism genome and from the mitotic spindle. Theoretically, it would be replicated and one copy would pass onto each daughter cell, but in reality, some cells may lose the vector during division. Over a series of divisions, a subpopulation of organisms emerges that is viable but does not carry the sequence (vector) of interest any longer and therefore is not effective in producing the desired product (Kelly 2014). Thus, the culture would become progressively "diluted" and less efficient in producing the substance of interest. The introduction of an antibiotic-resistance sequence in the vector provided a tool for selection of the cells that continued to carry the vector by providing a survival advantage when grown in the presence of the antibiotic. Cells that lost the vector would be sensitive to the antibiotic and eliminated. Therefore, the continuous presence of a considerable concentration of antibiotic in the transgene culture throughout the process was necessary to reduce or eliminate the likelihood that wild-type hosts that have lost the transgene would survive and therefore dilute the culture and reduce the production efficiency.

Current State of Technology. As a result of advances in biotechnology, host microorganisms are genetically modified to express a series of genes that form a biosynthetic pathway as it exists in the plant species that produce the substance in nature. Technological advances enabled the following: 1) uncovering of the biosynthetic pathway and the factors (enzymes, cofactors) involved in the synthesis of a natural substance in the plant of origin, as well as the biochemical and enzyme kinetic parameters that characterize the pathway; 2) engineering of microorganisms to express more than one external gene; and 3) incorporation of these sequences in the organism's genome instead of maintaining a separate vector with the necessary sequences. By incorporating the external genetic sequences in the host's genome, the requirement for antibiotic selection is eliminated. This last feature is a major difference between the previous and the new transgene technologies and has one direct implication in the assessment of safety of the biotechnology-derived food substances: It eliminates the requirement for use of antibiotics in the production process for the selection of transgenes because in the new technologies, the DNA sequences (genes) are reliably passed onto the daughter cells. However, antibiotic-resistance genes are often still incorporated into the host genome for the purpose of cloning the engineered organism. Once incorporated into the genome, these sequences are no longer mobile and cannot be transferred to other organisms. This difference eliminates the concern for inadvertent transfer of antibiotic-resistance genes to other organisms.

c) Structural Identity of Product

Previous Consideration. There were some concerns about whether the final product in a biotechnology-production system would be altered from the parent form due to reactions effected by endogenous proteins and host biochemical pathways.

Current State of Technology. The biosynthetic pathway of a natural substance as reconstructed in a host organism is designed to replicate the biochemical pathway as it exists in the plant of origin and only retains commercial value if the end product is identical to the conventionally produced flavor ingredient. The structural identity of the final product is characterized and verified during the method development process in small-scale enzymatic reactions *in vitro* and in the engineered host organism, with a combination of appropriate analytical methods such as GC-MS or LC MS/MS and spectral characterization by IR, NMR, MS, etc. Modifications and adjustments of the pathway also take place during method development to ascertain the efficient synthesis of the final product. Furthermore, during the design of the metabolic pathway and validation of the series of biochemical reactions, any deviation from the final product due to endogenous host processes or enzyme activities can be identified and removed through genetic engineering of

Update on Sensory Data Considerations in FEMA GRAS Evaluations

In its previous GRAS publication (Marnett et al. 2013), the Panel commented on the increasing need for sensory data for flavorings with modifying properties and its interest in having a set of best practices by which such data could be produced. The result of this request has been a recent publication that describes a set of tests designed to demonstrate if the function in food of the ingredient under conditions of intended use is flavoring (Harman et al. 2013). The guidance describes appropriate methodologies, analysis of the data, and reporting of the test for submission to the Panel. With a set of best practices in place, the Panel anticipates that future GRAS submissions for these types of flavor ingredients will include data consistent with such approaches.

host genes (deactivation or knockout). Secondary to this, the deactivation of endogenous host genes also reduces the number of endogenous host biosynthetic products.

d) Final Product Purity

Previous Consideration. Concerns related to the purity of the final product included 1) the presence of contaminants from the engineered organism such as toxins or products of endogenous processes that may present toxicological concern and 2) the presence of biosynthetic intermediates that co-purify with the final product.

Current State of Technology. The potential for introduction of contaminants during production and processing are assessed as part of the purity characterization of the final product. Once the identity of the product is verified during the method development phase, large-scale production is accomplished through microbial fermentation processes followed by purification. The final product is released from the organism into the media. It is purified following separation of the media from the organism and therefore it is free of the whole organism, organism fragments, and other components, including all genetic material. The large-scale production is identical to other fermentation processes.

The concerns specifically related to endogenous host products are largely addressed by the use of well-characterized hosts as described above. They are further addressed through the assessment of final product purity, including specific analysis for host organism toxins. With regard to the presence of biosynthetic intermediates, the reconstruction of the pathway using current biotechnology processes includes fine-tuning of transcription rates in order to facilitate efficient synthesis and avoid bottlenecks. For example, genetic regulatory controls are modified to align the rate of production of one biosynthetic intermediate to the rate of its utilization as substrate in the next enzymatic step. Therefore, the biosynthetic pathway in the biotechnology-based process can be as efficient, if not more so, than it is in nature. By controlling or eliminating inefficient metabolic flux, the biosynthetic intermediates are minimized or even absent compared to their presence in the parent plant source. Furthermore, considering that only a selected set of plant enzymes is coded for in the host organism, fewer possible biosynthetic intermediates are expected to be formed compared to those that may be formed through the activity of related enzymes or enzyme isoforms expressed in the plant. As a result, a substance produced by the new biotechnology processes is no more likely to contain biosynthetic intermediates than its counterpart extracted from the natural source. In principle, this results in final products of the same or higher purity.

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The final product purity must be documented with the same rigorous analytical methods as the conventionally produced substance, and this is reflected in the specifications. Therefore, any contaminants, regardless of whether they are intermediates from action of added genetic sequences or endogenous biochemical pathways or unrelated biochemical host products, are expected to be removed during the purification process so that the desired product meets specifications of purity.

Overall, it appears that refinement of biotechnology production processes reduces or, in some cases, eliminates some of the previous concerns related to host-derived toxins or other cell components, the presence of antibiotic-resistance genes, the use of selection antibiotics, and the presence of metabolic intermediates.

A revised decision tree for the evaluation of flavor ingredients produced using biotechnology is shown on page 47. It is based on the Hallagan and Hall publication from 1995, with revisions that address the current state of technology.

Identity Descriptions for Natural Flavor Complexes for Which Uses Are Considered to Be FEMA GRAS

The FEMA GRAS evaluation of a material under conditions of use as a flavoring ingredient in human food

includes the assessment of a number of elements, including the available safety data for the substance or for a closely related substance, the anticipated exposure, and the anticipated or known metabolism based on expert judgment or on available data (Smith et al. 2005a). In addition to these obvious factors, another critical component is an understanding of the specification of the substance—in other words, the identity of the substance, including its purity and any other parameters by which future production of the substance could be evaluated to confirm that the newly produced material is consistent with what was most recently evaluated by the Expert Panel.

However, the Panel recognizes that the initial set of specifications for a substance when its uses are first evaluated by the FEMA Expert Panel for FEMA GRAS status may vary to a minor extent with what is ultimately produced when the material enters into the marketplace. This is often due to the differences between small-scale initial production and larger-scale manufacturing as the substance enters the market. These types of differences in specifications are generally minor (e.g., specific gravity SD ± greater than 0.002 as described by the U.S. National Institute of Standards and Technology 2008) and do not affect the safety evaluation. In addition, the Expert Panel requires that any manufacturing change that significantly alters the specification beyond generally accepted variances for given purity and physical property parameters for the material reviewed for FEMA GRAS status be reevaluated by the Expert Panel. This is consistent with recent guidance published by the FDA on significant changes in manufacturing (FDA 2014).

For chemically defined substances, the Panel has approached naming and identity conventions using a rule similar to that used by other bodies (JECFA 2006; EFSA 2010) in that the assay value is generally >95% unless a secondary component or components are named and also evaluated for safety (JECFA 2006; EFSA 2010). This has resulted in a high degree of transparency and an ease of understanding of the identity of chemically defined FEMA GRAS substances. For natural flavor complexes (NFC), which are inherently mixtures of chemically defined substances, the Panel generally uses a congeneric group approach to conduct the safety evaluation (Smith et al. 2004, 2005b) and to describe the assessment of the FEMA GRAS NFC. However, the Expert Panel has concluded that the naming of NFCs for a variety of reasons does not provide a desirable level of transparency related to the constituents of which they are composed. Thus, the Expert Panel has concluded that there is value for the public, the industry, and others to better understand the identity of NFCs as evaluated by the Panel.

Beginning with GRAS 27 and available on the femaflavor.org website, the FEMA Expert Panel has now begun to include general chemical identity information for NFCs. This may include the identity and assay value for specific marker constituents or the identity and assay ranges for specific congeneric groups within the NFC. The Panel notes that when specifications for NFCs are ultimately set by standard-setting bodies (e.g., International Standards Organization, Food Chemicals Codex), then the Panel will review whether the information as reviewed during the FEMA GRAS evaluation remains consistent with the newly standard specification. It will also identify those situations for which a FEMA GRAS reevaluation should occur—meaning that the change is deemed significant.

Change in GRAS Status of Quinoline, Ethylene Oxide, and Styrene

The FEMA GRAS™ statuses of quinoline (FEMA No. 3470), ethylene oxide (FEMA No. 2433), and styrene (FEMA No. 3233) under their conditions of intended use as flavor ingredients were reviewed. For quinoline, the Expert Panel concluded that additional data, including *in vivo* genotoxicity and chronic toxicity testing, were required to support the continuation of its GRAS status. Until such data are available for review, the flavor ingredient quinoline has been removed from the FEMA GRAS list. There is little evidence that ethylene oxide or styrene are used for the technical effect of flavoring; based on this lack of evidence, the Panel concluded that both ethylene oxide and styrene should be removed from the FEMA GRAS list.

FEMA GRAS Evaluations of Palmitoylated Green Tea Extract Catechins (PGTEC)

One of the most abundant dietary sources of polyphenols is tea, and specifically, green tea. Green tea consumption is common in Asian countries and has been expanding globally for its purported health benefits and because of changes in the types of flavors that appeal to the taste of the general population. In the United States, green tea extract is used in two different formulations, a water-soluble green tea extract (GTE) used as a dietary supplement and palmitoylated green tea extract catechins (PGTEC) used as a

Decision Tree for the FEMA GRAS™ Safety Evaluation of Biotechnology-Derived Flavoring Substances

Table 1. Assessment of Final Product Identity and Purity.

Step	Question	If yes, then	If no, then
1)	Is the product currently approved for use in foods?	2	Develop specifications and safety evaluation, and move to 4.
2)	Is the product chemical structure identical to that of the currently approved product (or are there any modifications to the chemical structure compared to existing product, e.g. hydration, salt form)?[1]	3	Develop specifications and safety evaluation, and move to 4.
3)	Does the product meet existing specifications for [identity and] purity?	4	6
4)	Are (existing) specifications adequate to ensure the absence and control of novel potential constituents? (i.e., Do specs include the same list of potential contaminants? Are there any new possible contaminants resulting from the growth of microbial cultures or the purification process that are not reflected/foreseen in existing specs, e.g., different metals, toxins, etc.?)[1]	5	Revise specs, and go to 6.
5)	Proceed with FEMA GRAS procedure: Do the intended or reasonably expected conditions of use of the product result in a pattern of intake that is supported by the safety database?	Accept if safety profile indicates that there is no cause for concern.[2]	Accept with use limitations if safety profile indicates cause for concern, or do safety evaluation.
6)	Do all/secondary/new constituents pose no safety concern?	Revise specs, and go to 5.	7
7)	Can the constituents of concern be removed?	Remove, and go to 5.	Perform safety evaluation, revise specs, and go to 5.

Table 2. Assessment of Safety Concerns Related to the Host Organism and Introduced Genetic Sequences.

Step	Question	If yes, go to step	If no, go to step
1)	Is the host organism on the EU list of QPS agents?[1]	5	2
2)	Is the organism well-characterized, or does it have a history of use for production of food ingredients?[1]	5	3
3)	Does intact organism end up in food?	4	5
4)	Does the organism survive food processing, or does genetic engineering give it any advantage for survival in food or in the gastrointestinal tract?[1]	Does not meet requirements.[3]	5
5)	Does the organism contain vectors?[1]	6	7
6)	Are the vectors characterized and free of attributes that would render them unsafe for constructing microorganisms to be used to produce food-grade products?	7	Does not meet requirements.[3]
7)	Has there been an intermediate host?[1]	8	9
8)	Is the microbe free of the intermediate host DNA that could code for a toxic product?	9	Does not meet requirements.[3]
9)	Do the DNA inserts code for substances (enzymes)? Safe for use in food?	Accept, and continue with guidelines (Hallagan and Hall 1995b)	Does not meet requirements.[3]

[1] Note that this is a new question that did not appear in (Hallagan and Hall 1995b).
[2] No cause for concern is indicated by a margin of safety of greater than 100 based on a standard (GLP, OECD, etc.) oral toxicology study.
[3] Disposition of materials that fail any decision tree requirements: A negative answer to question 1, 2, or 3 signifies the presence of an undesirable substance, and the material is not acceptable for use in food. If the undesirable substance can be removed, the purified material must be passed through the system again beginning at the point of the original negative answer.

flavoring substance. The Expert Panel has determined that PGTEC is FEMA GRAS under conditions of intended use as a flavoring substance in selected food categories. GTE is consumed at much higher levels through use as a dietary supplement component, with typical doses of up to 2,000–4,000 mg/person/day, and has been reported to be the fourth most commonly consumed supplement ingredient in the United States (Sarma et al. 2008). As a flavor, PGTEC is used typically at very low levels (50–250 ppm; maximum use level up to 500 ppm) and consumer intake (eaters only) is estimated to be very low from consumption of foods containing this flavor ingredient (~0.1 mg/person/day). In its evaluation, the FEMA Expert Panel considered the available data for PGTEC as well as for the un-palmitoylated green tea catechins (GTC) to ensure a comprehensive review. The Panel anticipated that the review of data for GTC would be helpful in its assessment for PGTEC, since some metabolism of PGTEC to GTC would be expected.

Green tea extract is a complex mixture containing eight catechin monomers, collectively called "green

tea catechins" (GTC): (+)-catechin, (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin gallate (CG), (-)-epicatechin gallate (ECG), gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG), with EGCG making up between 50% to 60% of the extract by weight (Miyazawa et al. 2000). The ratios of catechins in green tea appear to vary depending on source, with the EGCG to ECG ratio of approximately 30:1 in green tea extract (Nakagawa et al. 1997) and the ratio of EGCG, EGC, EC, and ECG of 1:0.93:0.36:0.37, respectively, in decaffeinated green tea (Lee et al. 1995). Considerable differences among individuals and variations in the pharmacokinetic parameters were noted between repeated experiments with green tea, but not when EGCG was given in decaffeinated green tea or in pure form (Lee et al. 2002).

The absorption and fate of GTC in humans has been assessed in healthy subjects in a number of studies. Individual catechins are absorbed at different rates. Most studies have addressed the fate of water-soluble extracts, and only one has assessed the fate of palmitic acid esters of catechins. Generally, the lipid conjugates of catechins are absorbed at much higher levels (~70%) compared with their free (water-soluble) forms (up to 2%) (Chow et al. 2001; Lee et al. 1995, 2002; Nakagawa et al. 1997; Unno et al. 1996; Yang et al. 1998).

Following single oral ingestion of green tea extracts or green tea solids, absorption of free forms of EGCG and EGC is dependent on intake level, with up to 2% detected in plasma after 90 minutes (0.2%–2% of EGCG and 0.2%–1.3% of EGC) (Nakagawa et al. 1997; Yang et al. 1998), but it seems to reach saturation beyond a threshold (>4 g of green tea solids) (Yang et al. 1998). Although of similar scale relative to intake, absorption of phospholipid complexes of catechins is slightly higher than that of their free forms (Unno et al. 1996). The concentrations of EGCG, EGC, and epicatechin in the serum peak approximately 2 hours (1.5 hours and 2.5 hours) after ingestion of green tea extract or green tea solids (Unno et al. 1996; Yang et al. 1998; Lee et al. 2002), but the trace amounts of EGCG that appear in the blood are nominally lower than that of EGC and EC, all of which are less than 1% (Lee et al. 2002). Similarly, a dose-dependent increase in plasma levels of EGCG, as measured by AUC and C_{max} values, has been reported following administration of EGCG and Polyphenon E (a decaffeinated mixture of EGCG, EGC, epicatechin, and other tea polyphenols) to healthy volunteers (Chow et al. 2001). No significant differences in the pharmacokinetic characteristics of EGCG were found after administration of EGCG or Polyphenon E (the mean AUC and mean C_{max} plasma values of unchanged EGCG were similar) (Chow et al. 2001).

Tea polyphenols are eliminated predominantly via glucuronidation and sulfation pathways (Lee et al. 1995). Relative absorption parameters indicate that EGCG has a lower overall absorption or a larger volume of distribution than EGC. Catechins in plasma are detected as varying levels of free forms and glucuronide or sulfate conjugates (Lee et al. 1995; Chow et al. 2001). Plasma EGC is found mainly as glucuronide conjugates, epicatechin and EGCG mostly as sulfate conjugates, whereas epicatechin gallate (ECG) is not detected in human plasma (Lee et al. 1995; Yang et al. 1998; Lee et al. 2002). The urinary forms are predominantly (99% of EGC) or exclusively (epicatechin) conjugates, with maximum urinary excretion at 3–6 hours after intake and almost complete excretion by 9 hours while the conjugates are not detectable after 24 hours (Lee et al. 1995, 2002; Yang et al. 1998; Chow et al. 2001). EGCG and ECG are either not detected (Lee et al. 1995; Chow et al. 2001) or detected in trace amounts in urine (Lee et al. 2002). The ratio of urinary sulfate and glucuronide forms of both EGC and epicatechin is approximately 2:1, and the ratio of metabolites generally did not change over time. This ratio is likely dependent upon the dose applied. Since plasma EGC is detected mostly as the glucuronide form and urinary EGC mainly in the sulfate form, it is likely that EGC glucuronide undergoes biliary excretion in the feces. The total amount of EGC and epicatechin excreted in the urine accounted for 2% of the total polyphenol ingested (Lee et al. 1995). In one study, substantial amounts of 4′-O-methyl EGC metabolite, at levels higher than EGC, were detected in the urine and plasma, with peak plasma concentration at 1.7 hours

Expert Panel Member Changes

In June 2014, Dr. Lawrence J. Marnett of Vanderbilt University School of Medicine stepped down from his role as a member of the FEMA Expert Panel. Dr. Marnett spent more than a decade in service to the Expert Panel and flavor safety. His experience in biochemistry, medicinal chemistry, and molecular toxicology provided the Panel with expertise that contributed significantly to the long-standing success of the Panel. Dr. Marnett will continue as an ad hoc consultant to the Panel.

In January 2015, Dr. F. Peter Guengerich of Vanderbilt University School of Medicine joined the FEMA Expert Panel.

and a $t_{1/2}$ of 4.4 hours (Lee et al. 2002). The level of 4′-O-methyl EGC may be influenced by the polymorphism of catechol-O-methyl transferase, an enzyme that catalyzes the methylation of EGC (Weinshilboum et al. 1999; Zhu et al. 2000). Two ring-fission metabolites, (-)-5-(3′,4′,5′-trihydroxypheny1)-*gamma*-valerolactone (M4) accounting for 1.4% of the ingested EGC and (-)-5-(3′,4′-dihydroxyphenyl)-valerolactone (M6) accounting for 11.2% of the ingested epicatechin, have also been detected in significant amounts after 3 hours and peaked at 8–15 hours in the urine as well as in the plasma (Lee et al. 2002).

The kinetics of lipid conjugates are notably different compared to free forms of catechins, based on the fate of 3-palmitoyl-(+)-catechin as a representative. Radiolabeled 3-palmitoyl-(+)-[^{14}C]catechin, administered to male albino rats by gavage at two dose levels, 1.5 or 5 µCi (Hackett and Griffiths 1982), was more efficiently absorbed compared to catechin, with ~63% of the dose recovered in the urine, ~24% detected in the feces, and ~7.4% excreted as respired CO_2. A remaining ~3.7% of the dose persisted in the animal's body after 28 days, with the largest concentrations found in the liver (0.6% of dose) and the peritoneal fat (0.08% of dose). Glucuronide and sulfate conjugates of (+)-catechin and 3′-O-methyl-(+)-catechin accounted for ~80% of the radiolabeled urinary metabolites. Ring scission products of catechin from intestinal microorganism metabolism accounted for the remaining balance of labeled metabolites. Fecal metabolites included 3′-O-methyl-(+)-catechin-glucuronide and 3′-O-methyl-(+)-catechin. Results of *in vitro* experiments demonstrated that 3-palmitoyl-(+)-catechin is absorbed in the intestine and the ester is hydrolyzed by both plasma and liver enzymes (Hackett and Griffiths 1982).

Although water extracts of green tea and GTE catechins are poorly absorbed orally (~2%), lipid conjugates of catechins (lipid-soluble catechins) are efficiently absorbed (~70%) when taken orally and are readily hydrolyzed to release the free form of catechins systemically.

Palmitoylated green tea extract catechins (PGTEC) (no less than 74% mono-, di-, and tri-palmitate esters derived from green tea) have practically no acute toxicity to rodents (ICR mice and SD rats) following oral intake (Mei et al. 2010). In a short-term toxicity study, encapsulated PGTEC (35:65 in vegetable oil) was administered for 30 days by gavage to SD rats (10/sex/dose) at three dose levels: 1,670, 3,330, and 6,670 mg/kg bw per day, corresponding to 584, 1,165, and 2,334 mg/kg bw per day of PGTEC. No mortality or overt toxicity was observed.

Photo courtesy of FONA International

There were no significant differences in weekly food intake or body weight gain between the control groups and the treatment groups. Hematological testing revealed no differences in hemoglobin, erythrocyte, or leukocyte levels between the control and treatment groups and no differences in lymphocyte, monocyte, or granulocyte concentrations. Liver function tests, including serum glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, urea nitrogen, creatine, total cholesterol, triglycerides, glucose, total protein, albumin, and globulin, resulted in parameter values in the normal range and showed no differences between the treatment and control groups. At the end of the study, gross anatomical examinations found no abnormalities and no significant differences in organ weights (liver, spleen, kidney, and testis/ovary) or their respective organ to body weight ratios between treatment and control groups. Histopathological changes in the liver, kidneys, spleen, and stomach/intestines were not considered to be toxicologically relevant, since they

were observed in animals of the highest dose and control groups at similar frequencies (both vehicle and water control) (Xu et al. 2010).

Similar results were reported in a 90-day feeding study in which Sprague-Dawley rats (10/sex/dose) were fed a test diet containing PGTEC (no less than 74% mono-, di-, and tri-palmitate esters derived from green tea) at dose levels corresponding to 125, 250, and 500 mg/kg bw per day. Histopathological examination of the liver, kidney, spleen, stomach, duodenum, testis, and ovary showed no abnormalities in the treatment groups. No mortality and no significant differences were observed between the control and test groups, in overall signs of toxicity, body weight gain, food intake, or food utilization rate. There were also no differences upon gross examination and in the absolute and relative organ weights of the liver, kidney, spleen, and testis among the groups. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) is 500 mg/kg bw per day of PGTEC, the highest dose tested (Mei et al. 2010).

In a 90-day feeding study conducted in beagle dogs (5/sex/dose), the animals were fed a base diet for the first 7 days and a test diet of PGTEC blended into the same base diet (no further details reported) corresponding to doses of 10, 20, and 50 mg/kg bw per day of PGTEC for the remaining 84 days. Daily observations were made for adverse reactions, clinical signs, food consumption, and fecal observations, and body weights were measured weekly. Urinalysis, hematology, clinical chemistry, and physical examinations were performed prior to the study and at days 28, 56, and 84. With the exception of one dog that was euthanized due to hind leg paralysis unrelated to the test diet, no overt signs of toxicity were observed or clinical signs or adverse reactions attributed to the test article. There were no significant changes in body weight or food intake between control and treatment groups. Clinical chemistry and hematology results showed an increase in phosphorus levels in the middle- and high-dose groups when compared to control on days 28 and 56, but this trend was not observed on day 84. Decreases in red blood cell count, hemoglobin, and hematocrit were also observed in the middle- and high-dose groups on days 28, 56, and 84 and in the low-dose animals on days 56 and 84 when compared to concurrent controls. However, the values of these three parameters remained within normal historical limits throughout the study. Urine pH for the treated groups was increased compared to the concurrent controls on day 28 and was decreased compared to the control on days 56 and 84. Fecal observations found no abnormalities or changes. The authors concluded that the NOAEL in beagle dogs is 50 mg/kg bw/day of PGTEC incorporated in the diet (Stanford et al. 2011).

PGTEC has shown no evidence of genotoxicity *in vitro* or *in vivo*. *In vitro*, PGTEC was tested for mutagenicity to bacteria with *Salmonella typhimurium* tester strains TA97a, TA98, TA100, and TA102, with and without metabolic activation. PGTEC produced no increase in revertant mutants in any strain at five concentrations, up to 5,000 µg/plate (Mei et al. 2010). *In vivo*, PGTEC was administered twice to ICR mice (5/sex/group) at doses of 2,500, 5,000, or 10,000 mg/kg bw gavage, with a 24-hour interval between treatments. The mice were sacrificed 6 hours after the second treatment, and genotoxicity was assessed with the micronucleus assay. No differences in the induction of micronuclei were found in 1,000 polychromatic erythrocytes scored or in the toxicity based on the PCE/NCE ratio between the vehicle control and treatment groups (Mei et al. 2010).

To help clarify the differences between GTE and PGTEC, the Panel evaluated additional toxicology studies on the free GTC. In a 28-day repeat-dose study, three GTC preparations were tested: a heat sterilized preparation (GTC-H), a non-heat-treated (GTC-UN) preparation, and a decaffeinated heat-treated preparation (GTC-HDC) (Chengelis et al. 2008). GTC-H and GTC-UN were administered by gavage to Crl:CD(SD) rats (5/sex/dose) at three doses of 500, 1,000, and 2,000 mg/kg bw per day, and GTC-HDC was administered only at 2,000 mg/kg bw per day. Daily clinical observations and weekly physical examinations and food consumption measurements were made. Hematology, serum

In Memoriam

The Expert Panel notes with sadness the passing of two former FEMA Expert Panel members, Dr. William Waddell of the University of Louisville and Dr. Paul Newberne of Boston University. Dr. Waddell was an expert in pharmacology, medicine, and the study of mechanisms of toxicity. He retired from the Panel in 2010 after more than a decade of service, including as chair from September 2004 through February 2008. Dr. Newberne was a member of the Expert Panel from 1978 until 2000 and served for two terms as its chair. Dr. Newberne was an internationally recognized veterinary pathologist, expert in nutritional biochemistry and public health. Both Dr. Waddell and Dr. Newberne made numerous key contributions to the work of the Panel.

chemistry, and urinalysis were conducted at week 4. Baseline functional observational battery (FOB) and motor activity (MA) assessments were done one week prior to the beginning of the study and during week 3. Necropsies were performed on all animals, selected organs were weighed, and selected tissues were examined microscopically. There were two fatalities during the study, one female in the 500 mg/bw per day GTC-H group and one female in the 2,000 mg/bw per day GTC-UH group, that were not considered treatment related. There were no test article–related findings in the functional observational battery, locomotor activity, hematology, serum chemistry, and urinalysis tests in any treatment group. Neither gross necropsy examinations nor organ weights showed test article–related effects. Statistically significant lower mean body weights were recorded in the male rats of the high-dose GTC-H group compared to controls during the first week of the study, and statistically significant lower cumulative body weight changes were noted in males of the mid- and high-dose GTC-H groups throughout the study. Food consumption was also significantly lower in the high-dose GTC-H group during the first week. These body weight effects were not observed in the high-dose GTC-HDC group or in any of the GTC-UN dose groups nor were there any significant differences in feed consumption for these groups. Treatment-related minimal erosions of the glandular stomach were observed in one male and one female from the high-dose GTC-H group. It is postulated that both the body weight changes and the glandular stomach erosions were related to caffeine since these effects were not observed at any dose in the decaffeinated GTC-HDC preparation. This study resulted in a NOAEL for GTC-H of 1,000 mg/kg bw per day for local toxicity and 2,000 mg/kg bw per day for systemic toxicity. Both GTC-UN and GTC-HDC showed a NOAEL of 2,000 mg/kg bw per day (Chengelis et al. 2008).

In a 90-day feeding study, F344 rats (10/sex/dose) were fed diets containing GTC at concentrations of 0.3%, 1.25%, or 5.0%, corresponding to doses of 180, 764, and 3,525 mg/kg bw per day, respectively, in male rats and 189, 820, and 3,542 mg/kg bw per day, respectively, in female rats. Clinical signs of toxicity were recorded daily, and body weight and food consumption measurements were done weekly. Significant reductions of body weights compared to controls were found in top dose males from week 1 to the end of the study and in high-dose females at week 1 that corresponded to decreased food consumption. Serum biochemical analysis showed increased levels of aspartate transaminase and albumin in females and of alanine transaminase and alkaline phosphatase in both males and females in the high-dose group. Statistically significant increases in relative organ weights (brain, kidney, liver, and testis in males; kidney and liver in females) were observed but lacked corresponding histopathological changes and were not considered to be toxicologically significant. Decreases in triglycerides, total cholesterol, and creatinine levels were not considered to be adverse. Based on the results of this study, the NOAEL for GTC in F344 rats was 1.25% GTC in diet, which corresponds to 763 mg/kg bw per day for males and 820 mg/kg bw per day for females (Takami et al. 2008).

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Overall, the available evidence from toxicity studies indicates no concern for genotoxicity or other systemic toxicity from intake of PGTEC when used as a flavor substance. The estimated intake of 0.1 mg/person/day equivalent to 0.0017 mg/kg bw/day from use of PGTEC as a flavoring ingredient is at least 30,000 times lower than the most conservative NOAEL (50 mg/kg bw/day of PGTEC) derived from a subchronic study in beagle dogs (Stanford et al. 2011) and 300,000 times lower than the NOAEL (500 mg/kg bw/day of PGTEC) derived from a subchronic toxicity study in rats (Mei et al. 2010). The doses of no adverse effect are potentially higher (and the margin of safety larger) than those reported since both NOAELs in these studies were the highest doses tested. **FT**

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Flavoring Substances

Photo courtesy of Virginia Dare

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Corrections and Errata to Previous GRAS Publications

Isomeric clarification of FEMA 3818. In GRAS 25 (Smith et al. 2011) on Table 3, the name for FEMA No. 3818 should have read DL- and L-Alanine.

Use levels for FEMA 4309. In GRAS 26 (Marnett et al. 2013), there was a clerical error, and the Anticipated Maximum Usual Use Level for FEMA No. 4309 in hard candy should read 500 ppm.

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Key Findings for Safety Evaluation Decisions

Key findings of the FEMA Expert Panel GRAS determinations for each substance listed in Table 1 are available on femaflavor.org.

Tables with supporting data for this publication of GRAS 27 appear on the following pages. »»

TABLE 1. Primary Names & Synonyms

Primary Names (in boldface) & Synonyms (in lightface).

FEMA NO.	SUBSTANCE PRIMARY NAME AND SYNONYMS
4779	**(±)-2-Mercapto-5-methylheptan-4-one** (±)-5-Methyl-2-sulfanylheptan-4-one
4780	**Caryophylla-3(4),8-dien-5-ol** Mixture of 10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]-undecan-5-ol and 4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-en-5-ol
4781	***L*-Cysteine methyl ester hydrochloride** Methyl (*R*)-2-amino-3-mercaptopropanoate hydrochloride
4782	**2(3)-Hexanethiol**
4783	**Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde** Mixture of 1-Ethenyl-3-cyclohexene-1-carboxaldehyde and 4-Ethenyl-1-cyclohexene-1-carboxaldehyde
4784	**(±)-4-Hydroxy-6-methyl-2-heptanone**
4785	**2-Octyl-2-dodecenal**
4786	**2-Hexyl-2-decenal**
4787	***trans*-6-Octenal** (*E*)-6-Octenal (6*E*)-Octenal
4788	**(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide** (2*E*)-3-(1,3-benzodioxol-5-yl)-*N*,*N*-diphenylprop-2-enamide
4789	**2,6-Dimethyl-5-heptenol**
4790	**(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester** (±)-Ethyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (±)-5-Norbornene-2-carboxylic acid, ethyl ester
4791	**3-(Acetylthio)hexanal**
4792	**(±)-3-Mercapto-1-pentanol**
4793	**(*3R*,*3S*)-3-[[(4-Amino-2,2-dioxido-*1H*-2,1,3-benzothiadiazin-5-yl)oxy]methyl]-N-cyclopentyl-2-oxo-3-piperidinecarboxamide** (*3R*,*3S*)-3-[[(4-Amino-2,2-dioxido-*1H*-2,1,3-benzothiadiazin-5-yl)oxy]methyl]-*N*-cyclopentyl-2-oxopiperidine-3-carboxamide
4794	**(±)-1-Cyclohexylethanol** (±)-Methylcyclohexylcarbinol (±)-Cyclohexanemethanol
4795	**(±)-8-Methyldecanal**
4796	**Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 30%**
4797	**(±)-Naringenin** (±)-5,7-Dihydroxy-2-(4-hydroxyphenyl)-*4H*-chroman-4-one
4798	**2-(((3-(2,3-Dimethoxyphenyl)-*1H*-1,2,4-triazol-5-yl)thio)methyl)pyridine** 2-((5-(2,3-Dimethoxyphenyl)-*2H*-1,2,4-triazol-3-ylthio) methyl)pyridine 2-(((3-(2,3-dimethoxyphenyl)-*1H*-1,2,4-triazol-5-yl)thio)methyl)pyridine
4799	**(*2R*)-3',5-Dihydroxy-4'-methoxyflavanone**
4800	**Glucosylated *Rubus suavissimus* extract, 20-30% glucosylated rubusoside glycosides** Glucosylated Sweet Blackberry leaves extract, 20-30% glucosylated rubusoside glycosides
4801	**Olive Fruit Extract** *Olea europaea* fruit extract
4802	**(*S*)-1-(3-(((4-Amino-2,2-dioxido-*1H*-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one** 1-[(*3S*)-3-[[(4-Amino-2,2-dioxido-*1H*-2,1,3-benzothiadiazin-5-yl)oxy]methyl]-1-piperidinyl]-3-methyl-1-butanone
4803	**8-Methylnonanal** Isodecanal
4804	**Mixture of Ricinoleic acid, Linoleic acid, and Oleic acid**
4805	**Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 22%**
4806	**Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 22%**
4807	**Pinocarvyl acetate** 6,6-Dimethyl-2-methylenebicyclo[3.1.1]hept-3-yl acetate
4808	***N*-Ethyl-5-methyl-2-(1-methylethenyl)cyclohexanecarboxamide** *N*-Ethyl-5-methyl-2-(prop-1-en-2-yl)cyclohexanecarboxamide
4809	**2-(4-Methylphenoxy)-*N*-(*1H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl)acetamide** *N*-(*1H*-Pyrazol-5-yl)-*N*-(thiophen-2-ylmethyl)-2-(*p*-tolyloxy)acetamide
4810	**Ethyl-2-(4-hydroxy-3-methoxyphenyl)acetate** Ethyl homovanillate Ethyl 4-hydroxy-3-methoxyphenylacetate
4811	**Ginger Mint Oil (*Mentha x gracilis*)** Red stemmed mint oil Vietnamese mint oil
4812	**Palmitoylated Green Tea Extract Catechins** Palmitoylated *Camilla sinensis* Extract Catechins Lipid Soluble Green Tea Extract (Catechin Palmitate Esters)
4813	**2-(5-Isopropyl-2-methyl-tetrahydro-thiophen-2-yl)-ethanol**
4814	**Glucosylated *Rubus suavissimus* extract, 60% glucosylated rubusoside glycosides** Glucosylated Sweet Blackberry Leaves Extract, 60% glucosylated rubusoside glycosides
4815	**Sandalwood austrocaledonicum oil** Santalum austrocaledonicum oil
4816	**Sugar Cane Distillate**

Key Findings for Safety Evaluation Decisions

Key findings of the FEMA Expert Panel GRAS determinations for each substance listed in Table 1 are available on femaflavor.org.

TABLE 2. Average Usual Use Levels/Average Maximum Use Levels

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for new FEMA GRAS Flavoring Substances on which the FEMA Expert Panel based its judgments that the substances are generally recognized as safe (GRAS).

	(±)-2-Mercapto-5-methylheptan-4-one	Caryophylla-3(4),8-dien-5-ol	*L*-Cysteine methyl ester hydrochloride	2(3)-Hexanethiol	Mixture of 1-Vinyl-3-cyclohexenecarb-aldehyde and 4-Vinyl-1-cyclohexenecarb-aldehyde	(±)-4-Hydroxy-6-methyl-2-heptanone	2-Octyl-2-dodecenal	2-Hexyl-2-decenal	*trans*-6-Octenal
CATEGORY	**FEMA No. 4779**	**4780**	**4781**	**4782**	**4783**	**4784**	**4785**	**4786**	**4787**
Baked Goods	0.03/0.1	0.1/1	10/100	0.02/0.06		50/100	0.1/1	0.1/1	0.1/1
Beverages, Nonalcoholic	0.01/0.1	1/5		0.005/0.03	5/10	30/80	1/5	1/5	0.03/0.3
Beverages, Alcoholic	0.02/0.1	1/5		0.005/0.03		30/80	1/5	1/5	0.03/0.3
Breakfast Cereals	0.03/0.1	0.1/1		0.01/0.04		40/100	0.1/1	0.1/1	
Cheeses		0.1/1	10/100				0.1/1	0.1/1	0.1/1
Chewing Gum	0.1/0.2	1/5		0.02/0.06	100/1,000	60/100	1/5	1/5	0.1/1
Condiments and Relishes	0.01/0.1	0.1/1	10/100	0.02/0.06			0.1/1	0.1/1	
Confections and Frostings	0.05/0.1	1/5		0.02/0.06	5/50		1/5	1/5	0.1/1
Egg Products		0.1/1	10/100	0.015/0.05			0.1/1	0.1/1	0.05/1
Fats and Oils		0.1/1					0.1/1	0.1/1	0.03/0.5
Fish Products		0.1/1	10/100				0.1/1	0.1/1	
Frozen Dairy	0.05/0.1	0.1/1		0.02/0.06		50/100	0.1/1	0.1/1	0.1/1
Fruit Ices	0.005/0.05	1/5		0.01/0.04			1/5	1/5	
Gelatins and Puddings	0.03/0.1	1/5	10/100	0.015/0.04		40/100	1/5	1/5	0.1/1
Granulated Sugar		0.1/5					0.1/1	0.1/1	
Gravies	0.01/0.1	0.1/1	10/100	0.02/0.06			0.1/1	0.1/1	0.02/0.5
Hard Candy	0.1/0.2	1/5		0.02/0.05	5/50	50/100	1/5	1/5	0.05/1
Imitation Dairy		0.1/1					0.1/1	0.1/1	0.1/1
Instant Coffee and Tea		0.1/1	10/100	0.02/0.06		50/100	0.1/1	0.1/1	0.03/0.5
Jams and Jellies		1/5		0.02/0.06			1/5	1/5	
Meat Products		0.1/1	10/100	0.02/0.08			0.1/1	0.1/1	
Milk Products		0.1/1		0.005/0.04		40/80	0.1/1	0.1/1	0.2/2
Nut Products		0.1/1		0.01/0.04			0.1/1	0.1/1	0.05/0.2
Other Grains		0.1/1		0.01/0.06			0.1/1	0.1/1	
Poultry		0.1/1	10/100				0.1/1	0.1/1	
Processed Fruits	0.02/0.1	0.1/1		0.01/0.05			0.1/1	0.1/1	
Processed Vegetables		0.1/1	10/100				0.1/1	0.1/1	
Reconstituted Vegetables		0.1/1	10/100				0.1/1	0.1/1	
Seasonings and Flavors	0.005/0.2	1/5	10/100	0.01/0.1	10/100	50/100	1/5	1/5	0.05/0.5
Snack Foods	0.02/0.1	0.1/1		0.01/0.06			0.1/1	0.1/1	
Soft Candy	0.05/0.1	1/5		0.01/0.04	10/100	50/100	1/5	1/5	0.1/1
Soups	0.03/0.1	0.1/1	10/100	0.02/0.06			0.1/1	0.1/1	0.02/0.5
Sugar Substitutes		0.1/1					0.1/1	0.1/1	
Sweet Sauces	0.03/0.1	0.1/1	10/100	0.02/0.06		50/100	0.1/1	0.1/1	0.1/1

TABLE 2. *CONTINUED* Average Usual Use Levels/Average Maximum Use Levels

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for new FEMA GRAS flavoring substances on which the FEMA Expert Panel based its judgments that the substances are generally recognized as safe (GRAS)

	(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide	2,6-Dimethyl-5-heptenol	(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	3-(Acetylthio)hexanal	(±)-3-Mercapto-1-pentanol	(3*R*,3*S*)-3-[[(4-amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]methyl]-*N*-cyclopentyl-2-oxo-3-piperidinecarboxamide	(±)-1-Cyclohexylethanol	(±)-8-Methyldecanal	Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 30%	(±)-Naringenin
CATEGORY	**4788**	**4789**	**4790**	**4791**	**4792**	**4793**	**4794**	**4795**	**4796**	**4797**
Baked Goods	0.2/2	1/10	0.5/1	0.5/3	2/10	3/8		2/20	30/90	200/600
Beverages, Nonalcoholic	0.02/0.2	1/5	0.2/1	0.1/1	1/6	5/8		0.5/5	20/75	100/300
Beverages, Alcoholic	0.04/2	1/5	0.2/1			2/8		0.5/5	20/75	100/300
Breakfast Cereals	0.2/2	1/5	0.5/1	0.5/2	1/8	3/8		1/5	30/90	200/600
Cheeses										1,000/1,000
Chewing Gum	10/100	2/20	0.5/2	1/5	3/10	10/27	1,000/3,000	2/10	100/125	200/400
Condiments and Relishes										200/400
Confections and Frostings	0.5/5	2/10	0.4/2			3/8	50/100	2/10	20/75	200/400
Egg Products	0.2/2	0.2/2						0.2/2		
Fats and Oils	0.5/10	1/10	0.02/0.5					1/10		100/200
Fish Products										
Frozen Dairy	0.2/2	1/2	0.3/1	0.5/1	1/8	2/8		1/2	20/75	100/500
Fruit Ices	0.2/4	1/2	0.3/1			2/8	30/60	0.5/5	20/75	100/200
Gelatins and Puddings	0.2/4	1/10	0.2/1			2/8		1/10	20/75	100/400
Granulated Sugar	0.4/4									
Gravies				0.05/2	1/8	2/8			20/50	100/500
Hard Candy	1/10	1/10	0.4/2			5/8	100/300	1/10		100/400
Imitation Dairy	0.2/2	1/10						1/10	20/75	100/500
Instant Coffee and Tea	0.04/0.2	1/10		0.02/1	1/8	2/8		1/10		100/200
Jams and Jellies	0.2/2	1/10	0.4/2	0.02/1	1/8			1/10	20/75	100/400
Meat Products			0.05/0.5			2/8			10/20	100/200
Milk Products	0.2/2	1/5		0.02/1	1/8	3/8		1/5	30/90	100/500
Nut Products										50/100
Other Grains										
Poultry										100/200
Processed Fruits	0.2/2		0.1/1							50/400
Processed Vegetables										50/100
Reconstituted Vegetables										50/100
Seasonings and Flavors		1/10	0.2/1			2/8		1/10	20/50	500/1,000
Snack Foods		1/10		0.02/1	1/6	2/8		1/10		200/400
Soft Candy	0.5/10	1/10	0.3/2			5/8	50/200	1/10		100/400
Soups	0.5/10	1/10	0.05/0.8			2/8		1/10		100/300
Sugar Substitutes	0.4/8									100/200
Sweet Sauces						3/8				100/400

	2-((3-(2,3-Dimethoxyphenyl)-1H-1,2,4-triazol-5-yl) thio)methyl)pyridine	(2*R*)-3',5-Dihydroxy-4'-methoxyflavanone	Glucosylated *Rubus suavissimus* extract, 20-30% glucosylated rubusoside glycosides	Olive fruit extract	(*S*)-1-(3-(((4-Amino-2,2-dioxido-1*H*-benzo-[c][1,2,6] thiadiazin-5-yl)-oxy) methyl)-piperidin-1-yl)-3-methylbutan-1-one	8-Methylnonanal	Mixture of Ricinoleic acid, Linoleic acid, and Oleic acid	Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside A 22%	Steviol glycoside extract, *Stevia rebaudiana*, Rebaudioside C 22%	Pinocarvyl acetate
CATEGORY	**4798**	**4799**	**4800**	**4801**	**4802**	**4803**	**4804**	**4805**	**4806**	**4807**
Baked Goods	2/6			120/720	6/6	2/20	5/20	70/70	100/100	
Beverages, Nonalcoholic	1/2	20/25	150/350	120/720	2.5/6	0.5/10	1/5	70/70	110/110	0.1/5
Beverages, Alcoholic		20/25	75/200	120/720	2.5/6	0.5/10	1/5	70/70	100/100	0.5/7.5
Breakfast Cereals	5/10	20/25	150/400	120/720	6/6	1/10	1/5	70/70	100/100	
Cheeses	2/6			120/720			5/20			
Chewing Gum					6/6	2/20	1/5	70/70	100/100	0.5/7.5
Condiments and Relishes	5/10			120/720	6/6		5/10	70/70	100/100	
Confections and Frostings		20/25			6/6	2/20	1/5	70/70	100/100	0.5/7.5
Egg Products	2/6			120/720		0.2/2	1/10	70/70		
Fats and Oils	4/8			120/720	3/6	1/20	10/50	70/70	100/100	
Fish Products	4/10			120/720			1/5			
Frozen Dairy			200/300	120/720	3/6	1/5	1/5	70/70	100/100	0.1/5
Fruit Ices			100/300		3/6	0.5/5	1/5	70/70	100/100	
Gelatins and Puddings					3/6	1/10	1/5	70/70	100/100	
Granulated Sugar										
Gravies	4/10		100/150	120/720	3/6		5/20	70/70	100/100	
Hard Candy		20/25			3/6	1/20	1/5	70/70	100/100	0.5/7.5
Imitation Dairy					2.5/6	1/10	5/20	70/70	100/100	0.1/5
Instant Coffee and Tea		20/25	150/350		2.5/6	1/10	1/5	70/70	100/100	
Jams and Jellies		20/25			6/6	1/10	1/5	70/70	100/100	
Meat Products	4/10		100/150	120/720			5/20			
Milk Products		20/25	200/300	120/720	2.5/6	1/10	5/20	70/70	100/100	0.1/5
Nut Products	2/6			120/720	3/6		1/5	70/70	100/100	
Other Grains	2/6									
Poultry	2/6			120/720			5/25			
Processed Fruits					3/6		1/5	70/70	100/100	
Processed Vegetables	2/6						1/5	70/70	100/100	
Reconstituted Vegetables	2/6						1/5			
Seasonings and Flavors	10/20		100/150	120/720		1/10	5/50	70/70	100/100	
Snack Foods	10/20	20/25		120/720	6/6	1/10	5/50	70/70	100/100	
Soft Candy		20/25			6/6	1/10	1/5	70/70	100/100	0.5/7.5
Soups	4/8		100/150	120/720	3/6	1/10	5/25	70/70	100/100	
Sugar Substitutes		20/25					1/5	70/70	100/100	
Sweet Sauces		20/25		120/720	3/6		1/5	70/70	100/100	

TABLE 2. *CONTINUED* Average Usual Use Levels/Average Maximum Use Levels

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for new FEMA GRAS flavoring substances on which the FEMA Expert Panel based its judgments that the substances are generally recognized as safe (GRAS)

	N-Ethyl-5-methyl-2-(1-methylethenyl) cyclohexanecarboxamide	2-(4-Methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl) acetamide	Ethyl-2-(4-hydroxy-3-methoxyphenyl)acetate	Ginger Mint Oil (*Mentha x gracilis*)	Palmitoylated Green Tea Extract Catechins	2-(5-Isopropyl-2-methyltetrahydro-thiophen-2-yl)ethanol	Glucosylated *Rubus suavissimus* extract, 60% glucosylated *rubusoside glycosides*	Sandalwood Austrocaledonicum oil	Sugar Cane Distillate*
CATEGORY	**4808**	**4809**	**4810**	**4811**	**4812**	**4813**	**4814**	**4815**	**4816**
Baked Goods	20/30		25/200		100/200	0.2/2	30/150	5/10	0.01(2,250)/0.01(2,250)
Beverages, Nonalcoholic	5/10	1/3	10/75			0.05/2	30/150	1/2	
Beverages, Alcoholic	15/30	2/6	10/75			0.1/2	30/150	0.5/1	
Breakfast Cereals	10/20				50/75	0.2/2	30/150		
Cheeses									
Chewing Gum	2,000/3,000	75/150	100/500	4,000/8,000		0.5/5	30/150	3/3	
Condiments and Relishes			25/200		100/200				
Confections and Frostings	200/300	5/15		250/1,000	50/100	0.5/5	30/150		0.01(2,250)/0.01(2,250)
Egg Products						0.1/2			
Fats and Oils		1/3			200/500	0.2/5			
Fish Products					250/300				
Frozen Dairy		1/3	25/100	250/1,000		0.1/2	50/150	3/4	
Fruit Ices	200/300	1/3	25/100			0.05/2	50/150		
Gelatins and Puddings		1/3				0.2/5	30/150	0.3/1	
Granulated Sugar									
Gravies						0.2/5			
Hard Candy	200/300	5/15	25/100	2,500/5,000		0.5/5		90/90	
Imitation Dairy		1/3				0.2/5			
Instant Coffee and Tea	10/20	1/3	10/75			0.2/5	30/150		
Jams and Jellies	10/20					0.2/5			
Meat Products			25/100		250/300				
Milk Products	5/10	1/3	10/100			0.1/5	50/150		
Nut Products					50/100				
Other Grains					150/300				
Poultry					250/300				
Processed Fruits	5/10						30/150		
Processed Vegetables									
Reconstituted Vegetables									
Seasonings and Flavors	50/150		50/300			0.1/2			
Snack Foods	200/300		25/200		100/200	0.1/2	30/150		0.01(2,250)/0.01(2,250)
Soft Candy	500/1,000	5/15		2,500/5,000	50/100	0.2/5	30/150	5/10	
Soups		1/3	25/100		100/200	0.2/5	30/150		
Sugar Substitutes							30/150		
Sweet Sauces		5/15					30/150		0.01(2,250)/0.01(2,250)

**Figures in the parentheses represent the amount of diluted Sugar Cane Distillate in the commercial product as used in food.*

TABLE 3. Updated Average Usual Use Levels/Average Maximum Use Levels

Average Usual Use Levels (ppm)/Average Maximum Use Levels (ppm) for flavoring substances previously recognized as FEMA GRAS. Superscript 'a' represents a new use level.

	Potassium cinnamate	Quillaia extract	Glycine	*N*-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	3-[(4-Amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide	Glutamyl-valyl-glycine	Luo Han Fruit Concentrate
FEMA NO.	**2288**	**2973**	**3287**	**4232**	**4701**	**4709**	**4711**
GRAS PUBLICATION	**3**	**3**	**4**	**22**	**25**	**25**	**25**
CATEGORY							
Baked Goods	233/384	24/30	50/150	1/2	10/22	15/30	40/60
Beverages, Nonalcoholic	300/400	91.5/103	250/1,000	2/5	0/0	20/50	40/60
Beverages, Alcoholic	570/712	90/100		2[a]/5[a]	5[a]/22[a]		40/60
Breakfast Cereals		15[a]/75[a]			15/22	80/160	40/80
Cheeses		15[a]/75[a]		1/3		20/50	
Chewing Gum	224[a]/300	7.5[a]/30[a]			30/300	10[a]/30[a]	200[a]/400[a]
Condiments and Relishes		15[a]/75[a]	150/3,000[a]	2/4	3/22	30/60	5/40
Confections and Frostings		7.5[a]/30[a]			10/22		40/80
Egg Products		7.5[a]/30[a]		2[a]/5[a]		15/45	
Fats and Oils	746[a]/1,000[a]			2/4		30/60	
Fish Products		7.5[a]/30[a]		1/3		15/45	
Frozen Dairy	192/263	15[a]/75[a]			5/22	20/50	5/80
Fruit Ices		7.5[a]/30[a]			5/22	20/50	5/40
Gelatins and Puddings	459[a]/500[a]	7.5[a]/30[a]			5/22		40/80
Granulated Sugar							
Gravies	746[a]/1,000[a]	7.5[a]/30[a]	150/4,000[a]	2/4		30/60	5/40
Hard Candy	0.01/0.01	18/30[a]	25/150		15/75		40/80
Imitation Dairy		7.5[a]/30[a]	50/150			20/50	5/40
Instant Coffee and Tea	224[a]/300[a]	1.5[a]/30[a]	150/150			10/30	
Jams and Jellies	373[a]/500[a]	7.5[a]/30[a]			10/22		10/40
Meat Products		7.5[a]/75[a]		1/3		15/45	
Milk Products		1.5[a]/30[a]			3/22	15/45	40/80
Nut Products		30[a]/120[a]		2[a]/5[a]			5/40
Other Grains		7.5[a]/30[a]					
Poultry		15[a]/75[a]		1/3		15/45	
Processed Fruits	37[a]/50[a]	1.5[a]/30[a]					5/40
Processed Vegetables		7.5[a]/30[a]		1/3		15/45	
Reconstituted Vegetables		7.5[a]/30[a]		2[a]/5[a]		15/45	
Seasonings and Flavors		15[a]/75[a]		5/10		80/160	5/40
Snack Foods		15[a]/75[a]		5/10		80/160	5/40
Soft Candy	249/356	16[a]/30[a]	25/150		15/75		40/80
Soups		15[a]/75[a]	150/6,000[a]	2/4		20/50	
Sugar Substitutes	746[a]/1,000[a]	7.5[a]/30[a]				80/160	
Sweet Sauces	746[a]/1,000[a]	15[a]/75[a]			10/22	30/60	5/40