REVIEW

Safety assessment of botanicals and botanical preparations used as ingredients in food supplements: Testing an European Food Safety Authority-tiered approach

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This article describes results obtained by testing the European Food Safety Authority-tiered guidance approach for safety assessment of botanicals and botanical preparations intended for use in food supplements. Main conclusions emerging are as follows. (i) Botanical ingredients must be identified by their scientific (binomial) name, in most cases down to the subspecies level or lower. (ii) Adequate characterization and description of the botanical parts and preparation methodology used is needed. Safety of a botanical ingredient cannot be assumed only relying on the long-term safe use of other preparations of the same botanical. (iii) Because of possible adulterations, misclassifications, replacements or falsifications, and restorations, establishment of adequate quality control is necessary. (iv) The strength of the evidence underlying concerns over a botanical ingredient should be included in the safety assessment. (v) The matrix effect should be taken into account in the safety assessment on a case-by-case basis. (vi) Adequate data and methods for appropriate exposure assessment are often missing. (vii) Safety regulations concerning toxic contaminants have to be complied with. The application of the guidance approach can result in the conclusion that safety can be presumed, that the botanical ingredient is of safety concern, or that further data are needed to assess safety.

Keywords:
Botanicals / Botanical preparations / Guidance / Safety assessment

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Abbreviations: ADI, acceptable daily intake; **BD,** benchmark dose; **BMDL,** lower confidence bound of the benchmark dose; **EGCG,** epigallocatechingallate; **EFSA,** European Food Safety Authority; **ESCO,** EFSA Scientific Cooperation; **GMO,** genetically modified organisms; **MOE,** margin of exposure; **PAHs,** polycyclic aromatic hydrocarbons; ***p*-synephrine,** *para*-synephrine

1 Introduction

In June 2004, the Scientific Committee of the European Food Safety Authority (EFSA) adopted a discussion article on botanicals and botanical preparations widely used as food supplements and related products [1] (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902585418.htm). First, it was noted that the expanding market volume raises the need for a better characterization of botanicals and botanical preparations, and for harmonization of the scientific assessment of risks from exposure of consumers to these products. In addition, EFSA launched, *via* its Advisory Forum, a

Table 1. Overview of the selected cases tested using the guidance document and the possible safety issues expected to be linked with these examples

Botanical	Preparation	Possible safety issue
Triticum aestivum L.	Wheat bran	Low concern–presumption of safety
Citrus aurantium L. ssp. *aurantium* L.	Hydroalcoholic extract of dried peel	Misidentification/adulteration
Camellia sinensis (L.) O. Kuntze	Dried green tea extract	Liver toxicity
Foeniculum vulgare Mill. ssp. *vulgare* var. *vulgare*	Seeds and oil from the seeds	Carcinogenicity
Ocinum tenuiflorum L.	Dry leaves extract	Reproductive toxicity
Linum usitatissimum L.	Dried ripe seeds	Phytoestrogenic activity

questionnaire to the national food safety authorities of the European countries to get a clearer picture of the extent of the issue in Europe. After responses to the questionnaire were received, work was undertaken to (i) analyse the information provided by 25 European countries in response to the questionnaire, (ii) prepare a guidance document on how to assess the safety of botanicals and botanical preparations intended for use in food supplements, and (iii) establish a list of main categories of botanicals and used parts thereof (compendium) in order to prioritize the botanical preparations to be considered for a safety assessment. The draft safety guidance document and compendia thus prepared were revised following a public consultation, and the updated draft guidance document was published on the EFSA web site (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178669754855.htm). After the draft guidance document was published, EFSA concluded that it was necessary to test the proposed approach for the safety assessment of botanicals and botanical preparations to be used as ingredients in food supplements with a selected number of cases and to further update the compendia. To this end, on April 15, 2008, an EFSA Scientific Cooperation (ESCO) Working Group, composed of experts identified by EFSA and by the European Member States, was established, in order to (i) enlarge the information basis underlying the compendium of botanicals reported to contain toxic, addictive, psychotropic, or other substances of concern; (ii) test the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected number of botanicals as real case examples; and (iii) provide a report summarizing the outcome of the case studies as well as to advise on the adequacy of the proposed approach for the safety assessment of botanicals and botanical preparations.

This article aims at providing an overview of the outcome of the second above-mentioned task of this ESCO working group and describes especially the major issues emerging when testing the proposed tiered approach through a selected number of cases. Table 1 summarizes the case studies selected and presents an overview of the possible safety issues expected to be linked with these real case examples.

2 Materials and methods

Safety evaluation of the selected botanicals was performed using the proposed draft guidance document published on the EFSA web site (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812-1211902880131.htm) [2]. This guidance document indicates that data underlying a safety assessment of a botanical or botanical preparation should include technical data on the identity and nature of the source material, the manufacturing process, the chemical composition, specifications, stability of the botanical (preparation) used as ingredient in food supplements, proposed uses and use levels, information on existing assessments, exposure data including anticipated exposure and cumulative exposure, modality of use, as well as information on historical use and toxicological data. Data used when testing the guidance document for the safety evaluation of the selected botanicals were collected from the open literature. The work aimed at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with selected cases considering relevant constituents of concern within the botanical or botanical preparation. The evaluation was not aiming at providing a formal safety assessment of the botanical or its preparations, since each example focused on one type of preparation only. Once the outcome of this testing exercise has been considered for updating the draft guidance document for the safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements, EFSA intends to publish the reports summarizing the outcome of the case studies, together with the updated guidance document and the compendium on its website.

The conclusions and recommendations of this article reflect those of its authors as individual scientists and not necessarily represent the views of EFSA.

3 Results

3.1 *Triticum aestivum* L. (wheat bran)

Triticum aestivum L. (wheat bran) was chosen as an example of a botanical of low safety concern. Wheat bran is a by-product obtained in the manufacture of wheat flour from the grain. It consists mainly of the outer layers of the wheat kernel, including the aleuron layer, *i.e.* the husk, seed, coat, and germ. This example already reveals that when evaluating botanicals and botanical preparations, it is essential, though not always easy, to adequately define and characterize the actual preparation being evaluated. Some

selected wheat cultivars and varieties as well as genetically engineered wheat varieties exist [3]. The latter would fall under specific already existing European regulatory framework [4, 5]. Furthermore, selection of better tolerated wheat varieties by patients affected by gluten-induced entheropathy (celiac disease) has been described [6]. This illustrates that the botanical needs to be identified by its scientific name (binomial name, *i.e.*, genus, species, subspecies, author), including the part of the plant used. Wheat bran may be obtained, in addition to hexaploid wheats, also from tetraploid wheats, namely, *T. turgidum* L. ssp. durum (Desf.) Husn. (= syn. *T. durum* Desf., English common names: Durum wheat, hard wheat), which further supports the need to adequately define the botanical evaluated by its full scientific name.

Furthermore, there may be changes induced in bran composition by wheat-growing conditions including high-temperature stress and solar radiation [7, 8], and the composition of bran can vary depending on the milling process as well. Information on the milling process and the resulting size of the bran particles is of interest since these factors affect the biological properties, as illustrated by a particle size-dependency of the laxative effect and colonic fermentation [9]. Therefore, not only the scientific name but also the information on the manufacturing procedure and chemical specifications of the botanical or botanical preparation are essential to adequately define the preparation to be evaluated.

Another important issue emerging when evaluating this first example was the possible presence of contaminants in botanical preparations. Wheat bran must conform to the provisions of food regulations (Council Regulation 315/93/EEC) [10] (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = CONSLEG:1993R0315:20031120:EN:PDF), especially in terms of mycotoxins [11] arising from external fungal contamination (*Fusarium* spp.) [12], microbiology, and pesticides. For instance, a maximum level for the trichothecene mycotoxin deoxynivalenol of 500 μg/kg in cereal products was proposed by the Codex Alimentarius [13], and the maximum level for sclerotia of *Claviceps purpurea* is set at 0.05% m/m for wheat [14] (www.codexalimentarius.net/download/standards/62/CXS_199e.pdf). Fungal contamination and mycotoxin production cannot be totally eliminated at present [15]. In particular, mycotoxin contamination from *Fusarium* spp. is the result of a minor infection of grains and their envelopes by the fungi that may be transferred during the milling process [16–21].

Whereas for wheat bran, the situation with respect to these contaminants may be well recognized and is even regulated at some extent, this may not hold true for other botanicals and botanical preparations.

3.2 *Citrus aurantium* L. ssp. *aurantium* L. (bitter orange)

Citrus aurantium L. ssp. *aurantium* L. (bitter orange) is an example of a botanical, for which it is necessary to define the botanical or botanical preparation down to the subspecies level or even lower, given that different subspecies may vary in the constituents and the level of substances of concern. *C. aurantium* L. ssp. *aurantium* L. (bitter orange) as compared with *C. aurantium* L. ssp. *bergamia* (Risso & Poit.) Engl. (bergamot orange) produces different fruits that contain different levels of biologically active principles such as furanocoumarins and *para*-synephrine (*p*-synephrine) [22, 23].

The case of *C. aurantium* L. ssp. *aurantium* L. was chosen since it represents the issue of misidentification and/or adulteration which are matters for considerable concern with respect to the safety of botanical and botanical preparations.

Exposure to bitter orange peel and its constituents occurs primarily *via* ingestion of the fruit itself or its products (*e.g.*, orange juice, marmalade, and dietary supplements). Bitter orange peel is added to various foods (*e.g.*, beer, liquors and other beverages, and cakes). Moreover, bitter orange juice may be added in limited amounts to sweet orange juice. Exposure can also result from peel oil used in aromatherapy and flavouring. Several evaluations of *C. aurantium* L. ssp. *aurantium* L. have concluded that there is no safety concern related to the regular food use of bitter orange [24, 25]. However, more recent evaluations concerning preparations containing high amounts of the sympathomimetic alkaloid *p*-synephrine concluded that there may be a possible safety concern [26, 27]. Bitter orange extracts used in food supplements, such as weight-loss pills, are possibly enriched in *p*-synephrine, typically to an amount of 6–10% (but even extracts with a content of 95% *p*-synephrine are documented) [23, 28–32] (http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Bitterorange.pdf, http://content.balgram/articleview.asp?a = 2833&p = Y). Thus, extracts used in many dietary supplements and herbal weight-loss formulas as an alternative to *Ephedra* have concentrations of *p*-synephrine that are often much higher than the *p*-synephrine concentrations reported for traditional extracts of the dried fruit or peel. This reflects another important issue to be taken into account when assessing the safety of botanical ingredients, *i.e.*, that some preparations of a botanical may be marketed containing significantly higher levels of active (toxic) principles than those normally occurring in historical food uses of the same botanical.

Furthermore, the position isomer of synephrine found in bitter orange peel is *p*-synephrine, not *m*-synephrine. *m*-Synephrine and neo-synephrine are relatively rare synonyms of the compound named phenylephrine in the International Nonproprietary Name list of the WHO. Phenylephrine is used as a decongestant synthetic drug [33]. At least one product purportedly containing synephrine alkaloids from *C. aurantium* has been reported to contain both *p*-synephrine and *m*-synephrine [34, 35]. There is no evidence that octopamine or other phenethylamine alkaloids are present in bitter orange peel in any appreciable levels, although their increased content has been reported in some

extracts and herbal products on the market [29–32]. The presence of any amounts of *m*-synephrine, higher amounts of the (+)-*p*-synephrine stereoisomer, or higher amounts of octopamine in food supplements supposedly containing only extracts or alkaloid fractions of *C. aurantium* L. *ssp. aurantium* L. should be considered undesirable and suspicious of adulteration. The origin of these compounds is unlikely the natural botanical source (*C. aurantium* L. ssp. *aurantium* L.), thus strongly suggesting a requirement for a more efficient quality control.

3.3 *Camellia sinensis* (L.) O. Kuntze (green tea)

Dried green tea extracts prepared from young leaves and leaf buds from *Camellia sinensis* (L.) O. Kuntze are used as food, including beverages and food supplements, and as pharmaceuticals. Uses as a food include a stimulant drink in the form of ready-to-drink beverages or of beverages prepared by the consumer from instant green tea powder. Although the worldwide long-term consumption of traditional green tea infusions is assumed to be safe, a weight-loss product containing a high-dosed hydroalcoholic extract of green tea was marketed only until April 2003, when the French and Spanish authorities suspended the market authorization given its hepatotoxic side effects [36, 37]. Some data point at epigallocatechingallate (EGCG) as the ingredient of concern in relation to the hepatotoxicity of green tea extracts but this relationship is not firmly established. In green tea, EGCG is a major constituent in terms of quantity and a constituent useful to characterize the quality of the preparation, besides caffeine, theanine, and other catechins [38, 39]. Thus, the case of *C. sinensis* revealed that when evaluating the safety of a botanical or botanical preparation, it can be difficult to identify the constituent or group of constituents of concern. In other cases, it may be difficult to identify the active principle responsible for an effect, and therefore, it is concluded that the strength of the evidence underlying the concerns over a compound being reason for concern should be given in a safety assessment of the respective botanical.

Furthermore, the case of green tea reflects that different preparations from the same botanical source material can have a different outcome in the safety evaluation, especially since use of the different preparations may result in difference in composition and consequently in consumer exposure. Thus, regular intake of dried green tea extracts with food supplements or related products differs from the intake resulting from use of traditional green tea infusions (or beverages with identical composition). Dried aqueous green tea extracts, which are manufactured under the same extraction conditions as applied in the traditional preparation of green tea infusions and which are used to prepare solid or liquid food supplements, may be evaluated based on their EGCG content and the daily exposure resulting from their proposed uses and use levels. In food supplements and related products, the active green tea ingredients and particularly EGCG, which is associated with hepatotoxic concern, are available in a more concentrated form making higher dosage and bolus administration more likely than with the aforementioned beverages. Cases of liver disorders associated with intake of products containing dried aqueous green tea extract [37, 40, 41] have to be taken into consideration.

Furthermore, the green tea example indicates that the matrix effect should be taken into account. When given in a green tea extract to rats, EGCG appears to be eliminated less readily from the body [42] and to have a higher toxicity than when given as a pure compound [43]. In addition, studies in healthy volunteers point at a reduced bioavailability of EGCG in the presence of a food matrix, showing that administration of concentrated green tea extracts under fasting conditions lead to a significant increase of plasma concentrations of EGCG compared with administration with food [44]. Thus, the example of *C. sinensis* reflects the importance of the matrix effect that should be taken into account in the safety assessment of botanicals and botanical preparations. The use of dried green tea extracts in beverages or food supplements for weight reduction purposes under fasting conditions or reduced food intake might require adequate safety data accounting for the increased bioavailability in the absence of the food matrix effect. This applies as well to products containing dried green tea extracts as a part of the ingredient.

3.4 *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare*

Foeniculum vulgare Mill. ssp. *vulgare* var. *vulgare* (bitter fennel) was selected as one of the real cases to be evaluated given that it contains estragole, an ingredient that in animal experiments and at certain concentrations showed both genotoxic and carcinogenic activity. Fruits from *F. vulgare* Mill. ssp. *vulgare* var. *vulgare* contain 2–6% essential oil [45, 46]. The major constituent of the essential oil is *trans*-anethole at levels between 50 and 75% and estragole at levels amounting to 3.5–12% [46] (http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/Flavouring_substances/Active%20principles.pdf). Estragole is an alkenylbenzene that is of safety concern given its reported carcinogenic effect at high dose levels [47]. In the safety assessment of botanicals and botanical ingredients, a major issue is the question of how to deal with botanicals and botanical ingredients that contain chemicals that are both genotoxic and carcinogenic. The EFSA draft guidance document [2] states that in cases where the botanical ingredient contains substances that are both genotoxic and carcinogenic, the ''margin of exposure'' (MOE) approach [48] (http://www.efsa.europa.eu/en/science/sc_commitee/sc_opinions/1201.html) could be applied covering the botanical(s) under examination and any other dietary sources of exposure. The MOE approach compares animal toxic effect levels with human exposure

levels. The guidance document states that alternatively, it could be evaluated whether the expected exposure to the genotoxic and carcinogenic ingredient will not be significantly increased, compared with the intake from multiple sources. This implies that further data are required with respect to the assessment of the risk posed by the estragole levels present in bitter fennel fruits and their extracts including an estimate of the MOE. The MOE approach uses a reference point, usually taken from data from an animal experiment that represents a dose causing a low but measurable cancer response denoted the benchmark response. It can be, for example, the lower confidence bound of the benchmark dose ($BMDL_{10}$) that gives 10% (extra) cancer incidence (BMD_{10}). The MOE is defined as the ratio between the $BMDL_{10}$ and the estimated dietary intake in humans. To date, carcinogenicity data for estragole from which a $BMDL_{10}$, and thus an MOE, can be derived result from a long-term carcinogenicity study conducted in mice [49]. An accompanying article of the present special issue reports a BMD analysis of these data using BMDS version 1.4.1c software resulting in a $BMDL_{10}$ value for estragole that varies between 9 and 33 mg/kg bw and day [50]. This value can be compared to, for example, estimated intake levels resulting from the use of bitter fennel fruits for the preparation of fennel tea. The exposure to estragole from bitter fennel fruits can be estimated based on the assumption that 4.5–7.5 g (three times 1.5–2.5 g) of fennel fruits *per* day would be used for the preparation of fennel tea. Assuming that fruits contain 5% essential oil, that the extraction efficiency of the essential oil is 25–35%, and that there is 3.5–12% estragole in the oil, this would imply an intake of 1.9–15.8 mg estragole *per* day. This amounts to an estragole exposure from tea consumption that amounts to 33–263 μg/kg bw and day for a 60 kg person. Using the $BMDL_{10}$ values of 9–33 mg/kg bw and day for female mice as derived from the Miller *et al.* study [49, 50] one can calculate an MOE in the range of 34–1000 which indicates that use of bitter fennel fruits for the preparation of fennel tea could be considered a high priority for risk managers [48].

In addition, the example of bitter fennel reflects the possibility to use, in cases where the botanical ingredient of concern is not genotoxic and carcinogenic, the acceptable daily intake (ADI) for the safety assessment. The safety of the intake of *trans*-anethole from use of bitter fennel fruits can be judged using the temporary ADI of 0–2.0 mg/kg bw and day for *trans*-anethole derived by Joint FAO/WHO Expert Committee on Food Additives [51] (http://www.inchem.org/documents/jecfa/jeceval/jec_137.htm). The exposure to *trans*-anethole from bitter fennel fruits can be estimated based on the assumption that 4.5–7.5 g (three times 1.5–2.5 g) of fennel fruits *per* day would be used for the preparation of fennel tea. Assuming that fruits contain 5% essential oil, that the extraction efficiency of the essential oil is 25–35%, and that there is 50–75% *trans*-anethole in the oil, this would imply an intake of 28–98 mg *trans*-anethole *per* day. For a 60 kg person, this amounts to an intake of 0.5–1.6 mg *trans*-anethole/kg bw and day. This is below the above-mentioned ADI established by the Joint FAO/WHO Expert Committee on Food Additives. However, as the exposure to *trans*-anethole resulting from the use of bitter fennel fruits for the preparation of fennel tea already amounts to 25–80% of the ADI, a possibility exists for exceeding the ADI due to other sources of *trans*-anethole.

The case of bitter fennel further highlights the uncertainties associated with the kinetics as well as the expression of the inherent toxicity of a naturally occurring substance, *i.e.*, estragole, possibly related to effects induced by the matrix. The question may be raised, whether studies with pure compounds dosed by gavage without the normal food matrix being present represent a good starting point for the risk assessment of botanical ingredients. An illustrative example can be given for sweet basil that contains high amounts of estragole in the essential oil. Recently, Jeurissen *et al.* [52] demonstrated that the level of DNA binding of the proximate carcinogenic metabolite 1′-hydroxyestragole to DNA *in vitro* but also to DNA in intact HepG2 human hepatoma cells could be inhibited by a methanolic basil extract. It was demonstrated that the inhibition by the basil extract occurs at the level of the sulfotransferase-mediated bioactivation of 1′-hydroxyestragole to 1′-sulfoxyestragole [52]. Although it remains to be established whether a similar inhibition will occur *in vivo*, the inhibition of sulfotransferase-mediated bioactivation of 1′-hydroxyestragole by basil ingredients suggests that the possibilities for bioactivation and subsequent adverse effects may be lowered when estragole is dosed in a matrix of other basil ingredients than what would be expected on the basis of experiments dosing estragole as a single compound. Where a matrix effect is advocated to support the safety of specific levels of compounds (*e.g.*, that data from a pure compound may overestimate effects of the compound in the botanical matrix), testing and/or other data should be provided to demonstrate the occurrence of the matrix effect of the preparation and its magnitude. It is important to realize that when a matrix effect is demonstrated for an essential oil, this matrix effect will not be similar for the intact botanical. Thus, the example of bitter fennel containing estragole supports that the research on individual substance–matrix interactions cannot be used to draw general conclusions about intact botanicals, herbs, and spices under all conditions of use, ingestion, and metabolism and that the matrix effect should be judged on a case-by-case basis.

3.5 *Ocimum tenuiflorum* L. (holy basil)

Ocimum tenuiflorum L. (holy basil) was included in the evaluations representing an example of a botanical that may be of concern given its possible reproduction toxicity. There is, however, no information on actual constituents likely responsible for this effect. Only a few scientists attempted to look into the various changes in the reproductive system in

detail after feeding *O. tenuiflorum* L. leaf extract and there is considerable debate regarding the histopathological changes in reproductive organs following the feeding of *O. tenuiflorum* L. leaves [53–55].

In addition to the concerns over possible reproductive toxicity which need further testing, *O. tenuiflorum* L. contains methyleugenol, an alkenylbenzene known to be both genotoxic and carcinogenic [56] (http://ec.europa.eu/food/fs/sc/scf/out102_en.pdf). An *O. tenuiflorum* L. leaf extract may contain up to 86% methyleugenol [57–59]. Further details on an MOE assessment for methyleugenol, in line with what was done for estragole in the real case example on *F. vulgare* Mill. ssp. *vulgare* var. *vulgare*, can be found in the literature [60].

Finally, the case of *O. tenuiflorum* L. indicates once more the importance of defining the correct scientific name of a botanical to be evaluated. *O. tenuiflorum* L is the correct scientific name, but most publications still make use of the synonym *O. sanctum*.

3.6 *Linum usitatissimum* L. (flaxseed)

Flax is known to be the richest food source of plant lignans including secoisolariciresinol diglucoside. This plant lignan is a precursor of the mammalian lignans, enterodiol, and enterolactone and converted into these forms *via* the activity of colonic facultative aerobes [61]. Other lignans such as matairesinol and lariciresinol are also found in flaxseed. A 100 g dry flaxseeds contain about 300 mg lignans, including pinoresinol (~870 μg), syringaresinol (~48 μg), lariciresinol (~1780 μg), secoisolariciresinol (~165 mg), matairesinol (~529 μg), and hydroxymatairesinol (~35 μg), all expressed as aglycons [62, 63]. Phytoestrogens represent a family of plant compounds that have been shown to have both estrogenic and antiestrogenic properties. Lignans, similarly to isoflavonoids and coumestans, are often referred to as phytoestrogens, and may possess estrogen receptor agonistic or antagonistic properties, with unclear effects on hormone-sensitive cancers such as breast, uterine, and prostate cancer. Pharmacodynamic studies suggest that there might be an estrogenic or antiestrogenic effect of flaxseed [64]. Some authors therefore call mammalian lignans modulators of endogenous sex steroid hormones. Since 1981, when mammalian lignans were identified in human urine, evidence supporting their role as modulators of endogenous sex steroid hormones has increased. However, the most convincing results have come from *in vitro*, animal, and epidemiological studies, whereas results of the few intervention studies that have been conducted have been equivocal [65–69]. Therefore, further research, including in particular long-term intervention trials, is needed to provide clarification for this relationship [70, 71]. Thus, the case of flax seed demonstrates that even when the compounds of concern are clearly identified, the actual evidence for the effects may be controversial and requires further testing. So in this case, it is clear that the strength of the evidence underlying the concerns over a botanical ingredient should be included in the safety assessment and that an evaluation based on the available knowledge can result in the conclusion that further data are requested.

4 Discussion

Testing the EFSA draft guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements [2], through its application to several selected real cases, has revealed many specific issues to be taken into account in the safety evaluation of botanicals and botanical preparations intended to be used as ingredients in food supplements and has led to a set of suggested amendments of the guidance document, in addition to its validation.

The scheme proposed for safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements other than novel foods and Genetically Modified Organisms (GMO) (for which specific sectoral regulations exist) has been amended as shown in Fig. 1. The safety assessment approach is a tiered approach starting with the evaluation on available knowledge (level A) in compliance with the criteria described in the EFSA guidance document as amended on the basis of the results of the tests described in this article. A level A assessment can result in the conclusion that safety can be presumed based on available knowledge (like for *T. aestivum* bran or some of the *C. sinensis* extracts), but it could also lead to the conclusion that the ingredient is of safety concern. If needed, the assessment should continue with further experimental studies, following guidance provided in the EFSA docu-

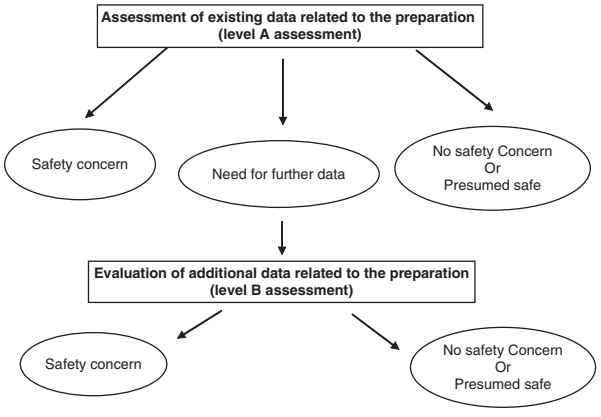

Figure 1. Scheme proposed for the safety assessment of botanicals and botanical preparations not regulated in the framework of specific regulations such as those on novel foods and GMOs. The safety assessment could include a tiered approach starting with a safety assessment based on available knowledge (level A) and if needed continuing with further testing to obtain additional data required (level B).

ment, to obtain additional data required to reach a conclusion on safety (level B). The level B assessment may result in the conclusion that either the product is of safety concern or that the botanical or botanical preparation is not of safety concern.

This guidance document is of importance to harmonize such an approach across Europe: In fact, in spite of the extensive harmonization that occurred through the EU Food Law, the safety assessment of food supplements based on botanicals and botanical preparations has remained a competence of each EU Member State.

The outcomes of the safety evaluations of the selected cases using the EFSA guidance document has been published on the EFSA web site as annexes of the advice of the ESCO Working Group on the adequacy of the proposed EFSA approach for the safety assessment of botanicals and botanical preparations (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902876819.htm). The most important issues emerging when performing the safety assessment on the selected botanicals are summarized in Table 2.

The botanical ingredient needs to be identified by its scientific name (binomial name, *i.e.*, genus, species, subspecies, author) and the part of the plant used. In most cases, it will be necessary to define the botanical down to the subspecies level or even lower given that different subspecies or varieties mostly vary in the constituents and the level of toxic principles. Examples are *F. vulgare* Mill ssp. *vulgare* var. *dulce* (sweet fennel) versus var. *vulgare* (bitter fennel) with the essential oil of the former containing about ten times lower levels of estragole than the latter, and *C. aurantium* L. ssp. *aurantium* L. (bitter orange) versus *C. aurantium* L. spp. *bergamia* (Risso & Poit.) Engl. (bergamot orange) producing fruits that contain different levels of active principles such as furanocoumarins and *p*-synephrine. In other cases, however, it is possible to evaluate a variety of subspecies on the basis of one representative species. For example, this would be the case for rose hips, the spurious fruits of dog rose (*Rosa canina* L.), alpine rose (*R. pendulina* L.), and other *Rosa* species, most commonly *R. rugosa* Thumb; the ripe hips of the different species are collected in late autumn and differ only slightly in their form as well as in the content of their main active constituent, ascorbic acid.

Many different preparations can be obtained from the chosen parts of a specific botanical, depending on a number of factors including, for example, the solvents used and the extraction process. The example of green tea preparations from *C. sinensis* demonstrates that while the consumption of traditional infusions is assumed to be safe, toxicological concerns have been associated with certain extracts intended for weight loss purposes. The composition of a botanical may vary significantly due to other factors that cannot be easily controlled; for example, concentrations of active ingredients measured in the plant material may show significant variation with geographical origin, plant maturity at harvest, harvesting techniques, storage conditions, processing (*e.g.*, drying), and method of detection. Therefore, adequate description is needed, not only of the botanical subspecies or variety evaluated, but also of the harvesting and manufacturing process. It is concluded that safety of a botanical ingredient cannot be assumed only relying on the long-term safe use of different preparations of the same botanical source but also it is necessary to rely on well-characterized preparation(s). Each safety evaluation should focus on a well-defined species (or subspecies or variety), a well-defined part of the plant, and a well-defined preparation.

Adulteration may occur. Manufacturers may add, for example, to *C. aurantium* L. ssp. *aurantium* L. preparations synthetic *p*-synephrine or isomers like *meta*-synephrine (also called phenylephrine or neosynephrine), which is not naturally occurring in bitter orange fruits. This will not become evident when in the specifications only known ingredients are listed and quantified. Furthermore, in some countries, restoration of botanical preparations is allowed and may be part of the manufacturing process, *i.e.* addition of volatile ingredients lost in the manufacturing process to a dry extract. Given these aspects, the establishment of adequate quality control methods is necessary.

It is often difficult to identify the constituent or group of constituents in a botanical or botanical preparation that is responsible for the safety concern. An example is EGCG from leaves of *C. sinensis* that is quantitatively a major constituent and useful to characterize the quality of the preparation, but for which, no firm link has been established with the hepatotoxicity of the dried green tea extracts. Furthermore, the case of the seeds of *Linum usitatissimum* L. (flaxseed) indicates that, even when compounds of concern have been clearly identified, the evidence for their effects may be controversial and require further testing. The strength of evidence underlying the concerns over a botanical ingredient should be, therefore, included in the safety assessment.

The matrix effect should be taken into account in the safety assessment of botanicals and botanical preparations. It is plausible that the kinetics as well as the expression of the inherent toxicity of a naturally occurring substance can be modified by the surrounding matrix. Depending on the mechanism of action of the substance and the nature of the matrix, this could result in the toxicity of the specific substance being unchanged, reduced or even increased. Research data on individual substances–matrix interactions, mainly available *in vitro* on specific botanical preparations, cannot be used to draw general conclusions applicable to intact botanicals or other preparations or *in vivo*. Where a matrix effect is advocated to support the safety of a botanical ingredient or specific levels of compounds, *ad hoc* test data should be provided to demonstrate the real occurrence of the matrix effect in that preparation and its magnitude. A matrix effect should be judged on a case-by-case basis as described in this article for *F. vulgare* and *C. sinensis*.

Table 2. Overview of issues emerging when testing the tiered approach for the safety assessment of botanicals and botanical preparations used as ingredients in food supplements

Recommendation	Rationale	Botanical example
Botanical ingredients must be identified by their scientific (binomial) name, in most cases down to the subspecies level or lower	Different subspecies or varieties mostly vary in the constituents and the level of toxic principles	*F. vulgare* Mill ssp. *vulgare* var. *dulce versus* var. *vulgare* *C. aurantium* L. ssp. *aurantium* L. *versus* *C. aurantium* L. spp. *bergamia* (Risso & Poit.) Engl.
Adequate characterization and description of the botanical parts and preparation methodology used is needed	Different preparations can be obtained from the chosen parts of a specific botanical	*C. sinensis*; different green tea preparations result in different outcome of the safety evaluation
Each safety evaluation should focus on a well-defined species (or subspecies or variety), a well-defined part of the plant, and a well-defined preparation	Composition of a botanical may vary significantly due to factors that cannot be easily controlled Safety of a botanical ingredient cannot be assumed only relying on the long-term safe use of other preparations of the same botanical	
Establishment of adequate quality control is necessary	Adulterations, misclassifications, replacements or falsifications, and restorations may occur	*C. aurantium* L. ssp. *aurantium* L. preparations containing *meta*-synephrine which is not naturally occurring in bitter orange fruits
The strength of the evidence underlying concerns over a botanical ingredient should be included in the safety assessment	It is often difficult to identify the constituent or group of constituents in a botanical or botanical preparation that is responsible for the safety concern	No firm link between hepatotoxicity and EGCG from leaves of *C. sinensis* Controversial evidence for adverse effects of phytoestrogens from seeds of *L. usitatissimum* L. (flaxseed)
The matrix effect should be taken into account on a case-by-case basis	The kinetics and toxicity of a naturally occurring substance can be modified by factors included in the matrix	*F. vulgare* *C. sinensis*
Adequate data and methods for exposure assessment are needed	Exposure assessment often plays a decisive role in the outcome of the safety assessment of the botanical or botanical preparations	MOE to the intake of estragole and margin of safety (MOS) to ADI for intake of *trans*-anethole from *F. vulgare*
Safety regulations concerning toxic contaminants have to be complied with	Some contaminants may arise from the manufacturing process and need to be kept within safety limits	Presence of PAH in dried preparations
Specifications should include maximum levels for possible contaminants, for example, pesticide residues, mycotoxins, heavy metals, and PAHs, according to existing guidelines for foods		

While working through the real cases, the outcomes of the exposure assessment most often appeared to play a decisive role in the outcome of the safety assessment of the botanical or botanical preparations. For example, the MOE to the intake of estragole from the consumption of *F. vulgare* as well as judging whether the intake of *trans*-anethole from the use of bitter fennel fruits for the preparation of fennel tea would remain below the ADI for *trans*-anethole also depend on the outcome of the exposure assessment. However, data on present uses and use levels of a botanical or botanical preparation may be sparse or lacking and other uncertainties may be in some cases unavoidable.

Botanical food ingredients must be obviously in compliance with regulations on contaminants. Some contaminants such as polycyclic aromatic hydrocarbons (PAHs) in dried preparations may arise from the manufacturing process and need to be kept within safety limits. Therefore, specifications should include maximum levels for possible contaminants, *e.g.*, pesticide residues, mycotoxins, heavy metals, and PAHs, according to existing guidelines for foods.

It should be pointed out that, although being outside the EFSA mission, there are other important issues that need to be considered to ensure safety of food supplements. These include: (i) the over-the-counter availability of food supplements through internet sites from countries where regulations are not in place or not aligned to European standards; and (ii) the fact that the control systems in place to guar-

antee the safety and quality of botanical supplements are not well harmonized among different countries. The latter is of particular concern as some products on the market are known to be of variable quality with high variation in the content of the active and/or the toxic principles, and due to the fact that already examples of replacement of a harmless variety with a toxic alternative have occurred [72–75]. Misidentification of plants harvested from the wild may add to the problem. The growing volume of products and sales call for a more formal premarketing assessment and better and stricter controls than at present. Regulatory bodies have become aware of the problem and are increasing their efforts to ensure the safety of botanical supplements [1].

A last consideration is related to consumer information and empowerment that would make it possible to reduce phenomena such as over-consumption of food supplements by particular groups and the fact that many consumers equate ''natural'' with ''safe'' when considering botanical food supplements.

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