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Updated procedure for the safety evaluation of natural flavor complexes used as ingredients in food

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ABSTRACT

An effective and thorough approach for the safety evaluation of natural flavor complexes (NFCs) was published in 2005 by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). An updated procedure is provided here, which maintains the essential concepts of the use of the congeneric group approach and the reliance on the threshold of toxicological concern (TTC) concept. The updated procedure emphasizes more rigorous considerations of unidentified constituents and the genotoxic potential of constituents. The update of the previously established procedure is the first step in a multi-year project to conduct safety re-evaluations for more than 250 NFCs that have uses that are currently considered Generally Recognized as Safe (GRAS) by the FEMA Expert Panel. In addition, this procedure can be more generally employed in the safety evaluation of NFCs.

1. Introduction

Natural Flavor Complexes (NFCs) are naturally occurring mixtures derived from plants and other natural sources that are used to flavor foods for human consumption. Many NFCs from commonly used spices and herbs, including black pepper, cinnamon, clove, rosemary, oregano and basil have been used to flavor food for centuries. By the beginning of the 20th century, NFCs were used for a variety of applications, such as use of peppermint and other mint oils for the flavoring of chewing gums and candy and use of citrus oils in soda fountain drinks. Today, NFCs remain important flavoring ingredients in almost all food categories.

The Flavor and Extract Manufacturers Association of the United States (FEMA) began a program in 1959 to assess the safety and generally recognized as safe (GRAS) status of flavoring ingredients under the authority provided by the 1958 Food Additives Amendments to the Federal Food, Drug, and Cosmetic Act (FFDCA). The FEMA Expert Panel published its first GRAS list in 1965 (Hall and Oser, 1965) including 265 NFCs that are also permitted at 21 Code of Federal Regulations Part 172.510 and 21 Code of Federal Regulations Part 182.20. The Expert Panel has since evaluated numerous chemically-defined flavoring materials for GRAS status including approximately 40 NFCs in recent years. As part of its mission, the Expert Panel continually reviews available safety data and use of all substances determined to be FEMA GRAS.

In the first FEMA GRAS evaluations for NFCs, conclusions on their safety were generally based on their long history of safe use in foods combined with their likely low exposure, based on the principle of self-limitation (i.e. flavor ingredients used at high concentrations are often unpalatable, and thus they are typically used at very low concentrations in food). Recognizing the need for a new safety evaluation procedure for NFCs that applied current scientific knowledge in the fields of

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toxicology, metabolism, biochemistry and analytical chemistry, a scientifically based procedure for the safety evaluation of NFCs based on their chemical composition was developed and published in 2005 (Smith et al., 2005). The procedure requires a comprehensive evaluation of the chemical and biological properties of the constituents. The safety evaluation of cardamom oil demonstrated the application of the procedure (Smith et al., 2004).

The Smith et al., 2005 procedure employs a congeneric group approach for the classification and evaluation of the identified (known) constituents of the NFC under consideration and compares the intake of each congeneric group to the threshold of toxicological concern (TTC) (Cramer et al., 1978; Kroes et al., 2000; Munro et al., 1996) a widely adopted and highly conservative approach to the safety evaluation of food ingredients. Both the European Food Safety Authority (EFSA) and the World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) use the TTC approach in their evaluation of flavoring substances (EFSA/WHO, 2016). In the Smith et al., 2005 procedure, the constituents of each congeneric group are related by chemical structure, biochemistry, metabolism and toxicologic potential. The 36 congeneric groups that were described in the original procedure, with some modifications, are listed in Appendix A. Comprehensive, quantitative chemical analyses of each NFC are considered, sorting each identified constituent into its appropriate congeneric group. The structure of each constituent is assessed for toxic potential using the Cramer decision tree (Cramer et al., 1978) which classifies chemical substances into the following classes: Class I (expected low oral toxicity), Class II (less innocuous than Class I but do not contain structural features that provide oral toxicity concern) or Class III (contains structural features which do not permit a presumption of safety). The Cramer decision tree class for each congeneric group is assigned using the highest structural class of any constituent present in the congeneric group. For the NFC under consideration, the range of concentrations of each congeneric group is determined based on multiple analyses. To determine the intake of each congeneric group resulting from consumption of the NFC, the highest percent concentration is multiplied by the NFC intake which is calculated in terms of daily *per capita* intake derived from annual volume of use surveys. The intake of each congeneric group is evaluated against the TTC thresholds for each Cramer Class, 1800 μg/person/day for Class I, 540 μg/person/day for Class II and 90 μg/person/day for Class III (Kroes et al., 2000). For the evaluation of the relatively small percentage of unidentified constituents of an NFC using the Smith et al., 2005 procedure, they are grouped, and approximations of intake of the unidentified constituents are determined in a similar way as for the known congeneric groups. The resulting intake is evaluated against the TTC threshold for Class III, 90 μg/person/day. For both known congeneric groups and unknown constituents of the NFC, if the intake is below the TTC threshold, there is no safety concern. When the intake exceeds the TTC threshold for the respective Cramer class, the procedure calls for the evaluation of the toxicological data for representative members of the congeneric group and/or the NFC. For the evaluation of the unidentified constituents, if their intake is greater via consumption of the food compared to their intake via use of the NFC as added flavoring, further consideration of the unknown portion is not needed and the evaluation of the NFC proceeds to other potential issues that may raise safety concerns.

This manuscript presents an update to the 2005 procedure for the safety evaluation of NFCs. A summary of the revised procedure is outlined in Fig. 1 but the full procedure described here should be used for evaluating NFCs. The original scope of the procedure was for the safety evaluation of essential oils derived from higher plants for the intended use as flavoring substances in food. However, the inherent flexibility and general applicability of the procedure has allowed for the evaluation of a wider range of complex mixtures including those that may be derived from non-botanical sources. While the general approach of the procedure remains the same as that published in 2005, the updated procedure reflects the knowledge obtained through its practical application over the last decade, including a more rigorous consideration of the unknown fraction and further consideration of the approach to genotoxicity evaluation of constituents, in addition to other minor changes.

2. The procedure for the safety evaluation of natural flavor complexes (NFCs)

Preamble

This procedure provides *guidance* for the safety evaluation of NFCs; it is not to be viewed as a rigid check-list.

The preamble identifies the data that must be available to successfully employ this safety evaluation sequence as described below:

A. It is essential to provide a complete analytical characterization of the chemical composition of the NFC to be used as a flavoring agent.
B. The description of the starting material and isolation method must take into account, where relevant:
   - all recognized botanical/natural sources,[1]
   - all relevant geographical sources,
   - all commercially used plant parts,
   - all commercially used degrees of maturity,
   - all commercially used methods of isolation, and
   - the variability inherent in each method of isolation.

These six factors can, and often do, have such an extensive influence on composition that their variation may result in a wholly distinctive product. Therefore, in all cases, it is essential to define these factors to ensure that commercial products conform to the identification that describes the evaluated product.

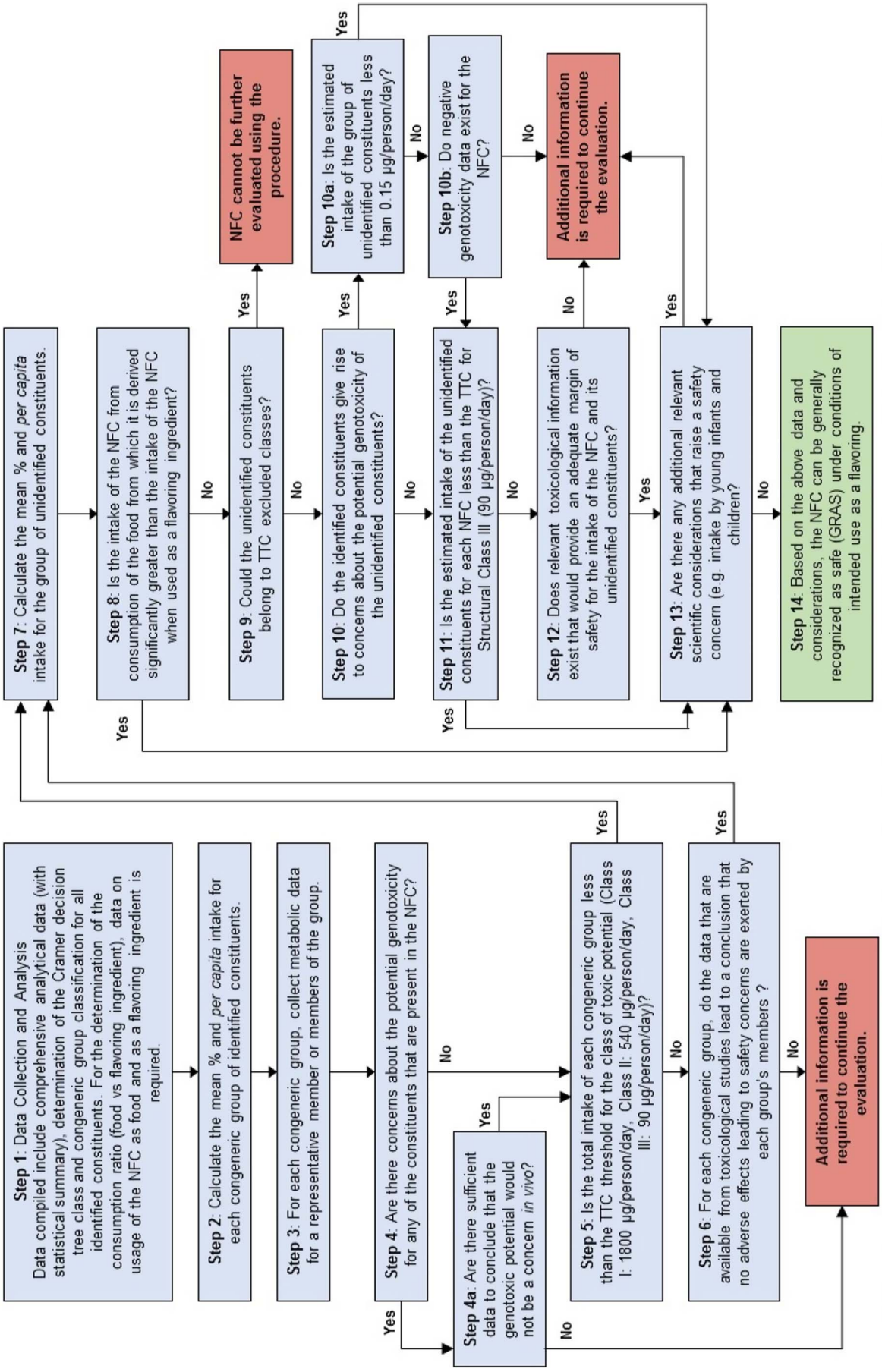
C. An NFC identification shall include existing relevant specifications and additional data that assure the identity, purity, technical effect and safety of the commercial product.
D. Data should be provided on the total exposure to the NFC that includes:
   a. History of use
   b. Intake of the natural source of the NFC when that source is itself consumed as a food, and
   c. Intake of the NFC when it is used as an added flavoring ingredient
   d. Any other relevant data on individual constituents

Step 1. To conduct a safety evaluation of an NFC, the Panel requires that comprehensive analytical data are provided. The analytical methodologies employed should reflect the expected composition of the NFC and provide data that identify, to the greatest extent possible, the constituents of the NFC and the levels (%) at which they are present. It is anticipated that GC-MS and LC-MS would be used for characterization of most NFCs, and that the chromatographic peaks based on peak area of total ion current will be almost completely identified. The percentage of unknowns should be low enough to not raise a safety concern. Other appropriate methods (e.g., Karl Fischer titration, amino acid analysis, etc.) should be employed as necessary. The analytical parameters should be submitted for each type of analysis, including the method of quantitation for both identified and unidentified constituents and libraries, databases and methodology employed for the identification of analytes. The Panel requires data from multiple batches to understand the inherent variability of the NFC.

a. Consumption of foods from which the NFCs are derived

Calculate the *per capita* daily intake (PCI) of the NFC based on the annual volume added to food.

[1] A botanical source should be described phytogenetically by family and by genus, species and variety within each family.



This scheme presents a summary of the revised procedure for the evaluation of NFCs to give an overall structural view. When applying the procedure, the full procedure described in the manuscript should be followed.

Fig. 1. Summary of the procedure for the safety evaluation of natural flavor complexes.

For NFCs with a reported volume of use greater than 22,700 kg (50,000 lbs), the intake may be calculated by assuming that consumption of the NFC is spread among the entire population, on a case-by-case basis. In these cases, the PCI is calculated as follows:

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

where:

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

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

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

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

If applicable, estimate the intake resulting from consumption of the commonly consumed food from which the NFC is derived. The aspect of food use is particularly important. It determines whether intake of the NFC occurs predominantly from the food of which it is derived, or from the NFC itself when it is added as a flavoring ingredient (Stofberg and Grundschober, 1987).[2] At this step, if the conditions of use[3] for the NFC result in levels that differ from intake of the same constituents in the food source, it should be reported.

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

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

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

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

Assign a decision tree structural class to each congeneric group. Within a congeneric group, when there are multiple decision tree structural classes for individual constituents, the class of highest toxicological concern is assigned to the group. In cases where constituents do not belong to a congeneric group, potential safety concerns would be addressed in Step 13.

Proceed to Step 2.

Step 2. Determine (a) the mean percentage (%) of each congeneric group in the NFC, and (b) the daily *per capita* intake,[4] of each congeneric group. The value (a) is calculated by summing the mean percentages of each of the constituents within a congeneric group, and the value (b) is calculated from consumption of the NFC and the mean percentage.

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

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

where:

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

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

Proceed to Step 3.

Step 3. For each congeneric group, collect metabolic data for a representative member or members of the group. Step 3 is critical in assessing whether the metabolism of the members of each congeneric group would require additional considerations in Step 13 of the procedure.

Proceed to Step 4.

Step 4. Are there concerns about potential genotoxicity for any of the constituents that are present in the NFC?

If Yes, proceed to Step 4a.

If No, proceed to Step 5.

Step 4a. Are there sufficient data to conclude that the genotoxic potential would not be a concern *in vivo*?

If Yes, proceed to Step 5.

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

Step 5. Is the total intake of each congeneric group less than the TTC for the class of toxic potential assigned to the group, i.e., Class I: 1800 μg/person/day, Class II: 540 μg/person/day, Class III: 90 μg/person/day (Kroes et al., 2000; Munro et al., 1996)? For congeneric groups that contain members of different structural classes, the class of highest toxicological concern is selected.

If Yes, proceed to Step 7.

If No, proceed to Step 6.

Step 6. For each congeneric group, do the data that are available from toxicological studies lead to a conclusion that no adverse effects leading to safety concerns are exerted by each group's members?

This question can commonly be answered by considering the database of relevant metabolic and toxicological data that exist for a representative member or members of the congeneric group, or the NFC itself. A comprehensive safety evaluation of the congeneric group and a sufficient margin of safety (MoS) based on the data available is to be determined on a case-by-case basis. Examples of factors that contribute to the determination of a safety margin include 1) species differences, 2) inter-individual variation, 3) the extent of natural occurrence of each of the constituents of the congeneric group throughout the food supply, 4) the nature and concentration of constituents in related botanical genera and species. Although natural occurrence is no guarantee of safety, if exposure to the intentionally added constituent is trivial compared to intake of the constituent from consumption of food, then this should be taken into consideration in the safety evaluation (Kroes et al., 2000).

If Yes, proceed to Step 7.

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

Step 7. Calculate the mean percentage (%) for the group of unidentified

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

[3] The focus throughout this evaluation sequence is on the intake of the constituents of the NFC. To the extent that processing conditions, for example, alter the intake of constituents, those conditions of use need to be noted, and their consequences evaluated in arriving at the safety judgments that are the purpose of this procedure.

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

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

Proceed to Step 8.

Step 8. Using the data from Step 1, is the intake of the NFC from consumption of the food[5] from which it is derived significantly greater than the intake of the NFC when used as a flavoring ingredient?

If Yes, proceed to Step 13.

If No, proceed to Step 9.

Step 9. Could the unidentified constituents belong to TTC excluded classes?[6] The excluded classes are defined as high potency carcinogens, certain inorganic substances, metals and organometallics, certain proteins, steroids, known or predicted bio-accumulators, nanomaterials, and radioactive materials (EFSA/WHO, 2016; Kroes et al., 2004).

If Yes, the NFC is not appropriate for consideration via this procedure.

If No, proceed to Step 10.

Step 10. Do the identified constituents give rise to concerns about the potential genotoxicity of the unidentified constituents?

If Yes, proceed to Step 10a.

If No, proceed to Step 11.

Step 10a. Is the estimated intake of the group of unidentified constituents less than 0.15 μg/person/day (Koster et al., 2011; Rulis, 1989)? A TTC of 0.15 μg/person/day has been proposed for potentially genotoxic substances that are not from the TTC excluded classes (Kroes et al., 2004).

If Yes, proceed to Step 13.

If No, proceed to Step 10b.

Step 10b. Do negative genotoxicity data exist for the NFC?

If Yes, proceed to Step 11.

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

Step 11. Is the estimated intake of the unidentified constituents (calculated in Step 7) less than the TTC (Kroes et al., 2000; Munro et al., 1996) for Structural Class III (90 μg/person/day)?[7]

If Yes, proceed to Step 13.

If No, proceed to Step 12.

Step 12. Does relevant toxicological information exist that would provide an adequate margin of safety for the intake of the NFC and its unidentified constituents?

This question may be addressed by considering data for the NFC or an NFC with similar composition. It may have to be considered further on a case-by-case basis, particularly for NFCs with primarily non-volatile constituents.

If Yes, proceed to Step 13.

If No, perform appropriate toxicity tests or obtain further analytical data to reduce the fraction of unidentified constituents. Resubmit for further evaluation.

Step 13. Are there any additional relevant scientific considerations that raise a safety concern (e.g. intake by young infants and children)?

If Yes, acquire and evaluate additional data required to address the concern before proceeding to Step 14.

If No, proceed to Step 14.

Step 14. Based on the above data and considerations, the NFC can be generally recognized as safe (GRAS) under conditions of intended use as a flavoring ingredient.

3. Discussion on significant revisions to the procedure

3.1. Consideration of intake

A discussion of the *per capita* intake PCI × 10 method (Rulis et al., 1984) was presented in the 2005 procedure and continues to be used in the revised procedure. The PCI × 10 method for the calculation of intake is used for NFCs except in instances where a large volume of use was reported and assumes that the volume of use for the NFC is consumed by 10% of the population. The PCI × 10 intake calculation factors are the volume of use, current population and a conservative correction factor of 0.8 to account for possible unreported volumes of use. FEMA currently conducts industry-wide surveys for volume of use data every five years use (Gavin et al., 2008; Harman et al., 2018; Harman et al., 2013; Lucas et al., 1999). In cases where the annual volume of an NFC exceeds 50,000 lbs (22,700 kg), it is highly unlikely that the NFC is consumed by 10% or less of the population (Lambe et al., 2002) and as a result, consumption is usually calculated based on the entire population. Calculations for *per capita* intake and consumption ratio for Coriander Oil (FEMA 2334) are shown as an example in Fig. 2.

Within the 2005 version of the procedure, the intake of each congeneric group and the group of unidentified constituents is determined from the maximum reported percentage (%) and the daily *per capita* intake of the NFC is calculated from the annual volume reported in industry surveys. The conservative use of the maximum % versus mean % was used, in part, to compensate for uncertainty in the analytical constituent data. This approach, however, results in an overestimation of the calculated intake for each congeneric group and, consequently, when the intakes of all the congeneric groups and the group of unidentified constituents of an NFC are summed, this sum is greater than the intake for the NFC, as calculated by the PCI × 10 method described above. The degree to which the intake is overestimated for each NFC depends on the variability in the collected composition data and may be biased by a single data set. In the revised procedure, this calculation was changed such that the intake of each congeneric group is determined from the mean reported percentage (%) in recognition of the fact that technological advances in the analysis of complex mixtures have greatly reduced the variability and uncertainty in the analysis of NFCs. Using this approach, the calculated intakes for the congeneric group and the group of unidentified constituents are a more accurate representation of the intake of the NFC as a whole. In Step 5 of the revised procedure, comparison of intake to TTC thresholds, remains a highly conservative evaluation due to the assignment of the most conservative Cramer decision tree class to the group and use of the inherently conservative TTC approach. When the congeneric groups are assessed, the decision tree class of the group is determined to be the highest class assigned to any one constituent. Thus, in many cases, the toxicological potential determined by decision tree class assigned to a

[5] Provided the intake of the unidentified constituents is greater from consumption of the food itself, the intake of unidentified constituents from the added NFC is considered trivial.

[6] This can be based on arguments including: expert judgement; nature of the identified ingredients; knowledge on the production/extraction process (see also Koster et al. (2011); EFSA/WHO (2016)).

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

Fig. 2. Calculation of *per capita* intake and consumption ratio for Coriander Oil FEMA 2334.

Coriander Oil (*Coriandrum sativum*) FEMA 2334

2015 Usage[8]: 14,100 kg

USA population in 2015[9]: 320,000,000 persons

per capita Intake Calculation[10]*:*

$$\text{PCI} \times 10 = \frac{14{,}100\ \text{kg} \times 10^9}{320{,}000{,}000\ \text{persons} \times 0.8 \times 365\ \text{days}} \times 10 = 1510\ \mu\text{g/person/day}$$

Consumption Ratio for Coriander oil consumed from coriander spice versus as added flavoring:

Coriander spice imported for food consumption in 2014[11]: 12,895,400 lbs

Percentage of coriander oil present in coriander spice[12]: 0.6%

Coriander oil consumed in the consumption of the spice: 77,400 lbs (35,100 kg)

$$\frac{\text{Consumption in food}}{\text{Consumption as added flavoring}} = \frac{35{,}100\ \text{kg}}{14{,}100\ \text{kg}} = 2.5$$

The consumption of coriander oil from food (coriander spice) is estimated to be 2.5 times higher than the consumption of the NFC coriander oil as flavoring in food.

[8] Harman, C.L., et al. 2015 Poundage and Technical Effects Survey, 2018, Flavor and Extract Manufacturers Association of the United States (FEMA) Washington DC, USA, *in preparation.*

[9] Source: https://www.census.gov/data/tables/2016/demo/popest/nation-total.html accessed on October 31, 2017

[10] For usage less than 22,700 kg/year, the PCI × 10 calculation is used, which assumes that consumption is distributed among only 10% of the total US population.

[11] Source: Calculated by ERS/USDA based on data from various sources (see https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/food-availability-documentation/). Data last updated Feb. 1, 2017.

[12] Anitescu, G., Doneanu, C., Radulescu, V. (1997) "Isolation of *Coriander* Oil: Comparison Between Steam Distillation and Supercritital CO2 Extraction", *Flavour and Fragrance Journal*, 12, 173 – 176.

congeneric group is higher than that for one or more constituents within a congeneric group. In addition, in the following step, these intake values are compared to the TTC thresholds. The TTC threshold values are based on the 5th percentiles of the NOAEL of each class with an additional 100-fold safety factor, resulting in a highly conservative threshold for each class (Kroes et al., 2000; Munro et al., 1996). In brief, use of the reported mean percentage (%) to calculate the intake for each congeneric group will still result in a conservative safety evaluation.

An additional consideration of intake by young infants and children is considered within Step 13. In cases where intake for a congeneric group is within the range of the TTC value, a further evaluation will be conducted to consider possible exposure to children and infants, given their lower body weights and the potential for differences in toxicokinetics and toxicodynamics as compared to adults.

3.2. Consideration of metabolism within the updated procedure

The progression from Step 3 to Step 4 in the revised procedure differs from the original procedure where if metabolic data could not adequately indicate that the constituents of each congeneric group would be metabolized to innocuous products, the original procedure did not evaluate the congeneric groups against the TTC thresholds. In the revised procedure, the consideration of metabolic data in Step 3 does not preclude application of the TTC concept in Step 5. It is recognized that metabolism is an inherent consideration within the structural class assignments made by the Cramer Decision Tree (Cramer et al., 1978; EFSA/WHO, 2016). For substances for which a metabolic pathway could not be predicted with reasonable confidence, or that would be predicted to metabolize to products of potentially higher toxic concern, additional considerations are made in Step 13 of the procedure. This approach is well-aligned with recent amendments to the evaluation of flavoring substances through the JECFA evaluation procedure (JECFA, 2016).

3.3. Consideration of genotoxicity of the identified constituents in the updated procedure

Although consideration of genotoxicity data for the identified constituents was included in the original procedure, more specific guidance has been added to this revised procedure. In new Steps 4 and 4a if there are concerns about potential genotoxicity for any of the congeneric groups or specific constituents that are present (in Step 4), the material is evaluated specifically for the potential *in vivo* genotoxicity in Step 4a, before continuing to Step 5. All relevant data should be considered in these steps. A weight of evidence approach based on expert judgement is used to conclude whether a potential for genotoxicity exists for any constituent of the NFC, and whether this potential is biologically relevant *in vivo*. If there is an *in vivo* genotoxicity concern in Step 4a.

additional information is required to address the concern before continuing the evaluation. A manuscript on the FEMA Expert Panel's approach to consideration of potential genotoxicity in the evaluation of flavoring ingredients is in preparation.

3.4. Changes in the safety evaluation of unidentified constituents

In Step 9, a new step in the revised procedure, the evaluation considers the possibility of the presence of constituents belonging to the TTC excluded classes among the unidentified constituents. The TTC excluded classes contain high potency carcinogens, as well as certain metals, proteins, steroids, bio-accumulators, nanomaterials and radioactive materials (Kroes et al., 2004). This step is answered based on the identified constituents of the NFC under consideration, source of material and process of preparation (Koster et al., 2011). For example, many NFCs are produced by distillation of the botanical or botanical extract and thus contain only a small percentage of non-volatile constituents. Thus, metals and non-volatiles such as aflatoxins, proteins and steroids are not typically found in this type of NFC. If the possibility of the presence of constituents belonging to the TTC excluded classes among the unidentified constituents is excluded, the evaluation continues to Step 9. If not excluded, the NFC is not appropriate for consideration using the procedure.

Steps 10, 10a and 10b are new steps in the procedure that evaluate the genotoxic potential of the unidentified constituents of the NFC based on its identified constituents. The identified constituents provide information on the relevant biosynthetic pathways active in the botanical from which the NFC is derived. The constituents of an NFC are generally derived from the isoprene pathway, the shikimic acid pathway, the photosynthetic pathway and the lipoxygenase oxidation of lipids, resulting in chemical profiles with predictable structural variation (Schwab et al., 2008) and generally lacking structural alerts for genotoxicity. However, if the identified constituents of an NFC have a biologically relevant structural alert for genotoxicity, further evaluation is conducted in Steps 10a and 10b. If it is determined that there is no concern for genotoxic potential from the unidentified constituents, the evaluation proceeds to Step 11.

Step 10a tests whether the *per capita* intake of the unidentified constituents in the NFC exceeds 0.15 μg/person/day, the TTC threshold previously established for chemicals that would be considered potential genotoxic compounds (Kroes et al., 2004; Rulis, 1989). If the intake of the unidentified constituents is less than 0.15 μg/person/day and thus of negligible concern, the evaluation progresses to Step 13. If the intake is greater than this threshold, the evaluation progresses to Step 10b. In Step 10b, genotoxicity data on the NFC are considered and if negative, provide adequate evidence to exclude concerns of genotoxicity and the evaluation proceeds to Step 11. If negative genotoxicity data are not available for the NFC or for NFCs of similar composition, the evaluation cannot proceed until additional data become available to address the concern.

Steps 11 and 12, which are identical to Steps 9 and 10 in the original procedure, test whether the *per capita* intake of the unidentified constituents in the NFC exceeds 90 μg/person/day, the TTC threshold for Class III substances (Kroes et al., 2000; Munro et al., 1996). As in the original procedure, the group of unidentified constituents, if not of concern for genotoxicity, are treated as a group and assigned the highest toxicity potential, Class III in the Cramer classification scheme. If the intake is below the 90 μg/person/day TTC threshold, the evaluation continues to Step 13. If the intake exceeds this threshold, the evaluation proceeds to Step 12 and the toxicological data are analyzed for the NFC or closely related NFCs with a similar composition. If an adequate margin of safety can be determined in Step 12, the analysis proceeds to Step 13.

The safety evaluation concludes in Steps 13 and 14, which are identical to Steps 11 and 12 in the original guide. In Step 13, any relevant data on the NFC not previously examined are considered. For example, unique metabolic considerations from Step 3, studies on potential interactions of the NFC or its constituents, or constituents that would present a unique potential safety concern, would be considered in this step.

4. Conclusions

An effective and thorough approach for the safety evaluation of NFCs was published in 2005 and has been applied for the evaluation of NFCs for GRAS status by the FEMA Expert Panel. The revised procedure reported here retains the core approaches of organizing constituents into congeneric groups and comparing the intake of those groups relative to established TTC values. It updates the previous procedure by including a more rigorous consideration of the unidentified constituents, by utilizing mean versus maximum percentage when determining intake for each congeneric group, and by further assessing the genotoxic potential of constituents. The revised procedure is being utilized in a multi-year project to conduct safety re-evaluations of more than 250 NFCs that have uses currently considered FEMA GRAS. The scope of the project includes botanically derived essential oils, extracts and oleoresins of various origin and will be covered in future publications. In addition, future NFC evaluations for FEMA GRAS status under conditions of intended use as flavoring ingredients will rely on this revised procedure. Finally, the updated procedure described could be more generally employed in the safety evaluation of complex mixtures.

Declaration of interests

Drs. Cohen, Eisenbrand, Fukushima, Gooderham, Guengerich, Hecht, and Rietjens, are members of the Expert Panel of the Flavor and Extract Manufacturers Association. Authors Davidsen, Harman, and Taylor are employed by Verto Solutions which provides scientific and management support services to FEMA.

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Transparency document

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Appendix A

Congeneric Groups

1	Saturated aliphatic, acyclic, linear primary alcohols, aldehydes, carboxylic acids and related esters
2	Saturated aliphatic, acyclic, branched-chain primary alcohols, aldehydes, carboxylic acids and related esters
3	Aliphatic linear and branched-chain alpha, beta-unsaturated aldehydes and related alcohols acids and esters
4	Aliphatic allyl esters
5	Unsaturated linear and branched-chain aliphatic, non-conjugated aldehydes, related primary alcohols, carboxylic acids and esters

6	Aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups
7	Saturated alicyclic primary alcohols, aldehydes, acids and related esters
8	Saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and related esters
9	Aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroxyketones
10	Alicyclic ketones, secondary alcohols and related esters
11	Pulegone and structurally and metabolically related substances
12	Aliphatic and aromatic tertiary alcohols and related esters
13	Aliphatic, alicyclic, alicyclic-fused and aromatic-fused ring lactones
14	Benzyl derivatives
15	Hydroxy- and alkoxy-substituted benzyl derivatives
16	Cinnamyl alcohol, cinnamaldehyde, cinnamic acid and related esters
17	Phenyl-substituted primary alcohols, aldehydes, carboxylic acids and related esters
18	Phenyl-substituted secondary alcohols, ketones and related esters
19	Aliphatic and aromatic hydrocarbons
20	Phenol derivatives
21	Hydroxyallylbenzenes and hydroxypropenylbenzene derivatives
22	Phenethyl alcohol, phenylacetaldehyde and related acetals and esters
23	Aliphatic and aromatic ethers
24	Furfuryl alcohol, furfural and related substances
25	Furan derivatives
26	Aliphatic and aromatic sulfides and thiols
27	Sulfur-substituted furan derivatives
28	Sulfur-containing heterocyclic and heteroaromatic derivatives
29	Aliphatic acyclic diols, triols and related substances
30	Aliphatic and aromatic amines and related amides
31	Nitrogen containing heterocyclic and heteroaromatic substances
32	Pyrazine derivatives
33	Anthranilate derivatives
34	Amino acids
35	Maltol derivatives
36	Epoxide derivatives

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