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Safety evaluation of natural flavour complexes

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Abstract

Natural flavour complexes (NFCs) are chemical mixtures obtained by applying physical separation methods to botanical sources. Many NFCs are derived from foods. In the present paper, a 12-step procedure for the safety evaluation of NFCs, 'the naturals paradigm', is discussed. This procedure, which is not intended to be viewed as a rigid check list, begins with a description of the chemical composition of the commercial product, followed by a review of the data on the history of dietary use. Next, each constituent of an NFC is assigned to one of 33 congeneric groups of structurally related substances and to one of three classes of toxic potential, each with its own exposure threshold of toxicological concern. The group of substances of unknown structure is placed in the class of greatest toxic potential. In subsequent steps, for each congeneric group the procedure determines the per capita intake, considers metabolic pathways and explores the need and availability of toxicological data. Additional toxicological and analytical data may be required for a comprehensive safety evaluation. The procedure concludes with an evaluation of the NFC in its entirety, also considering combined exposure to congeneric groups. The first experiences with the use of this procedure are very promising. Future safety evaluations of larger numbers of NFCs will indicate the usefulness of the system, either in its present form or in a form modified on the basis of experience.

Keywords: Natural flavour complexes; Mixtures; Safety evaluation

1. Introduction

Natural flavour complexes (NFCs) are mixtures of chemicals obtained by applying physical separation methods to botanical sources such as pulp, bark, peel,

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leaf, bud, berry, and flower of fruits, vegetables, spices and other plants. The processing methods include fractional distillation, topping (removal of volatile parts), solvent extraction, supercritical extraction, thin-film evaporation and molecular distillation. The essential oils obtained represent the aroma part and are distinguished from the 'fixed' or non-volatile oils, usually triacyl glycerides, that have virtually no aroma or flavour value. Redistillation of the essential oils is used to remove colour, water, resinous matter and unpleasant initial aroma and taste perception. Often, different batches of oil from the same botanical species are blended to provide a commercial product exhibiting consistent technical function as a flavouring.

Many of the approximately 300 NFCs currently in use are derived from sources normally consumed as food, e.g. lemon, basil and celery oils. These NFCs are composed predominantly of terpenoid hydrocarbons, esters, aldehydes, alcohols, acids and ketones produced by major biosynthetic pathways in the higher plants (Roe and Field, 1965). The vast majority of NFCs are used in food at extremely low levels (<0.001%) and are present naturally at similar levels in food, facts that should be kept in mind when evaluating the safety of NFCs.

It is widely accepted that standards of safety for naturally occurring substances that have a long history of use in food should differ from those for chemical substances intentionally added to food. NFCs occupy an intermediate position between the major traditional foods, which are themselves sources of many NFCs, and single chemical entities. Because NFCs are considered neither direct food additives nor a food themselves, no current standard can be applied to their safety evaluation. To date no system that allows the safety evaluation of NFCs has been developed by any agency, governmental or non-governmental. The present paper presents a procedure for such an evaluation.

2. The naturals paradigm

2.1. Background and starting points

'The naturals paradigm' is a procedure for the safety evaluation of NFCs. It is intended to be applied only to NFCs derived from higher plants for intended use as flavouring substances. Fermentation products, process flavours, substances derived from fungi, micro-organisms, or animals and direct food additives are explicitly excluded. The procedure should be viewed as a tool that prioritises constituents of NFCs according to their chemical structure and intake. Thus, safety evaluation of the constituents becomes an integral part of the evaluation of the NFC itself.

The procedure does not use conventional criteria for the safety evaluation of individual chemicals. The procedure ultimately focuses on those constituents and congeneric groups which due to their structure and intake might pose some significant health risk from their consumption as a component of an NFC. In these cases, a detailed analysis of relevant scientific information is performed for the constituents and congeneric groups as part of the overall safety evaluation of the NFC. Major elements used by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to evaluate the safety of NFCs include product description (chemical composition including percentage of unknowns), exposure (including history of use), structural analogy (including classes of toxic potential and congeneric groups), and toxicology (including the threshold of toxicological concern and consideration of additivity and synergistic interactions). The use of these elements combined with professional judgement and scientific expertise of the Expert Panel provides a thorough safety evaluation of NFCs (Adams et al., 1996, 1997; Newberne et al., 1998).

2.2. Scientific basis

In this section, the key elements of the procedure are discussed.

2.2.1. Product description

The following six factors can, and often do so extensively influence the composition of an NFC as to result in a wholly distinctive product:

- all recognised commercially used botanical sources;
- all relevant geographical sources and differences in harvesting time;
- all commercially used plant parts;
- all commercially used degrees of maturity;
- all commercially used methods of isolation;
- variability inherent to each method of isolation.

Therefore, for each NFC, it is essential to define these factors in order to ensure that the commercial product conforms to the composition ranges used in the safety evaluation. Furthermore, the expected range of concentrations of each constituent of known structure of the NFC intended for commerce must be provided. These data may be obtained by using analytical data from currently or recently available commercial products derived only from the botanical source named plus carefully reviewed literature data and supplemented as necessary with new analytical data. Finally, data should be provided on impurities and the range of concentrations of the total of all constituents of unknown structure in the NFC intended for commerce; the way these data were obtained and calculated should be discussed. The range of the concentration of each known constituent is presented and the safety evaluation is based upon a consideration of the highest level of each. Similarly, the range of unknowns is presented, and the highest level of the unknown fraction in thoroughly characterised NFC preparations is used for the safety evaluation.

In brief, it is essential to provide a detailed characterisation of the chemical composition of the commercial product to be used as a flavouring agent.

2.2.2. Exposure

Data should be provided on the total exposure to the NFC. If the NFC is derived from a commonly consumed food and is added to food in a manner and at levels comparable to those encountered by consumers of that food, the daily per capita intake of the NFC resulting from consumption of the food itself as well as from consumption of the NFC as an added flavouring ingredient should be calculated. History of food use is particularly important; it determines whether exposure to the NFC occurs predominantly from intake of the parent botanical when it is used as a food, or from the NFC itself when it is added as a flavouring ingredient.

The known constituents of an NFC are assigned to their respective congeneric groups and prioritised according to decreasing concentration. The daily per capita intake of each congeneric group is then calculated from the highest credible concentration of each congeneric group in the NFC and the reported annual volume of use of the NFC. In this manner, intake is determined for the combined constituents of the same congeneric group (i.e. those constituents exhibiting similar metabolic fate and toxicologic potential).

2.2.3. Structural analogy

Each known constituent of an NFC is classified in one of three structural classes (classes I, II and III) reflecting a presumption of low, moderate or serious toxicity, respectively. Each constituent of unknown structure will, as a conservative default assumption, be assigned to the class of highest toxic potential (viz. class III). This classification system was developed and published by Cramer et al. (1978). In brief, a decision tree of 33 questions leads to a final classification in one of three classes. The logic of the tree rests heavily on known data on metabolism and toxicity. Class I substances contain structural features and related data suggesting low order of toxicity. If combined with low human exposure, class I substances should enjoy a very low priority for testing. Class II substances are clearly less innocuous than class I substances but contain neither the structural features nor the indication of toxicity characteristics of class III. Class III substances contain structural features, for example the epoxide functional group or unsubstituted heteroaromatic chemicals that permit no presumption of safety but rather may suggest significant toxicity. Class III substances deserve the highest priority for investigation, particularly when human exposure is high.

In addition to classification in one of the three above described classes of toxic potential, each of the known constituents is also classified into one of some 33 different congeneric groups defined so far (European Commission, 2000). Each congeneric group contains structurally related substances expected, on the basis of established data, to exhibit consistently similar pathways for metabolism and excretion as well as common toxicological endpoints. The next step is to assign to each congeneric group a structural class of toxicity. If constituents of a congeneric group exhibit different classes of toxic potential, the highest class of toxicity of one or more of the constituents is assigned to the entire congeneric group.

2.2.4. Toxicology

'The naturals paradigm' ultimately focuses on those constituents or congeneric groups of constituents which because of structure or concentration might be deemed to pose some significant health risk

from consumption of the NFC in which they occur. A database of relevant toxicological data including a 'no-observed-adverse-effect-level' (NOAEL) may be required for a representative member or members of a congeneric group that would allow for a comprehensive safety evaluation and would provide a sufficient margin of safety for that particular congeneric group. However, in many instances there is no need for such a toxicological database because of structural characteristics and low level of exposure to the congeneric group, namely when the intake of that total congeneric group falls below the 'threshold of toxicological concern' (TTC). The TTC for each of the three classes of toxic potential has been defined and quantified by Munro et al. (1996) and further refined by Kroes et al. (2000). In brief, an extensive toxicity database has been compiled for substances in each structural class, and conservative NOAELs (5th-percentile NOAELs) have been determined for each class. The 5th-percentile NOAELs in each class are then converted to TTCs for each class by applying a 100-fold safety factor and correcting for mean body weight i.e. NOAEL X 60/100. The TTCs for the three classes of toxic potential are 1,800, 540 and 90 μg per person per day for classes I, II and III, respectively.

The TTC concept is also crucial for the safety evaluation of the fraction of constituents of unknown structure in an NFC. As a conservative default assumption, the unknowns are placed in the structural class of greatest toxic potential, i.e. class III, and the total intake of all unknowns is considered and compared to the most conservative TTC, viz. 90 μg per person per day. If the intake of the total of unknown constituents is greater than this threshold, additional analytical data should be produced to reduce the fraction of unknown constituents. In some cases, it may be necessary to perform toxicity studies on the NFC itself. The power of the safety evaluation procedure relies on the premise that no significant part of the NFC should go unevaluated.

Finally, the procedure also considers that the possibility of additivity or synergistic interaction of the individual compounds and the different congeneric groups to generally not be of concern. The level of exposure to the congeneric groups is relevant to the question of the possibility of additive or synergistic effects presenting any significant hazard. The vast majority of NFCs is used in food in extremely low concentrations; obviously, also resulting in very low exposure levels of the different congeneric groups (and of the fraction of unknowns). Moreover, major constituents of NFCs representative of each congeneric group have been tested individually and pose no toxicological threat even at dose levels that are often orders of magnitude greater than those experienced through normal levels of intake of NFCs. Based on the results of toxicity studies on such major constituents of different congeneric groups in the NFC and the NFC itself, it can be concluded that the toxic potential of these major constituents is representative of that of the NFC itself, indicating the likely absence of additivity and synergistic interaction. Therefore, as a rule the margin of safety is so wide and the possibility of additivity or synergistic interaction so remote that combined exposure to the different congeneric groups and the unknowns—as long as they are present at levels at or below their respective TTCs—is considered of no health concern, even if expert judgement cannot fully rule out additivity or synergism. However, case-by-case considerations are appropriate. Where possible combined effects might be considered to have toxicological relevance, additional data may be needed for an adequate safety evaluation of the NFC.

2.3. Guide for the safety evaluation of NFCs

'The naturals paradigm' is to be viewed as a flexible procedure, not a simple checklist to evaluate the safety of novel and traditional NFCs under conditions of use as flavouring agents. As an aid for the safety evaluation process, a 12-step guide has been developed, pursuant to the key elements discussed in the previous section. This system, presented in Fig. 1, is highlighted in this section.

The first three steps deal with history of use (step 1) and classification of constituents into classes of toxic potential (step 2) and into congeneric groups (step 3). In step 4, which addresses the per capita intake of all constituents, the scheme diverges into two routes, one dealing with known constituents (step 4a proceeding with steps 5–7) and the other dealing with constituents of unknown structure (step 4b proceeding with steps 8–10). Steps 5–7 address the metabolism and potential toxicity of congeneric groups while steps 8–10 consider the safety aspects of the group of constituents of unknown structure. Both routes converge again in

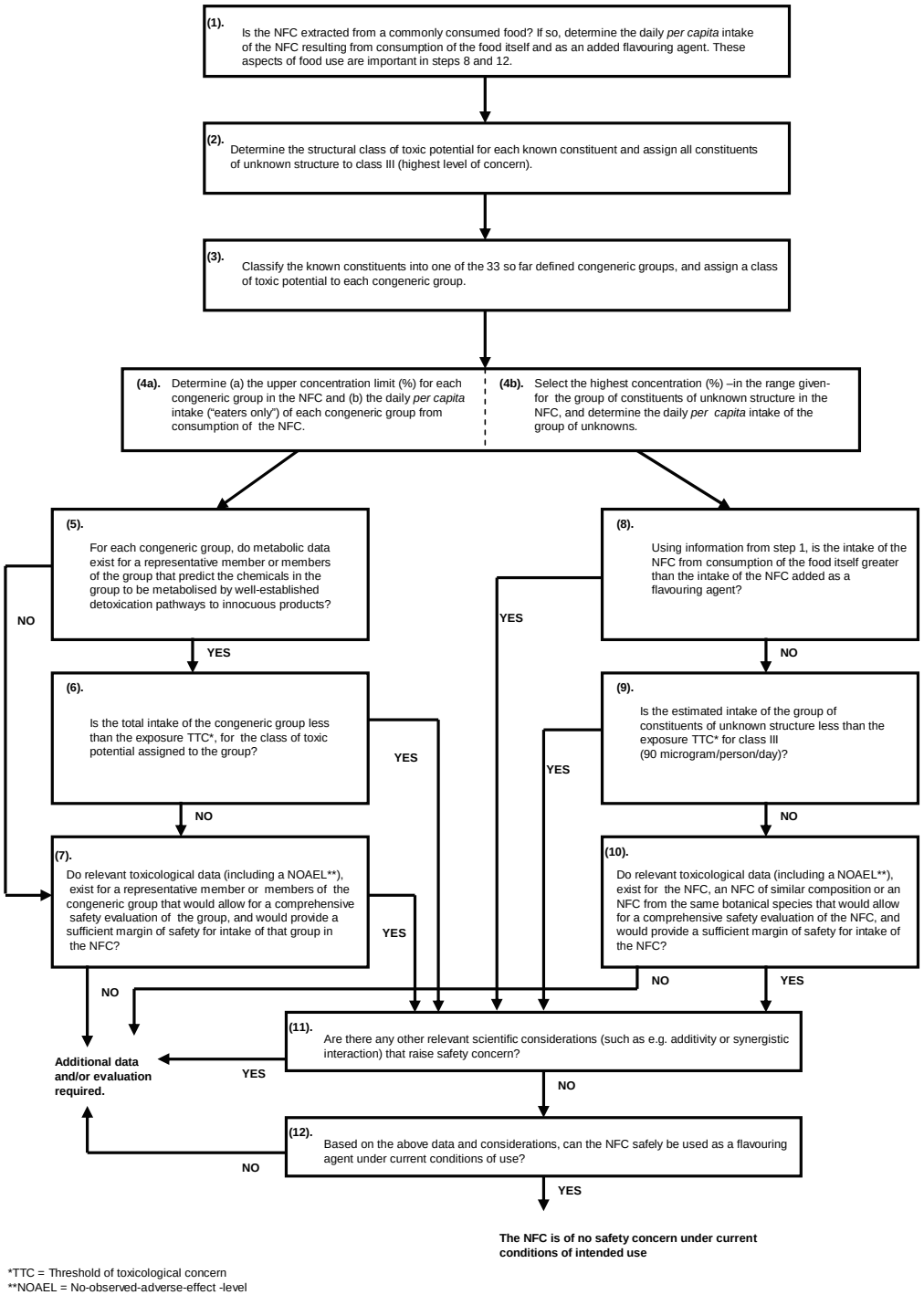

Fig. 1. Guide for the safety evaluation of a natural flavour complex (NFC).

the two concluding final steps (steps 11 and 12) that address the ultimate overall question on the safety of the NFC as a whole.

The safety evaluation procedure begins with a description of the chemical composition of the NFC based largely on data from recent commercial products. These data define the product to be evaluated. The different aspects that should be taken into account in defining the accepted composition ranges are listed above (see Section 2.2.1). Once the NFC has been characterised chemically, the question of whether or not the NFC is derived from a commonly consumed food is addressed (in step 1). If the NFC is extracted from a common food, the daily per capita intake of the NFC resulting from consumption of the food itself and as an added flavouring agent is determined, and the two intakes are mutually compared. If the NFC is not derived from a commonly used food, this should be clearly stated. This information on food use is particularly important in steps 8 and 12.

In step 2, each known constituent is classified into one of the three classes of toxic potential (see Section 2.2.3), and the group of constituents of unknown structure is assigned to class III (highest level of concern).

In step 3, each known constituent is assigned to one of some 33 congeneric groups, and to each congeneric group a class of toxic potential is assigned (see Section 2.2.3).

Step 4 consists of two parts, step 4a and step 4b. In step 4a, a limit concentration is determined for each group of congeneric substances in the NFC. The upper limit represents the highest anticipated concentration of the congeneric group in the NFC intended for commerce. Based on the upper limit concentration for each congeneric group in the NFC, and the reported annual volume of consumption of the NFC, the daily per capita intake (‘eaters only’) is determined for each congeneric group from consumption of the NFC. In step 4b, the highest concentration, in the range given, of the group of constituents of unknown structure in the NFC intended for use as a flavouring substance, is selected. Then, the daily per capita intake of the group of unknowns is determined.

Step 5 addresses the question of metabolic fate of substances in a congeneric group. The key question is, do metabolic data exist for a representative member or members of the group, that indicate, in the context of current estimated levels of intake, that the group is anticipated to be metabolised by well-established detoxication pathways to innocuous products?

In step 6, the question has to be answered whether the total intake of the congeneric group is less than the TTC for the class of toxic potential assigned to the group. It may be stressed that in case the group contains members of different classes of toxicity, the TTC for the class with the highest level of concern should be selected for comparison with the group intake.

Step 7 deals with the question about the availability of relevant and adequate toxicity data (including data on genotoxic potential, metabolism, and a NOAEL). These data should exist for a representative member or members of the congeneric group and should allow for a comprehensive safety evaluation of the congeneric group and should also provide a sufficient margin of safety for intake of the congeneric group from the NFC.

Step 8 is the follow-up step of step 4b and uses data collected in step 1. It deals with the question of whether the intake of the NFC (derived from a food) is significantly greater than the intake of the added NFC. Clearly, when the intake of the group of constituents of unknown structure is significantly greater from consumption of the food itself, the intake of the unknown constituents from the added NFC is considered trivial.

Step 9 deals with the question of whether the intake of the group of unknown constituents is less than the TTC for class III, the class of the highest level of toxicological concern and thus, with the lowest TTC viz. 90 μg per person per day (also see Section 2.2.4).

Step 10 addresses the availability of relevant and adequate toxicological data (including a NOAEL) for the NFC, an NFC of similar composition or from the same botanical species and prepared in the same fashion as the NFC of interest. These data should allow for a comprehensive safety evaluation of the NFC (including the group of unknowns) and should provide a sufficient margin of safety for intake of the NFC.

Step 11 addresses the possibility that there might be relevant scientific considerations that raise a safety concern and that have not been covered in the 10 preceding steps. For example, one such consideration could be the question about additivity or synergistic interaction among congeneric groups (also see Section 2.2.4).

The procedure concludes with step 12 which asks the key question whether, in view of the data and considerations discussed in the 11 preceding steps, the NFC can safely be used as a flavouring agent under current conditions of intended use.

2.4. Safety evaluation of cardamom oil

As an example to illustrate the use of the procedure, the safety evaluation of cardamom oil is described. Data relevant to the safety evaluation of cardamom oil are listed in Tables 1 and 2. The data presented in these tables only constitute a succinct summary of the available data but are considered sufficient to demonstrate the use of this procedure.

The flowchart (Fig. 1) is followed step by step:

Step 1: Is cardamom oil extracted from a commonly consumed food? If so, determine the daily per capita intake of cardamom oil resulting from consumption of the food itself and as an added flavouring agent. These aspects of food use are important in steps 8 and 12.

Yes, cardamom seeds are used as food seasoning.

Data from the American Spice Trade Association on the use of cardamom spice and from the flavour industry on the use of cardamom seed oil indicate that the consumption of cardamom oil from the use of cardamom seeds as a food seasoning is roughly nine times higher than the consumption of cardamom oil as an added food flavour.

Step 2: Determine the structural class of toxic potential for each known constituent and assign all constituents of unknown structure to class III (highest level of concern).

The great majority of constituents is assigned to class I (lowest level of concern). Some constituents such as the major one 1,8-cineole are class II chemicals. By default the group of chemicals of unknown structure is assigned to class III (see Table 2).

Step 3: Classify the known constituents into one of the 33 so far defined congeneric groups, and assign a class of toxic potential to each congeneric group.

The known constituents are classified into four different congeneric groups: alpha, beta-unsaturated aliphatic primary alcohols/aldehydes/acids/acetals/esters (class I, total of 12 constituents); aliphatic, terpene tertiary alcohols and related esters (class I, total of 18 constituents); aliphatic and alicyclic ethers (class II, total of 5 constituents); aliphatic and aromatic hydrocarbons (class I, total of 25 constituents) (see Table 2).

Step 4a: Determine (a) the upper concentration limit (%) for each congeneric group in cardamom oil, and (b) the daily per capita intake ('eaters only') of each congeneric group from consumption of cardamom oil.

The upper concentration limits of the four congeneric groups (as obtained from complete analyses) mentioned in Table 2 are 4.31, 61.1, 31.9 and 11.32%, respectively. The per capita intake ('eaters only') for cardamom oil amounts to 171.4 μg per person per day (see Table 1), leading to a per capita intake of 7.4, 104.7, 54.7 and 19.4 μg per person per day for the four congeneric groups, respectively. For the other congeneric groups, the per capita intake ('eaters only') is less than 90 μg per person per day (highest level of concern) (data not presented).

Proceed to step 5.

Step 4b: Select the highest concentration (%)—in the range given—for the group of constituents of unknown structure in cardamom oil, and determine the daily per capita intake ('eaters only') of the group of unknowns.

The highest concentration of constituents of unknown structure is 1.47% (see Table 2), leading to a daily per capita intake of the group of unknowns ('eaters only') of 3 μg per person per day (see also step 4a).

Proceed to step 8.

Step 5: For each congeneric group, do metabolic data exist for a representative member or members of the group that predict the chemicals in the group to be metabolised by well-established detoxication pathways to innocuous products?

Yes, for each of the four congeneric groups, data exist for a representative member or members of the group that predict the chemical in the group to be metabolised by well-established detoxication pathways to innocuous products.

Table 1
Identity, method of isolation and purification, specifications and use of cardamom oil

I. Identity	
NFC[a] cluster	Cardamom
Common name(s)	Cardamomum Pai Tou K'Ou
Botanical family	Zingiberaceae
Genus and species	*Elettaria cardamomum (Linnaeus) Maton*
Synonyms	*Elettaria cardamomum* var. *minuscula* Burkill, α-*Minor*.
Geographical source	India, Guatemala, Sri Lanka
Description of botanical source	Plants with leaves more than 6 ft tall. The short stalks which bear the white flowers and fruit emerge at the base from the rhizomes.
Degree of maturity	Mature green seeds
FEMA[b] number(s)/FDA[c] citations(s)/CoE no.	2240, 2241/182.20/180
II. Method of isolation and purification	
Plant parts used	Comminuted green seed
Derivatives used (e.g. oil, extract, etc.)	Spice (FEMA 2240), essential oil (FEMA 2241), oleoresin (NAS 6366), extract (NAS 6694)
Yield (%, based on original botanical)	Cardamom oil yield is dependent on seeds enclosed in hulls until immediately prior to distillation. The so-called 'green' cardamom typically yields 4–6% oil. Cardamom oleoresin use has not been reported since 1982 and current industrial information is lacking (see FCC[d] specification).
Method of isolation	The essential oil is produced by steam distillation of the comminuted, mature, green seed.
Solvents used	The oleoresin is produced by extraction of the seed with volatile solvent. Permissible FDA-approved solvents are used, e.g. acetone, chlorinated hydrocarbons, ethyl alcohol, hexane, isopropanol, and methanol.
III. Typical analysis/specifications for cardamom oil	
Appearance	Colourless or pale yellow to light brownish liquid with a warm spicy aromatic odour. The flavour of cardamom oil is rich, aromatic, warm and spicy with a somewhat burning or pungent flavour at high concentrations.
Acid value	6.0 (maximum)
Angular rotation	FCC: +22 to +44 °C; industry: +22 to +41 °C; India:+22 to +41 °C; Guatemala: +24 to +39 °C; Sri Lanka: +22 to +41 °C
Heavy metals	FCC: passes test (as Pb)
Specific gravity	FCC: 0.917–0.947 @25 °C; industry: 0.919–0.936 @20 °C; India: 0.919–0.936 @20 °C; Guatemala: 0.925–0.935 @20 °C; Sri Lanka: 0.919–0.935 @20 °C
Refractive index	FCC: 1.462–1.466 @20 °C; Industry: 1.462–1.468 @20 °C; India: 1.462–1.468 @20 °C; Guatemala: 1.460–1.467 @20 °C; Sri Lanka: 1.462–1.468 @20 °C
Distillation range	Not applicable
Solubility in alcohol	FCC: miscible, 1:2–1:5 in 70% alcohol
Major components assay (if applicable)	Refer to ISOTC54 specifications given below.
Other	Ester value: India: 92–150; Guatemala: 92–150; Sri Lanka: 92–150
IV. Use[f]	
History of use	The use of cardamom spice in medicine dates to the 4th century BC. Large quantities of cardamom are reported to have been imported into Rome in 2nd century AD. Reports of Valerius Cordus, 1540, describes the oil and its distillation is outlined (Arctander, 1960)
Earliest reported use by flavour industry	Cardamom (FEMA 2240): 1880; seed oil (FEMA 2241): 1900
Earliest reported 'general' use by flavour industry	Cardamom (FEMA 2240): 1890; seed oil (FEMA 2241): 1926
1995 annual volume	Cardamom (FEMA 2240): 23042.5; seed oil (FEMA 2241): 1301.0; oleoresin: 0.0; extract: 0.1
Corrected 1995 annual volume	Cardamom (FEMA 2240): 28803; seed oil (FEMA 2241): 1626; oleoresin: 0; extract: 0.1
Per capita consumption (μg per person per day):	Cardamom (FEMA 2240): 3,035; seed oil (FEMA 2241): 17; oleoresin: 0; extract: 0.001

Table 1 (*Continued*)

Eaters only, per capita consumption × 10 (μg per person per day):	Cardamom (2240): 30, 350; seed oil (2241): 171.4; oleoresin: 0; extract: 0.01
Major food categories:	Baked goods, beverages, condiments and relishes, and meat products

ISOTC54 specifications[e] Constituent	India		Guatemala		Sri Lanka	
	Minimum (%)	Maximum (%)	Minimum (%)	Maximum (%)	Minimum (%)	Maximum (%)
1,8-Cineole	23.00	33.00	27.00	35.00	23.00	33.00
Limonene	3.00	7.00	2.00	3.00	3.00	7.00
Linalyl acetate	4.00	9.00	4.00	6.00	4.00	9.00
Myrcene		2.50		2.50		2.50
(E)-Nerolidol	1.00	2.00	0.50	1.00	1.00	2.00
Terpinen-4-ol	1.50	3.00	0.50	0.15	1.00	3.00
Alpha-terpineol	3.00	7.00	1.50	2.50	5.00	9.00
Alpha-terpinyl acetate	32.00	42.00	35.00	44.00	30.00	42.00

[a] NFC: natural flavour complex.

[b] FEMA: Flavor and Extract Manufacturers' Association.

[c] FDA: Food and Drug Administration.

[d] FCC: Food and Chemical Codex.

[e] International Organisation for Standardisation Technical Committee 54 (ISOTC 54) is active in developing specifications for essential oils of commercial importance to the flavour and fragrance industry. Those specifications include relevant physical properties as well as GC information on specific components responsible for type recognition and the satisfactory performance of the respective essential oils. Gaschromatography is carried out on chiral capillary columns (General method, Document ISO/DIS 22972) (ISO TC54).

[f] This section provides both the history of use of cardamom oil, the calculated per capita intake in the U.S. in mg per person per day, and the calculated 'eaters only' intake in the U.S. (per capita intake × 10), and major food categories in which used. Intake (μg per person per day) calculated as follows: [[(annual volume, kg) × (1×10^9 μg/kg)]/[population × survey correction factor × 365 days]], where population (10%, 'eaters only') = 32×10^6 for Europe and 26×10^6 for the USA; where correction factor is 0.8 for the Lucas et al. USA survey representing the assumption that 80% of the annual flavour volume was reported in the poundage surveys (Lucas et al., 1999).

Proceed to step 6.

Step 6: Is the total intake of the congeneric group less than the TTC for the class of toxic potential assigned to the group?

Yes, for each of the four congeneric groups the intake is clearly lower than the TTC for the toxic class assigned to the different groups, viz 1800 μg per person per day for the three class I congeneric groups and 540 μg per person per day for the class II congeneric group (for the intake figures see step 4a).

Proceed to step 11.

Step 7: Do relevant toxicological data (including a NOAEL) exist for a representative member or members of the congeneric group that would allow for a comprehensive safety evaluation of the group, and would provide a sufficient margin of safety for intake of that group in cardamom oil?

Yes, but not required to complete this evaluation at step 6 of the guide.

Step 8: Using information from step 1, is the intake of cardamom oil from consumption of cardamom seeds greater than the intake of cardamom oil added as a flavouring agent?

Yes, cardamom oil is consumed as a food. The consumption ratio is about 9.

Proceed to step 11.

Step 9: Is the estimated intake of the group of constituents of unknown structure less than the TTC for class III, the highest level of concern (90 μg per person per day)?

Yes, the intake of the group of unknowns amounts to 3 μg per person per day.

Proceed to step 11.

Step 10: Do relevant toxicological data (including a NOAEL) exist for cardamom oil, an NFC of similar composition or an NFC from the same botanical species, that would allow for a comprehensive safety evaluation of cardamom oil and would provide a suffi-

Table 2
Constituents of cardamom oil organised by toxicity class and congeneric group[a]

Toxicity class	FEMA no.	CAS no.	Constituent	Composition[b]			
				Target analysis[c]	Complete analysis[d]	Target analysis[e]	Complete analysis[f]
I	2303	141-27-5	Geranial		0.54		0.68
I	2507	106-24-1	Geraniol	0.99	1.22	1.8	2.66
I	2509	105-87-3	Geranyl acetate	0.67	0.52	1.01	0.68
I	Congeneric group represented		α,β-Unsaturated ali primary alc/ald/aci/acetal/est (total of 12 constituents)	1.41	2.89	3.33	4.31
I	2635	78-70-6	Linalool	2.07	3.57	3.3	5.91
I	2636	115-95-7	Linalyl acetate	5.68	5.32	6.1	1.96
I	3045	98-55-5	Alpha-terpineol	2.12	3.5	1.6	2.97
I	3047	80-26-2	Alpha-terpinyl acetate	38.12	43.6	42.4	39.10
I	Congeneric group represented		Aliphatic, terpene 3° alc and related est (total of 18 constituents)	52.11	61.1	55.1	53.66
II	2465	470-82-6	1,8-Cineole	26.43	21.69	32.55	31.8
II	Congeneric group represented		Aliphatic and alicyclic ethers (total of 5 constituents)	26.4	21.95	32.55	31.9
I	2633	138-86-3	Limonene	2.41	1.85	1.5	0.22
I	2762	123-35-3	Myrcene	3.0	1.55	1.6	2.21
I	2903	80-56-8	Alpha-pinene	1.78	1.71	1.45	1.51
I		3387-41-5	Sabinene	5.44	4.15	3.78	3.55
I	Congeneric group represented		Aliphatic and aromatic hydrocarbons (total of 25 constituents)	13.3	11.32	10.55	10.81
			Total	93.5	98.53	99.8	100.56
III			Total constituents of unknown structure		1.47		0

[a] Only four of the 31 analyses available are listed here.
[b] The composition data cited above is from industry sources and published literature data.
[c] Industry Target Analyses: routine quality control analysis for key constituents responsible for technical flavour function. Private communication to FEMA, Washington D.C. from industry (1999–2002).
[d] Industry Complete Analyses: complete analysis for all constituents in cardamom oil intended for commerce. Private communication to FEMA, Washington D.C. from industry (1999–2002).
[e] Literature Target Analyses: published limited analysis for constituents in cardamom oil based on the objective (Bernhard et al., 1971).
[f] Literature Complete Analyses: published complete analysis for constituents in cardamom oil (Chou, 1974).

cient margin of safety for intake of cardamom oil?

No, but not required at step 9 of the guide.

Step 11: Are there any other relevant scientific considerations (e.g. additivity or synergistic interaction) that raise safety concern?

No.

Proceed to step 12.

Step 12: Based on the above data and considerations, can cardamom oil safely be used as a flavouring agent under current conditions of use?

Yes, cardamom oil is of no safety concern under current conditions of intended use.

3. Concluding remarks

The usefulness of a method for the safety evaluation of a complex chemical mixture (such as for in-

stance an NFC) depends on factors such as the nature of the mixture (e.g. novel food product, pollution at waste sites, occupational atmosphere), availability of the mixture for testing in its entirety (e.g. readily available or virtually unavailable), and also on the amount, type and quality of the available data on the chemistry and toxicity of the mixture (Feron et al., 1998; Groten et al., 2001; Feron and Groten, 2002; HCN, 2002). A common characteristic of NFCs is that, in the vast majority of cases, they are derived directly from commonly consumed foods, and thus, human exposure through the food itself is often much greater than that through the NFC added to food as flavouring material. Since the use-levels of many NFCs are rather low, there is inadequate economical base to support extensive standard toxicity testing. Also, because these naturals are mixtures, it is difficult to identify a standard constituent for testing. This results in a paucity of data regarding the safety of the NFC per se. Against this background, 'the naturals paradigm' was developed.

As is apparent from this paper, the procedure focuses on the identification of those constituents that may constitute a health concern. In fact, it is a 'bottom up' approach starting with chemical analysis and characterisation of the NFC followed by safety evaluation of the constituents classified according to congeneric groups and toxic potential, using the human TTC that has been defined for each of the three classes of toxic potential. The procedure concludes with an overall evaluation considering the safety of the NFC in its entirety. With the developed strategy, the overall objective of the system can be attained: that no reasonably significant health risk associated with the intake of an NFC goes unevaluated.

The first experiences of the FEMA Expert Panel with the use of the system are very promising, but they are still too few to warrant final conclusions about the usefulness of this new procedure for the safety evaluation of NFCs. Using the new procedure, the Panel will evaluate many types of NFCs in the years to come, implying that the near future will tell us about the applicability and adequacy of the procedure, either in its present form or in a form modified on the basis of experience.

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