EFSA SCIENTIFIC COOPERATION (ESCO) REPORT

Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies[1]

ESCO WORKING GROUP ON BOTANICALS AND BOTANICAL PREPARATIONS[2, 3]

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SUMMARY

In June 2008, after an initial public consultation, the Scientific Committee of EFSA published a guidance document for the safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements. Recommendation was made at the same time to test the proposed approach for safety assessment with a number of examples, and consider amending the Compendia i) of botanicals reported to contain toxic, addictive or psychotropic substances and ii) of botanicals reported to have also a medicinal use.

An EFSA Scientific Cooperation (ESCO) Working Group composed of experts identified by the members of the Advisory Forum, and by the Scientific Committee was created to fulfill this request, and advise on the adequacy of the proposed approach for the safety assessment of botanicals and botanical preparations for EFSA and the European Member States' needs. In addition the Working Group was asked to update the Compendia.

The ESCO Working Group considered six botanical preparations to test the science-based framework described the guidance document: i) hydroalcoholic extract of dried peel of *Citrus aurantium* L. ssp. *aurantium* L., ii) dried green tea extract of *Camellia sinensis* (L.) Kuntze, iii) dried leaves extract of *Ocimum tenuiflorum* L., iv) dried fruits water extract of *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare*, v) dried ripe seeds of *Linum usitatissimum* L., and vi) wheat bran from *Triticum aestivum* L. These examples were selected in order to address various safety issues, such as misidentification / adulteration, liver toxicity, possible presence of genotoxic and carcinogenic compounds. Based on the experience acquired through the examples, the ESCO Working Group identified a number of possible amendments and additions for the guidance document of the Scientific Committee.

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1 On request of EFSA, Question No EFSA-Q-2008-388a, issued on 30 April 2009.

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3 This scientific technical report of EFSA is based on major contributions from (i) Experts nominated by the EFSA Scientific Committee: Robert Anton, Angelo Carere, Luc Delmulle (Chair Subgroup Compendium), Corrado L. Galli, Ivonne Rietjens (Chair Subgroup Real Cases), Vittorio Silano (Chair Overarching Group) and Gerrit Speijers, (ii) Experts nominated by the members of the EFSA Advisory Forum: Ilze Abolina, Judith Amberg-Müller, Ulla Beckman-Sundh, Birgit Dusemund, Marie-Hélène Loulergue, Andrea Lugasi, Martijn Martena, Maria Nogueira, Kirsten Pilegaard, Mauro Serafini, Jaroslav Toth, Arnold Vlietinck and Magdalini Zika.

For citation purposes: EFSA Scientific Cooperation (ESCO) Working Group on Botanicals and Botanical Preparations; Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies on request of EFSA. EFSA Journal 2009; 7(9):280. [104 pp.]. doi:10.2903/j.efsa.2009.280. Available online: www.efsa.europa.eu

Taking into account comments received during the public consultation, the ESCO Working Group merged the two compendia into a single one, after removing references made to possible medicinal use of botanicals. The resulting Compendium now focuses on toxicity aspects, listing botanicals reported to contain toxic, addictive, psychotropic, or other substances of concern. The Compendium aims at flagging plants or parts of plants or compounds of possible concern for human heath naturally present in the listed botanicals and that therefore require specific attention while assessing the safety of the product(s) containing such botanical(s). It is recommended that EFSA keeps updating the Compendium on a regular basis.

After possible update of the EFSA guidance document for the safety assessment of botanicals and botanical preparations by the Scientific Committee, based on the recommendations made in this advice, the guidance document and the Compendium will be made publicly available on the EFSA website.

KEY WORDS

Botanicals, botanical preparations, safety assessment, food supplement, toxicological properties, ESCO.

DISCLAIMER

The conclusions and recommendations of this report reflect those of the experts involved in the ESCO Working Group on Botanicals and Botanical Preparations and not necessarily represent the views of EFSA.

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BACKGROUND AS PROVIDED BY EFSA

Following the discussion paper of the Scientific Committee on botanicals and botanical preparations adopted on 23 June 2004 and the mandate received by the Scientific Committee in August 2005 from EFSA, the Scientific Committee developed a two-level approach for the safety assessment of botanicals and botanical preparations. A guidance document was published focussing on botanicals and botanical preparations intended for use as food supplements[4].

A conceptual framework for safety assessment was advocated, in which botanicals or botanical preparations for which an adequate body of knowledge exists could benefit from a "presumption of safety" without any need for further testing (first level of the framework). Issues that should be carefully considered in order to reach such a conclusion were discussed in detail in the guidance document. Botanicals and botanical preparations for which a presumption of safety is not possible would be subject to a more extensive safety assessment with the methodology described in the second level of the proposed framework.

As a follow up, it was proposed to test the approach described in the guidance document for safety assessment with a selected number of cases including botanicals known to contain toxic substances, botanicals with an established history of food use, and botanicals that are known to contain potentially genotoxic/carcinogenic substances.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The ESCO Working Group on Botanicals and Botanical Preparations is requested by EFSA to:

- enlarge the information basis underlying the compendia, and therefore improve their values
- test the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected number of cases.
- provide the EFSA Executive Director with the updated compendia, a report summarising the outcome of the case studies, and advise on the adequacy of the proposed tiered approach for the safety assessment of botanicals and botanical preparations for EFSA and the Member States' needs.

The present advice addresses the 3rd point of the Terms of Reference. The reports on the 6 examples selected to test the proposed approach (2nd point of the Terms of Reference) are annexed.

The 1st point of the Terms of Reference was also addressed by the ESCO Working Group and resulted into one updated compendium. This compendium is, together with the present advice, one of the deliverables of this activity.

ACKNOWLEDGEMENTS

This technical report was prepared by the ESCO Working Group with the support of the Scientific Committee & Advisory Forum Unit.

The EFSA Scientific Committee wishes to thank all the members of the WG for their contribution during the meetings and in the preparation of this report.

[4] See http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_draftguidance_botanicals_public_cons_update_en.pdf?ssbinary=true

INTRODUCTION

The overarching ESCO Working Group on Botanicals defined the following 6 examples for the testing of the science-based framework described in the guidance document. The Table also lists the possible safety issues expected to be linked with the real case examples. The opinions prepared on each of the 6 case studies are included as annexes to the present opinion.

Botanical	Preparation	Possible safety issue	For opinion see
Citrus aurantium L. ssp. *aurantium* L.	Hydroalcoholic extract of dried peel	Misidentification / adulteration	Annex 1
Camellia sinensis (L.) O. Kuntze	Dried green tea extract	Liver toxicity	Annex 2
Ocimum tenuiflorum L.	Dry leaves extract	Reproduction toxicity	Annex 3
Foeniculum vulgare Mill. ssp. *vulgare* var. *vulgare*	Dried fruits (water extract)	Genotoxic carcinogen	Annex 4
Linum usitatissimum L.	Dried ripe seeds	Phytoestrogens	Annex 5
Triticum aestivum L.	Wheat Bran	Low concern – presumption of safety	Annex 6

The next sections are organised according to the structure described in the draft guidance for the safety assessment of botanicals and botanical preparations used as foods supplements. The present document provides an advice on whether the proposed framework is suitable for the safety assessment of botanicals and botanical preparations used as food ingredients.

The present document presents an overview of matters encountered when using the proposed tiered approach and proposes modifications/text to update relevant sections of the guidance document.

1. Safety assessment of botanicals and botanical preparations intended for use as food supplements

1.1. Proposed data requirements for safety assessment of food supplements

The guidance document states that the following sections aim at identifying additional data and information considered as necessary or desirable to assess the safety of food supplements. These data are of: (i) technical; (ii) exposure and (iii) toxicological nature. The guidance document states that the lists below are meant to give guidance on possible data requirements. They have been made as exhaustive as possible and should be adapted on a case-by-case basis, depending on the nature of the botanical or botanical preparation. This implies that not all the information listed would be needed in all cases.

1.1.1. Technical data

1.1.1.1. Identity of the botanical or botanical preparation

The Working Group stressed 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. Synonyms can also be included.

In some cases it can be necessary to define the botanical or botanical preparation down to the subspecies level or lower because different subspecies may vary in the constituents and the level of toxic principles. Examples are *Foeniculum vulgare* Mill ssp. *vulgare* var. *dulce* (sweet) versus var. *vulgare* (bitter) with essential oil of the first one containing about 10 times lower levels of estragole, and *Citrus aurantium* L. ssp. *aurantium* L (bitter orange) versus *Citrus aurantium* L. spp. *bergamia* (Risso & Poit.) Engl. (bergamot orange) producing different fruits that contain different levels of the active principles such as furanocoumarins and *p*-synephrine.

In other cases however it appears possible to evaluate a wide variety of subspecies on the basis of one representative species. This would be the case of e.g. rose hips, the spurious fruits of dog rose (*Rosa canina* L.), alpine rose (*Rosa pendulina* L.) and other *Rosa* species, most commonly *Rosa rugosa* Thunb. 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 a specific botanical, and depending on a number of factors including the solvents, and process of extraction, different components of the botanical may be extracted. It is therefore needed to adequately describe the preparation(s) considered. The Working Group stressed the difficulty of dealing with different uses and starting materials, and concludes that 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. The identity of the botanical and botanical preparation evaluated needs to be clearly described.

The Working Group recommends the insertion of the following text in the guidance document:

> "It is recognized that identification of the botanical source and botanical preparation may in some cases be more complicated than expected. It is then recommended to stick as much as possible to the European Pharmacopeia. Additional nomenclature sources are as follows:
> World Checklist of Selected Plant Families (Royal Botanic Garden, Kew); the books by Hanelt (2001) also available on the Internet as Mansfeld's World Database of Agricultural and Horticultural Crops; and the database by United States Department of Agriculture. If a scientific name is not found in any of the above-named references, its existence may be checked in The International Plant Names Index.
> Since there have been many instances where species have been reclassified, a same species may be known by different scientific names. There is therefore an entry for synonyms that are still in use. Common (vernacular) names (in English, French, German, and Italian, if known) are also given, but it should be noted that a common name used in one region to refer to a particular plant may be used elsewhere to refer to another quite unrelated species. Hence common names may not uniquely identify a species and are not as reliable as the systematic names.
> The following scheme summarizes the requirements for description of the identity of the botanical:

Scientific (Latin) name: *full systematic species name incl. author (according to reference source)*

Synonyms: *botanical name(s) according to other references*

Common names: v*ernacular name(s)*

Family: *botanical family*

Part used: *e.g. root, leaf, seed …*

Geographical origin: *continent, country, region*

Growth and harvesting conditions: *wild or cultivated, cultivation practices, time of harvest in relation to both season and stage of the plant growth."*

1.1.1.2. Manufacturing process

The Working Group advises to refer to Hazard Analysis Critical Control Point (HACCP) requirements that guarantee that all the manufacturing steps are properly carried out, including the careful identification of the botanical used, the latter, to avoid adulteration, miss-classification or switching of species.

The guidance document already indicates that the following information is considered necessary for assessing the safety of botanicals and botanical preparations:

i) Information on the method(s) of manufacture (e.g. the process by which the raw material is converted into a preparation, such as extraction or other procedure(s), and plant extract ratio).

ii) Information on substances entering the manufacturing process, e.g. identity of the extraction solvent, reagents, special precautions (e.g. light and temperature).

iii) Standardization criteria (e.g. see European Pharmacopoeia); with respect to standardization criteria, the Working Group advises to clearly define the criteria needed to guarantee quality.

The Working Group also noted that adulteration may occur. Manufacturers may add for example to *Citrus aurantium* preparations synthetic synephrine or isomers like meta-synephrine (also called phenylephrine or neosynephrine) which is not naturally occurring in citrus fruit. This will not become evident when in the specifications only known ingredients are listed and quantified.

Furthermore the Working Group noted that 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 of adulterations, misclassification, switching of species, and restoration, the Working Group concludes that, although remarks on quality control were taken out of the guidance document upon the public consultation round, some sentences referring to quality control seem to be required.

All together the Working Group advises to add the following text to the guidance document in the section on manufacturing process:

> "Botanicals or botanical preparations might become hazardous as a result of deviations in the production process (e.g. misclassification, switching of species). Therefore the safety of botanicals and botanical preparations should be ensured by following a Hazard Analysis and Critical Control Point (HACCP) approach (Codex Alimentarius 1997). The whole production

chain, from primary production of botanicals to the storage and commercialisation of the botanical preparations should be taken into consideration. The HACCP system must be applied with the necessary flexibility and adapted to each botanical preparation on a case-by-case basis."

1.1.1.3. Chemical composition

The guidance document states under this heading only the following: "Data on the chemical composition of the botanical ingredient and preparation to be evaluated should be provided with emphasis on compounds of relevance for the safety assessment."

The Working Group suggests to extend this section so that it reads as follows:

"Data on the chemical composition of the botanical ingredient should be provided with emphasis on the concentrations of constituent of relevance for the safety assessment; this includes the concentrations of:

- Major compounds of concern in terms of quantity: Compounds should be classified according to their chemical structure (e.g. flavonoids, terpenoids, alkaloids, etc.). Levels at which the constituents are present in the respective part of the botanical or botanical preparation should be given where available.
- Constituents to characterise the quality, chemical fingerprint, production process and/or biological activity of the preparation (markers).
- Constituents that provide reasons for concern due to their chemical, physiological or toxicological properties.

In some cases, it may be difficult to identify the active principle responsible for an effect. Therefore the strength of the evidence underlying the concerns over a compound being reason for concern should also be given."

The Working Group recognizes that it is often difficult to identify the constituent or group of constituents of concern. As an example one might consider epigallocatechingallate (EGCG) from *Camellia sinensis* which is a major constituent in terms of quantity and also a constituent useful to characterise the quality of the preparation but for which the link to the hepatotoxicity of the dried green tea extracts is not firmly established (Annex 2).

1.1.1.4. Specifications

The guidance document states that specifications of the botanicals or botanical preparations are required. They may be based on nutritional or biologically active components or, when these are not known, on selected chemical markers. Limits for or absence of specific undesirable / toxic substances should be specified.

The Working Group noted that including all preparations from one botanical in a single document seems unrealistic. This is why in the examples in the annexes, specific preparations were defined to be evaluated according to the guidance document.

The Working Group also indicated that the guidelines should better define how to identify the nature of the botanical and its preparation to be assessed. Specifications in combinations with defined manufacturing processes should be used. The specifications in the annexes can be used as examples on how it should be done.

The Working Group stressed that, as already outlined in the guidance document, the proposed specifications should be modelled on recent European or other internationally accepted specifications. Where the proposed specifications differ from internationally recognised specifications, the latter specifications should be set out alongside the proposed new specifications, and any differences pointed out. Validated and well-established methods should be preferably used for the analysis of compounds considered in the specifications.

The Working Group noted that a reference to possible contamination aspects is missing in the section on specifications of the guidance document and that to this end, existing regulations may be used.

The Working Group also noted that the composition of a botanical or botanical preparation may vary significantly due to factors that cannot be easily controlled.

The Working Group considered it appropriate to also define requirements on pesticide residues, mycotoxins, heavy metal and PAH (polycyclic aromatic hydrocarbon) residues in the specifications. Although this may refer to quality control, the Working Group considered this an important issue in the field of botanicals and botanical preparations that cannot be ignored. It was noted that some contaminants (e.g. PAHs in dried preparations) may arise form the manufacturing process and need to be kept within safety limits.

Taking this all together the Working Group proposes to extend the section on specifications with the following text:

> "The specifications should include concentrations of major groups of constituents present in the botanical preparation including for example: amino acids, lipids, polysaccharides, volatile oil, anorganic ions, polyphenols, alkaloids, terpenes, alkenylbenzenes, lignin, saponins etc. as well as the major constituents within these classes.
> In addition, maximum levels for possible contaminants including e.g. heavy metals, mycotoxins, pesticide residues, and polycyclic aromatic hydrocarbon (PAH) residues should be proposed in line with existing guidelines."

1.1.1.5. Stability of the botanical or botanical preparation used as ingredient in food supplement

The guidance document states that stability of the botanical ingredient should be demonstrated over the shelf-life time. Any information concerning possible degradation should also be provided.

The Working Group noted that data on stability are often missing and that stability will depend to a high extent on the storage conditions.

1.1.1.6. Proposed uses and use levels

The guidance document states only that information should be provided on any intended uses and recommended intakes for each product.

The Working Group noted that further specifications may be needed on the types of uses. The Scientific Committee may consider whether the focus is only on food uses or if other uses like fragrance or skin exposure should be considered.

The Working Group also noted that it is useful to specifically mention uses for the following categories:

- Common foods
- Supplements
- Medicinal products

Special attention should be given to population groups with specific uses like for example use of tea from *Foeniculum* by young children. For food use, quantitative information is often lacking.

Finally the Working Group noted that uses and use levels should also specify the duration of use (see section 1.1.2) because for example use as an herbal medicine may be for short term periods whereas use in food may represent a life time exposure.

The Working Group suggests to extend the text of this section in the guidance document with the following paragraph:

> "Information on intended uses and recommended intakes for a product should specifically mention uses and use levels for the following categories:
>
> - Common foods
> - Supplements
> - Medicinal products
>
> Special attention should be given to population groups with specific uses like for example young children. Information on the duration of the proposed uses and use levels should also be provided".

1.1.1.7. Information on existing assessments

The guidance document states that information on any existing assessments by national competent authorities or other bodies should be provided

The Working Group suggests to consider adding "European/International" bodies in the guidance document, because existing assessments should preferably come from established (inter)national bodies. In that case the text would read as follows:

> "Information on any existing assessments by International bodies or national competent authorities should be provided."

1.1.2. Exposure: extent and duration

The Working Group suggests to modify the heading of this section in the guidelines from: "Exposure: extent and time" to "Exposure: extent and duration", and to replace "time" by "duration" whenever relevant in this section.

Furthermore the Working Group would like to stress that while working through the examples the outcomes of the exposure assessment often appeared to play a decisive role in the outcome of the safety assessment of the botanical or botanical preparations. For example the margin of exposure (MOE) to the intake of estragole from the consumption of *Foeniculum vulgare* strongly depends on the exposure assessment (Annex 4).

The Working Group also noted that often adequate data and even adequate tools to make an appropriate exposure assessment are missing. One could question for example if the presently available tools are able to estimate the intake of synephrine from *Citrus aurantium* as a result of its use in marmalade because 15 broad food categories in the EFSA European Concise Database may not be refined enough to get an estimation on the exposure to marmalade made of *Citrus aurantium*.

The Working Group noted that adequate strategies for exposure assessment of botanical and botanical preparations may have to be developed and/or agreed upon, as in most cases the ingredients are present as a totum and not isolated.

The Working Group also stresses that data on present uses and use levels of a botanical or botanical preparation may be sparse or lacking.

The Working Group noted that this uncertainty in the exposure assessment is already taken into account by the requirement stated in the guidance document that "uncertainties associated with the food consumption data considered and anticipated exposure ranges should be clearly described."

The Working Group noted that clear distinction should be made between intake of a botanical itself, intake of its essential oil and other preparations made of it, since levels and compounds may largely differ between various preparations.

To take this better into account the text of the guidance document in this section sub i) stating; "Anticipated human exposure to the botanical ingredient, including amount (e.g. maximum and average daily intake or exposure), frequency and duration. It is important to characterize as much as possible the expected human exposure to the botanical ingredient according to the recommended modalities of use in terms of extent and duration." could be extended with the following sentence:

> "Clear distinction should be made between intake of a botanical itself, intake of its essential oil and other preparations made of it."

A matter to be specifically addressed in the evaluation is whether the proposed use and use levels will increase already existing human exposure. This can be especially of importance when use of the botanical or botanical preparation would result in exposure to a compound that is genotoxic and carcinogenic and is further illustrated by the example of *Foeniculum* containing the alkenylbenzene estragole (Annex 4). To take this issue into account, at the end of the whole section the following sentence could be added:

> "A matter to be specifically addressed in the evaluation is whether the proposed use and use levels will significantly increase already existing human exposure."

Finally the Working Group noted that exposure data should also specify the duration of exposure.

1.1.3. Toxicological data

The Working Group did not identify any need for amending the current section of the guidance document.

1.2. Proposed general framework for evaluating the safety of botanicals and botanical preparations used as food supplements

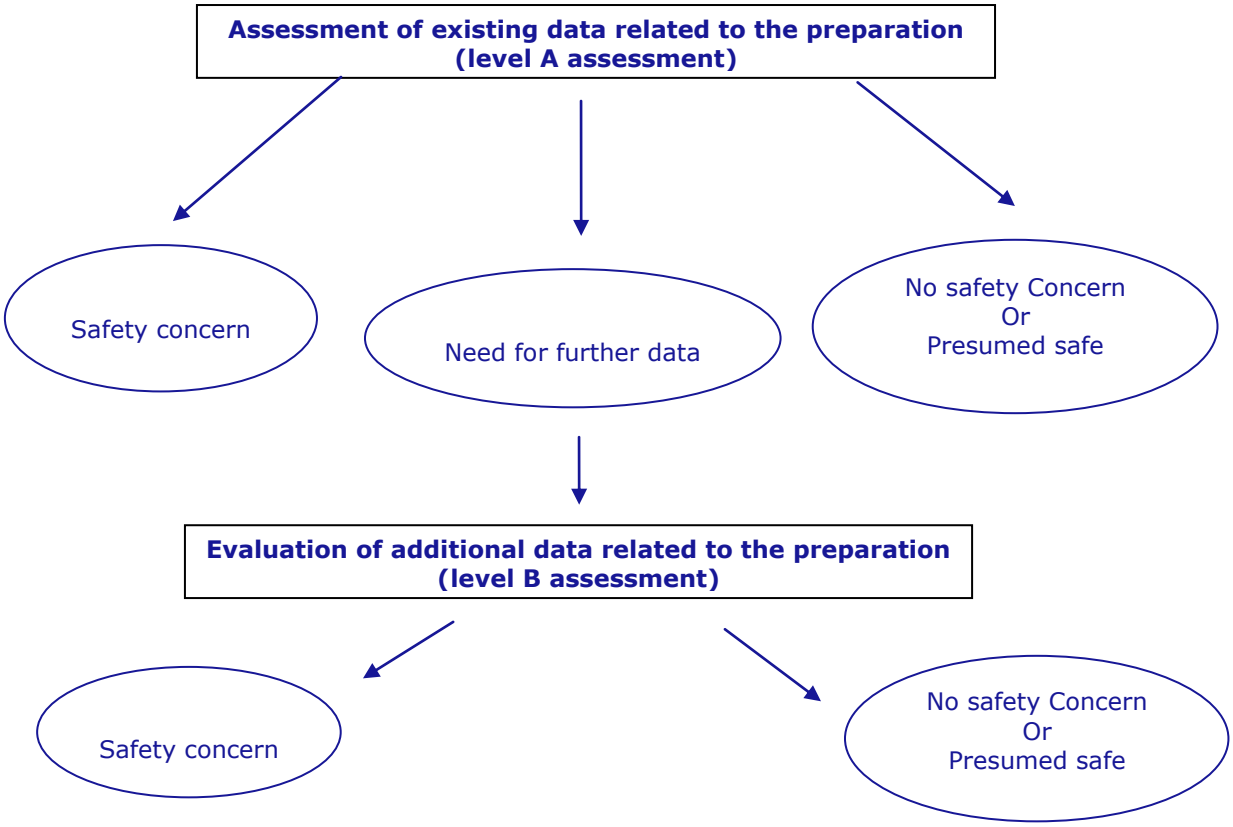
The approach proposed in the guidance for the safety assessment of botanicals and botanical preparations not regulated in the framework of specific regulations such as those on novel foods and GMOs, consists of the two following levels:

- Level A: Safety presumed based on available knowledge.
- Level B: Further testing and/or data required.

While testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected number of cases, it became clear that the heading of level A was confusing. A level A assessment could indeed result in the conclusion reflected in the heading, namely that safety can be presumed based on available knowledge (like for *Triticum aestivum* in Annex 6 or some of the *Camellia sinensis* extracts in Annex 2) but at the same time an evaluation on the basis of existing knowledge (level A assessment) could lead to the conclusions that either the product is of safety concern or that further testing and/or data are requested. This implies that the original heading of level A reflects only one of the three possible outcomes of an assessment of the available knowledge Therefore the Working Group suggests to modify the heading of level A to

"Level A: safety assessment based on available knowledge",

and to include the following flow chart into the guidelines:



1.2.1. Level A: Assessment of available data - no further testing required (presumption of safety based on long term food use in Europe)

Given the above remarks, the heading of this section in the guidance document should be modified to:
"Level A: safety assessment based on available knowledge".

The Working Group stresses that a botanical can have several compounds of concern regarding consumer safety. The guidance document should clearly indicate which to consider / ignore. The Working Group noted that it may not be always straight forward to identify the compound responsible for toxicity. To give an example: in the case of green tea extract, EGCG is taken as a marker compound but it is not unequivocally established that it is the only compound responsible for the adverse liver effects induced by the green tea preparations (Annex 2).

In the section on chemical composition it was already suggested to add a sentence stating: "In some cases, it may be difficult to identify the active principle responsible for an effect. Therefore the strength of the evidence underlying the concerns over a compound being reason for concern should also be given." This statement might be repeated in a somewhat modified form in the section on the level A assessment stating:

> "If compounds of concern can be well defined, evaluations can focus on these specific compounds. In some cases, it may be difficult to identify the active principle responsible for an effect. In such cases the strength of the evidence underlying the concerns over a compound taken as the reference compound for the safety evaluation should also be given."

Another issue that should be dealt with in further detail in the guidance document is to what extent we can extrapolate from one preparation to another and/or from one botanical to another (with the same compounds of toxicological concern).

The Working Group concludes that toxicological data on a certain dried extract cannot be transferred to another without giving evidence of their equivalent pattern of composition. This should be stressed in the guidance document by including a sentence stating:

> "Extrapolating from one preparation to another and/or from one botanical to another with the same compounds of toxicological concern can only been done when accompanied by evidence of their equivalent pattern of composition and consumption."

The guidance document stated that in cases where no health-based guidance values are available or where the botanical or botanical preparation contains substances that are both genotoxic and carcinogenic, the "Margin of Exposure" (MOE) approach[5] could be applied covering the botanical(s) under examination and any other dietary sources of exposure. The MOE approach compares toxic effect levels with human exposure levels. The Working Group concludes that it should be made more explicit in the guidance document how risk assessors should proceed with applications / preparations that would imply no or a minor exposure increase to the substance of concern. Furthermore some text should be added to outline what would be the alternative approach to the MOE calculation.

To this end the following text could be included in the guidance document:

[5] See http://www.efsa.europa.eu/en/science/sc_commitee/sc_opinions/1201.html

> "Botanical or botanical preparations for which the MOE approach indicates a low priority for risk management of the genotoxic and carcinogenic constituent of concern, or for which the exposure to this constituent resulting from the proposed use and use levels of the botanical or botanical preparation would be negligible compared to normal dietary intake of the constituent from other dietary sources, might be considered of no safety concern, given that the database on other toxicological endpoints also does not give reason for concern."

The guidance document stated that 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. The Working Group notes that the importance of the matrix effect should be further stressed and that it may require a more extended section in the guidance document. It should be stated for example that a matrix effect should be judged on a case-by-case basis and that it may either reduce toxicity, but that it can also increase toxicity (e.g. EGCG toxicity appears to be higher when given in green tea extract than when given as a pure compound - Annex 2).

The Working Group proposes that a separate paragraph needs to be inserted on the possible matrix effect and interactions with other compounds. To this end the sentence in the guidance document referred to above can be extended in line with a previous statement the AFC Panel (http://www.efsa.eu.int/EFSA/efsa_locale-1178620753812_1211902090253.htm), and presented as a separate paragraph reading as follows:

> "It is plausible that the kinetics as well as the expression of the inherent toxicity of a naturally occurring substance could be modified by the matrix in which it was present. Depending on the mechanism of action, this could result in the toxicity being unchanged, reduced or even increased. Research on individual substance/matrix interactions or botanical preparations cannot be used to draw general conclusions about intact botanicals, herbs and spices under all conditions of use, ingestion and metabolism. 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. A matrix effect should be judged on a case-by-case basis."

The Working Group indicates that when a matrix effect is demonstrated for an essential oil this matrix effect will not be similar for the intact botanical. This is taken care of by stating in the above section that research on individual substance/matrix interactions or botanical preparations 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.

The Working Group also noted that the toxicological section should provide data on known interactions (e.g. herbal-drug), since such interactions have frequently been documented.

To this end the following statement could be added:

> "If available, data on the presence of possible interactions (e.g. herbal-drug) should also be provided."

1.2.2. Level B: Further testing and/or data required

In line with the draft proposal concerning the data required for the risk assessment of flavouring substances, currently being discussed by the EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF), the Working Group suggests replacing the section of the guidance document on Genotoxicity testing with the following:

"- a test for induction of gene mutations in bacteria (Ames test; OECD guideline 471)

- a test for induction of gene mutations and chromosomal effects in mammalian cells (the mouse lymphoma *tk* assay with colony sizing; OECD guideline 476)

The *in vitro* chromosomal aberration test (OECD guideline 473) or the *in vitro* micronucleus assay (OECD guideline 487, in preparation) would also be acceptable as alternative to the mouse lymphoma assay

There may be circumstances under which it may be justified to deviate from the above-mentioned core set. In such cases a scientific justification should be provided and additional types of considerations or mechanistic studies may be needed.

One or more positive *in vitro* tests normally require follow-up by *in vivo* testing, unless it can be adequately demonstrated that the positive *in vitro* findings are not relevant for the *in vivo* situation. The choice of the appropriate *in vivo* test is critical, due to different sensitivities, different endpoints and other variables. It requires expert judgement based on all available information, to be applied case-by-case. For this reason, a flexible approach is preferable to a fixed decision tree. Guidance for the follow-up of *in vitro* positive results could be taken from section 3.10.5.6 of the Technical Guidance Document on Risk Assessment of Chemical Substances and Biocides (TGD) (EC, 2003). According to this guidance, a mouse micronucleus assay and a rat liver unscheduled DNA synthesis (UDS) assay are recommended for substances with adequate systemic availability (i.e. evidence for adequate availability to the target cells). For substances that are short lived, direct acting *in vitro* genotoxic or for which no indication of systemic availability have been presented, a strategy involving studies with tissues at initial sites of contact is recommended. The main options for the latter substances are, according to the TGD, the *in vivo* Comet assay, gene mutations tests with transgenic animals and *in vivo* DNA adduct studies. In particular, the Panel recommends an *in vivo* Comet assay in addition to a mouse micronucleus assay. The Comet assay has the advantage that it enables the investigation of tissues at initial sites of contact with the body and that several tissues could be analysed in parallel. Furthermore, it has recently been shown that the Comet assay is able to detect several carcinogens which were negative in the *in vivo* micronucleus assay and that the sensitivity (ability to detect carcinogens as positive) of the Comet assay is clearly higher than the sensitivities of the *in vivo* UDS assay and the transgenic animals assays, while the specificity (ability to give negative results with non-carcinogens) of the Comet assay is slightly higher than that of the transgenic animals assays (Kirkland and Speit, 2008). A combination of the *in vivo* micronucleus assay and the Comet assay in a single study as suggested by Pfuhler et *al.* (2007) would also be acceptable.

Studies should be conducted using internationally agreed protocols. Test methods described by OECD or in European Commission Directives are recommended. The most up-to-date edition of any test guideline should be followed. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directives 87/18/EEC and

88/320/EEC and accompanied by a statement of GLP compliance. Use of any methods differing from internationally agreed protocols should be justified. An OECD guideline is not yet available for the Comet assay. However, recommendations for an appropriate performance of the assay using OECD guidelines for other *in vivo* tests have been published and a standard protocol and acceptance criteria for this assay have been developed through the International Workshop on Genotoxicity Working Parties and International Comet assay workshops (Tice et al., 2000; Hartman et al., 2003; Burlinson et al., 2007). Additional information could be taken from a website on the Comet assay (http://cometassay.com)"

CONCLUSIONS AND RECOMMENDATIONS

The ESCO Working Group identified a number of possible amendments and additions for the guidance document for the safety assessment of botanicals and botanical preparations, in order to make it more comprehensive and detailed. Detailed suggestions for amendments of the text are given throughout this document.

The ESCO Working Group recommends the flow diagram for the outcome of the level A and B safety assessment to be included in the guidance document.

As already suggested in the guidance document, the ESCO Working Group recommends updating on a regular basis the Compendium of botanicals reported to contain toxic, addictive, psychotropic or other substances of concern. The ESCO Working Group recommends also extending the current content of the compendium to exotic botanicals (primarily from South America, China (currently addressed by EMEA HMPC), Africa, and ultra-peripheric regions). Indeed, the currently ongoing revision of the Novel Food Regulation proposes that "history of safe use in the third country of origin should be taken into account. … If Member States and/or the Authority have not presented any reasoned safety objections, based on scientific evidence, … it will be permissible to place the food on the Community market after notification of the intention to do so…" Such extension would provide EFSA and the Member States with a useful source of information on compounds of possible concern present in botanicals.

The ESCO Working Group finally recommends harmonising the approaches on gentoxicity testing and therefore to update the guidance document with the approach currently developed by the EFSA CEF Panel for the risk assessment of flavouring substances.

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APPENDIX A: *CITRUS AURANTIUM* L. SSP. *AURANTIUM* L.

The present report focuses on the hydro-alcoholic extract of the (unripe) fruit standardized to 6 % of *p*-synephrine

Disclaimer:

The present document aims at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected case and considers relevant constituents. The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated. The document is not, and cannot be interpreted as being, a policy document or a decision allowing the classification of a certain botanical or botanical preparation as a foodstuff or as a medicinal product. The document is focusing on one type of preparation and is not intended to evaluate all possible preparations of this botanical or all possible constituents, which should normally be part of a full safety assessment of a botanical. Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete. Contaminants such as heavy metals, mycotoxins, dioxins, pesticides, microbial contamination or PAHs are not evaluated.

It is outside the remit of this Working Group to evaluate possible beneficial effects and associated claims

1. Identity and nature of the source material

Scientific name:	*Citrus aurantium* L. ssp. *aurantium* L.
Synonyms:	*C. aurantium* L. ssp. *amara* Engl. [Notes: *Citrus* × *aurantium* is possibly an ancient *Citrus reticulata*-based hybrid; non-botanical scientific literature often confuses species, subspecies and varieties of the genus *Citrus*]
Common names:	Engl: Neroli, bitter/sour/Seville/Bigarade orange tree Fr: Orange amère, bigaradier Germ: Bitterorangenbaum, Pomeranzenbaum Ital: Arancio amaro, neroli
Family:	Rutaceae
Parts used:	In food supplements relevant to this case report: Dried whole immature fruit, Dried peel of the immature fruit (Traditional Chinese Medicine (TCM) name: *zhi shi*), Dried peel of the mature fruit (TCM name: *zhi qiao*), and their hydro-alcoholic extracts, usually standardized to the content of *p*-synephrine (e. g. 6 % of *p*-synephrine)

other parts of the plant used in food, pharmacy and ethnomedicine: Leaf, Bud, Twig, Whole dried unopened flower (*Aurantii amari* flos Ph. Eur. 6), Whole fresh mature fruit, Juice of the fresh mature fruit, Peel of the fresh mature fruit, Dried epicarp and mesocarp of the ripe fruit partly freed from the white spongy tissue of the mesocarp and endocarp (*Aurantii amari* epicarpium et mesocarpium Ph. Eur. 6), Essential oil of the leaves and buds (Petitgrain oil), Essential oil of the fresh flowers (*Neroli aetheroleum* Ph. Eur. 6), Essential oil of the fresh fruit peel

Geographical origin: Native to the southern slopes of the Himalaya.

Growth and harvesting conditions: Cultivated in southern and subtropical Europe and America, Mediterranean countries, Caribbean; imported from Spain, Portugal, Italy, Israel, Mexico, and islands of the Caribbean. In the United States, the trees are grown as a crop in Arizona, where the peak of production is in January. In China, fruits are harvested mature in July (though still green) or immature (May through June, not fully developed).
Flowers are harvested before they bloom.

2. Manufacturing process

The peel of the fruit is used mostly dried. The outermost part of the pericarp is peeled in helical strips. Officinal quality requires that the peel of the ripe fruit be partly freed from the white spongy tissue (i.e. *flavedo* with rests of the *albedo*). The drying process should be quick, yet the temperature should not exceed 50 °C. The dried peel is hard and brittle when dry; tough and supple when exposed to a moist atmosphere. The outer surface is rough, dark orange-red and shows numerous small circular depressions or pits above the oil glands; the inner surface is whitish and pithy (Wallis 1946, NTP/NIEHS 2004, Blaschek et al. 2006).

An officinal tincture is produced from 1 part of the freshly powdered dried material and 5 parts of alcohol (70 per cent V/V/V) (Aurantii amari epicarpii et mesocarpii tinctura Ph. Eur. 6). Another traditional recommended formula for bitter orange peel fluid extract or tincture consists of a menstruum composed of two parts alcohol and one part water used for the extraction of the botanical (NTP/NIEHS 2004).

Many suppliers of powdered *Citrus aurantium* peel extracts sell their products as *Zhi Shi* with 6% or 10% *p*-synephrine content in bulk quantities up to 25-kg. Information on the manufacturing process of these extracts, where the synephrine concentration is increased compared to levels usually found in plant material, was not described. Despite the advertised bitter orange peel extract, it is more likely that the actual starting material for these dried alcohol-water extracts is the immature fruit that is sold as a powder in bulk quantities as well. The TCM name *zhi shi* can refer to the immature bitter orange fruit and/or the peel of the immature fruit (NTP/NIEHS 2004, Blumenthal 2005).

The chemical substance of (±)-*p*-synephrine is available on the market as well, in quantities up to such as 25 kg, e.g. from Boehringer Ingelheim Pharma (Germany).

3. Chemical composition

The main constituent of the bitter orange peel is the volatile oil (1.0-2.5 % (V/m)). The main component of the essential oil is limonene (up to 90 %); several other monoterpenes are present (citral, linalool, linalyl-, neryl-, geranyl-, citronellyl-acetate); aliphatic aldehydes and methyl-anthranilate determine the fragrance of the oil (Wallis 1946, NTP/NIEHS 2004, Hänsel & Sticher 2007).

The bitter substances of the bitter orange peel are flavonoids (flavanones glycosylated with neohesperidose, e.g. naringin, neohesperidin, neoeriocitrin) and tetranortriterpenes (limonoids). A non-identified amorphous bitter principle, aurantiamarin, is commonly mentioned in older literature. Other flavonoids of the peel include hesperidin, narirutin, eriocitrin (non-bitter flavanone rutinosides), isohesperidin, and naringenin. The absence of quercetin (flavonol) derivatives is a typical marker of *Citrus aurantium* (Wallis 1946, NTP/NIEHS 2004, Blaschek et al. 2006, Hänsel & Sticher 2007, Chuang et al. 2007).

In the peel of immature fruits, the chief phenolic constituents are the flavonoids naringin and hesperidin, while in the fruit flesh it is the coumarin umbelliferone. The juice of the fruit also contains small concentrations of furanocoumarins (bergamottin, 6',7'-dihydroxybergamottin and bergapten (5-36 µmol/l)) (Malhotra et al. 2001, Gurley et al. 2004, NTP/NIEHS 2004).

p-Synephrine and *p*-octopamine are the most frequently mentioned biogenic amines found in bitter orange peel, however, there is no evidence that octopamine or other phenethylamine alkaloids are present in bitter orange peel in any appreciable levels. For each of these hydroxyphenethylamine alkaloids there are six position- and stereoisomers known, but *Citrus* plants usually contain only the respective (–)-*para*-isomers. The dried herbal material (peel, mature/immature fruit) contains about 0.25-0.35 % (–)-*p*-synephrine (HPLC), but the levels vary greatly, from 0.1 % to 2.0 %. The content of this substance in fresh juice can be even 1000-times lower (3-60 ppm). *p*-Synephrine and related alkaloids appear to be present in slightly higher quantities in the unripe fruit than in the ripe fruit (NTP/NIEHS 2004, Blumenthal 2005, Pellati et al. 2002, Pellati & Benvenuti 2007, Avula et al. 2005 & 2007).

Other constituents of the peel include pectin, carotenoids, cyclic peptides; the juice contains saccharides, vitamins, organic acids etc. (NTP/NIEHS 2004, Blaschek et al. 2006).

The compound selected for this safety assessment is *p*-synephrine, which is supposed to be the ingredient responsible for the adrenergic effect of the bitter orange preparation.

4. Specifications

Several analytical methods have been developed for the quantification of the content of phenethylamine alkaloids (e.g. synephrine, octopamine, tyramine) in plant materials, extracts, foods and food supplements; most of them use chromatographic (HPLC, GC) and electrophoretic (CE) methods, including enantioselective methods (for a review see Pellati & Benvenuti 2007).

Extracts used in many dietary supplements and herbal weight-loss formulas as an alternative to *Ephedra* have concentrations of the sympathomimetic alkaloid synephrine that are often much higher than the synephrine concentrations reported for traditional extracts of the dried fruit or peel. Commercial extracts are marketed with a content of 6-10 %, but can contain up to 95 % synephrine (Avula et al. 2005 & 2007, Pellati et al. 2002, Pellati & Benvenuti 2007, NTP/NIEHS 2004, Blumenthal 2005).

Synephrine has been incorrectly characterized in some of the published literature. The position isomer of synephrine found in bitter orange peel is *p*-synephrine (*para*-synephrine), not *m*-synephrine, which is said to be present in bitter orange by various authors (e.g. Penzak et al. 2001). *Meta*-synephrine (*m*-synephrine) and neo-synephrine are relatively rare synonyms of the compound named phenylephrine in the International Non-proprietary Name (INN) list of the WHO[6]. Phenylephrine is used as a decongestant synthetic drug (The Merck Index, O'Neill 2008). At least one product purportedly containing synephrine alkaloids from *Citrus aurantium* has been reported to contain both *p*-synephrine and *m*-synephrine (Allison et al. 2005, Santana et al. 2008).

The application of the enantioselective analysis of synephrine in food originating from *Citrus* plants confirmed that the fruits contain (−)-*p*-synephrine only. The (+)-*p*-synephrine enantiomer may be formed during the production of juices and marmalade with *Citrus* fruits. In dry extracts and dietary supplements, small amounts of (+)-*p*-synephrine were observed as well, which may be formed during the production of *Citrus aurantium* extracts, using high temperatures and long periods of refluxing (Pellati et al. 2002, Pellati & Benvenuti 2007).

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 (Pellati et al. 2002, Pellati & Benvenuti 2007, NTP/NIEHS 2004, Blumenthal 2005).

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 *Citrus aurantium* should be considered undesirable, and suspicious of adulteration. The origin of these compounds is unlikely the natural botanical source (*Citrus aurantium* L. ssp. *aurantium* L.). Moreover, potential additive effects of the *p*-synephrine, *m*-synephrine and/or octopamine are to be considered in such cases (Jordan et al. 1987, Brown et al. 1988, Evans et al. 1988, NTP/NIEHS 2004, Blumenthal 2005).

Other biologically active constituents present in the hydro-alcoholic extract of the (unripe) fruit of *Citrus aurantium* include flavonoid glycosides and low levels of furanocoumarins (Avula et al. 2005 & 2007).

A dry hydro-alcoholic extract does possibly not contain large amounts of essential oil, even though its content is the criterion of quality in the officinal herbal drug. A yield of minimum 20 ml/kg of essential oil from the dried peel (determined using hydrodistillation) is required by the European Pharmacopoeia (Aurantii amari epicarpium et mesocarpium Ph. Eur. 6).

5. Stability of the botanical ingredient

The shelf-life of herbal drugs containing essential oils strongly depends on storing conditions, but does generally not exceed 36 months. No information is available on the shelf-life of bitter orange extracts used in food supplements or of these preparations.

The crystalline form of (±)-*p*-synephrine is stable to air and light according to The Merck Index (O'Neill 2008).

Pellati et al. (2002) state that aqueous solutions of (±)-*p*-synephrine, (±)-*p*-octopamine and (±)-*p*-tyramine as well as water extracts of fresh and dried *Citrus aurantium* fruits, and aqueous solutions of some commercially available extracts and herbal products were found to be stable. These authors

[6] See http://www.who.int/medicines/services/inn/en/

have performed a stability test to evaluate the eventual optical isomerization of synephrine induced by heat and observed no significant changes of the relative percentage of synephrine enantiomers in the course of a 24 h experiment where homogenized fruit pulp was refluxed with water at 100 °C.

6. Proposed uses and use levels

The bitter orange tree is small and produces an extremely sour and bitter citrus fruit. It is generally not considered an edible fruit, but it is eaten in some countries. Due to the fruit's acidity and tart flavour it is popular for making preserves (especially, marmalade), syrup and liquors such as triple sec, grand marnier, cointreau, and curacao. The dried peel of the fruit is also used as a seasoning. The essential oils are used to add fragrance to beverages and liqueurs, sweet foods, soaps, detergents, cosmetics, and perfumes.

In the herbal medicine of western countries, the dried bitter orange peel has historically been used to stimulate appetite, for the treatment of dyspepsia and related conditions. The German Commission E (at the Federal Institute for Drugs and Medical Devices – Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) recognizes the medicinal value of bitter orange peel for loss of appetite and dyspeptic complaints; daily dosages are given in ranges of 4-6 grams for the dried peel, 2-3 grams for the tincture, and 1-2 grams for the extract. Bitter orange is also reported to have been rarely used as an expectorant, laxative, hypertensive, nervine, tonic, and diuretic (no therapeutic doses available) (BGA/BfArM 1987, Blumenthal 2005).

In Traditional Chinese Medicine (TCM), the two traditional medicines, *zhi shi* and *zhi qiao*, both based on bitter orange peel, are used for several indications that can be summed up in the Western medicinal term indigestion. The dosage ranges from 3 to 10 grams of the dried plant material in decoctions per day (Blumenthal 2005).

These historical indications are in surprising contrast to bitter orange's primary use today as a component of weight loss products.

Extracts (water/alcohol) of dried immature fruit and/or peel have been added to many dietary supplements and herbal weight loss formulas (as an alternative to *Ephedra*). Synephrine is believed to be the active ingredient and to act as a stimulant, agonist of adrenoceptors. Products are claimed to produce and/or maintain weight loss, improve physical fitness, and increase lean muscle mass. Such weight loss formulas usually contain 100-200 mg of bitter orange extract (NTP/NIEHS 2004, Blumenthal 2005).

p-Synephrine and *p*-octopamine are weak adrenergic agonists, active on both α- and β-adrenoceptors, but generally orders of magnitude less active than norepinephrine. The *meta* isomers of phenethylamine alkaloids, e.g. *m*-synephrine (phenylephrine) and *m*-octopamine (norfenefrine), are generally more potent adrenergic agonists than their *para* counterparts. *p*-Octopamine was reported to be more active than *p*-synephrine in some studies and less or equally active in other studies. The adrenergic activities of (+)-isomers are at least 1 to 2-fold lower than those of their (–)-counterparts. The activities of the respective synephrine and octopamine isomers towards α-adrenoceptors are about 1 or 2 orders of magnitude higher compared to their activities towards β-adrenoceptors (Jordan et al. 1987, Brown et al. 1988, Evans et al. 1988, Pellati et al. 2002, Pellati & Benvenuti 2007, Avula et al. 2005 & 2007).

Octopamine was reported to be a selective β3-adrenoceptor agonist, stimulating fat cell lipolysis by β3-adrenoceptor activation rather than by activation of other adrenoceptor subtypes and is being marketed as a weight-loss product having thermogenic properties and as an appetite suppressant (NTP/NIEHS 2004, Blumenthal 2005).

7. Information on existing assessments

A Swedish list concerning plants, which are considered as not suitable in foods states that extracts of *Citrus aurantium* L. ssp. *aurantium* L. with high content of synephrine are not suitable as a food (level not specified)[7].

A Danish list (Drogelisten) concerning toxicological evaluation of plants in food supplements states that extracts that concentrate synephrine are not acceptable (level not specified)[8].

The Council of Europe assessed plants as flavourings and classified infusions and the essential oil of the rind (peel) of *Citrus aurantium* L. ssp. *aurantium* L. in Category 3[9], with restrictions for furocoumarins (Council of Europe, 2000).

According to the Belgian Regulation (Arrêté Royal 29/8/1997 – annex list 3 and following acts), food supplements with a synephrine content up to 20 mg/daily dose are subject to notification before marketing as food supplement. Products with a synephrine content higher than 20 mg/daily dose are considered as medicinal products.

Health Canada issued two warnings towards consumers in Canada concerning the suspected cardiovascular adverse reactions of products containing bitter orange (*Citrus aurantium*) or synephrine and used for their claims of promoting weight loss. Health Canada states that such products are not authorized for sale in Canada (Jordan et al. 2004, Jack et al. 2007).

Citrus aurantium is listed in the Poisonous Plants Database of the U.S. Food and Drug Administration (US FDA)[10].

On the other hand, *C. aurantium* oil, extract, peel, flowers, and leaf are listed in the food additive database EAFUS ("Everything" Added to Food in the United States) with an indicator of the status of the toxicology information available for the substance *fully up-to-date toxicology information has been sought*[11].

Bitter orange peel is Generally Recognized as Safe (GRAS) as a direct additive to food, and the oils, extracts, and oleoresins of flowers and peel of bitter orange, and petitgrain oil (essential oil from leaves and twigs of the bitter orange plant) are also designated as GRAS (FDA 21CFR182) [12].

In frozen concentrated orange juice, the volume of bitter orange juice that may be added shall not exceed 5 % (FDA 21CFR146.146)[13].

FDA's guidance for the preparation of orange marmalade is suggesting that bitter orange marmalade be prepared by mixing at least 25 weight parts of fruit (peel and juice) to each 75 weight parts of sweetening ingredient (FDA/ORA CPG 7110.17)[14].

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[7] See http://www.slv.se/upload/nfa/documents/food_safety/lists_plants_may_05.pdf
[8] See http://www.dfvf.dk/Default.aspx?ID=11322
[9] Plants, animals and other organisms, and parts of these or products thereof, normally consumed as food items, herbs or spices in Europe which contain defined "active principles" requiring limits on use levels. Flavouring preparations, which are not themselves consumed as food but which are derived from plants, animals and other organisms, and parts of these or products thereof, normally consumed as food items, herbs or spices in Europe which contain defined "active principles" requiring limits on use levels. These source materials and preparations are not considered to constitute a risk to health in the quantities used provided that the limits set for the "active principles" are not exceeded.
[10] See http://www.cfsan.fda.gov/~djw/plantox.html
[11] See http://www.cfsan.fda.gov/~dms/eafus.html
[12] See http://www.cfsan.fda.gov/~lrd/fcf182.html
[13] See http://www.cfsan.fda.gov/~lrd/FCF146.html
[14] See http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg550-575.html

Most of these safety regulations have been created by the US FDA decades ago when bitter orange extracts standardized to concentrations of *p*-synephrine as high as 6 % did not exist on the market (NTP/NIEHS 2004, Blumenthal 2005).

The World Anti-Doping Agency (WADA) does not consider synephrine or phenylephrine as *prohibited substances* in The Prohibited List for neither 2008 nor 2009 (as compared to ephedrine), but both these substances are included in WADA's monitoring program of stimulants. Besides, synephrine is considered a banned substance by some national athletic associations (WADA 2007, 2008, NTP/NIEHS 2004).

The Working Group notes that several evaluations conclude that there is no safety issue related to the regular food use of bitter orange, while recent evaluations of preparations with high content of *p*-synephrine concluded that there may be a possible safety concern.

8. Exposure

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 (beer, liquors and other beverages, cakes, etc.). 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.

p-Synephrine is present in most *Citrus* fruits, both in their juice and peel. The presence of *p*-synephrine is hence probably more widespread in the conventional food supply than generally recognized. On the other hand, *p*-synephrine is not broadly distributed in higher plants; apart from the genus *Citrus*, it is present in only a few non-food plants (Blumenthal 2005).

Calculations based on HPLC measurements suggest that the percentage of *p*-synephrine in marmalade is in the range of 0.01%, an extremely low level of synephrine; the amount of marmalade usually consumed per serving is also quite low (20 – 30 g). Higher concentrations of *p*-synephrine are found in some *Citrus* juices, e.g. orange juice (ranges from 15 to 27 mg/l); tangerines (125 mg/l) and mandarins (280 mg/l) seem to be the richest source (Blumenthal 2005). Dragull et al. (2008) studied Satsuma mandarins and found that the content of *p*-synephrine in juice varies greatly upon several influences (73 to 158 mg/l). Avula et al. (2005 & 2007) found synephrine levels from 3 to 60 mg/l in 48 different *Citrus* juices and amounts of 0.018-1.02 mg per serving in 32 *Citrus* jams. As for mandarins, fresh or canned fruits are prevailing on the market, whereas mandarin juice products are rare, because of certain off-flavours as well as changes in flavour that occur during storage of the juice.

Weight loss formulas usually contain 100-200 mg bitter orange extract, which provides 6-40 mg *p*-synephrine per dose (NTP/NIEHS 2004, Blumenthal 2005).

The historical medicinal use of bitter orange peel – to stimulate appetite and for the treatment indigestion – is similar in both Western medicine and TCM. The following recommended daily dosages are found in the literature: 4-6 g (dry peel) in drugs, 2-3 g in tincture, and 1-2 g in extract (German Commission E, BGA/BfArM 1987), 3-10 grams of the dried peel in decoctions (TCM, Blumenthal 2005).

A theoretical calculation of an exposure to *p*-synephrine from food for a person with an individual preference for a *Citrus*-rich diet could be estimated based upon the values given above as high as 54 mg of *p*-synephrine (2 litres of orange juice)/day/person or even 280 mg *p*-synephrine (more than 1 kg of mandarins containing 1 litre of juice)/day/person. The intake of orange marmalade adds only

about 1 mg of *p*-synephrine per serving to these values. There is no information on *p*-synephrine levels in other foodstuffs (alcoholic beverages, liquors, sweets, bakery, seasoning).

It could be hypothesized that a dose of e.g. 30 mg of *p*-synephrine/day/person that occurs from the intake of 500 mg of bitter orange extract (standardized to 6 % *p*-synephrine) in weight loss pills equals to the daily intake of 1.1-2 litres of orange juice (containing 15-27 mg *p*-synephrine per litre depending on the variety), or of 0.1-0.4 litres of mandarin juice, or of 30 servings of marmalade, or of decoctions from 8-12 grams of dried bitter orange peel (as used for traditional medicinal purposes, e.g. indigestion).

9. Toxicological data

Food supplements claimed for weight loss and containing extracts of bitter orange peel with higher amounts of synephrine, one of the plant's natural components, appeared on the world market to a higher extent after the US FDA banned similar food supplements containing extracts of *Ephedra* and its alkaloid ephedrine in 2004[15].

Citrus aurantium has been suggested as a *safe* alternative to *Ephedra* and food supplements are often sold with remarks such as *Ephedra-free*. The actual safety of such extracts and their components has been under investigation since that time. Some experts have hailed the potential therapeutic value of *Citrus aurantium*, while others have warned about possible safety concerns. Reservations mainly surround potentially adverse cardiovascular and cerebrovascular effects. Safety-related information primarily comes from animal studies, clinical trials, acute physiologic studies in humans and case reports. To date, no large epidemiologic studies of the safety of *Citrus aurantium* preparations exist (Haaz et al. 2006, Fugh-Berman & Myers 2004).

Oral administration (single daily gavage) of *C. aurantium* fruit hydroalcoholic extracts standardized to two different concentrations of *p*-synephrine, 4 % or 6 %, each given in 4 different doses (2.5, 5, 10, or 20 mg/kg bw, respectively) to male Sprague-Dawley rats for 15 days (i.e. 8 groups of test animals) caused a significant and dose-dependent decrease in food intake and body weight gain. Deaths of the animals occurred in all treatment groups: at the lowest dose, 10% mortality was seen with both extracts; at the highest dose, 30 and 50% mortalities were reported for extracts standardized to 4 % and 6 % of *p*-synephrine, respectively. No marked changes were seen in blood pressure; however, ventricular arrhythmias with enlargement of the QRS complex were observed, their occurrence increased dose-dependently (Calapai et al. 1999).

The highest dose given in this study (i.e. 20 mg/kg bw of the extract standardized to 6 % *p*-synephrine) corresponds to 1.2 mg *p*-synephrine/kg bw. As 50 % of the test animals died in the course of the experiment when given such a dose of the extract, this dose can be considered an approximation of a possible LD_{50} value for rats.

Acute oral administration of C. aurantium extracts (2.5% *p*-synephrine, 300-5000 mg/kg bw) in mice produced reduction of locomotor activity, *p*-synephrine (150-2000 mg/kg bw) produced piloerection, gasping, salivation, exophtalmia and reduction in locomotor activity, which was confirmed in spontaneous locomotor activity test. All the effects were reversible and persisted for 3-4 h (Arbo et al. 2008).

A huge amount of experimental studies has been conducted on the pharmacology and toxicology of isolated bitter orange alkaloids, *p*-synephrine and *p*-octopamine.

[15] See http://www.fda.gov/oc/initiatives/ephedra/february2004/

In humans, rats, guinea pigs, cats, and/or dogs, synephrine, octopamine, and extracts of *C. aurantium* have been evaluated for effects on the cardiovascular system that include changes in blood pressure, cardiovascular toxicity, contractility and excitability of the heart muscle, and/or adrenergic activity. Pressor effects and the ability to restore contractility and excitability to heart muscle were reported for synephrine and octopamine (NTP/NIEHS 2004, Blumenthal 2005, Jordan et al. 1987, Brown et al. 1988, Evans et al. 1988)

In China, in cases of shock, *p*-synephrine is administered intravenously, together with *N*-methyltyramine, at doses of 20 – 60 mg each, depending on the severity of the shock, diluted in normal saline or glucose solution. It was suggested that synephrine could be considered a stimulant of cardiac performance, usually when administered via injection. A direct relationship, however, cannot be assumed between the activities of intravenously administered, isolated *p*-synephrine to the orally consumed bitter orange extracts found in dietary supplements (Blumenthal 2005).

Studies evaluating the effect of bitter orange on cardiovascular parameters have reported mixed results. Some clinical research suggests that bitter orange, in combination with caffeine, can increase systolic and diastolic blood pressure and heart rate in otherwise healthy normotensive adults (Haller et al. 2005 & 2008). Taking a single dose of bitter orange extract (900 mg), standardized to 6 % *p*-synephrine (54 mg), seems to increase diastolic and systolic blood pressure and heart rate for up to 5 hours in young, healthy adults (Bui et al. 2005). But using half that dose of bitter orange and providing half as much *p*-synephrine, does not seem to significantly affect the blood pressure or the QT interval in healthy adults (Min et al. 2005).

Health Canada published two summaries of the case reports on adverse reactions suspected of being associated with bitter orange and *p*-synephrine-containing natural health products. From 1998 to 2006 there were 37 reports, 31 of these were cardiovascular adverse reactions (tachycardia, cardiac arrest, ventricular fibrillation, transient collapse and blackout), of which 26 were serious. Two of the patients died, both of whom had taken products containing *Ephedra*/ephedrine and caffeine in addition to bitter orange. There was another case of myocardial infarction. It is concluded that an increased risk of adverse reactions is associated with the use of synephrine-containing products by people with heart conditions, diabetes, thyroid disease, central nervous system disorders, glaucoma, pheochromocytoma, hypertension, known risk factors for cardiovascular disease, or enlarged prostate, by underweight people, people taking thyroid hormones, monoamine oxidase inhibitors, medications to control heart rate or blood pressure, or caffeine-containing products (Jordan et al. 2004, Jack et al. 2007, Firenzuoli et al. 2005). Other case reports of serious adverse reactions have been published in the meantime, including cases of coronary thrombosis and severe exercise-induced rhabdomyolysis (Smedema & Müller 2008, Burke et al. 2007).

Only one of the case reports (Jordan et al. 2004, Jack et al. 2007) is associated with a suspect product containing bitter orange but no caffeine nor *Ephedra*/ephedrine (no data is given of possible other ingredients).

In most of the studies combined exposure with other ingredients may have occurred and it is beyond the present safety evaluation to analyse the combined effects. NTP is presently performing an evaluation of the combined effects of bitter orange (6% extract) and caffeine[16].

No studies evaluating disposition, metabolism, or kinetics of administered bitter orange products were available. Chronic exposure, cytotoxicity, carcinogenicity, or tumor initiation/promotion studies were not available (NTP/NIEHS 2004).

Reproductive and teratological effects: In rats, daily intramuscular injection of *p*-synephrine (55 or 110 mg/kg [0.33 or 0.66 mmol/kg]) on days 7-16 of pregnancy decreased the number of uterine

[16] See http://ntp.niehs.nih.gov/go/TS-M050092

implants and viable foetuses, increased mean foetal weight and the number of micro foetuses, and retarded cranial and thoracic ossification. Additionally, renal and intestinal haemorrhage, brain hypoplasia, and unilateral microphthalmia were reported in some foetuses (NTP/NIEHS 2004).

Genotoxicity: In the L5178Y mouse lymphoma assay, *p*-synephrine (20-3600 µg/ml [120 µM-21.53 mM]) was inactive (NTP/NIEHS 2004).

6',7'-Dihydroxybergamottin and naringin, found in grapefruit juice and to a lesser extent in some orange juices, inhibits the metabolism of substrates for enzymes of the CYP3A subfamily. Due to the very low content of this compound in *Citrus aurantium* juice, peel and extracts, the risk of CYP-mediated herb-drug interactions in humans is considered minimal (Malhotra et al. 2001, Gurley et al. 2004, Fugh-Berman & Myers 2004).

10. Safety assessment based on available knowledge (Level A)

Bitter orange fruits, peels, and the oil from peel are all used to make orange marmalade. The average consumption of culinary products containing bitter orange peel (mostly marmalade, liqueurs, beer and sweets) may be considered safe on the historical level. It can be expected that the amount of *p*-synephrine from such a dose does not affect physiological functions significantly. The exposure to *p*-synephrine from the average consumption of such products is comparable to the exposure that occurs from the consumption of other *Citrus* products like orange fruits or juices that have become a normal part of a common European diet (e.g. the amount of 1 mg of synephrine is being consumed in 1 serving of *Citrus* marmalade or in 37 – 67 ml of orange juice).

While *Citrus aurantium* extracts have been used in a variety of cultures for thousands of years, they have not been traditionally utilized for long time periods, nor specifically for weight loss (Haaz et al. 2006).

Bitter orange extracts in food supplements, such as weight-loss pills, however, have been reported to be enriched in the content of *p*-synephrine, typically to an amount of 6 – 10 % (but even extracts with a content of 95 % *p*-synephrine are documented). This fact represents a significant increase of intake compared to historical levels due to the intended levels of use in food supplements.

It is concluded that for traditional food uses of bitter orange, there is no presumed safety concern, whereas for the safety assessment of the use of bitter orange preparations with *p*-synephrine content above 6%, additional data would be required (Level B). The Working Group notes that other evaluations have recommended that additional data are required when the estimated maximum intakes of synephrine would exceed 20 mg/day (Belgian Arrêté Royal 29/8/1997 – annex list 3 and following acts). The average exposure to *p*-synephrine from a normal balanced diet that includes occasional consumption of *Citrus* fruits and juices would probably not exceed the mentioned value, i.e. 20 mg *p*-synephrine per day

11. Further testing and/or data required for the assessment (Level B)

In cases where estimated exposure would exceed traditional intake levels, additional data would be required. Data requirements would be in line with existing guidelines e.g. for food supplements or novel foods, and include studies evaluating disposition, metabolism, or kinetics of administered bitter orange products (see guidance). This may also hold for genotoxicity, long term toxicity and developmental toxicity.

p-Synephrine is considered an agonist of adrenoceptors. As an isolated pure drug, *p*-synephrine is often administered via injection. It has been stated however, that *p*-synephrine is not well absorbed in the gastrointestinal tract. This issue of mode of administration and of the relatively poor oral absorption of *p*-synephrine is obviously significant, particularly for dietary supplements, which, by legal definition are consumed orally (Blumenthal 2005).

It is difficult to confidently extrapolate from short-term studies of substances used for one indication in one population (e.g. several days for relief of nasal congestion among the general population) to long-term use for another indication in another population (e.g. several months or years for weight loss among obese individuals). Although some safety-related data exists for bitter orange preparations in general, there is no published human trial of *Citrus aurantium* extracts for weight loss with more than 20 people or for more than 7 weeks, limiting current conclusions (Haaz et al. 2006, Fugh-Berman & Myers 2004, Bent et al. 2004, Colker et al. 1999).

Many studies addressing the safety of *Citrus aurantium* extracts are performed using normotensive subjects. Because hypertension is more common in overweight and obese subjects than in normal-weight individuals, more studies are warranted looking at the effects of *Citrus aurantium* extracts in a hypertensive population. Additionally, there are no case–control or epidemiologic cohort studies of *Citrus aurantium* extracts with respect to hard safety endpoints such as myocardial infarction or stroke (Haaz et al. 2006).

Most of the data that concern case reports and results of conducted studies of food supplements that contain *p*-synephrine is controversial, as subjects ingested multicomponent dietary supplements, containing e. g. ephedrine in *Ephedra*, or caffeine in botanicals such as guarana and maté, next to *p*-synephrine in bitter orange. Some report that the use of bitter orange with stimulants such as *Ephedra* causes cardiotoxicity. Similarly, the combination of *p*-synephrine and caffeine has been reported to have the potential in inducing cardiac arrhythmias, hypertension, heart attacks, and strokes (NTP/NIEHS 2004, Blumenthal 2005, Jordan et al. 2004, Jack et al. 2007).

The U. S. National Toxicology Program (NTP) is currently performing tests of short-term toxicity, organ systems toxicity and conventional teratology of a 6% bitter orange extract on Sprague Dawley rats (doses: 10, 25, 50, and 100 mg/kg bw), as well as of a combination of bitter orange extract and caffeine (50 mg/kg bw 6% bitter orange extract, plus 25 mg/kg bw caffeine).

The testing status (assigned/on test) and the standard methodology of the tests can be found on the NTP webpage so far[17]. The specification of the bitter orange extract would be of interest, as the question arises if the given information "6% extract" refers to the drug/liquid-extract ratio, or to the content of *p*-synephrine in the dried extract, as the standardisation value of 6 % seems to be popularly declared amongst bulk suppliers and food supplements producers.

[17] See http://ntp.niehs.nih.gov/go/TS-M040019 and http://ntp.niehs.nih.gov/go/TS-M050092

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APPENDIX B: *CAMELLIA SINENSIS* (L.) O. KUNTZE

The present report focuses on "Dried Green Tea Extracts".

Disclaimer:

The present document aims at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected case and considers relevant constituents. The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated. The document is not, and cannot be interpreted as being, a policy document or a decision allowing the classification of a certain botanical or botanical preparation as a foodstuff or as a medicinal product. The document is focusing on one type of preparation and is not intended to evaluate all possible preparations of this botanical or all possible constituents, which should normally be part of a full safety assessment of a botanical. Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete. Contaminants such as heavy metals, mycotoxins, dioxins, pesticides, microbial contamination or PAHs are not evaluated.

It is outside the remit of this Working Group to evaluate possible beneficial effects and associated claims

1. Identity and nature of the source material

Scientific name:	*Camellia sinensis* (L.) O. Kuntze
Synonyms:	*Camellia thea* Link, *Thea sinensis* L., *Thea viridis* L, *Theaphyla lanceolata* Raf. , *Theaphyla laxa* Raf. , *Theaphyla viridis* Raf.,
	Varieties: *Camellia sinensis* (L.) Kuntze var. *sinensis* (Engl.: China-Tea, small-leaved tea plant) *Camellia sinensis* (L.) Kuntze var. *assamica* (J.W. Mast.) Kitam. (Syn. *Camellia assamica* (Mast.) Hung T. Chang, *Camellia theifera* Griff. , *Thea assamica* J.W. Masters, *Camellia assamica* (J. W. Mast.) W. Wight; Engl.: Assam-Tea, Ceylon-Tea, Indian-Tea, large-leaved tea plant)
Common names:	Engl.: Tea plant, French: Thé, théier, Germ.: Teestrauch, Ital.: Te
Family:	Theaceae
Parts used:	Young leaves and leave buds used to produce traditional "green tea" as the basis for traditional aqueous green tea infusions. Leaves and leaf buds are also used to produce the so called "dried green tea extracts".

Geographical origin: The tea plant originated in Southeast Asia and is presently cultivated in over 30 countries (e.g. Burma, China, India (Assam), Indonesia, Japan, Sri Lanka). Green tea is produced e.g. in China, Japan, Indonesia, Vietnam, GUS, Taiwan, India.

Growth and harvesting conditions: Much hybridization has occurred. Breeding, vegetative propagation, and selection have resulted in the emergence of thousands of lines with varying properties, including compositional differences (e.g. Graham, 1992).

2. Manufacturing process

Depending on the manufacturing process mainly three different traditional tea products are prepared from the leaves and leaf buds of *C. sinensis*: Green tea, black tea and oolong tea. While "green tea" is produced without fermentation and thus preventing oxidation of the polyphenolic components, "black tea" manufacture is carried out by fermentation ensuring a high degree of enzymatically catalyzed aerobic oxidation of the polyphenols followed by a series of chemical condensations. In "oolong tea", a semifermented tea, polyphenols are partially oxidized. The present opinion evaluates "dried green tea extracts" from *C. sinensis* and refers also to the data of traditional green tea infusions for reasons of comparison. Thus the following green tea extracts are taken into account:

2.1. Traditional green tea infusions:

Traditional green tea infusion are made from "traditional green tea" that is prepared as follows: To preserve the leaf catechins after harvesting the tea leaves, an enzyme deactivation is performed by rapid steaming (Japanese green tea) or pan firing/roasting (Chinese green tea) before rolling and high temperature air drying (e.g. Graham, 1992).

Depending on the quality of green tea the recommendations for preparing traditional green tea infusions vary in amounts of green tea and water used (usually 0.75 - 1.5 g green tea /100 ml), temperature of water (50 - 100°C, usually sub-boiling), brewing time (30 sec – 3 min) and the possibility of a repeated extraction (e.g. recommendation to discard the first and consume the second extraction) (e.g. Scholz, 1995; Astill et al., 2001).

2.2. "Dried green tea extracts":

Commercial preparations use various extraction techniques and manufacturing procedures and are not uniform. They may differ from the traditional green tea infusion not only in the deprivation of water but also e.g. in the solvent being different from water, in the source (e.g. fresh leaves instead of green tea), in extraction conditions (e.g. degree of comminution, concentration ratios, temperature, duration, stirring) and in fractionation procedures concentrating active compounds. Some tea extract powders or dry extracts are made by spray drying strong infusions obtained by soaking tea leaves in ethanol/water mixtures after they have been concentrated to 40-50 % solids (e.g. Sarma et al., 2008; Wang et al., 2000; Lin et al., 2003; Liebert et al., 1999).

3. Chemical composition

In the present section first the chemical composition of traditional green tea is given, which identifies the material from which the green tea extracts evaluated are prepared, followed by the chemical composition for traditional green tea infusions and dried green tea extracts.

3.1. Traditional green tea

The plant variety, growing environment, season, age of the leaves, and manufacturing conditions have been shown to have a pronounced impact on the green tea composition.

Polyphenols: Green tea contains a diversity of polyphenolic compounds, which account for up to 30% of the dry weight of green tea leaves. Most of the polyphenols in green tea are flavanols, commonly known as catechins. The primary catechins in green tea are (-)epicatechin (EC), (-)epicatechin-3-gallate (ECG), (-)epigallocatechin (EGC), and (-)epigallocatechin-3-gallate (EGCG). Furthermore (+)catechin (C), (+)gallocatechin (GC), (-)gallocatechingallate (GCG), (-)catechingallat (CG), occur in green tea (Table 1). Young leaf green tea contains lower levels of EGCG and total catechins than old leaf green tea (Lin et al., 2003).

Purine alkaloids: caffeine (previously referred to as theine or teine; depending upon the development stage of the leaves, 2.9-4.2%, content declining with age), theobromine (0.15-0.2%), theophylline (0.02-0.04%).

Amino acids: The total amino acids content in green tea amounts to 4%, including the tea characteristic L-theanine as a major component (2% of green tea).

Proteins: 15%.

Triterpene saponins: theafolia saponins, aglycones including, among others, barringtogenol.

Caffeic acid derivatives: including, among others, chlorogenic acid, theogallin.

Lignin: 6.5%.

Lipids: 3%.

Polysaccharides: 13%.

Anorganic ions: fluoride (130-160 mg/kg), content depending on soil (quality), potassium and aluminum ions.

Volatile oil: chief components linalool (Mitscher et al., 1997; Scholz, 1995; Gruenwald, 1998; USDA Database, 2007).

Table 1: USDA database for the flavonoid content of dry green tea leaves (USDA, 2007)

Subclass of Component	Flavonoid	Mean (mg/100g)	Standard Error (mg/100g)	N	Min (mg/100g)	Max (mg/100g)
Flavan-3-ols	(-)-Epicatechin	811.72	21.10	68	190.00	2000.00
	(-)-Epicatechin 3-gallate	1491.29	112.42	68	340.00	4630.00
	(-)-Epigallocatechin	2057.98	103.55	68	100.00	5477.40
	(-)-Epigallocatechin 3-gallate	7115.98	632.06	68	1600.00	20320.00
	(+)-Catechin	57.12	3.40	38	0.00	252.88
	(+)-Catechin 3-gallate	7.07	1.83	6	0.00	14.14
	(+)-Gallocatechin	258.11	80.69	6	69.46	446.76
	Theaflavin	1.64	0.74	10	0.00	6.2451
	Theaflavin-3,3´-digallate	1.08	0.63	4	0.00	2.3947
	Theaflavin-3´-gallate	0.44	0.26	4	0.00	0.9933
	Theaflavin-3-gallate	0.47	0.32	10	0.00	2.7387
	Thearubigins	131.91	131.91	4	0.00	527.62
Flavones	Apigenin	12.03	2.86	9	0.00	23.76
	Luteolin	0.17	0.17	3	0.00	0.50
Flavonols	Kaempferol	147.55	4.40	18	77.61	77.611
	Myricetin	104.76	7.94	18	31.16	164.41
	Quercetin	223.97	9.60	18	54.36	405.00

3.2. Traditional green tea infusions:

Aside from influences due to differences in the botanical source the concentrations of components in a traditional green tea infusion have been shown to be strongly dependent on the way the consumer prepares it (amounts of tea and water used, the brewing time (Table 2) and the degree of agitation), and to be affected by the grade of comminution of the tea leaves and whether they are contained in a teabag.

Table 2: Effect of brew time on the caffeine and total catechins extracted from a teabag containing 3.125g tea leaves (probably black) with 200 ml of water (Astill et al., 2001)

brew time (s)	mg/l in aqueous extract	
	catechins	**caffeine**
30	22.1 (19.9%) [a]	108.0 (34.6%)
60	36.5 (32.9%)	159.2 (50.9%)
120	62.9 (56.7%)	227.3 (72.7%)
300	90.5 (81.6%)	285.9 (91.5%)

[a] Extraction efficiencies in parentheses are calculated from leaf content of 2.21% caffeine and 0.71 % catechins.

Table 3 gives the range and Table 4 gives examples of flavonoid contents of traditionally prepared green tea infusions.

Table 3: USDA database for the flavonoid content of brewed green tea infusions (USDA, 2007)

Subclass of component	Flavonoid	Mean (mg/100g)	Standard Error (mg/100g)	N	Min (mg/100g)	Max (mg/100g)
Flavan-3-ols	(-)-Epicatechin	8.29	0.49	67	1.90	26.00
	(-)-Epicatechin 3-gallate	19.73	2.76	67	4.30	139.60
	(-)-Epigallocatechin	16.71	1.41	67	1.00	54.40
	(-)-Epigallocatechin 3-gallate	77.81	6.97	67	2.31	203.20
	(+)-Catechin	2.55	1.53	39	0.00	44.40
	(+)-Gallocatechin	1.54		3	1.54	1.54
	Theaflavin	0.05	0.01	4	0.02	0.08
	Theaflavin-3,3´-digallate	0.01	0.01	4	0.00	0.03
	Theaflavin-3´-gallate	0.01	0.00	4	0.00	0.01
	Theaflavin-3-gallate	0.01	0.01	4	0.00	0.03
	Thearubigins	1.08	1.08	4	0.00	4.30
Flavones	Apigenin	0.17	0.17	3	0.00	0.50
	Luteolin	0.17	0.17	3	0.00	0.50
Flavonols	Kaempferol	1.42	0.22	12	0.67	3.31
	Myricetin	1.10	0.11	12	0.52	1.60
	Quercetin	2.69	0.26	12	1.40	4.10

The large concentration range given for EGCG in Table 3 is assumed to reflect differences due to variation in extraction methods. The data are in accordance with the range published by Bronner et al. (1998) compiling data from literature (EGCG in green tea infusions: 5 - 190 mg/100ml). Using a defined extraction method (stirring of 0.25 g green tea leaves with 80 ml boiling water for 3 min or 20 min) Bronner et al. (1998) determined 6 mg EGCG /100 ml and 9 mg EGCG /100 ml infuse, respectively (averages of 6 measurements; 2 batches of tea each analyzed in triplicate). These data are consistent with results of Khokar et al., (1997) (Table 4) using a higher concentration of green tea leaves for extraction.

Table 4: Contents (mean values of 3-5 determinations) of catechins in a Chinese and a Japanese green tea infusion (preparation: 1g tea leaves were brewed with 100 ml boiling water and decanted after 5 minutes. Prior to this it was shaken for 30 seconds). The (+)-catechin contents are below the detection limit (10 µg/ml) (1). a = percentage referred to total catechins (Khokar et al., 1997).

	EC (mg/100 ml)	ECG (mg/100 ml)	EGC (mg/100 ml)	EGCG (mg/100 ml)	Total catechins (mg/100 ml)
Green tea infusion					
China	4.7 (9.1 %)[a]	4.4 (8.5 %)[a]	16.3 (31.7 %)[a]	26.3 (51.1 %)[a]	51.5
Japan	9.4 (11.1 %)[a]	5.9 (6.9 %)[a]	28.7 (33.8 %)[a]	40.8 (48.1 %)[a]	84.9

3.3. "Dried green tea extracts":

The concentrations of components in "dried green tea extracts" vary in a wide range depending on the source material and the extraction procedure. Commercial preparations are now available that have been decaffeinated and that contain enriched quantities of polyphenolics (60-80% or more of dry weight) with EGCG particularly prominent in the mixture (Mitscher et al., 1997).

4. Specifications

The specifications for "dried green tea extracts" should apply to the different catechins (above all to EGCG), caffeine and L-theanine representing the major biologically active components. The specifications should also refer to contamination with pesticides and benzo(a)pyrene. Analysis for pesticides of 38 Chinese green teas, 22 Japanese green teas and 8 green teas of different origin commercial available in Germany revealed 15, 17, and 4 samples, respectively, containing pesticide residues above the admissible maximum concentration (Stiftung Warentest, 1999). Determination of benzo(a)pyrene in 22 food supplement products (capsules) containing green tea preparations revealed contamination of 18 products with benzo(a)pyrene at levels in the range of 0.39 - 144.7 µg /kg leading to daily intakes up to 224.8 ng benzo(a)pyrene/person. This contamination is thought to be due to heating processes in the manufacture of green tea preparations (Martena, 2008).

5. Stability of the botanical ingredient

A stability study demonstrated that green tea catechins are stable in water at room temperature. When pure EGCG was autoclaved at 120° C for 20 min, epimerization of EGCG to (-)gallocatechin gallate (GCG) was observed. The relatively high amount of GCG found in some tea drinks was most likely due to the epimerization of EGCG during autoclaving.

It is suggested that other ingredients used in production of tea drinks might interact with green tea catechins and affect their stability. When canned and bottled tea drinks are produced, stored, and transported, the degradation of green tea catechins must be taken into consideration (Chen, 2001).

The epimeric isomers of (-)-epicatechin, (-)-epicatechin-3-O-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-O-gallate (EGCG), and (-)-epigallocatechin-3-O-(3-O-methyl)-gallate in green tea extracts increase with the extraction time at 90° C. The epimerization rates of authentic tea catechins in distilled water are much lower than those in tea infusion or in pH 6.0 buffer solution (Suzuki et al., 2003).

The epimerization of the catechins was inhibited when the tea catechins extract was heated as solid powder and the dry tea was extracted in 50% (v/v) ethanol or in water at 80° C or below. In order to reveal profiles of tea catechins in teas to be analyzed, it is recommended that they are extracted with 50% (v/v) ethanol for 10 min, while fresh tea leaves should be extracted with 75% (v/v) ethanol for 10 min (Liang et al., 2007).

6. Proposed uses and levels

Dried green tea extracts are used as food including beverages and food supplements, and as pharmaceuticals.

6.1. Uses as food:

As a stimulant drink in form of infusions, of ready-to-drink beverages on the basis of "dried green tea extract" or of beverages prepared by the consumer from instant green tea powder (for use levels see data presented in the section on exposure).

As non stimulant beverage e.g. in form of decaffeinated green tea beverages.

As food supplements or related products in solid form or as a drink in many cases on the basis of dried green tea extracts and advertised with the following arguments (e.g. list of claims submitted under Article 13 of Reg. (EC) 1924/2006[18]):

- to help reducing weight and to support lipid metabolism (daily dose: the equivalent of minimum 150 mg caffeine, 115-270 mg EGCG, and 375 mg catechins),

- to protect body tissues from oxidative damage, to contribute to healthy ageing by maintaining intact cell DNA, and to help to maintain healthy bones, skin health, or normal blood glucose levels, (daily doses: the equivalent to 1 - 3 cups of tea per day delivering 360 - 1080 mg tea solids),

- to support natural sleep and relaxation (daily dose: equivalent of 45-200 mg L-theanine).

[18] See http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_article13.htm

6.2. Pharmaceutical use:

Traditional green tea infusions

Traditional green tea infusions are used for treatment of stomach disorders, vomiting, and diarrhea (Gruenwald et al., 1998 – Physicians' Desk Reference (PDR) for Herbal Medicine).

Dried green tea extracts

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 because of hepatotoxic side-effects (AFSSAPS, 2003, Sarma et al., 2008).

Clinical trials to evaluate the possible therapeutical efficacy of green tea extracts, often enriched in EGCG, in patients suffering from advanced cancer or other chronical diseases (e.g. prostate cancer, breast cancer, bladder cancer, lymphocytic leukemia, Parkinson's disease, type 2 diabetes, ulcerative colitis, relapsing-remitting multiple sclerosis) are underway or in the recruiting stage (Clinical Trials - NIH, 2008).

Green tea extract is used topically in the form of an ointment for the treatment of external genital and perianal warts (FDA, 2006).

7. Information on existing assessments

The AFSSAPS (French Health Products Safety Agency) published a press release on 7 April 2003 stating that the market authorization of a phytotherapeutical drug, recommended as adjunct to weight-loss programs, was suspended because the product was suspected of having caused hepatic disorders in 13 cases (9 in France, 4 in Spain), of which 4 cases were serious. One capsule of the phytotherapeutical drug contained 375 mg of a patented hydroalcoholic green tea extract, which was obtained using 80% ethanol as the extraction agent and which was standardised to 25% EGCG. Furthermore the extract contained 5-10 % caffeine. The drug was reported to be used at a recommended dose of two capsules twice a day (daily dose corresponding to 375 mg EGCG). AFSSAPS declared that its decision did not apply to other medicinal products composed of green tea (weak hydroalcoholic extract, aqueous extract and leaf powder) authorised in France and that it does not challenge the traditional use of green tea in phytotherapy or in food (AFSSAPS, 2003). Some new cases of liver damage have been reported to AFSSAPS since 2006 with aqueous green tea food supplements, one of which with a high causality (AFSSA, 2009).

In response to communicated hepatotoxicity concerns the US Pharmacopeia (USP) Dietary Supplement Information Expert Committee (DSI EC) systematically reviewed the safety information for green tea products. A total of 216 case reports on adverse side effects of green tea products were analysed, including 34 reports concerning liver damage. Twenty-seven reports pertaining to liver damage were categorized as possible causality and seven as probable causality. Clinical pharmacokinetic and animal toxicological information were also taken into consideration. The available data indicate that consumption of green tea concentrated extracts on an empty stomach is more likely to lead to adverse effects than consumption in the fed state. DSI EC suggested that USP green tea extract monographs carry the following labelling caution statement: "Take with food. Discontinue use and consult a health care practioner if you have a liver disorder or develop symptoms of liver trouble such as abdominal pain, dark urine, or jaundice." The Committee proposal does not pertain to traditional green tea infusions or other beverage preparations (Sarma et al., 2008).

Regarding a health claim petition FDA concluded that there was no credible evidence to support qualified health claims for green tea consumption and a reduced risk of gastric, lung, colon/rectal, esophageal, pancreatic, ovarian, and combined cancers. Furthermore, FDA concluded that there was very limited credible evidence for qualified health claims specifically for green tea and a reduced risk of breast cancer and for green tea and a reduced risk of prostate cancer (FDA, 2005).

On evaluation of data on clinical pharmacology and toxicology the FDA approved an herbal ointment, containing 15% of green tea ingredients for the treatment of external genital and perianal warts caused by certain strains of human papillomavirus. These ingredients were a partially purified fraction of the water extract of green tea leaves from *Camellia sinensis* (L.) O. Kuntze, representing a mixture of catechins and other green tea components. Catechins constitute 85 to 95% (by weight) of the total drug substance which includes more than 55% of EGCG, other catechin derivatives such as EC, EGC, ECG and some additional minor catechin derivatives i.e. GCG, GC, CG and C. In addition to the known catechin components, it also contains gallic acid, caffeine, and theobromine which together constitute about 2.5% of the drug substance. The remaining amount of the drug substance contains undefined botanical constituents derived from green tea leaves (FDA, 2006).

8. Exposure

8.1. Traditional green tea infusion

According to the data from the RKI nutrition survey in Germany (RKI, 1998; Mensink, 2002) the mean value for the consumption of green tea infusions is 362 g/person/day and the 95th percentile is 1097 g/person/day. Referring to the catechin contents in samples of Chinese and Japanese green tea (Table 4) exposure estimates are calculated as given in table 5.

Table 5: Daily exposure to catechins from the consumption of traditional green tea infusion with mean (mean: 362 g infusion/day) and high exposure (95th percentile: 1097 g infusion/day) (RKI, 1998).

		EC (mg/day)	ECG (mg/day)	EGC (mg/day)	EGCG (mg/day)	Total catechins (mg/day)
Green tea China	Intake of 362 g of the infusion/day	17.0	15.9	59.0	95.2	186.4
	Intake of 1097 g of the infusion/day	51.6	48.3	178.8	288.5	565.0
Green tea Japan	Intake of 362 g of the infusion/day	34.0	21.4	103.9	147.7	307.3
	Intake of 1097 g of the infusion/day	103.1	64.7	314.8	447.6	931.4

8.2. Food supplements

Exposure with green tea components from food supplements may vary considerably.

According to the proposed use levels for green tea preparations in food supplements offered for weight reduction purposes, daily doses of 150 mg caffeine, 115-270 mg EGCG, and 375 mg catechins may be exceeded. With products indicated to protect body tissues from oxidative damage, to

contribute to healthy ageing by maintaining intact cell DNA, and to help to maintain healthy bones, skin health, or normal blood glucose levels, exposures to daily doses equivalent to 1 - 3 cups of tea per day delivering 360 - 1080 mg tea solids (residue after drying a liquid extract) have to be expected.

9. Toxicological data

The consumption of traditional green tea infusions leads to intake of caffeine and is therefore associated with the known intended stimulant effects but also with the known adverse effects of caffeine in a dose-dependent way (Jellin et al., 2007). Concerning this, reference is made to the relevant pharmaceutical literature (e.g. Martindale, 2008) and to the assessment of caffeine as a constituent of so-called "energy" drinks by the SCF (SCF, 1999). Considering that green tea is often consumed daily in Asian cultures without having been associated with significant adverse effects, there are no safety concerns reported as regards traditional consumption of green tea infusions. No health hazards are known in conjunction with the proper administration of designated therapeutic dosages of traditional green tea infusions (Gruenwald et al., 1998 – Physicians' Desk Reference (PDR) for Herbal Medicine). The PDR for Herbal Medicine specifies, that side effects of tea consumption are possible for persons who have sensitive stomachs. Hyperacidity, gastric irritation, reduction of appetite, as well as obstipation or diarrhea can result from intense consumption. Concerning the caffeine content of green tea the PDR for Herbal Medicine suggests that care should be taken with persons who have weakened cardiovascular systems, renal diseases, thyroid hyperfunction, elevated susceptibility to spasm and certain psychic disorders, e.g. panicky states of anxiety. With long term intake of dosages above 1.5 g caffeine/day, non-specific symptoms occur, such as restlessness, irritability, sleeplessness, palpitation, vertigo, vomiting, diarrhea, loss of appetite and headache. Overdosage (quantities corresponding to more than 300 mg caffeine, or 5 cups of tea as a beverage) can lead to restlessness, tremor and elevated reflex excitability. The first signs of poisoning are vomiting and abdominal spasm. Infants whose nursing mothers consume beverages containing caffeine could suffer from sleep disorders. According to the PDR for Herbal Medicine pregnant women should not exceed a dosage of 300 mg/day (5 cups of tea spread out over the course of a day) or avoid caffeine.

Reference has to be made to a perspective cohort study, which has been published in 2008. It involves 1,063 women and records the course of pregnancy up to the 20th week of pregnancy. The investigators observed that compared to pregnant women with no caffeine intake, the women who were exposed daily to 200 mg caffeine were at increased risk of miscarriage (15% versus 12%) and that the corresponding risk for pregnant women with caffeine intakes of more than 200 mg was considerably higher (25% versus 12%). This result was independent of the type of caffeine-containing preparation (Weng et al., 2008).

Regarding new results from a large prospective observational study on maternal caffeine intake during pregnancy and the risk of fetal growth restriction (CARE Study Group, 2008), the Food Standard Agency gave advice to pregnant women to limit their daily caffeine intake, ideally keeping this below 200 mg a day (FSA, 2008).

Green tea appears to reduce the absorption of non-heme iron from foods (e.g. Jellin et al., 2007, Wang et al., 2000).

Jellin et al. (2007) summarize, that cases of hepatotoxicity, which is a concern with certain green tea extract supplements/medicaments, have not been reported in people consuming traditional green tea infusions.

Concerning L-theanine as an ingredient of green tea reference is made to the compilation of available safety data in a BfR report regarding the use of isolated L-theanine in beverages (BfR, 2003).

"Dried green tea extracts" and products on the basis of "dried green tea extracts"

Only selected exemplary data estimated to have relevance for the risk assessment on "dried green tea extracts" are presented in the following sections.

Human data

Toxicokinetic and safety studies

After oral absorption, tea catechins undergo glucuronidation, sulphatation and O-methylation (e.g. Chow et al., 2003; Lu et al., 2003; Higdon and Frei, 2003). Following ingestion of green tea extract by human volunteers, plasma EGCG is mainly found in the free form (64-77 %), while plasma EGC and EC were conjugated to a higher degree (Lee et al., 2002). Data on tissue distribution and excretion of green tea catechins and EGCG in humans are not available.

According to a randomized, placebo-controlled study in healthy volunteers (eight subjects per group) EGCG or a decaffeinated extract of green tea containing 60 % EGCG, orally administered once daily at a dosis of 800 mg EGCG/day for 4 weeks, were regarded as safe and well tolerated. Adverse events reported during the 4-week treatment period include nausea, stomach ache, dizziness, and muscle pain. All of the reported events were rated as mild events. No significant changes were observed in blood counts and blood chemistry profiles after repeated administration of green tea polyphenol products.Both catechin formulations exhibited similar EGCG kinetics on the last treatment day (e. g. for EGCG from the decaffeinated extract: C_{max} 287.6 ± 124.2 ng/ml; t_{max} 248 ± 184.9 minutes; halflife 163.0 ± 56.2 minutes). There was a >60% increase in the systemic availability (area under the plasma EGCG concentration-time curve, AUC) of free EGCG after chronic green tea polyphenol administration at a high daily bolus dose (800 mg EGCG or the decaffeinated extract once daily) for 4 weeks of treatment as compared to the onset of the studies. A corresponding testing at a dose of 800 mg EGCG/day administered in 2 doses/day (400 mg twice a day) did not result in a significant increase in bioavailability after 4 weeks. The mechanism responsible for the observed increase in the AUC of free EGCG after repeated treatment of EGCG or the decaffeinated extract at a high daily bolus dose remains to be studied. The elimination half lives of EGCG do not indicate accumulation (Chow et al., 2003). Inhibition of nonenzymatic degradation, intestinal flora metabolism, methylation, and/or intestinal efflux of EGCG are plausible contributing factors (Chow et al., 2003).

Studies in healthy volunteers (ten subjects per group) also showed that the decaffeinated extract administered as a single dose (800 mg EGCG/day), was rather well tolerated (reports of mild and transient nausea) when taken with food. Notably, the plasma C_{max} of free EGCG in the fasting condition was more than five times that obtained after administration of the same dose with food (Chow et al., 2005).

In a randomized, double-blind, placebo-controlled study the kinetic parameters of single doses of EGCG (50 mg, 100 mg, 200 mg, 400 mg, 800 mg, 1600 mg) or placebo in 50 healthy male subjects were investigated. It was reported that 1600 mg EGCG administered under fasting conditions produced a peak plasma concentration of 3300 ng/ml after 1.3-2.2 hours (Ullmann et al., 2003).

In a second randomised double-blind, placebo-controlled study with repeated oral administration male test persons obtained 1 capsule with EGCG (purity: 94%) every day (doses: 0, 200, 400 or 800 mg/day) for 10 days after overnight fasting. On treatment day 1 and day 10 kinetic parameters were determined. The EGCG was quickly absorbed. Despite very high inter-individual variability of the free EGCG plasma concentrations, a linear increase in systemic availability depending on the dose was observed for the tested dose range after the first application (day 1). After repeated administration (day 10) a dose proportionality was only observed for the two lower dose groups whereas rising the dose to 800 mg/day led to a steeper increase in systemic availability. The increase in elimination half-

life and the accumulation factor in the 800-mg dosage group indicated dose-dependent saturation of capacity-limited excretion routes or an increase in hepatoduodenal re-circulation. As an accumulation factor < 1 was calculated for all dose groups, it is assumed that EGCG does not accumulate. Comparison of the kinetic parameters measured on day 1 and day 10 showed that the mean values of systemic availabilities and maximum plasma concentrations for free EGCG with repeated administration decreased at the dose of 200 mg/day (C_{max} (ng/ml): 327.4 (day 1); 259.5 (day 10)) and increased at doses of 400 mg/day (C_{max} (ng/ml): 504.0 (day 1); 704.5 (day 10) and 800 mg/day (C_{max} (ng/ml): 2268.8 (day 1); 2800.2 (day 10)). The authors indicated that the treatment was well tolerated by the test persons; that minor changes in various physiological parameters were not deemed to be clinically relevant and that only one test person in the highest dose group had manifested a slight reversible increase in alanine aminotransferase (ALT) (Ullmann et al., 2004).

The abovementioned results show that administration of concentrated green tea extracts under fasting conditions and as a bolus lead to a significant increase of plasma concentrations and bioavailability of EGCG compared to administration with food or in split doses respectively. The Working Group noted that the design of the studies (e.g. small number of persons per group, short exposure period) did not allow to detect any adverse effects other than those that are very common. The Working Group also noted that although these clinical trials with dose levels up to 800 -1600 mg EGCG /day did not reveal serious adverse effects the studies were not designed to investigate the safety of EGCG.

Case reports

In the following reference is made to the evaluation of the DSI EC, who critically analysed the adverse event reports on liver damage using the Naranjo causality algorithm to assess the likelihood that exposure to green tea products caused hepatotoxicity (Naranjo et al., 1981, Sarma et al., 2008). The Naranjo scale analyses adverse event reports according to several criteria: the patient's previous experience with the substance, alternative aetiologies, temporal correlation, correlation with intake, and de-challenge/re-challenge information. The likelihood of causation is estimated on a scale ranging from 0 (doubtful or unlikely), to 1-4 (possible), 5-8 (probable) and 9-13 (definitive or certain).

In April 2003, French and Spanish authorities suspended market authorization of a phytotherapeutical drug which was suspected of having caused liver disorders in 13 subjects (9 cases reported in France and 4 cases in Spain). One capsule of the phytotherapeutical drug contained 375 mg of a patented hydroalcoholic green tea extract, which was obtained using 80% ethanol as the extraction agent and which was standardised to 25% EGCG. Furthermore the extract contained 5-10 % caffeine. At a recommended dose of two capsules twice a day (daily dose corresponds to 375 mg EGCG), the phytotherapeutical drug was said to facilitate weight-loss as part of a calorie-controlled diet. The estimated frequency of the hepatotoxic effects was 1 case per 100 000 boxes of the drug sold from 1999 to 2003. Liver toxicity appeared on average following 50 days of use. The time of onset of liver damage ranged from 9 days to 5 months with usage of 2-5 capsules/day (187.5 – 468.75 mg EGCG/ day), mostly 4 capsules /day (375 mg EGCG/ day). In 12 of the 13 cases of hepatic damage, of which 4 were serious, discontinuation of the phytotherapeutical drug had led to a favourable recovery. However, in the remaining patient (with reported co-administration of other drugs and regular alcohol intake), the disorder progressed to liver failure (AFSSAPS, 2003; Sarma et al., 2008; Gloro et al., 2005; Seddik et al., 2001; Vial et al., 2003; Pedros et al., 2003). Reports of liver toxicity with use of this drug were mostly cases of non-uniform hepatites, cytolytic and cholestatic liver disorders as well as jaundice (Molinari et al., 2006; Sarma et al., 2008). The phytotherapeutical drug was the only therapy in four cases. Patients were admitted to hospital in six cases. When the Naranjo scale was applied to these 13 reports involving this drug, two case reports scored "probable causality" because no alternative causal factors were reported. However, information was incomplete in these two reports, with patient medical histories not being available. "Possible causality" was assigned in the 11 remaining cases (Sarma et al., 2008). Among these were the only two cases, in which the lowest

reported daily dose of 2 capsules of this drug (187.5 mg EGCG/ day corresponding to 3.13 mg EGCG/kg body weight per day for a 60 kg person) taken for longer than a month had led to liver disorders (hepatitis and cytolytic and cholestatic liver disturbances, respectively) (Sarma et al., 2008).

In addition to the 13 cases, that led to the recall of the abovementioned drug, 2 cases of hepatitis and 2 cases of rise in transaminases are reported, that involved a product containing an aqueous extract of green tea, white tea and red bush tea (rooibos tea) with 40-50% catechins (Kantelip et al., 2003). One case reported positive re-challenge ("probable causality" on the Naranjo scale); the other 3 cases involved use of multiple medications (possible causality) (Sarma et al., 2008).

Furthermore one case of cytolytic hepatitis with positive re-challenge was reported for a most widely sold green tea leaf powder. In this case, a 19-year old female reportedly consumed 1800 mg of the product daily for 1 month and developed cytolytic hepatitis. No further information about co-administration of other medications or patient history is available (Naranjo score: 6, "probable causality") (Sarma et al., 2008).

AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé) monitoring recently draw the attention of AFSSA (Agence Française de Sécurité Sanitaire des Aliments) on new cases of liver damage with aqueous green tea food supplements since 2006, one of them with a high causality (AFSSA, 2009).

Regarding the US FDA MedWatch reports on liver damage associated with green tea products during the period from January 2001 to July 2006 5 cases are listed, all associated with intake of polyherbal formulations and categorized as "possible causality" according to the Naranjo scale (Sarma et al., 2008).

In the Australian Therapeutic Goods Administration Report 3 cases of abnormal liver function were associated with green tea products all of them being polyherbal formulations and resulting in "possible causality" ratings according to the Naranjo scale (Sarma et al., 2008).

In the Canadian Adverse Drug Reaction Monitoring Program Reports only one case of liver damage associated with a product containing a hot water extract of green tea (100 mg green tea catechins/capsule, 65% EGCG) was reported involving a 42-year-old female patient who was presented with stomach discomfort. The patient reportedly was taking the capsules (recommended dose, 6/day) for 6 months for weight-loss prior to admission. The patient's liver function enzymes were elevated, her ammonia levels were increased and she was negative for hepatitis B and C serology (direct bilirubin 234 µmol/l, total bilirubin: 423 µmol/l, alanine aminotransferase (ALT): 432 IU, aspartate aminotransferase (AST): 217 IU). She was reported to be taking concomitant medication (medroxyprogesterone injections, 150 mg every 3 month) over the previous few years. One of the known serious adverse effects of parenteral medroxyprogesterone is abnormal liver function. The patient subsequently underwent liver transplantation and reportedly recovered. When the available information was applied to the Naranjo scale, it resulted in a "possible causality" rating (Sarma et al., 2008, Canadian Adverse Reaction Newsletter, 2007).

Below, literature reports on cases of hepatotoxicity associated with green tea-based products, which were published since 2005, are shortly summarized. In most cases no detailed information is available on the type of extraction agent used or the composition of the preparation. Duplicate reporting with regard to the abovementioned cases of monitoring programs cannot be excluded.

After five-day or five-week treatment with a plant food supplement to support weight-loss, which contained other ingredients besides green tea (no further dose information available), two men were diagnosed as having severe hepatotoxicity (Stevens et al., 2005).

Bonkovsky et al. (2006) also reported on hepatotoxicity in a woman following the 4-month taking of a food supplement for weight-loss. Besides other ingredients, it contained a green tea extract. The symptoms disappeared once the preparation was no longer taken but reappeared when exposure was resumed. Referring to other published cases of green tea-associated liver damage, the authors came to the conclusion that the intake of larger amounts or concentrated preparations of green tea would be dangerous and should be avoided.

Molinari et al. (2006) report a case of acute liver dysfunction caused by consumption of a food supplement containing 720 mg of a green tea extract (no further information) over a six-month period for weight-loss (direct bilirubin: 224 µmol/l, total bilirubin: 275 µmol/l, ALT: 3583 U/l, AST: 2393 U/l and γ-glutamyl transferase (γ-GT): 112 U/l). The authors recommend to include a careful evaluation of the history of herbal product use when evaluating a patient with acute hepatitis or liver failure.

The growing concerns of experts (e.g. Lambert et al., 2007) about the regular consumption of high-dose green tea preparations were also highlighted in two letters to the publisher of the Journal of Hepatology (Jimenez-Saenz and Martinez-Sanchez, 2006; Javaid and Bonkovsky, 2006). Javaid and Bonkovsky (2006) describe a case in which hepatocellular damage (ALT: 1100-3962 U/l, total bilirubin: 91-505 µmol/l) occurred following the seven-month intake of an extract of Chinese green tea (no further information). They consequently advised against high-dosed green tea extracts as the cause of rare but severe "drug-induced liver injury" (DILI).

Jimenez-Saenz and Martinez-Sanchez (2006) describe the case of a 45-year-old man who developed acute hepatitis (ALT: 1613 U/l, AST: 1033 U/l, γ-GT: 394 U/l, direct bilirubin: 102 µg/dl, total bilirubin: 119 µg/dl) after the 4-month consumption of 6 cups a day of an unknown marketed green tea infusion (no further information regarding ingredients or manufacturing). After discontinuing the consumption the serum levels returned to normal after two months. When the patient resumed his green tea consumption after 6 weeks, elevated serum concentrations were again observed for ALT, AST, γ-GT and total bilirubin one month later.

Furthermore, there is a report on a 26-year-old woman who developed acute hepatocellular hepatitis with icterus and asthenia (ALT: 3314 U/l, AST: 1813 U/l, direct bilirubin: 6.5 mg/dl) after the four-month consumption of a green tea beverage for "weight-loss" called "Hacendado" (75% green tea leaves, 25% mint leaves in the starting material). She drank 2 cups daily. After halting consumption of the beverage the symptoms disappeared but reappeared after re-exposure. The authors point out that pulegon contained in mint leaves is also hepatotoxic but that they ascribe the liver damage observed here to the green tea portion in the starting material of the beverage (Martinez-Sierra et al., 2006).

Reference is made to adverse effects observed for the medicinal use of (+)-catechin as a liver-protecting substance with peroral doses of 3 g/day over 6 months (febrile attacks, haemolytic anaemia, immunoallergic liver damage) (Arznei-telegramm, 1992; Bar-Meir et al., 1985).

"Benefits-versus-Risk" considerations in literature

Based on the knowledge that green tea catechins and their derivatives have diverse biological activity, e.g. interacting with a number of endogenous enzymes and other structures, and have been shown to be effective antioxidants *in vitro* and in animal models, numerous epidemiological studies were conducted in order to examine the benefits of these substances in preventing chronic disease (for review see e.g. Higdon and Frei, 2003; Moyers and Kumar, 2004). The assumption that the long-term intake of high doses of these products can prevent carcinogenesis in humans could not be proven up to now. Overall the findings of the epidemiological studies regarding an association between tumour incidence and exposure to green and black tea products were contradictory (most studies did not

establish an association between tea exposure and tumour incidence). For the same organ localisation (e.g. stomach, pancreas or rectum), some studies established an association between tea exposure and lower tumour incidence, while other studies established an association between tea exposure and higher tumour incidence (Higdon and Frei, 2003; IARC, 1991). Given these unclear findings and the absence of a life-long animal experimental study, there are reservations based on the current level of knowledge about the long-term ingestion of high-dose green tea preparations for the prevention of disease (Verschoyle et al., 2007).

Possible reactions of green tea catechins, and particularly EGCG, with endogenous substances, which may be responsible for possible toxicological effects, cannot be addressed here in detail. Reference is made to the available overviews (e.g. Higdon and Frei, 2003, Moyers and Kumar, 2004). However there are indications not only of the potential for possible preventive uses (e.g. inhibition of oxidative damage to DNA, lipid peroxidation or of the release of free radicals) or for therapeutic use (e.g. growth inhibition and apoptose induction in the case of tumour cell lines) (e.g.:, Higdon and Frei, 2003; Verschoyle et al., 2007; Lee et al., 2005; Muto et al, 2001, Paschka et al., 1998; Chung et al., 1999; Aktas et al., 2004) but also of possible adverse effects such as the ones described hereafter (e.g. Lambert et al., 2007).

EGCG and ECG bind for instance to α- and β- oestrogen receptors and increase the 17β-estradiol-induced reactions in mice (Goodin et al., 2002). Furthermore, EGCG showed a distinct inhibition of catechol-O-methyl transferase (COMT), which catalyses the metabolisation of endogenous (e.g. oestrogens, catecholamines) and exogenous compounds (Lu et al, 2003). In corresponding experiments there was a strong *in vitro* inhibition of O-methylation of 2- and 4-hydroxyestradiol which is seen as a detoxification reaction (Nagai et al., 2004; Lakhani et al., 2003). Furthermore, it could be demonstrated that the use of COMT inhibitors increased EGCG-induced cell death. In this context, it is assumed that metabolisation by COMT is one of the main biotransformations of green tea catechins and that COMT could play a key role in protecting against ECGG-induced liver toxicity. Hence, there is a hypothesis that the elevated sensitivity of certain individuals to green tea catechins could be linked to low COMT activity as a consequence of a polymorphism (Lambert et al., 2007).

Animal data and *in vitro* data

Toxicokinetics and subchronic toxicity

After oral administration two distinct pathways for metabolism of EGCG are described: it is degraded in the gut by microorganisms; and it is also conjugated to form glucuronides, sulphates and O-methylated derivatives. Considerable evidence suggests that EGCG can undergo enterohepatic cycling. Following the oral administration of ^{14}C-labelled EGCG to rats at a dose of 50 mg/kg body weight, 11.9% of cumulative radioactivity was found in urine and 78.3% in faeces within 96 hours (e.g. Ullmann et al., 2003; Higdon and Frei, 2003).

The toxicokinetics of radiolabelled EGCG (intravenous, single dose, 25 mg/kg; oral, single dose, 250 mg/kg) have been investigated in beagle dogs (Swezey et al., 2003). This study found that approximately 20% of orally administered EGCG is absorbed systemically in beagle dogs compared with 1.6% (Chen et al., 1997) to 14% (Zhu et al., 2000; Zhu et al., 2001) in rats.

Suganuma et al. (1998) present biodistribution data obtained after gastric intubation of mice with (^{3}H)EGCG. The authors describe that EGCG is widely distributed into various organs and that the liver is one of the target organs of EGCG. A second administration of the substance after a 6 h interval enhanced tissue levels of radioactivity in blood, brain, liver, pancreas, bladder and bone 4-6 times above those after a single administration.

The effect of food on green tea bioavailability and related safety parameters has been investigated in studies by Isbrucker et al. (2006a): No adverse effects were noted when 500 mg green tea preparation (91.8% EGCG)/kg body weight per day was administered for 13 weeks to pre-fed dogs in divided doses. However 500 mg green tea preparation (80% EGCG)/kg body weight per day caused morbidity, linked to severe liver damage and lethality when administered to fasted dogs as a single bolus for 13 weeks. Accordingly, the no observed adverse effect level (NOAEL) was determined to be 500 mg green tea preparation/kg body weight per day for pre-fed dogs and 50 mg green tea preparation/kg body weight per day for fasted dogs. In addition, maximum plasma levels of free EGCG were approximately ten times greater in fasted than in pre-fed dogs at the dose level of 500 mg/kg body weight per day (C_{max} = 55.6 µg/ml in fasted male dogs after 81 days of administration versus 5.75 µg/ml in pre-fed dogs after 78 days of administration). In the following some details of the studies of Isbrucker et al. (2006a), which include also a study in rats, are described.

In a 13-week feeding study, Sprague-Dawley rats (10 animals/gender and dose) received 0, 50, 150 or 500 mg EGCG/kg body weight per day. The EGCG was administered in the form of a green tea preparation containing 77% EGCG. Changes in feed intake, body weight, clinical and haematological data observed in the treated rats, were judged not to be treatment related. The only statistically significant haematological effect in female rats in the highest dose group at the end of the recovery phase was the increase in total bilirubin. Unconjugated EGCG could not be detected in the plasma of the animals in the low dose and medium dose groups (exception 2 male animals). In the highest dose group the mean plasma values during treatment were between 3.8 and 11.5 ng unconjugated EGCG/ml. The authors refer to the findings of other investigators according to which the oral bioavailability of EGCG in rats is only 0.003-0.45% of the ingested dose. Thus the reduced oral toxicity has to be interpreted in conjunction with the low bioavailability. The authors concluded that the no-adverse effect level (NOAEL) for EGCG in rats was 500 mg EGCG/kg body weight per day. (Isbrucker et al., 2006a).

In a 13-week oral study "pre-fed" beagles (6/gender and group) were given capsules containing an EGCG preparation (91.8 % EGCG purity) in doses of 0, 50, 300 or 500 mg/kg body weight per day (though not expressively explained by the investigators it is assumed that these doses delivered 0, 46, 275 or 460 mg EGCG /kg body weight per day). These doses were administered divided into two doses per day. Vomiting was the most obvious dose-dependent effect which decreased in the course of the study. The haematological and clinical parameters were normal. There were no clinical-pathological effects and no effects on feed consumption or body weight. The only treatment-related histopathological effect described by the authors was the occurrence of pigmentation in the villous tips in the duodenum of treated dogs. The same brown pigment was found in the liver Kupffer cells of one female in the highest dose group and associated with EGCG phagocytosis. As there were no histopathological changes in the corresponding tissues, the finding was not deemed to be toxicologically relevant by the authors and a NOAEL of 500 mg/kg bodyweight/day was derived from the study for the EGCG preparation, corresponding to 460 mg EGCG /kg body weight per day. The plasma levels of free EGCG showed considerable variations with, in some cases, higher values at the end of the treatment period (e.g. mean values for females in the highest dose group: C_{max} on day 1: 4972 ng/ml, C_{max} on treatment day 78: 5752 ng/ml) (Isbrucker et al., 2006a).

Groups of 4 male and 4 female fasting beagles, which had been given no food for at least 15 hours, were given an EGCG preparation (80% purity) at doses of 0, 50, 150 or 500 mg/kg body weight per day (referred to the pure substance 0, 40, 120 or 400 mg EGCG/kg body weight per day) in capsules for 13 weeks. They were treated 3-4 hours prior to food intake. Depending on the dose, vomiting and diarrhoea were observed in the medium and highest dose groups. Three animals (1 male, 2 female) in the highest and 2 females in the medium dose group died or were killed because of a moribund condition. These animals showed considerably reduced bodyweight and were anorexic for three weeks prior to death. In the highest dose group the male manifested severe proximal tubular necrosis in the kidneys and one female showed haemolytic anaemia, liver necrosis, fairly severe gastric erosions and basophilic degeneration of the renal tubules. One of the females in the medium dose

group also manifested liver necrosis and gastric erosion and myocardial necrosis. In the two upper dose groups all animals manifested elevated total serum bilirubin values and more or less marked elevations of the AST and ALT values. None of the surviving animals manifested liver necrosis, but in the highest dose group one male was identified with proximal tubular necrosis of the kidneys and in one female degenerative renal tubules were observed. From the study the authors derived a NOAEL of 40 mg EGCG/kg body weight per day (Isbrucker et al., 2006a).

The corresponding toxicokinetic studies show that the bioavailability (determined as the AUC) increased in a linear manner with the doses, that this occurred at the two time points of measurements (day 1 and day 81 of the study) and that bioavailability particularly in the highest dose group was higher at the end of the study than at the beginning (e.g. mean values for males in the lowest dose group: C_{max} on day 1: 1047 ng/ml, C_{max} on day 81: 3832 ng/ml; in the medium dose group: C_{max} on day 1: 5781 ng/ml; C_{max} on day 81: 13,370 ng/ml; in the highest dose group: C_{max} on day 1: 25,350 ng/ml; C_{max} on day 81: 55,560 ng/ml) (Isbrucker et al., 2006a).

In one additional study with fasting (treatment after overnight fasting and 4 hours prior to feeding) and pre-fed beagles which were given an EGCG preparation (78% purity) at a dose of 300 mg/kg body weight per day in gelatine capsules for two weeks, 10-fold higher maximum plasma concentrations of EGCG were observed on day 14 of treatment in the fasting animals than in the pre-fed animals (Isbrucker et al., 2006a).

Based on the absence of any treatment-related toxicological or histopathological effects, using the NOAEL from 13-week-studies in rats (500 mg EGCG /kg body weight per day) (Isbrucker et al., 2006a) and pre-fed dogs (500 mg EGCG preparation (91.8 % EGCG purity)/kg body weight per day) (Isbrucker et al., 2006a) and applying an uncertainty factor of 100 Isbrucker et al. (2006a) suggest an acceptable daily intake (ADI) of 5 mg EGCG (preparation)/kg body weight per day, equivalent to 300 mg EGCG (preparation)/day for an adult weighing 60 kg. Regarding the study in pre-fed dogs this calculation has to take into account that the purity of the administered EGCG preparation was only 91.8 % EGCG. The resulting ADI value amounts to 4.6 mg EGCG /kg body weight per day, equivalent to 276 mg EGCG (preparation)/day for an adult weighing 60 kg.

According to a published abstract Sprague-Dawley rats (20/sex and group) received oral doses of 0, 45, 150, or 500 mg EGCG/kg/day via gavage for 13 weeks. Toxicological endpoints included survival, body weight, food consumption, clinical signs, clinical pathology, ophthalmology, organ weights, gross pathology, and microscopic pathology. Early deaths in the high dose group and a dose-related suppression of body weight gain were seen in both sexes of rats. Microscopic findings suggesting possible alterations in gastrointestinal function included pancreatic necrosis and hepatic (periacinar) degeneration and necrosis; modest elevations in alkaline phosphatase (ALP), aspartate-aminotransferase (AST) and alanine aminotransferase (ALT) were seen in female rats in the high dose group. High dose rats of both sexes also demonstrated necrosis/atrophy of the thymus. According to the authors histopathologic data suggest that the NOAEL of EGCG is 150 mg/kg body weight per day in both male and female rats. However, body and organ weights appear to be more sensitive indicators of EGCG toxicity in male rats. On the basis of reduced body weight gain and decreased absolute and relative thymus weights, the investigators derive a NOAEL for EGCG in male rats of 45 mg/kg/day (Abstract, McCormick et al., 1999).

In a 13-week-study by the same investigators, in which beagle dogs (4 animals/sex and group) were treated by oral doses of 0, 30, 100, or 300 mg EGCG/kg body weight per day via capsules, a NOAEL of ≥ 300 mg EGCG / kg body weight per day was determined (Abstract, McCormick et al., 1999).

In a 13-week gavage study in Sprague-Dawley rats (20 animals/sex and group) a polyphenol fraction from green tea (53.4% EGCG, 11.4% EGC, 9.1 % EC, 5.1 % GCG and 4.9% ECG) was administered in doses of 0, 48, 160, 534 mg EGCG/kg body weight per day by the same investigators. Early mortality was observed in both genders in the highest dose group. Body weight gain, feed

consumption, relative and absolute weight of the spleen and thymus were reduced dose-related. Histopathological findings were pancreatic necrosis, hepatic degeneration/necrosis, thymic necrosis/atrophy. The authors derived a NOAEL of 48 mg EGCG/kg body weight per day (Abstract, Johnson et al., 1999).

In a 13-week-study by the same investigators, in which beagle dogs (4 animals/sex and group) were treated orally with the same polyphenol fraction by capsules in doses corresponding to 0, 32, 107, or 320 mg EGCG/kg body weight per day, a NOAEL of ≥ 320 mg EGCG/kg body weight per day was determined (Abstract, Johnson et al., 1999).

Bun et al. (2006) examined the serological parameters of liver function (amongst others alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, ɣ-glutamyl transferase, total bilirubin) in a 12-week study in female Wistar rats (9 animals/group). Green tea extracts, which had been obtained with water or 80% ethanol as a solvent were administered by gavage in doses of 1400 mg/kg body weight per day or 2000 mg/kg body weight per day, respectively. The ethanol extract was the same one contained in the oral phytotherapeutical drug mentioned in section 7 whereby the dosage given was 80 times higher than the therapeutical daily dosage of the drug. The authors did not observe any characteristic signs of hepatotoxicity. The only significant change in the animals treated with the ethanol extract was an increase in total bilirubin values and, in the case of those treated with the aqueous extract, a reduction in total bilirubin values.

In a 13-week feeding study in F344/Du Cij rats (10 animals /sex and group) a polyphenol fraction from green tea containing 32.1% EGCG, 17.7 % EGC, 8.5 % EC, 3.3% GCG, 10.7% ECG, and 1.4% CG and a total of 66.2% catechins was administered by the diet in concentrations of 0, 0.625, 1.25, 2.5 and 5 % of the polyphenol fraction, corresponding to EGCG doses of 0, 141, 283, 566 and 1132 mg EGCG/kg body weight per day. The mean thyroid weight of the rats fed a diet containing 5.0% of the polyphenol fraction (5.0%-group) significantly increased to 444% of the control in males and to 304% of the control in females. Histological examinations of the thyroid of the 5.0%-group revealed marked hypertrophy and/or hyperplasia of the follicles. Slight hypertrophy of follicular cells was observed in male rats of the 1.25%-group and female rats of the 2.5%-group. Degree and incidence of thyroid lesions were higher in males than in females in the 1.25 %-, 2.5%- and 5.0 %-groups. The induction of goitres by green tea extract catechins at high doses may be due to antithyroid effects of catechins. The NOAEL of the polyphenol fraction based on histological changes of the thyroid was considered to be 0.625% in males and 1.25% in females in the diet, corresponding to 141 and 283 mg EGCG/kg body weight per day, respectively (Sakamoto et al., 2001).

Reproduction and developmental toxicity

An embryo-fetal development study was conducted in rats. Oral administration of a green tea preparation (catechins constitute 85 to 95% (by weight) of the preparation which includes more than 55% of EGCG, other catechin derivatives such as EC, EGC, ECG and some additional minor catechin derivatives i.e. GCG, GC, CG and C) during the period of organogenesis (gestational days 6 to 15) did not cause treatment related effects on embryo-fetal development at doses of up to 1,000 mg/kg body weight per day (FDA, 2006; Faqi et al., 2001).

Other developmental studies using subcutaneous or vaginal administration of green tea preparations (FDA, 2006) were considered by the Working Group to be not relevant for the risk assessment of orally applied green tea extracts.

EGCG preparations of >91% purity were administered to pregnant rats during organogenesis and development. Feeding pregnant rats diets supplemented at 1400, 4200 of 14,000 ppm during organogenesis was non-toxic to dams or foetuses. A two-generation study in rats fed 1200, 3600 or 12,000 ppm EGCG preparation showed no adverse effects on reproduction or fertility. The highest

dose reduced the growth rate of offspring, and there was a slight increase in pup loss. A growth effect among pups was also seen at 3600 ppm, but in the second generation only. The lowest dose was considered the overall no-observed adverse effect level (NOAEL). The authors derived a NOAEL equivalent to 200 mg/kg body weight per day EGCG preparation. Because dams consumed twice the amount of feed during the crucial lactation period, in which effects occurred, twice the lowest dose which would normally have been 100 mg/kg body weight per day was calculated (Isbrucker et al., 2006b).

Genotoxicity and carcinogenesis

A green tea preparation (catechins constitute 85 to 95% (by weight) of the preparation which includes more than 55% of EGCG, other catechin derivatives such as EC, EGC, ECG and some additional minor catechin derivatives i.e. GCG, GC, CG and C) was negative in the Ames test, the rat micronucleus assay, the UDS test, and the transgenic mouse mutation assay, but positive in the mouse lymphoma mutation assay (FDA, 2006; Chang et al., 2003). In an oral (gavage) carcinogenicity study, the same preparation was administered daily for 26 weeks to p53 transgenic mice at doses up to 500 mg/kg/day. The treatment was not associated with an increased incidence of either neoplastic or non-neoplastic lesions in the organs and tissues examined (FDA, 2006).

The effects of dietary administration of green tea catechins (GTC) were examined using a multi-organ carcinogenesis model. Groups of 15 F344 male rats were initially treated with a single i.p. administration of 100 mg/kg body weight N-diethylnitrosamine, 4 i.p. administrations of 20 mg/kg body weight N-methylnitrosourea, 4 s.c. doses of 40 mg/kg body weight 1,2-dimethylhydrazine, together with 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine for 2 weeks and then 0.1% 2,2´-dihydroxy-di-n-propylnitrosamine for 2 weeks, both in the drinking water, for a total initiation period of 4 weeks. GTC in the diet, at doses of 1.0 or 0.1%, was administered from 1 day before and during carcinogen exposure, after carcinogen exposure or both during and after carcinogen exposure. Further groups of animals were treated with carcinogen, 1% GTC or basal+ diet alone as controls. All animals were killed at the end of week 36, and all major organs examined histopathologically. The numbers of small intestinal tumors (adenomas and carcinomas) per rat were significantly reduced in the groups treated with 1% GTC during (0.13 ± 0.35) and after carcinogen exposure (0.31 ± 0.48) and in those receiving 1% and 0.1% GTC both during and after carcinogen exposure (0.14 ± 0.36, 0.46 ± 0.97 respectively) as compared with the carcinogen alone group (1.07 ± 1.21). On the other hand, numbers of glutathione S-transferase placental form positive liver foci per cm^2 were slightly but significantly increased in the groups treated with 1 and 0.1% GTC during carcinogen exposure, 1% GTC after carcinogen exposure and 1% GTC both during and after carcinogen exposure. According to the authors the results indicated that while GTC inhibits small intestinal carcinogenesis it slightly enhances hepatocarcinogenesis in a dose dependent manner when applied both during and after carcinogen exposure (Hirose et al., 1993).

In vitro experiments with hepatocytes

The cytotoxicity of green tea components was tested in rat hepatocytes *in vitro* and turned out to be highest with EGCG and to decline in the following order: EGCG > propylgallate > ECG > bile acid > EGC > EC (Galati et al., 2006).

In vitro studies by Schmidt et al. (2005) it was shown that comparatively high concentrations of green tea extracts obtained with 80% ethanol can damage rat hepatocytes whereby EGCG was identified as the responsible key compound. "Typical hepatotoxins" would, however, show toxicity which is 1-2 orders of magnitude higher. Dried green tea extracts, from which the lipophilic fraction had been removed, manifested a similar cytotoxicity as the untreated dried extracts. This seems to indicate that

EGCG, a polar substance that is soluble in water and water-ethanol mixtures, was responsible for cytotoxicity.

10. Safety assessment based on available knowledge (Level A)

The present document evaluates "dried green tea extracts" including also decaffeinated preparations. The document takes into account that the composition of the extracts will differ according to the various extraction techniques. The evaluation considers the qualitative and quantitative equivalence of the extracts to the traditional green tea infusions. Furthermore it is evaluated if under the conditions of use for the extract an equivalent level and mode of exposure can be assumed as for the traditional green tea infusions. For beverages which are quantitatively equivalent to traditional green tea infusions, reference can be made to the presumed safety, based on the knowledge derived from the broad long-term food use of the latter.

In the following assessment at level A the Working Group evaluates four different types of preparations and/or uses for dried green tea extracts:

1) Dried aqueous green tea extracts manufactured by the traditional infusion procedure for preparation of beverages

2) Dried aqueous green tea extracts manufactured by the traditional infusion procedure for preparation of food supplements

3) Other "dried green tea extracts" manufactured by procedures not in accordance with the conditions of the traditional preparation of green tea infusions

4) Dried green tea extracts used in products recommended as adjunct to weight loss programs or weight-loss purposes.

10.1. Level A assessment of dried aqueous green tea extracts manufactured by the traditional infusion procedure for preparation of beverages

Green tea-containing products are claimed to have various health-promoting effects and are increasingly spreading to areas which extend beyond the traditional consumption of green tea infusions as a stimulant. With regard to the human cases of hepatotoxicity questions arise, why liver damage occurs particularly with weight-loss products containing green tea extracts in high daily doses, which components of green tea extracts have to be causally associated with hepatotoxicity and which influences have to be attributed to matrix effects due to components of the green tea extracts and which to food components in general.

The analysis of the available human data clearly shows that, in recent years, in more than 30 cases an association was seen between the intake of larger amounts of green tea-derived products and the occurrence of, in some cases, severe liver damage. In many cases of these liver disorders green tea extracts with high EGCG contents were taken in capsule form over several months as a medicinal product or food supplement to support weight-loss. Concerning the treatment with an oral phytotherapeutical drug the daily intake of 2 capsules to 5 capsules (187.5 – 468.75 mg EGCG/ day), mostly 4 capsules (375 mg EGCG/ day) was associated with liver damage (Sarma et al., 2008). A causal relationship has to be regarded as probable in 7 cases and as possible in 27 cases (Sarma et al., 2008). Given the considerable distribution of corresponding products around the world, the hepatotoxicity described is deemed to be a relatively rare adverse effect (AFSSAPS, 2003; Molinari et al., 2006; Javaid and Bonkovsky, 2006). Nevertheless, assessing the frequency of reported cases the limitations of the dietary supplement adverse event reporting systems have to be taken into

consideration (Sarma et al., 2008). Hepatological expert circles issued an urgent warning recently because of the possible severity of liver damages associated with green tea extracts (Molinari et al., 2006; Javaid and Bonkovsky, 2006; Jimenez-Saenz, and Martinez-Sanchez, 2006).

Based on these case studies, assumptions that green tea extracts, which are obtained with water as the extraction agent, are not linked to toxic effects, e.g. on the liver, have recently been rejected; indeed in some cases of liver disorder, products containing a dried aqueous green tea extract had been ingested (Kantelip et al., 2003; Sarma et al., 2008). AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé) monitoring also draw the attention of AFSSA on new cases of liver damage with aqueous green tea food supplements since 2006, one of them with a high level of causality (AFSSA, 2009). Furthermore, Jimenez-Saenz and Martinez-Sanchez (2006) reported on the occurrence of acute hepatitis after the 4-month consumption of 6 cups a day of an unknown marketed green tea infusion, with a renewed increase in the liver enzyme values after re-exposure. It is unknown if the beverage was manufactured according to the traditional way to prepare an infusion or was a ready-to-drink beverage on an extract base. Given these facts, the Working Group concludes, that even liquid aqueous green tea preparations can trigger severe liver damage in predisposed individuals. Since no corresponding findings have been published up to now for intake of traditionally manufactured green tea infusion (Jellin et al., 2007), the risk of liver damage in conjunction with the intake of traditionally manufactured green tea infusions is regarded by the Working Group to be very low given the available knowledge derived from the many centuries of global intake of green tea infusions.

Thus, based on the current knowledge the worldwide long-time consumption of traditional green tea infusions leading to intake of catechins as specified in table 5 is assumed to be safe on level A (safety presumed based upon available knowledge derived from the long-term food use). The Working Group notes that this conclusion holds provided that pregnant and nursing mothers as well as children and other caffeine sensitive persons consume the beverage in moderation or abstain from it (general recommendation for caffeine containing beverages postulated to be common knowledge).

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 produce beverages, which have a similar composition and do not exceed the concentrations of polyphenols compared to a traditional green tea infusion are also classified to be safe on level A (safety presumed based on available knowledge) according to the criteria outlined in the EFSA Guidance on the safety assessment of botanicals and botanical preparations[19].

As for traditional green tea infusions, it is provided that pregnant and nursing mothers as well as children and other caffeine sensitive persons consume the beverage in moderation or abstain from it (general recommendation for caffeine containing beverages postulated to be common knowledge).

Conclusions:

The resulting beverages (ready to drink or prepared from instant preparations) are under qualitative and quantitative aspects equivalent to traditional green tea infusions, since they have a similar composition and do not exceed the concentrations of polyphenols in traditional infusions. Presumed the intake levels of these beverages do not exceed that of the traditional tea infusions as shown in table 5, they are considered to be as safe on level A as the latter, for which safety is presumed basing upon the knowledge derived from the broad long-term food use.

[19] See http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_draftguidance_botanicals_public_cons_update_en.pdf?ssbinary=true

The Working Group also notes that the use of these green tea extracts in beverages for weight reduction purposes cannot be presumed to be safe on level A since this indication suggests intake under fasting conditions or reduced food intake without adequate safety data being available at present. This applies also to the use of beverages for weight reduction purposes containing green tea extracts as part of a herbal mixture. Generally for these products safety issues may arise concerning a possible synergistic effect between green tea catechins and botanical ingredients of other origin having hepatotoxic potential (e.g. other catechins and tannins).

10.2. Level A assessment of:

- **Dried aqueous green tea extracts manufactured by the traditional infusion procedure for preparation of food supplements**
- **Other "dried green tea extracts" manufactured by procedures not in accordance with the conditions of the traditional preparation of green tea infusions**
- **Dried green tea extracts used in products recommended as adjunct to weight loss programs or weight-loss purposes**

For food supplement-use of dried green tea extracts case reports on liver toxicity associated with these or related products have to be taken into account. These findings question the safety of use even for aqueous green tea extracts.

Therefore dried green tea extracts for food supplement-use as well as "other dried green tea extracts" cannot be considered safe based upon the knowledge derived from long-term food use and further evaluation on level A based on existing data available is necessary.

The safety of extracts for food supplement-use as well as the safety of "other dried green tea extracts" may be assessed focussing on their contents of EGCG and its assumed hepatotoxicity taking the above mentioned uncertainties (e.g. additional active ingredients, other target organs, matrix effects) into account by applying the MOS (margin of safety) approach. This should guarantee that the MOS, which is defined as ratio between the NOAEL and the daily intake for EGCG, amounts at least to 100, this being the routinely used uncertainty factor for non genotoxic compounds.

The pathogenesis of the liver damage described is said to be unknown (Molinari et al., 2006; Javaid and Bonkovsky, 2006; Jimenez-Saenz and Martinez-Sanchez, 2006). There are various assumptions which components of the green tea preparations cause hepatotoxicity. The fact that initially mainly liver damage was reported after taking the oral phytotherapeutical drug mentioned in section 7, led to the hypothesis that the potentially hepatotoxic compounds were to be found in the lipophilic fraction of the green tea extracts obtained with the ethanol-water mixtures (Schmidt et al., 2005). However, this hypothesis was rejected on the basis of the findings by Schmidt et al. (2005): Green tea extracts obtained with 80% ethanol showed unchanged cytotoxicity in the rat hepatocyte cultures even after removal of their lipophilic fraction. EGCG was identified as the main component in the green tea extract probable responsible for hepatotoxicity. As a polar substance, it is soluble both in water and in ethanol-water mixtures. Countering the assumption that cytotoxicity caused by EGCG was the reason for the observed liver damage in humans, it is specified, that the cytotoxicity of EGCG in hepatocytes *in vitro* was comparatively weak, that EGCG only led at high doses to liver necroses in animals and that the bioavailability of EGCG in man was relatively low (Molinari et al., 2006; Jimenez-Saenz and Martinez-Sanchez, 2006; Schmidt et al., 2005). In this context it is also discussed whether other mechanisms, e.g. allergic reactions to green tea components or metabolic idiosyncrasy, are possible causes of liver damage (Molinari et al., 2006; Jimenez-Saenz and Martinez-Sanchez, 2006; Schmidt et al., 2005). There have been no reports up to now of allergic reactions following the oral intake of green tea preparations; however asthmatic reactions to green tea dust were described for workers in a tea factory where EGCG was involved (Shirai et al., 1994). The rare occurrence of the hepatotoxic

effects points to a genetically based higher sensitivity of some individuals (Javaid and Bonkovsky, 2006). Similarly, Lambert et al. (2007) suspect that sensitive individuals have a polymorphism in a decisive biotransformation pathway of green tea polyphenols which could lead to elevated plasma levels of toxicologically relevant parent substances. It has been considered unlikely that weight-loss caused by the green tea extract could play a causal role (Molinari et al., 2006; Javaid and Bonkovsky, 2006; Javaid and Bonkovsky, 2006; Javaid and Bonkovsky, 2006 et al., 2006).

Concerning the studies in beagles by Isbrucker et al. (2006a) the Working Group questions if the effects observed in rats and pre-fed dogs (female rats: significant increase in total bilirubin at the end of the recovery phase; pre-fed dogs: vomiting as a dose-dependent effect) observed at the dose of 500 mg/kg body weight per day can rightly be seen not to be treatment related. Comparable effects were namely associated in humans and animals following intake of EGCG-containing preparations (Bun et al., 2006; Chow et al., 2005; Chow et al., 2003; Jimenez-Saenz and Martinez-Sanchez, 2006; Javaid and Bonkovsky, 2006; Molinari et al., 2006). The studies in beagles by Isbrucker et al. (2006a) appear to be relevant for the interpretation of the results observed in humans. The finding that administration of green tea extracts to fasting dogs lead to a NOAEL for EGCG being more than a factor 10 lower than that derived from the study in pre-fed dogs (Table 6) may suggest that conditions in which green tea extracts are taken combined with food consumption may minimize possible risks (see also Sarma et al., 2008)

Table 6: Effects of subchronic exposure to green tea extracts in experimental animals

Material tested (GTE = dried Green Tea Extract)	**Species** animals /group	**Dose and Route** mg EGCG/kg b.w./day	**Duration of observation**	**NOAEL**	**References**
GTE containing 77 % EGCG	rat (Sprague-Dawley), 10 males, 10 females	0, 50, 150, 500 (feeding), additional groups with a four week recovery without GTE treatment in this period: 0, 500 (feeding)	13 weeks	NOAEL for EGCG in rats = 500 mg EGCG/kg b.w./day. If the increase of total bilirubin is regarded to be treatment-related a NOAEL of 150 mg/kg b.w./day would have to be derived.	Isbrucker et al., 2006a
GTE containing 91.8 % EGCG	"pre-fed" Beagle dogs, 6 males, 6 females	0, 46, 275, 460 (oral per capsules, each dose divided into two doses per day)	13 weeks	A NOAEL of 460 mg EGCG/kg b.w./day was derived, corresponding to 500 mg GTE /kg b.w./day.	Isbrucker et al., 2006a
GTE containing 80 % EGCG	"fasting" Beagle dogs, no food for at least 15 hours prior to treatment, 4 males, 4 females	0, 40, 120, 400 (one oral bolus dose as capsule per day 3-4 hours prior to food intake	13 weeks	NOAEL of 40 mg EGCG/kg b.w./day.	Isbrucker et al., 2006a
GTE not specified	rat (Sprague-Dawley), 20 males, 20 females	0, 45, 150, 500 (gavage)	13 weeks	Histopathologic data suggest that the NOAEL of EGCG is 150 mg/kg b.w./day in both male and female rats. On the basis of reduced body weight gain and decreased absolute and relative thymus weights, a NOAEL of EGCG in male rats of 45 mg/kg b.w./day was derived	McCormick et al., 1999 (Abstract only)
GTE containing 53.4% EGCG, 11.4% EGC, 9.1% EC, 5.1% GCG and 4.9% ECG	rat (Sprague-Dawley), 20 males, 20 females	0, 48, 160, 534 (gavage)	13 weeks	NOAEL of 48 mg EGCG/kg b. w./day.	Johnson et al., 1999 (Abstract only)
GTE containing 32.1% EGCG, 17.7% EGC, 8.5% EC, 3.3% GCG, 10.7% ECG, 1.4% CG and a total of 66.2% catechins	rat (F344/Du Cij), 10 males, 10 females	0, 0.625, 1.25, 2.5 and 5 % GTE in the diet, corresponding to EGCG doses of 0, 141, 283, 566 and 1132	13 weeks	NOAEL of the GTE based on histological changes of the thyroid considered to be 0.625% in males and 1.25% in females in the diet, corresponding to 141 and 283 mg EGCG/kg b.w./day, respectively.	Sakamoto et al., 2001

The fact, that the occurrence of liver toxicity and other organ damage (e.g. kidneys and gastrointestinal tract) is associated with elevated bioavailability values of EGCG in dogs treated in a fasting state, whereas pre-fed dogs treated in the same dose range at a far lower bioavailability of EGCG did not show these effects, is another important indication for the causal involvement of EGCG in the hepatotoxicity induced by green tea extracts. Secondly, the findings of the dog studies suggest a plausible explanation, why in particular weight-loss products on basis of green tea extracts were associated with an increased incidence of liver toxicity: It has to be assumed, that patients undergoing weight-loss programs are also exposed to the green tea extract during fasting days, periods of food restriction or reduced food intake in consequence of diets. Assuming higher bioavailability upon fasting, as observed in the beagle studies, far higher EGCG bioavailability could result in these patients than for people on a normal diet. This applies in particular to ingestion spanning several weeks, because, as shown by kinetic studies in humans and beagles, after repeated oral intake higher EGCG blood level result than at the beginning of exposure (Isbrucker et al., 2006a; Ullmann et al., 2004). Taken together these considerations may contribute to explain, why uptake of 187.5 – 468.75 mg EGCG/ day (2 to 5 capsules of the phytotherapeutical drug) in form of weight-loss products could have caused liver disorders, while intake of green tea infusions in high amounts (95^{th} percentile: 1097 g infusion/day equivalent to 288.5 to 447.6 mg EGCG/day, cf. Table 5) has not been associated with liver damage until now.

Possible mechanisms leading to an increased bioavailability of EGCG under fasting conditions (e.g. increase of absorption rate, modified biotransformation) have not been investigated. When interpreting toxicokinetic and toxicodynamic data on purified EGCG preparations the hypotheses of Chen et al. (1997) are to be taken into account according to which EGCG from green tea extracts, as a consequence of assumed interactions with other polyphenols (e.g. competition for binding sites in metabolizing enzymes), may show slower elimination than when used as an isolated compound. This is in accordance with the results of Johnson et al. (1999) who found that the mortality pattern in an oral rat study indicated that a green tea extract was more toxic than would be predicted based on its EGCG content alone. This illustrates why toxicological data obtained investigating a certain dried green tea extract cannot be transferred to another one without giving evidence of their equivalent pattern of composition.

The genotoxicity and limited carcinogenicity data obtained with concentrated green tea extracts do not raise safety concern.

The Working Group notices, that NOAELs derived from the five subchronic oral animal studies with green tea extracts containing not more than 80 % EGCG lie between 40 and 150 mg EGCG/kg body weight per day (Table 6). For further assessments the Working Group refers to a NOAEL ranging from 40 to 50 mg EGCG/kg body weight per day according to the results of three out of the five studies (McCormick et al., 1999; Johnson et al., 1999; Isbrucker et al., 2006a (fasting dogs)).

The Working Group concludes that the regular intake of dried green tea extracts with food supplements or related products, which differs from the intake of traditional green tea infusions (or beverages with identical composition), may carry the risk of severe hepatotoxicity for a small proportion of the population. These risks may be higher under conditions of reduced food intake or starving as occurring during efforts to lose weight. According to present knowledge there is uncertainty in the risk assessment of these dried green tea extracts

- about which ingredients in green tea extracts besides EGCG possibly contribute to hepatotoxicity,
- how the bioavailability of EGCG is influenced by interactions with accompanying catechins (possible elevation of EGCG bioavailability),
- about the influence of extraction conditions on hepatotoxicity,

- about the influence of fasting or reduced food intake on the toxicity of green tea extracts
- which consumer groups are predisposed for induction of the described liver damage by green tea extracts,
- whether other organ damage (e.g. to kidneys, the gastrointestinal tract or the thyroid) seen besides hepatotoxicity in animal studies is also of relevance for man,
- about the underlying mechanisms of the hepatotoxicity.

Taking these data and conclusions into account the Working Group makes the following evaluation of "dried aqueous green tea extracts manufactured by the traditional infusion procedure for preparation of food supplements" for "other dried green tea extracts" and for "dried green tea extracts used in products recommended as adjunct to weight-loss programs or weight-loss purposes":

Dried aqueous green tea extracts manufactured by the traditional infusion procedure for preparation of food supplements

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 should 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 are of 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 extracts have to be taken into consideration.

Conclusion:

The Working Group states that the MOS (margin of safety) resulting from intake of food supplements and related products, as compared to the NOAEL ranging from 40 to 50 mg EGCG/kg body weight per day derived from the 13 weeks-study of McCormick et al. (1999), Johnson et al. (1999) and Isbrucker et al. (2006a; fasting dogs) should lie above the routinely used 100-fold uncertainty factor for non genotoxic compounds. Based on these considerations it is concluded that when the daily dose of EGCG gives rise to an adequate margin of safety, these extracts are considered to be safe on level A (safety presumed based on available knowledge) according to the criteria outlined in the EFSA Guidance on the safety assessment of botanicals and botanical preparations[20].

The Working Group also notes that the use of these green tea extracts in food supplements for weight reduction purposes cannot be presumed to be safe on level A since this indication suggests intake under fasting conditions or reduced food intake without adequate safety data being available at present.

[20] See http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_draftguidance_botanicals_public_cons_update_en.pdf?ssbinary=true

"Other dried green tea extracts"

Dried green tea extracts manufactured by procedures not in accordance with the conditions of the traditional preparation of green tea infusions, enriched dried green tea extracts, isolated green tea catechins, mixtures of green tea extracts with botanical preparations of different origin with possible hepatotoxic potential cannot be assumed to be safe based on available knowledge because they may differ in their composition from that of traditional green tea infusions.

Conclusion:

Dried green tea extracts differing in their composition from traditional green tea infusions are not considered to be safe on level A. They have to be evaluated at level B meaning that further testing and/or data are required according to the criteria outlined in the EFSA Guidance on the safety assessment of botanicals and botanical preparations.

Dried green tea extracts used in products recommended as adjunct to weight-loss programs or weight-loss purposes

For dried green tea extracts used in products (e.g. beverages or food supplements) recommended as adjunct to weight-loss programs or weight-loss purposes, the Working Group considered that these products could be taken regularly in the fasting state in which the bioavailability of EGCG may be considerably higher than in the non-fasting state.

Conclusion:

Regarding case reports on liver toxicity associated with the intake of dried green tea extracts for weight-loss purposes, these extracts cannot be presumed to be safe on level A. They should therefore undergo a level B evaluation, meaning that further testing and/or data are required according to the criteria outlined in the EFSA Guidance on the safety assessment of botanicals and botanical preparations[21].

11. Further testing and/or data required for the assessment (Level B)

As outlined above, further testing and/or data are required for "other dried green tea extracts" and also for "dried green tea extracts used in products recommended as adjunct to weight-loss programs or weight-loss purposes".

"Other dried green tea extracts"

The intake of these extracts results in an exposure with green tea components, which is qualitatively and quantitatively not equivalent to that resulting from the consumption of traditional green tea infusions. In view of reported cases of hepatotoxicity following the consumption of green tea extracts,

[21] See http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_draftguidance_botanicals_public_cons_update_en.pdf?ssbinary=true

the assumed hepatotoxic potential of EGCG as their major component, and existing uncertainties (e.g. questions of underlying mechanisms of hepatotoxicity, of possible contributions of other green tea ingredients to over-all toxicity, of possible elevation of EGCG bioavailability by interactions with accompanying catechins, and of the influence of the extraction conditions on the toxicity of the extract) these green tea extracts should undergo adequate experimental and clinical testing in animals and humans. As bioavailability of EGCG is considered to be a decisive parameter for the toxicity of green tea extracts, corresponding kinetic data should be recorded in the course of the studies regarding also fasting conditions. Extracts of identical manufacturing procedure using the same kind of green tea could share the same results.

Dried green tea extracts used in products recommended as adjunct to weight-loss programs or weight-loss purposes

Since this use implies regular daily administration under conditions of fasting, food restriction or diets the toxicological relevant components of green tea, particularly EGCG, may undergo toxicokinetic processes, which differ from those in the non starving organism to which the safe use of traditional green tea infusions refers. There are indications from experimental studies, that EGCG is at least partly responsible for liver toxicity and that its bioavailability, when taken in a fasting state, is considerably higher than after food intake, which means correspondingly lower doses have a toxic effect. This is assumed to be related to the reported cases of human hepatotoxicity following the intake of weight-loss products containing dried green tea extracts, gained by aqueous or hydroalcoholic extraction. A green tea extract, for use in products (e.g. beverages or food supplements) recommended for weight-loss purposes should therefore, regardless of its manufacturing procedure and composition, undergo adequate experimental and clinical testing in fasting animals and fasting humans, including examination of kinetic parameters. This also applies to herbal mixtures containing green-tea extracts for use in weight-loss products.

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APPENDIX C: *OCIMUM TENUIFLORUM* L.

The present report focuses on leaf extracts and their possible reproduction toxicity.

Disclaimer:

The present document aims at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected case and considers relevant constituents. The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated. The document is not, and cannot be interpreted as being, a policy document or a decision allowing the classification of a certain botanical or botanical preparation as a foodstuff or as a medicinal product. The document is focusing on one type of preparation and is not intended to evaluate all possible preparations of this botanical or all possible constituents, which should normally be part of a full safety assessment of a botanical. Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete. Contaminants such as heavy metals, mycotoxins, dioxins, pesticides, microbial contamination or PAHs are not evaluated.

It is outside the remit of this Working Group to evaluate possible beneficial effects and associated claims

1. Identity and nature of the source material

Scientific name:	*Ocimum tenuiflorum* L.
Synonyms:	*O. sanctum* L. (=main synonym), *Geniosporum tenuiflorum* (L.) Merr. *Moschosma tenuiflorum* (L.) Heynhold, *Ocimum album* Blanco, *O. anisodorum* Muell., *O. brachiatum* sensu Hasskarl (non Blume), *O. flexuosum* sensu Blanco, *O. frutescens* sensu Burm. f. (non L.), *O. gratissimum* sensu Lour (non L.), *O. hirsutum* Benth., *O. inodorum.* Burm. f., *O. monachorum* L., *O. nelsonii,* Zipp ex Span., *O. sanctum* L. var. hirsute (Benth.) Hook.f., *O. villosum* Roxb., *O. virgatum Blanco* , *O. virgatum, O. tomentosum* Lam., *Lumnitzera tenuiflora* (L.) Spreng., *Plectranthus monachorum* (L.) Spreng., *P. striatus* sensu Meschler et Hosseus. (non Benth.), (Blaschek et al, 1998; MMPND, 2008; Suddee, 2001).
Common names:	Engl.: Holy basil, Sacred basil, Monks basil, Fr.: Basilic sacré, Basilica thailandais, Germ: Heiliges Basilikum. Königsbasilikum, Indisches Basilikum, Ital.: Basilico sacro, Sanskrit: Tulsi, Tulasi.
Family:	Labiatae (Lamiaceae)
Parts used:	Leaf or seed.
Geographical origin:	Tropical and subtropical regions of Asia and North Australia.

Growth and harvesting conditions: *O. tenuiflorum* L. is an annual herb commonly found in India. It has been widely grown throughout the world and is commonly cultivated in gardens.

2. Manufacturing process

No data available

3. Chemical composition

The main components of *Ocimum tenuiflorum* L. dry leaf extract are tannins like gallic acid and chlorogenic acid (up to 4.6 %) and essential oil (up to 2 %) (Materia Medika Indonesia, 1995). The major active constituents include also ursolic acid, a pentacyclic triterpenoid ((3β)-3-hydroxy-urs-12-en-28-oic acid) (1.5% in extract) (Silva et al., 2008). The amounts of the primary constituents of the essential oil vary according to the geographical distribution and variety of the source plant material: eugenol (up to 62 %), methyleugenol (up to 86 %), and α- and β-caryophyllene (up to 42 %). Also present are methylchavicol (= estragole), linalool and 1,8-cineole (=eucalyptol) (Blaschek et al., 1998; Sukari and Takahashi, 1988; Brophy and Jogia, 1984; Maheshwari et al., 1987; Lal et al., 1978, WHO, 2002). *Ocimum tenuiflorum* L. leaves are also reported to contain alkaloids, glycosides, and saponins.

The presence of phenolic compounds was recorded in the extract (no further specifications given) and rosmarinic acid was also detected. The extract of the leaves contained ocimumosides A and B and ocimarin, as well as flavones and flavone derivatives such as apigenin, apigenin-7-O-beta-D-glucopyranoside, apigenin-7-O-beta- D-glucuronic acid 6'-methyl ester, luteolin-7-O-beta-D-glucuronic acid 6'methyl ester, luteolin-7-O-beta-D-glucopyranoside, luteolin-5-O-beta-D-glucopyranoside, and 4-allyl-1-O-beta-D-glucopyranosyl-2-hydroxy benzene, and two known cerebrosides (Gupta et al., 2007).

The Working Group notes that *Ocimum tenuiflorum* L. was on the list of examples to be evaluated because of its possible reproduction toxicity but that there is no information on the actual constituent responsible for this effect.

The Working Group also notes that the extract may contain up to 86% of methyleugenol, a compound known to be genotoxic and carcinogenic (SCF 2001). Because evaluation of an example of a botanical containing the related alkenylbenzene estragole can be found in Annex 4 (evaluation of *Foeniculum vulgare* Mill.), the present evaluation does not focus on this genotoxic carcinogen. Further details on a Margin of exposure assessment for methyleugenol, in line with what was done for estragole in Annex 4, can be found in the literature (Rietjens et al. 2008).

Although *Ocimum tenuiflorum* L. is the correct and preferred name, most publications still use the synonym *Ocimum sanctum.*

4. Specifications

The evaluation in this document focuses on the leaf extract of *Ocimum tenuiflorum* L. There are no specifications on the leaf extracts used in the reported studies and evaluated in the present document.

5. Stability of the botanical ingredient

No data available.

6. Proposed uses and levels

O. tenuiflorum L. is a sacred plant (Holy basil) in India. The plant has mosquito-repellent action. Traditionally, the fresh leaf juice was commonly used in cough, mild upper respiratory infection, bronchospasm, general stress syndrome, skin infections and many other medical purposes (Khanna and Bhatia, 2003).

Preclinical and clinical studies have also suggested an anti-stress and immunostimulant property of *Ocimum tenuiflorum* L. A study supporting this involved the evaluation of the effect of *Ocimum tenuiflorum* L. extract, on the mouse swimming performance. The extract of *Ocimum tenuiflorum* L. increased the swimming time suggesting a central nervous system stimulant and/or antistress activity. Also the effect produced by the *Ocimum tenuiflorum* L. extract was comparable to that of desipramine, an antidepressant drug. In another study the effect of *Ocimum tenuiflorum* L. extract was studied on the noise stress induced changes in albino rats. Pre-treatment with the *Ocimum tenuiflorum* L. extract brought the stress-altered values like leucopenia, increased corticosterone level and enhanced neutrophil functions back to normal levels indicating the stress alleviating effect of *Ocimum tenuiflorum* L. Ether and alcohol extracts of leaves of *Ocimum tenuiflorum* L. were also shown to possess significant activity against *Escherichia coli*. *Ocimum tenuiflorum* L. has been also extensively studied for its therapeutic potential in various areas like immune stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, anti-inflammatory, analgesic and antidiabetic and more.

No quantitative data are available or proposed. The extract of the leaves is claimed for several medical purposes in many publications. These beneficial effects include anti-stress in case of exposure to noise, anti-helminic, anti-diabetic, anti-carcinogenic, anti-inflammatory, wound healing, immunomodulation and cardioprotective effects (e.g. Kamboj, 1988; Mokhasmit et al., 1971; Sharma et al., 2001; Shetty et al., 2008; WHO, 2002; Mediratta et al., 2002; Adhvaryu et al., 2007).

7. Information on existing assessment

No official assessments on the botanical by national or international bodies are available. WHO (2002) prepared monographs on selected medicinal plants, including *Ocimum tenuiflorum* L., which did not include any safety assessment.

8. Exposure

For medical and other uses recommended doses are based on historical practice. However, with natural products it is often not clear what the optimal doses are to balance efficacy and safety. Preparation of botanicals may vary from manufacturer to manufacturer, and from batch to batch within one manufacturer. Because it is often not clear what the active components of a product are, standardisation may not be possible, and the clinical effects of different brands may not be comparable.

Dosages given are based on traditional use and are not supported by clinical evidence. General use is 300-2000 mg of dried leaves of *Ocimum tenuiflorum* L. taken daily as single dose for preventive therapy and 600 -1800 mg in multiple doses daily for curative therapy. Infusions are prepared with 2 g of dried leaves per cup of water. Other reported uses lead to an intake of 10-20 ml of fresh leaf juice

or 1 oz (approx. 28 g) of dried herb in 16 oz (approx. 450 g) of water, three times daily in 5 oz doses (approx. 140 g; containing 3-6 ml of fresh juice or 8.7 g of dried herb per dose), or specifically for diabetic individuals the intake of 2.5 g of dried leaf powder by mouth every morning or 1 teaspoon dried herb brewed in one cup of water three times a day. In the traditional treatment of diabetes mellitus dietary supplementation of fresh *Ocimum tenuiflorum* L. leaves in a daily dose of 2 g/kg bw for 30 days were recommended as effective to reduce blood glucose level (Sethi et al, 2008). Limited data on the intake of *Ocimum tenuiflorum* L. leaves, both as medical preparations or food supplement, can be retrieved also from the internet. Recommended intakes of commercial products range from 300-600 mg of fresh leaves person per day, equivalent to 5-10 mg/kg bw/day.

9. Toxicological data

Several data on health beneficial effects have been reported, which at best provide only some indirect data on the safety of extract or oil extract of *Ocimum tenuiflorum* L. There are a lot of anecdotic and local history reports about the benefits and sometimes indirectly the safety of *Ocimum tenuiflorum* L.

The toxicity or related (pharmacological) data are for the majority special studies on certain effects and only a few studies examined the dose response related toxic effects as requested by most guidelines such as the EFSA or JECFA for e.g. food additives or in the guideline for botanical preparations as adopted by the SCF of EFSA.

No data for safety assessment from acute, subacute, subchronic and chronic and or carcinogenicity studies are available.

Effects on the reproduction and fertility in animals and human

Various beneficial effects have been ascribed to the different parts of the plant *Ocimum tenuiflorum* L. but only a few scientists attempted to look into the various changes in the reproductive system in detail after feeding *Ocimum tenuiflorum* L. leave extract. There seems to be debates among various scientists regarding the histopathological changes in reproductive organs following the feeding of *Ocimum tenuiflorum* L. leaves (Ahmed et al., 2002a&b; Reghunandan et al., 1997).

Fresh leaves of *Ocimum tenuiflorum* L. were given (1 g/kg bw) twice a week for one month to sexually matured rabbits (both male and female). Significant changes in the histology of the testis, epididymis, uterus and ovary were observed in animals fed the *Ocimum tenuiflorum* L. leaves. In testis spermatogenetic elements were degenerated and the number of spermatozoa was significantly reduced in the epididymis. In female rabbits fed with *Ocimum tenuiflorum* L. leaves the uterus showed oedema and, congestion in all the layers of the wall, in addition an increased vascularity, and hemorrhagic corpora luteum were seen in the ovary. Pregnancy and subsequent delivery occurred only in those rabbits which were allowed to mate one month after the stoppage of the *Ocimum tenuiflorum* L. leaves feeding period as compared to those rabbits allowed to mate directly after the stoppage of the feeding period of the leaves (Reghunandan et al., 1997). This indicates that the effect under certain condition might be reversible.

Ahmed et al. (2002) studied the anti-fertility effects in rats, however, this was performed with a benzene extract of *Ocimum tenuiflorum* L. leaves (250 mg/kg bw) given for 48 days. A decreased total sperm count, sperm motility, and forward velocity were observed. The percentage of abnormal sperms increased in the caudal epididymal fluid, and the fructose content decreased in the caudal plasma of the epididymis and the seminal vesicles. The results suggest that such effects are due to the androgen deprivation, caused by the anti-androgenic property of *Ocimum tenuiflorum* L. leaves. This

effect was reversible because all parameters returned to normal levels within 2 weeks after the withdrawal of treatment.

Genotoxicity and carcinogenic effects

A hot aqueous extract of fresh leaves of *O. tenuiflorum* L. was negative in *Bacillus subtilis* H-17(rec) and M-45 (rec) at a concentration of 0.5 ml/disc (Ungsurungsie et al., 1982). There were no further data available.

The genotoxicity and carcinogenicity of the methyleugenol constituent has been well documented (SCF, 2001; Rietjens et al., 2008)

Special studies on toxic effects

The effects of *Ocimum tenuiflorum* L. leaf extract on the changes in the concentrations of serum triiodothyronine (T_3), thyroxine (T_4) and serum cholesterol; in the activities of hepatic glucose-6-phosphatase (G-6-P), superoxide dismutase (SOD) and catalase (CAT); hepatic lipid peroxidation (LPO) and on the changes in the weight of the sex organs were investigated in male mice. While the plant extract at the dose of 0.5 g/kg bw for 15 days significantly decreased serum T4 concentrations, hepatic LPO and G-6-P activity, the activities of endogenous antioxidant enzymes, SOD and CAT were increased by the extract. No marked changes were observed in serum T_3/T_4 ratio and in the concentration of serum cholesterol. It was concluded that *O. tenuiflorum* L. leaf extract is antithyroidic as well as antioxidative in nature (Panda and Kar, 1998).

10. Safety assessment based on available knowledge (Level A)

There are no acute, subacute, subchronic and chronic, nor carcinogenicity studies available. The genotoxicity studies performed are inadequate. The studies on reproduction and fertility are also inadequate. One study showed some effects (decrease) on thyroid hormones, although the ratio T3/T4 was unchanged and some liver enzymes were increased; this study was however not designed to study the safety of *Ocimum tenuiflorum* L. It is therefore concluded that further data are needed to assess the safety of *Ocimum tenuiflorum* L. and its preparations.

The Working Group notes that this safety assessment does not cover methyleugenol, a compound known to be genotoxic and carcinogenic (SCF 2001), and present to up to 86 % in dry leaf extracts of *Ocimum tenuiflorum* L.

11. Further testing and/or data required for the assessment (Level B)

Some clarification is needed on the teratogenic effects and reproduction effects. As there are data indicating an effect on the fertility, an adequate reproduction and developmental effect study is required for the safety assessment. Furthermore at least a subchronic toxicity study with special focus on the thyroid function and morphology is needed.

As there are no adequate genotoxicity data available, additional studies as indicated in the guidance document for the safety assessment of botanicals and botanical preparation and OECD guidelines are also required.

When a full evaluation of *Ocimum tenuiflorum* is made, the presence of methyleugenol should be taken into account in the assessment.

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APPENDIX D: *FOENICULUM VULGARE* MILL. SSP. *VULGARE* VAR. *VULGARE*

The present report focuses on dried fruit extracts from *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare* and their estragole and *trans*-anethole content.

Disclaimer:

The present document aims at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected case and considers relevant constituents. The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated. The document is not, and cannot be interpreted as being, a policy document or a decision allowing the classification of a certain botanical or botanical preparation as a foodstuff or as a medicinal product. The document is focusing on one type of preparation and is not intended to evaluate all possible preparations of this botanical or all possible constituents, which should normally be part of a full safety assessment of a botanical. Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete. Contaminants such as heavy metals, mycotoxins, dioxins, pesticides, microbial contamination or PAHs are not evaluated.

It is outside the remit of this Working Group to evaluate possible beneficial effects and associated claims.

1. Identity and nature of the source material

Scientific name:	*Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare*
Synonyms:	*Anethum foeniculum* L., *Anethum foeniculum* L. var. *vulgare* Schkuhr, *Foeniculum officinale* All. var. *silvestre* Alef., *Foeniculum vulgare* subsp. *silvestre* (Brot.) Janchen, *Foeniculum vulgare* var. *silvestre* Presl, *Foeniculum vulgare* var. *vulgare* (non-scientific)
Common names:	Engl.: Bitter fennel, Common Fennel, Fr.: Fenouil amer, Germ.: Bitterfenchel, Wilder Fenchel, Dunkler Fenchel, Ital.: Finoccio comune, F. forte, F. selvatico, Span.: Hinojo amargo
Family:	Umbelliferae (Apiaceae)
Part used:	Dried whole cremocarps and mericarps (fruit) (water extracts)
Geographical origin:	Native to the Mediterranean region, and temperate regions of Asia, introduced and distributed to subtropical regions, Europe and North America; cultivated worldwide.

Growth and harvesting conditions: The whole plants are cut when the fruits are ripe.

2. Manufacturing process

After harvesting the ripe fruits are dried in the sun and separated by trashing.

In the manufacturing process the fruits yield up to 5% of an essential oil (Grieve, 1984; Uphof, 1959).

3. Chemical composition

Foeniculum vulgare Mill. ssp. *vulgare* var. *vulgare* (bitter fennel) contains 2-6% essential oil (European Pharmacopoeia, 2005).

The major constituent of the essential oil is *trans*-anethole at levels between 50-75%. Other constituents of the essential oil are estragole (3.5 to 12%), fenchone (12 to 25 %), limonene (1-2%), alpha-pinene (1-5%), and other monoterpenes at levels generally below 1% each.

The composition of the essential oil may vary with place of origin and ripening stage of the fruits.

Other constituents of the oil are fatty oil (10-20%) (high content of cis-6-octadecenoic acid), phenol carboxylic acids, and flavonoids).

Table 1 presents the occurrence of estragole in natural sources of flavourings (Council of Europe, 2006). The data presented in Table 1 illustrate that *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare* is not the only source of estragole in the regular diet.

Table 7: Main occurrence of estragole in natural sources of flavourings (Council of Europe, 2006)

Botanical name	Common name	Essential oil in plant (%)[b]	Estragole in essential oil (%)[a]	Estragole in part of plant used (%)[b]
Artemisia dracunculus L. (Asteraceae)	Tarragon	0.25-1 in herb	60-75	0.7
Ocimum basilicum L. (Lamiaceae)	Sweet basil	0.8 in herb	20-89	approx. 0.4
Foeniculum vulgare Mill. subsp. *vulgare* var. *dulce* (Mill.) Batt. (syn. *Foeniculum vulgare* Mill. var. *dulce* (Mill.) Batt. et Trab.) (Apiaceae)	Sweet fennel, Roman fennel		1.5-5.0	
Foeniculum vulgare Mill. subsp. *vulgare* var. *vulgare* (syn. *Foeniculum vulgare* var. *vulgare*) (Apiaceae)	Bitter fennel, Common fennel	2-6 in fruit	3.5-12.0	0.3
Pimpinella anisum L. (Apiaceae)	Anise, Sweet cumin	1-4 in fruit	1-5	max. 0.04
Illicium verum Hook. f. (Magnoliaceae)	Star-anise	5 in fruit	5-6	max. 0.25
Agastache foeniculum (Pursh.) Ktze. (syn. *Lophantus anisatus. A. anethiodora, A. anisata*) (Lamiaceae)	Anise hyssop, Giant hyssop, Liquorice mint		74	
Anthriscus cerefolium (L.) Hoffm. ssp. *cerefolium* (Apiaceae)	(Garden) chervil	0.9 in fruit	up to 85	max. 0.8
Melissa officinalis L. (Lamiaceae)	Lemon balm		6.3	
Myrrhis odorata (L.) Scop. (Apiaceae)	Sweet chervil		up to 75	

[a] According to Council of Europe (1998a) and secondary literature
[b] According to Leung (1980), Council of Europe (1997a) and Council of Europe (1995)

The flavour of fennel oil depends upon its two main constituents, fenchone and *trans*-anethole. Fenchone is a bitter tasting element whilst *trans*-anethole is an aromatic compound that accounts for the distinctive "liquorice" flavour of anise, fennel, star anise, and anise myrtle. It may also be referred to as p-propenylanisole, anise camphor, isoestragole, or oil of aniseed (Bown, 1995). The proportions of these two ingredients vary according to strain and region. Plants growing in the Mediterranean and southern Europe usually have a sweet oil, whilst plants growing in central and northern Europe usually produce a more bitter oil (Bown, 1995). The quality of the oil also depends upon how well the fruit has been dried - the oil from fully ripened and dried fruits being much sweeter and more fragrant.

The concentration of *trans*-anethole and estragole in fennel fruits may show significant variation with geographical origin, plant maturity at harvest, harvesting techniques, storage conditions, processing (e.g. drying) and method of extraction (e.g. extraction with CO_2 vs. CH_2Cl_2)(Smith et al., 2002).

The major compounds present in fruits and the essential oil of bitter fennel that are of toxicological concern are estragole and *trans*-anethole. The safety assessment can therefore be based on these constituents.

4. Specifications

The estragole content of the essential oil has been reported to amount to 2 to 8% (European Pharmacopoeia, 2005); taking into account that the fruits may contain up to 6% essential oil (European Pharmacopoeia, 2005) this amounts to a level of estragole in the fruits of 1200 – 4800 mg/kg. The composition of the essential oil may vary with geographical origin, plant maturity at harvest, harvesting techniques, storage conditions, processing (e.g. drying) and method extraction (e.g. extraction with CO_2 vs. CH_2Cl_2)(Smith et al., 2002).

The level of *trans*-anethole in the essential oil amounts to 50-75% (European Pharmacopoeia, 2005). Taking into account that the fruits contain 2-6% essential oil this amounts to a level of *trans*-anethole in the fruits of 10000 – 45000 mg/kg.

5. Stability of the botanical or botanical preparation

It is known that under influence of UV, *trans*-anethole in essential oil can isomerise to *cis*-anethole, which is more toxic. Such isomerisation is not expected to occur in the fruits.

6. Proposed uses and levels

In some countries herbal tea beverages on the basis of bitter fennel fruits or bitter fennel oil, partly in the form of instant teas with reduced estragole content, are used for babies and infants as food for carminative purposes.

The EMEA monograph on the fructus/seeds of bitter fennel (EMEA, 2007b) indicates use in adults of single doses amounting to 1.5 to 2.5 g (freshly) comminuted fruits with 0.25 l of boiling water (brew for 15 minutes) three times a day as a herbal tea.

For children the monograph lists an average dose of 3-5 g of (freshly) comminuted fruits as a herbal tea, in three divided doses, for short term use (less than one week), and also indicates that use is not recommended in children under 4 years of age.

Reported uses of bitter fennel fruits for flavouring purposes are as follows (Burdock, 1995): baked goods 130 mg/kg, fats and oils 260 mg/kg, meat products 1200 mg/kg, snack foods 700 mg/kg, alcoholic beverages 300 mg/kg, gravies 190 mg/kg (rounded figures). Information from Leung and Foster (1996) corroborates that the information given above on the use levels refers to the use of the fruits.

7. Information on existing assessments

Bitter Fennel

Bitter fennel was evaluated by EMEA in 2007 and monographs on both the essential oil (EMEA, 2007a) and the fructus/seeds (EMEA, 2007b) are available.

Foeniculum vulgare is allowed for food supplement use in Belgium (Royal Decree) and evaluated in Italy for food supplement use.

Trans-anethole

JECFA derived a temporary ADI of 0-2.0 mg/kg bw for *trans*-anethole and concluded that there is no safety concern at current levels of intake when used as a flavouring agent (JECFA, 1998).

Scientific data relevant to the safety evaluation of *trans*-anethole (i.e. 4-methoxypropenylbenzene) as a flavouring substance were evaluated by the FEMA Expert Panel (Newberne et al., 1999). It was concluded that because *trans*-anethole undergoes efficient metabolic detoxification in humans at low levels of exposure, the neoplastic effects in rats associated with dose-dependent hepatotoxicity are not indicative of any significant risk to human health from the use of *trans*-anethole as a flavouring substance.

Trans-anethole is currently permitted by the U.S. Food and Drug Administration (FDA) for direct addition to food for human consumption as a flavouring substance and is considered by the Flavour and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavouring substance (Newberne et al., 1999).

Estragole

The SCF and Council of Europe published scientific evaluations on estragole (SCF, 2001; Council of Europe, 2006) in which it was concluded that estragole is genotoxic and carcinogenic, and recommended restrictions in use of the compound.

EMEA published a public statement on the use of herbal medicinal products containing estragole (EMEA, 2005) concluding that estragole is a naturally occurring genotoxic carcinogen, but that at the low levels of exposure resulting from consumption of herbal medicinal products (short time use in adults at recommended posology) it does not pose a significant cancer risk. This conclusion is in line with that of Smith et al. (2002) who published a safety assessment of allylalkoxybenzene derivatives, including estragole, used as flavouring substances. The Working Group notes that these were qualitative safety assessments.

The Working Group notes that the EMEA monograph concludes on the safety of the preparations evaluating infusion from the fruits (water extracts), whereas lipophilic extracts and essential oils are expected to contain estragole in higher concentrations.

The EMEA monograph on the fructus/seeds from bitter fennel (EMEA, 2007b) also concludes that the genotoxic risk related to estragole is not considered to be relevant in the specified conditions of use due to the small amount present in herbal infusions prepared from fennel, although a quantitative risk assessment was not presented.

Estragole is currently permitted by the U.S. Food and Drug Administration (FDA) for direct addition to food for human consumption as a flavouring substance and is considered by the Flavour and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavouring substance (Hall and Oser, 1965).

Recently the European Union has updated existing legislation on flavourings in the light of technical and scientific developments. The resulting Regulation No 1334/2008, which shall apply from 20 January 2011, now bans the addition of estragole as such to food. For certain compound foods the Regulation establishes maximum levels for estragole resulting from the use of flavourings or food ingredients with flavouring properties, which naturally contain this substance (table 2). The Regulation does not apply to raw foods and non-compound foods and mixtures such as, but not exclusively, fresh, dried or frozen spices and/or herbs, mixtures of tea and mixtures for infusion as such as long as they have not been used as food ingredients (European Union, 2008).

Table 8: European maximum levels for estragole (1-allyl-4-methoxybenzene), naturally present in flavourings and food ingredients with flavouring properties, in certain compound foods as consumed to which flavourings and/or food ingredients with flavouring properties have been added

Compound food in which the presence of estragole is restricted	Maximum level (mg/kg)
Dairy products	50
Processed fruits, vegetables (incl. mushrooms, fungi, roots, tubers, pulses and legumes), nuts and seeds	50
Fish products	50
Non-alcoholic beverages	10

Ref.: European Union, 2008

8. Exposure

Intake of estragole and trans-anethole from bitter fennel fruits

Common fennel (e.g. bitter fennel) appears to be the more commonly used fennel whenever the spice is called for. However, according to the National Formulary (USA) and the Food Chemicals Codex (USA) bitter fennel oil is used only to a limited extent, mainly in cosmetics (Leung and Foster, 1996).

The exposure to estragole and *trans*-anethole from bitter fennel fruits can be estimated based on the assumption that 4.5 to 7.5 g (3 times 1.5 to 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 and 50-75 % *trans*-anethole in the oil, this would imply an intake of 1.9 to 15.8 mg estragole per day and of 28 to 98 mg *trans*-anethole per day. For a 60 kg person this amounts to an intake of 33 to 263 μg estragole/kg bw/day and 0.5 to 1.6 mg *trans*-anethole/kg bw/day.

9. Toxicological data

Data from previous assessments

Trans-anethole

The pharmacologic effects of *trans*-anethole most often noted are reduction in motor activity, lowering of body temperature and hypnotic, analgesic, and anticonvulsant effects. The toxicology of *trans*-anethole was evaluated by JECFA and an ADI of 0-2 mg/kg was set. JECFA concluded that there is "no safety concern at the current levels of intake when used as a flavouring agent (JECFA, 1998).

The EMEA monograph (EMEA, 2007a) states that because of its estrogenic activity, excessive doses of fennel oil may affect hormone therapy, oral contraceptives and hormone replacement therapy. It is also stated however that data on the estrogenic activity of *trans*-anethole and an acetonic extract of fennel and of the antifertility activity of *trans*-anethole demonstrated *in vitro* and in laboratory animals at high concentrations, are not considered relevant to human exposure given the recommended levels and conditions of use (EMEA, 2007a).

Estragole

Estragole is an alkenylbenzene that might be of safety concern because of its reported carcinogenic effect at high dose levels (SCF, 2001).

EMEA published a public statement on the use of herbal medicinal products containing estragole (EMEA, 2005) concluding that estragole is a naturally occurring genotoxic carcinogen, but that it does not pose a significant cancer risk at the low levels of exposure resulting from consumption of herbal medicinal products (short time use in adults at recommended posology). This conclusion is in line with that of Smith et al. (2002) who published a safety assessment of allylalkoxybenzene derivatives, including, used as flavouring substances.

The EMEA monograph on the fructus/seeds from bitter fennel (EMEA, 2007b) also concludes that the genotoxic risk related to estragole is not considered to be relevant in the specified conditions of use due to the small amount present in herbal infusions prepared from fennel.

Carcinogenicity of estragole

The SCF opinion presented an overview of the toxicological data on estragole (SCF, 2001). It was concluded that the compound is both genotoxic and carcinogenic. The induction of hepatomas was observed in different species. Of the data listed in the SCF opinion one experiment appears useful for further assessment of bitter fennel and its fruits.

Groups of circa 50 CD-1 female mice, approximately 8 weeks old, were maintained for 12 months on grain diets containing 2300 or 4600 mg/kg estragole and the incidence of hepatomas was quantified (Miller et al., 1983). Table 3 summarizes the results (see also SCF, 2001). Table 4 presents the results from a Benchmark Dose (BMD) analysis of these data using the EPA BMD software (version 1.4.1c).

Table 9: Overview of the data from Miller et al. (1983) on the incidence of hepatomas in female mice exposed for 12 months via the diet to estragole

dose	Estimated dose mg/kg bw/day	No of animals	No of mice with hepatomas	incidence
0	0	43	0	0
0.23% in diet	150-300	48	27	56
0.46% in diet	300-600	49	35	71

Table 10: Results of a BMD analysis of the data from Miller et al. (1983) on the incidence of hepatomas in female mice exposed for 12 months via the diet to estragole (Table 3), using BMDS version 1.4.1c and the default settings of extra risk, a Benchmark Response (BMR) of 10% and a 95% confidence limit. To make a worst case estimate the lowest dose levels of the range were used (i.e. 150 and 300 mg/kg bw respectively.

Mice gender	model	No of parameters	Log likelihood	accepted	BMD_{10} mg/kg bw/day	$BMDL_{10}$ mg/kg bw/day
females	null	1	-96.1243			
females	full	3	-62.2103			
females	two-stage	1	-62.7403	yes	22.4	18.1
females	gamma	1	-62.7403	yes	22.4	18.1
females	log-logistic	1	-62.2124	yes	13.1	9.2
females	log-probit	1	-62.7928	yes	40.7	32.7
females	Weibull	1	-62.7403	yes	22.4	18.1

From this it is concluded that the $BMDL_{10}$ values vary between 9 and 33 mg/kg bw/day for female mice.

10. Safety assessment based on available knowledge (Level A)

Fennel is a commonly used household remedy, having been claimed to be useful in the treatment of a variety of complaints, especially those of the digestive system (Wicht, 1989; Leung & Fosters, 1996). The fruits, leaves and roots can be used, but the fruits are most active medicinally and are the part normally used. An essential oil is often extracted from the fruits.

The plant is said to be analgesic, anti-inflammatory, antispasmodic, aromatic, carminative, diuretic, emmenagogous, expectorant, galactogogous, hallucinogenic, laxative, stimulant and stomachic (Emboden, 1979; Chiej, 1984; Grieve, 1984; Launert 1981; Bown, 1995). Bitter fennel is often added to purgatives in order to allay their tendency to cause gripe, and also to improve the flavour. The essential oil is bactericidal, carminative and a stimulant (Duke and Ayensu, 1985). The plant was formerly used as a strewing herb[22]

[22] See http://www.pfaf.org/database/plants.php?Foeniculum+vulgare+azoricum

Trans-anethole

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 for *trans*-anethole derived by JECFA who concluded that there is no safety concern at current levels of intake when used as a flavouring agent (JECFA, 1998).

The exposure to *trans*-anethole from bitter fennel fruits can be estimated based on the assumption that 4.5 to 7.5 gram (3 times 1.5 to 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 to 98 mg *trans*-anethole per day. For a 60 kg person this amounts to an intake of 0.5 to 1.6 mg *trans*-anethole/kg bw/day. This is below the ADI of 0-2.0 mg/kg bw established by JECFA. The Working Group notes that this exposure to *trans*-anethole resulting from the use of bitter fennel fruits for the preparation of fennel tea would already amount to 25 to 80% of the ADI while also other sources of *trans*-anethole exist.

Estragole

Bitter fennel fruits and their extracts also contain estragole which is a compound know to be both genotoxic and carcinogenic. This excludes that safety can be presumed based on available knowledge and a history of safe use.

An important issue is the question on how to deal with botanicals and botanical ingredients that contain chemicals that are both genotoxic and carcinogenic.

The EFSA guidance document[23] states the following:

> "In cases where no health-based guidance values are available or where the botanical ingredient contains substances that are both genotoxic and carcinogenic, the "Margin of Exposure" (MOE) approach (EFSA, 2005) could be applied covering the botanical(s) under examination and any other dietary sources of exposure. The MOE approach compares toxic effect levels with human exposure levels. Alternatively, it could be evaluated whether the expected exposure to the genotoxic and carcinogenic ingredient will not be significantly increased, compared to the intake from other sources.
>
> Where a matrix effect is advocated to support the safety of particular 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."

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 as well as possible information on the matrix effect.

[23] See http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_draftguidance_botanicals_public_cons_update_en.pdf?ssbinary=true

Margin of Exposure

The Margin of Exposure (MOE) approach was developed by EFSA as a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic (EFSA, 2005). 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. It can be for example the $BMDL_{10}$, the lower confidence bound of the Benchmark Dose that gives 10 % (extra) cancer incidence (BMD_{10}). The MOE is defined as the ratio between this reference point, the $BMDL_{10}$, and the estimated dietary intake (EDI) in humans.

EFSA has already clarified that the reference point is to be compared to various dietary intakes estimates in humans, taking into account different consumption patterns (EFSA, 2005).

The exposure to estragole from bitter fennel fruits estimated based on the assumption that 4.5 to 7.5 gram of fennel fruits per day would be used for the preparation of fennel tea, amounts to 33 to 263 µg estragole/kg bw/day for a 60 kg person.

Using the $BMDL_{10}$ values of 9 to 33 mg/kg bw/day for female mice as derived from the Miller et al. study (Miller et al., 1983) (see above Table 10) one can calculate a MOE of about 34 to 1000 which indicates that use of bitter fennel fruits for preparation of fennel tea could be considered a high priority for risk management.

Matrix effect

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.

Recently, Jeurissen et al. (2008) 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. A similar effect could be obtained by adding the SULT inhibitor pentachlorophenol to the incubation medium. Because the inhibition by basil extract resembles the inhibition by the SULT inhibitor pentachlorophenol and because the inhibition was not observed in incubations with the direct electrophile 1'-acetoxyestragole, it was concluded that the inhibition by the basil extract occurs at the level of the SULT mediated bioactivation of 1'-hydroxyestragole to 1-sulfoxyestragole (Jeurissen et al., 2008). Although it remains to be established whether a similar inhibition will occur in the *in vivo* situation, the inhibition of SULT mediated bioactivation of 1′-hydroxyestragole by basil ingredients suggests that the possibilities for bioactivation and subsequent adverse effects may be lower 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.

In contrast to the findings with this methanolic basil extract, Müller et al. (1994) showed that the genotoxic potential of estragole is not masked by ingredients of basil oil. The genotoxic potentials of basil oil and estragole were compared in the unscheduled DNA synthesis (UDS) test, using basil oil with an estragole content of 88%, and it was concluded that basil oil induced UDS in the same dose range as estragole (Müller et al., 1994). This lack of protective effect in basil oil may be related to the high ratio between estragole (88%) and other herbal oil ingredients (12%) in the essential oil fraction of basil compared to the ratio between estragole and other herbal ingredients in the total herb. The Working Group notes that this last example may not be indicative for the matrix effects on estragole in bitter fennel itself since the concentration of estragole in basil oil is much higher and that of other ingredients probably much lower than in the bitter fennel fruits or tea derived from it.

From these examples it becomes clear that when a matrix effect is advocated to support the safety of a botanical or a botanical ingredient, experimental and/or other data need to be provided that support the occurrence of the matrix effect *in vivo* at relevant levels of intake and with the botanicals or botanical preparation of interest.

Based on the Margin of Exposure of about 34 to 1000, it is concluded that based on the existing data, the use of infusions of bitter fennel fruits as tea is of high priority for risk management.

11. Further testing and/or data required for the assessment (Level B)

The Working Group notes that the issue requiring further data relate in first instance to the carcinogenicity of estragole within the bitter fennel fruits especially focussing on the importance of the matrix effect and the actual exposure levels for different uses and use levels. The Working Group notes that with the presently available data the MOE approach is driven by the accuracy of the intake estimates rather than by the $BMDL_{10}$ derived from the carcinogenicity data, although more accurate determination of this $BMDL_{10}$ may also be required.

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APPENDIX E: *LINUM USITATISSIMUM* L.

The present report focuses on dried ripe seeds (also referred to as linseed, flaxseed) and their lignan content.

Disclaimer:

The present document aims at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected case and considers relevant constituents. The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated. The document is not, and cannot be interpreted as being, a policy document or a decision allowing the classification of a certain botanical or botanical preparation as a foodstuff or as a medicinal product. The document is focusing on one type of preparation and is not intended to evaluate all possible preparations of this botanical or all possible constituents, which should normally be part of a full safety assessment of a botanical. Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete. Contaminants such as heavy metals, mycotoxins, dioxins, pesticides, microbial contamination or PAHs are not evaluated.

It is outside the remit of this Working Group to evaluate possible beneficial effects and associated claims

1. Identity and nature of the source material

Scientific name:	*Linum usitatissimum* L.
Synonyms:	*Linum crepitans* (Boenn.) Dumort., The most important cultivated subspecies are: *Linum usitatissimum* ssp. *usitatissimum* var. *vulgare*, *Linum usitatissimum* ssp. *crepitans*, *Linum usitatissimum* ssp. *humile*
Common names:	Engl.: Flax, Common flax, Fr.: Lin, Germ.: Dreschlein, Saatlein, Flachs, Ital.: Lino
Family:	Linaceae
Parts used:	Dried ripe seeds (whole seeds)
Geographical origin:	Flax is native to the region extending from the eastern Mediterranean to India, and extensively cultivated since ancient Egypt. Today significant areas of land are devoted to oilseed and fiber flax cultivation in more than 16 countries distributed throughout the temperate zones of the world, Russia (fiber) and Canada (oil) are the major flaxseed production areas.

Growth and harvesting conditions: Flax is an erect annual plant growing to 1.2 m tall, with slender stems. The leaves are glaucous green, slender lanceolate, 20-40 mm long and 3 mm broad. The flowers are pure pale blue, 15-25 mm diameter, with five petals. The fruit is a round, dry capsule 5-9 mm diameter, containing several glossy brown seeds shaped like an apple pip, 4-7 mm long. In addition to referring to the plant itself, "flax" may refer to the unspun fibres of the flax plant.

Flax is planted in early spring and harvested in autumn after flowering. The best time to harvest is when there are a few flowers in bloom and a few green leaves on the plant. A rule of thumb is to harvest when 90 % of the seed capsules are brown. Flax can directly be combined, but sometimes it is swathed and allowed to dry in the field before picking it up with a combine.

2. Manufacturing process

No manufacturing process is necessary to get whole flaxseed.

3. Chemical composition

Main compounds

Flaxseed is mainly composed of mucilage (3-10 %), dietary fibre (4-7 %), fatty oil (20-45 %), proteins (20-25 %), water (5-14 %), and minerals (3-5 %). The typical fatty acid composition of the fatty oil is the following: palmitic acid (6 %), stearic acid (2.5 %), oleic acid (19 %), linoleic acid (24.1 %), α-linolenic acid (47.4 %), and other fatty acids around 0.5 % (Hänseler et al., 1993-95; Morris, 2003; USDA, 2008).

Mucilage is composed of neutral and acidic polysaccharides which after hydrolysis yield galactose (8–10 %), arabinose (9–12 %), rhamnose (13–29 %), xylose (25–27 %), and galacturonic and mannuronic acids (approx. 30 %) (EMEA, 2006).

Lignans

100 g dry flaxseed contain about 300 mg lignans, including pinoresinol (~870 µg), syringaresinol (~48 µg), lariciresinol (~1780 µg), secoisolariciresinol (SEC) (~165 mg), matairesinol (MAT) (~529 µg), and hydroxymatairesinol (HMR) (~ 35 µg), all expressed as aglycons. (Smeds et al., 2007; Milder et al., 2005a)

Cyanogenic glycosides

100 g dry flaxseed contain about 1-500 mg total cyanide incorporated in cyanogenic glycosides, depending on the cultivars including linustatin as a diglycoside (210-350 mg, 54-76 % of total cyanogenic glycosides), neolinustatin as a diglycoside (90-200 mg) and linamarin as a monoglycoside (<32 mg) (Oomah et al., 1992; Kobaisy et al., 1996).

The major compounds present in flaxseed that are of toxicological concern are the lignans and the cyanogenic glycosides.

The present evaluation focuses on the lignans because of their oestrogenic effects.

4. Specifications

Flaxseed products are generally not standardized based on specific chemical components, but rather are evaluated with a number of identity and quality tests. Tests may include microscopic/macroscopic inspection and sensory evaluation (Natural Standard Monograph, 2008).

5. Stability of the botanical ingredient

Based on chemical structure it is unlikely that the amount of lignans significantly changes during storage. It is known that inappropriate storage of flaxseed may lead to a higher content of cyanogenic glycosides. Fatty acids, especially polyunsaturated ones are sensitive to oxidation, but in whole unbroken flaxseed the outer layers of the seed prevent oxidation.

Whole flaxseed can be stored for up to one year in a dry location. Ground flaxseed can be kept in a refrigerator for up to three months or in a freezer for six months. At high temperatures, such as during cooking, flaxseed powder/flour will degrade.

There is no available information about the stability of lignans in foods and/or food supplements.

6. Proposed uses and levels

Flaxseed is used for various purposes which are explained in some detail in the following sections.

6.1. Phytoestrogens – lignans

Flax is noted to be the richest food source of plant lignans including secoisolariciresinol diglucoside (SDG, MW 686.7). 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 (Thompson et al., 1991). Other lignans such as matairesinol and lariciresinol are also found in flaxseed.

Phytoestrogens represent a family of plant compounds that have been shown to have both oestrogenic and antioestrogenic properties. Lignans, similarly to isoflavonoids and coumestans, are often referred to as phytoestrogens, and may possess oestrogen receptor agonist or antagonist properties, with unclear effects on hormone-sensitive cancers such as breast, uterine, and prostate cancer. Pharmacodynamic studies suggest that there might be an oestrogenic or antioestrogenic effect of flaxseed (Adlercreutz, 2002). Some authors therefore call mammalian lignans modulators of endogenous sex steroid hormones. Since the identification of mammalian lignans in human urine in 1981, 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. Results of the few intervention studies that have been conducted have been mixed; therefore, further research, in particular long-term intervention trials, is needed to provide clarification for this relationship (Adlercreutz, 1984; Adlercretz et al., 1987).

A number of epidemiological studies and animal experiments indicate that phytoestrogens could play a role in the prevention of a number of sex-hormone dependent cancers. Indicators of a protective activity are the following observations (Adlercreutz, 2002; Haggans et al., 1999 & 2000):

- high phytoestrogen excretion in the urine is accompanied by high levels of sex hormone binding globulin (SHBG);
- high phytoestrogen excretion in the urine is accompanied by a lower incidence of certain cancers, e.g. breast cancer (BC) in Asian women and prostate cancer in Asian men;
- lignan deficient diets are correlated with increased BC risk.

Hutchins et al. (2001) conducted a randomized crossover trial in 28 postmenopausal women (aged 52-82 years). During three seven-week feeding periods, subjects consumed their normal diets with or without the addition of ground flaxseed (5-10 g). It was found that flaxseed diets significantly reduced serum 17-beta-estradiol and estrone sulfate levels, and increased serum prolactin levels.

Thompson et al. (2005) conducted a randomized double-blind placebo-controlled clinical trial in 32 women to assess the effects of flaxseed on breast cancer in menopausal women. Patients were randomized to daily intake of either a 25 g flaxseed-containing muffin (N=19) or a control (placebo) muffin (N=13). In a randomized trial of 28 post-menopausal women given 5-10 g per day of ground flaxseed for seven weeks, the urinary excretion of 2-hydroxyestrogen increased significantly and the urinary 2-hydroxyestrogen and 16-alpha-hydroxyestrone ratio was also increased, in a dose-dependent fashion (Haggans et al., 1999). Brooks et al. (2004) similarly observed in a study of 46 postmenopausal women an increase in urinary 2-hydroxyestrogen to 16-alpha hydroxyestrone ratio in those fed muffin with 25 g flaxseed daily compared with those fed placebo muffin for 16 weeks.

In a study of 18 women, Phipps et al. (1993) found that subjects who supplemented their diets with 10 g of flaxseed daily (vs. a low fibre diet) for three menstrual cycles experienced prolongation of their luteal phase and had fewer anovulatory cycles. Kurzer et al. (1995) conducted a small study in 13 women over the course of three menstrual cycles. Daily diets were supplemented with 10g of ground flaxseed. It was found that flaxseed consumption significantly increased the excretion of enterodiol, enterolactone, and matairesinol (compared to baseline values).

The recommended dosage of ground, bruised or milled whole flaxseed as a laxative for adults is 10–15 g, 2–3 times daily, as n-3 fatty acid source between 15-50 g and for decreased blood glucose level 50 g/day.

As a source of phytoestrogen lignans flaxseed alone or in test meals was used between 5-25 g/day in controlled trials corresponding to 15-75 mg lignans intake per day (lignan in flaxseed: 300 mg/100 g dry seed).

6.2. Laxative effect

The laxative effects of flaxseed have long been recognized empirically, and then shown in animal and clinical investigations. These effects are attributed to the bulk materials and in particular to the mucilage that binds with water and swells to form a demulcent gel in the intestine (Schilcher et al., 1986; Wirths et al., 1985; Schulz et al., 1983). In a randomised investigator-blinded trial with two parallel treatment groups, 55 patients suffering from constipation predominant irritable bowel syndrome received 6 – 24 g/d either flaxseed (roughly ground partly defatted) or psyllium seed for 3 months (Tarpila et al., 2004). The recommended dosage as a laxative for adults, elderly and adolescents over 12 years of age (10 – 15 g, 2 – 3 times daily) is supported by general evidence and by clinical investigations (Schilcher et al., 1986; Willuhn, 1989).

6.3. n-3 fatty acid source

Flaxseed can be used as a source of n-3 fatty acids. In a three-month feeding trial, the effect of flaxseed supplementation on atherogenic risk reduction was investigated. The researchers reported the effects on serum lipids of a flaxseed supplement; supplementation levels were between 15-50 g ground bruised, or milled raw seeds in test meal (Bierenbaum et al., 1993; Cunnane et al., 1993) or 1-10 g, of bruised, cracked, ground or milled seed 3 times per day (Blumenthal et al., 2000; Fischer et al., 1984).

6.4. Effect on blood lipids levels

Flaxseed may affect blood lipid levels. Consumption of 50 g/day ground, raw flaxseed by healthy female volunteers for 4 weeks lowered serum total cholesterol by 9 % and low-density-lipoprotein (LDL)-cholesterol by 18 % (Cunnane et al., 1993).

In an investigation concerning 32 subjects of an elderly/nursing home (special test meals beside usual diet for 84 days), the effect of fibre-rich test meals on blood lipids was studied. They received 39 g whole flaxseed two times a day for 4 weeks, then 34 g dietary fibre mix of fruits two times a day for 4 weeks, then 46 g fruit muesli two times a day for 4 weeks.-The identified reductions in blood lipids were considered insignificant in regard to the effect on an increased serum triglyceride level (Wirths et al., 1985).

7. Information on existing assessments

- The ESCOP Monograph (2003) shows the therapeutic indications, pharmacodynamic properties, and some preclinical and clinical safety data of the whole flaxseed or mucilage prepared from the seed. The monograph does not give any information on safety of lignans in the seed.

- EMEA (2006) states that herbal medicinal products containing flaxseed have a well-established use for the treatment of habitual constipation or in conditions in which easy defaecation with soft stool are desirable. The recommended dosage as a laxative for adults, elderly and adolescents over 12 years of age (10–15 g, 2–3 times daily) is supported by general evidence and by clinical investigations. Flaxseed is traditionally used as a demulcent preparation for the symptomatic relief of mild gastrointestinal discomfort. Linseed has a long history of medicinal use, its main effects being as a laxative and expectorant. The seeds, one or two 5-ml spoonfuls in a tumbler of water, may be taken to increase the bulk of intestinal contents in the treatment of constipation. Mucilage has also been used as a demulcent drink. The poultice mass may be prepared by gradually adding 100 g of broken linseed to 250 g of boiling water. Available data of blood cholesterol and glucose lowering effects, anti-tumour and oestrogenic effects are not sufficient. There is no evidence of possible harmful effect of thiocyanate originated from cyanogenic glycosides in human beings, in the amount generated by flaxseed consumption. Flaxseed preparations are claimed to produce a gentle and safe laxation and to have an acceptable level of safety when the special warnings for such bulk forming agents are followed.

- The Natural Standard Monograph on flaxseed and flaxseed oil (2008) concludes that flaxseed is likely safe when used orally in recommended doses for under four months by healthy individuals. Although long-term studies on the safety of flaxseed is scarce in the available literature, a major component of flaxseed, α-linolenic acid, has been well tolerated for up to five years as part of the Mediterranean diet. The safety of flaxseed when used for >4 months is not clear due to lack of

data. Flaxseed is possibly unsafe when used in patients with prostate cancer based on conflicting reports on alpha linolenic acid and prostate cancer development but recent reports both in humans and animals reveal benefit in the use of flax in prostate cancer. In theory, flaxseed may increase the risk of bleeding, lower blood pressure, or cause hypoglycemia, although scant human evidence is currently available. Flaxseed is likely unsafe when it is used in patients with acute or chronic diarrhea, irritable bowel disease, diverticulitis, or inflammatory bowel disease

- According to the MedlinePlus website (http://www.nlm.nih.gov/medlineplus/druginfo/natural/patient-flaxseed.html), evidence from human studies suggests that flaxseed can be used as a laxative. However, more information is needed to compare effectiveness and dosing to more commonly used agents. There are few studies on flaxseed safety in humans. Flaxseed supplements do appear to be well tolerated in the available research, and there is long-standing historical use of flaxseed products without many reports of side effects. Due to the possible oestrogen-like effects of flaxseed, it should be used cautiously in women with hormone sensitive conditions such as endometriosis, polycystic ovary syndrome, uterine fibroids, or cancer of the breast, uterus, or ovary. Until more information is available, men with prostate cancer or at risk for prostate cancer should avoid flaxseed supplements. Flaxseed may stimulate menstruation or have other hormonal effects and could be harmful to pregnancy.

Existing assessments aforesaid do not evaluate the safety of flaxseed as a source of phytoestrogen plant lignans.

8. Exposure

8.1. Dietary intake of lignans

An American case-control study used a Block Food Frequency Questionnaire and several published databases to estimate phytoestrogen intake. The mean lignan intake was 6 mg/day, the highest ever reported (Fink et al., 2006). A French cohort study used a FFQ and published phytoestrogen databases to estimate isoflavone, lignan and coumestan intake. The median intake of phytoestrogens was low - 1 mg/day with 97 % of this from lignans (Touillaud et al., 2006). Horn-Ross et al. (2001) found 29 µg/day matairesinol and 121 µg/day secoisolariciresinol and total lignan 150 µg/day intakes in 1272 pre- and postmenopausal, and 1616 control women.

A survey of 4660 Dutch men and women during 1997-1998 found that the median total lignan intake was 0.98 mg/day (Milder et al., 2005b). Lariciresinol and pinoresinol contributed about 75 % to the total lignan intake while secoisolariciresinol and matairesinol contributed only about 25 %. The daily phytoestrogen intake of postmenopausal women in the US was estimated to be less than 1 mg/day with 80 % from lignans and 20 % from isoflavones (de Kleijn et al., 2001). McCann et al. (2002) found total lignan intake as 0.46-0.67 mg/day in 207 pre- and postmenopausal, and 188 control women, and 0.47-0.50 mg/day in 122 pre- and postmenopausal and 2036 control women, while Keinan-Boker et al. (2002 & 2004) reported 0.67 and later 1.1 mg/day intake in Dutch women.

8.2. Intake from food supplements containing flaxseed

There are several products marketed in Europe and USA; their composition is listed below:

- 66 mg lignans per capsule; recommended intake of 1 capsule 2-3 times a day (132-198 mg/day),

- 78 mg secoisolariciresinol diglucoside (SCG) per capsule; recommended intake of 1 to 2 capsules 2 times daily (156-312 mg/day). The product contains 90% pure flax seed lignans and 10% flax seed oil.
- one capsule containing 200 mg lignan extract from flaxseed, and 50 mg secoisolariciresinol diglycoside, among other constituents; recommended intake of one capsule per day.
- 70 mg lignan per capsule in 350 mg of a high lignan flax hull extract 20 %; recommended intake of 2 capsules daily (140 mg/day).
- flax seed powder: recommended daily serving of 15 g containing 300 mg lignan.
- 35 mg secoisolariciresinol diglucoside per capsule, recommended intake of 1 capsule per day.
- 20 mg SDG flaxseed lignans per serving of 6 tablets, 1 to 6 capsules/day
- 40 mg lignans per capsule, 1 capsule/day
- 20 mg SDG flaxseed lignans per softgel, 1-2 softgels/day
- 20 mg SDG flaxseed lignans per 3 softgels, 3 softgels/day
- 24 mg SDG flaxseed lignans per 2 tablets, 2 tablets/day
- A number of products with unknown lignan contents are also marketed.

Food supplement's consumers' intake of flaxseed lignans varies between 35-312 mg/day, this value is 5 to 52 times higher than the level of dietary intake.

9. Toxicological data

The U.S. Food and Drug Administration (FDA) has not granted a GRAS ("generally regarded as safe") status to flaxseed, although it does allow up to 12 % flaxseed in food by weight (Natural Standard Monograph, 2008).

9.1. Reproductive toxicity, genotoxicity and carcinogenicity of lignans

It can be stated that no studies have been performed in relation to the phytoestrogens secoisolariciresinol and matairesinol. Muir and Westcott (2003) in their review work said that flaxseed is essentially free of compounds that cause acute toxicity.

The values for red blood cell characteristics including red blood cell counts, haemoglobin and haematocrit levels, and also platelet volume were similar in lignan complex-treated or untreated normo- and hypercholesterolemic rabbits. It is concluded that chronic use of lignan complex had no adverse effects on the hemopoietic system (Prasad, 2005).

In a study of Mahmoud et al (1992) no toxic effects of linseed were observed in a brine shrimp lethality bioassay and linseed did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 102.

Kulling et al. (1998) tested mammalian lignans enterolactone and enterodiol, and plant lignans matairesinol and secoisolariciersinol on cell-free microtubule assembly and at the following genetic

endpoints in cultured male Chinese hamster V79 cells: disruption of the cytoplasmic microtubule complex, induction of mitotic arrest, induction of micronuclei and their characterization by CREST staining, and mutagenicity at the HPRT gene locus. The lignans were tested at concentrations of 200 μM in the cell-free system and 100 μM in cultured cells, which represents the limit of solubility in each assay. The established aneuploidogen diethylstilbestrol and the clastogen 4-nitroquinoline-N-oxide were used as positive reference compounds. As none of the four lignans had any activity at the endpoints studied, the authors concluded that the mammalian and plant lignans tested were devoid of aneuploidogenic and clastogenic potential and did not exhibit any mutagenic activity at concentration of 100 μM under the experimental conditions used in the study.

In rat experiments flaxseed affected the reproductive development of the offspring, depending on the dose and timing of exposure. Exposure to 5 or 10 % flaxseed during lifetime or during gestation and lactation induced structural changes in the mammary gland. The authors concluded that caution is suggested when consuming flaxseed during pregnancy and lactation (Tou et al., 1998 & 1999a & 1999b). Increased mammary cancer in rats exposed to flaxseed through a maternal diet in utero or lactation was observed in a 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis model, but the question is whether it was caused by cadmium present in flaxseed, or that the reduced mammary oestrogen-receptor-beta content was causally linked to increased mammary cancer risk among the offspring (Khan et al., 2007).

Dietary 10 % flax chow was without long-term effect on growth, development and behaviour in male and female Fischer 344 rats since plasma levels of alanine aminotransferase and gamma-glutamyltranspeptidase (γ-GT) were the same as those of regular chow-fed rats. The observed increase in liver γGT was considered as a hepatobeneficial effect induced by the flax. The results of the study indicated the absence of developmental toxicity and a possible hepatoprotective effect of flaxseed (Hemmings et al., 2004).

Maternal feeding of flaxseed during lactation appears to be safe with respect to reproductive indices among offspring, but the authors also concluded that future investigation would be required to elucidate whether there are any long-term implications with respect to fertility. Exposing male rats to a diet containing 10 % flaxseed or an equivalent quantity of lignan either during suckling only or through to early adulthood did not reveal adverse effects on bone health, as measures of bone mass and strength were similar to control rats (Ward et al., 2001a & 2001b).

The plant lignan hydroxymatairesinol (HMR) is a precursor of the mammalian lignan enterolactone. A 13 week toxicity study at dietary levels of 0, 0.25, 1, and 4 % (w/w) of potassium acetate complex of HMR was conducted in the Wistar rat. These dietary levels resulted in an average daily intake of 160, 640, and 2600 mg HMR lignan/kg body weight/day, respectively. HMR lignan exposure did not significantly affect clinical signs, ophthalmoscopy or neurobehavioural observations, and motor activity. Transient reductions in food intake and body weight gain in the mid-and high-dose group were ascribed to decreased palatability of the test feed. Only in males of the high-dose group the body weights remained slightly reduced throughout the study. In the high-dose group the number of thrombocytes (females), and total white blood cell count (males) were increased. Plasma triglycerides were dose-dependently depressed in males of all test groups and in females of the mid- and high-dose group, while plasma total cholesterol and phospholipids were decreased in high-dose males. These changes were not considered to represent adverse effects. The relative weight of the kidneys was increased in males of the high-dose group. The weight of the full and empty caecum showed dose-related increases in males of all treatment groups and in females of the high-dose group. Absolute ovary weights were decreased in all treatment groups while decreases in relative ovary weights were confined to the mid- and high-dose group. In addition, a marginal lengthening of the oestrus cycle was noted in high-dose females. Apart from prevention of hyaline droplet nephropathy in all high-dose male rats, there were no treatment-related histopathological alterations. It was concluded that HMR lignan showed weak antioestrogen-like activity which may be mediated through the HMR metabolite enterolactone. Based on declined ovary weight, the no observed adverse effect level (NOAEL) of

HMR lignan was set at 0.25 % in feed corresponding to 160 mg/kg body weight/day (Lina et al., 2005).

10. Safety assessment based on available knowledge (Level A)

The present document evaluates the lignans as phytoestrogens of flax seed origin. The present evaluation is focussed on lignans as one of the essential ingredients but does not take into account other ingredients that may eventually have to be taken into account in a full evaluation (e.g. mucilage, n-3 fatty acids, cyanogenic glycosides, cadmium).

The similarity in the chemical structure of mammalian lignans and oestradiol led to the suggestion that lignans may have weak oestrogenic/antioestrogenic properties, and several studies, many *in vitro*, have subsequently demonstrated that lignans have hormonal effects.

While lignan exposure during pregnancy and lactation may influence reproduction, it may also reduce the risk of breast cancer. Enterolactone, however, can stimulate growth of oestrogen-dependent breast cancer cell lines (Welshons et al., 1987). Evidently, the hormonal effects of flaxseed and lignans seen *in vitro* and in animal models can result in beneficial or adverse effects on cancer and reproduction depending on the dose, timing and duration of lignan exposure. Meanwhile, one should be cognisant not only of the potential health benefits, but also of any potential adverse effects that a certain level of lignan intake might have in the human lifecycle.

Based on the genotoxicity tests with mammalian lignans, lignans in flaxseed are supposed to be non-genotoxic, however it should be underlined that there are no carcinogenicity studies on lignans available. Secoisolariciresinol and matairesinol did not exhibit any mutagenic activity in cultured cells at concentration of 100 μM (Kulling et al., 1998). In case of lignans there are no structural alerts for the genotoxicity or carcinogenicity.

In an animal study, a lignan complex had no adverse effects on the hemopoietic system (Prasad, 2005). Dietary 10 % flax chow was without long-term effect on growth, development and behaviour in male and female Fischer 344 rats (Hemmings et al., 2004). In other rat experiments flaxseed affected the reproductive development of offspring, could potentially alter reproduction depending on the dose and timing of exposure, and lifetime or gestation and lactation, and exposure to 5 or 10 % flaxseed in the diet induced structural changes in the mammary gland (Tou et al., 1998, 1999a, 1999b). Exposing male rats to a diet containing 10 % flaxseed or an equivalent quantity of lignan either during suckling only or through to early adulthood did not show any effect on bone health, as measures of bone mass and strength were similar to control rats (Ward et al., 2001a, 2001b).

In the 13 week toxicity study conducted in the Wistar rats, a no observed adverse effect level (NOAEL) of HMR lignan, based on declined ovary weight, was set at 0.25 % in feed corresponding to 160 mg/kg body weight/day (Lina et al., 2005).

Dietary daily lignan intake varied between 0.15-6 mg, which corresponds to 0.05-2 g flaxseed or 0.0025 – 0.10 mg lignans/kg bw/day for a 60 kg person.

Based on the NOAEL level of 160 mg/kg bw/day from the 13 week toxicity study the Margin of Safety for lignan intake form the diet amounts to 1600 to 64000.

Based on these considerations it is concluded that the daily dietary dose of lignans gives rise to an adequate Margin of Safety, and is considered to be safe on level A (safety presumed based on

available knowledge) according to the criteria outlined in the EFSA Guidance on the safety assessment of botanicals and botanical preparations[24].

Consumer intake of lignans from food supplements was estimated to vary between 35-312 mg/day which is equal to 0.58 to 5.2 mg/kg bw/day for a 60 kg person.

Based on the NOAEL level of 160 mg/kg bw/day from the 13 week toxicity study the Margin of Safety for lignan intake from food supplements would amount to 31 to 275, and cannot be assumed to be safe based only on the evaluation of existing data. Intake of lignans from food supplements has to be evaluated at level B meaning that further testing and/or data are required.

The Working Group also notes that the safety of lignan supplements in pregnant or lactating women has not been established.

Due to the possible oestrogen-like effects of lignans, it should be used cautiously in women with hormone sensitive conditions such as endometriosis, polycystic ovary syndrome, uterine fibroids, or cancer of the breast, uterus, or ovary. Lignans should be avoided by women who had diagnosed hormone-dependent breast cancer.

11. Further testing and/or data required for the assessment (Level B)

Additional studies are required to evaluate the safety of the intake of lignans from food supplements at levels only 31-275 fold above the NOAEL of 160 mg/kg bw/day. This NOAEL was derived from a 13 week study in rats dosed with HMR and revealing declined ovary weight at the higher dose level of 640 mg HMR/kg bw/day. Additional studies required should be selected according to the criteria outlined in the EFSA Guidance on the safety assessment of botanicals and botanical preparations, and should focus especially on reproduction and developmental toxicity of not only HMR, the lignan tested in the 13 week study, but also of other lignans contributing to the estimated intake from food supplements.

For a full safety assessment, also the exposure to cyanogenic glycosides and possible anaphylaxis resulting from this intake should be evaluated (Alonso et al., 1996). Long-term consumption of flaxseed has been shown to increase plasma levels and urinary excretion of thiocyanate. Regular consumption of flaxseed causes an accumulation of thiocyanate, which is comparable to the blood level of thiocyanate in heavy smokers. However, up to 100g of flaxseed has been consumed without alterations of serum cyanide levels (Frehner et al., 1990). It is concluded that the available scientific data are not sufficient to substantiate such a risk associated to the cyanide content in flaxseed (Schultz et al., 1983). Furthermore, for use levels and durations above the traditional use, additional safety data are required.

[24] See http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/sc_draftguidance_botanicals_public_cons_update_en.pdf?ssbinary=true

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APPENDIX F: *TRITICUM AESTIVUM* L.

The present report focuses on wheat bran.

Disclaimer:

The present document aims at testing the proposed tiered approach for the safety assessment of botanicals and botanical preparations with a selected case and considers relevant constituents. The document is not intended to provide a formal safety assessment of the botanical or botanical preparation, and therefore the outcome of the assessment cannot be used to legally support the safety of the botanicals and botanical preparations evaluated. The document is not, and cannot be interpreted as being, a policy document or a decision allowing the classification of a certain botanical or botanical preparation as a foodstuff or as a medicinal product. The document is focusing on one type of preparation and is not intended to evaluate all possible preparations of this botanical or all possible constituents, which should normally be part of a full safety assessment of a botanical. Data evaluated were collected for the purpose of this testing exercise and are not intended to be complete. Contaminants such as heavy metals, mycotoxins, dioxins, pesticides, microbial contamination or PAHs are not evaluated.

It is outside the remit of this Working Group to evaluate possible beneficial effects and associated claims

1. Identity and nature of the source material

Scientific name:	*Triticum aestivum* L. emend. Fiori et Paol.
Synonyms:	*T. aestivum* L. ssp. *aestivum*, *T. hybernum* L. emend. Mérat, *T. sativum* Lam., *Triticum vulgare* Vill., *T. cereale* Schrank;
Common names:	Engl. Breadwheat, Common wheat, Fr.: Blé, blé ordinaire, Germ. Saatweizen, gemeiner Weizen, Ital.: Frumento, frumento tenero
Family:	Gramineae (Poaceae)
Parts used:	Bran (outer layers of the caryopsis/wheat kernel)
Geographical origin:	Cultivated throughout countries with moderate climate
Growth and harvesting conditions:	There exist some selected wheat cultivars and varieties. Genetically engineered wheat production (Peterson and Shama, 2005) and the potential for selection of special non-toxic varieties for special patients (celiac disease) have also been studied (Spaenij-Dekking et al., 2005). Effects of growing conditions including high temperature stress, solar radiation on antioxidant properties were evaluated (Zhou et al., 2004a; Zhou and Yu, 2004b).

Remark: Wheat bran may also be obtained from other *Triticum* species, namely *T. turgidum* L. ssp. durum (Desf.) Husn. (= syn. *T. durum* Desf., Engl.: Durum wheat, hard wheat, Fr.: Blé dur, froment dur, Germ.: Hartweizen, Ital.: Frumento duro, grano duro)

2. Manufacturing process

Milling: Information on the size of the wheat bran particles is of interest because of possible influences of the particle size on the effect of wheat bran on different biological properties. For example, wheat bran preparations of different particle size will have a different effect on laxation and colonic fermentation (Jenkins et al., 1999)

3. Chemical composition

Wheat bran is a by-product formed in the manufacture of wheat flour from the grain. It mainly consists of the outer layers of the wheat kernel, including the aleuron layer i.e. the husk, seed, coat and germ. There is no natural demarcation between the starch containing endosperm and the bran layers, consequently the composition of the bran can vary somewhat, depending on the growing conditions and the milling process.

The main components present in wheat bran are: carbohydrates (starch 15-20 %; insoluble fibres (cellulose and hemicelluloses) 43-45 %), proteins (15-17 %), water (10 %), lignin (8 %), vitamins and minerals (7 %), lipids (5 %).

Wheat bran also contains the following minor substances: phenolic compounds, waxes, saponins and phytates.

4. Specifications

This report is focussed on wheat bran.

Wheat bran must conform to the provisions of food regulations (Council Regulation 315/93/EEC), especially in terms of mycotoxins (WHO, 1990) arising from external fungal contamination (*Fusarium* spp.) (Mueller et al., 1994), microbiology and pesticides. Exposure to these substances may present a risk (Luo et al., 1990). For instance, the maximum levels for deoxynivalenol (DON) mycotoxins proposed by the Codex alimentarius in cereal products was 500 µg/kg (Visconti et al., 2004), and the maximum levels for sclerotium of *Claviceps purpurea* is set at 0.05 % m/m (Codex Alimentarius, 1995)

Contamination by fungal and mycotoxin production cannot be totally eliminated at present (Codex alimentarius, 2003). In particular, mycotoxin contamination from *Fusarium* spp. is the result of a minor infection of grains and their envelopes by the fungi. These mycotoxins are widespread in cereals: 57 % of 11,444 tested wheat samples were contaminated with maximum levels of 30µg/g of DON (trichothecene mycotoxin deoxynivalenol) according to a FAO/WHO survey and 57 % out of 11,022 samples of foods in the EU (quoted by Visconti et al., 2004) were contaminated with DON. Bran was the fraction of wheat most contaminated with DON (Visconti et al., 2004). The concentration of DON in the bran was on average 160 % of that found in the total wheat grain but could represent more than 200 % in some batches of wheat (for instance, 800 µg/kg DON was found

in bran compared with 350 µg/kg in the whole grain). Similarly high concentrations in wheat bran were reported for other mycotoxins and high concentration of mycotoxins were also reported for cereals (Brera et al., 2006; Brera et al., 2004; Broggi et al., 2002; Ryu et al., 2002). In the USA, 37 % of wheat bran samples contained DON at levels higher than 1000 µg/kg (Trigo Stockli et al., 1996), which is higher than the limit recommended by the Codex for cereal products (500 µg/kg) (quoted by Visconti et al., 2004). Similarly, in Hungary (Rafai et al., 2000), the percentage of non-acceptable samples of bran was two to three times higher than that for wheat and corn.

Toxic contamination related to the development of *Claviceps purpurea* is not considered because this fungus is mainly a parasite of *Secale cereale* L. (rye). Nevertheless about 50 different species of *Claviceps* should be considered as contaminants of various Poaceae species. These *Claviceps*, also referred to as ergot, produce toxic alkaloids derived from lysergic acid, but nowadays cultivation and production of wheat is well supervised and specific chemical tests are available to detect these alkaloids (WHO, 1990 and Mueller et al., 1994). Ergot sclerotia, the fruiting structures of the fungus, are often larger than cereal grains and can be removed mechanically by conventional grain cleaning equipment such as the sieves and separators used in the harvesting process. Up to 82 % of ergot sclerotia can generally be removed by this process with the ergot sclerotia being in the 'dockage'. Ergot sclerotia remain intact when cereal grains are in storage but tend to break into smaller fragments during transport, making cleaning procedures less reliable (EFSA, 2005).

There are only few data available on the occurrence of ergot in cereals and specifically feeding stuffs (EFSA, 2005). In most instances the occurrence is reported on a weight (%) basis because this relates more directly to controls, which range from 0.1 to 0.3 % in grains. Previous surveys have suggested that in central Europe the total alkaloid concentrations in cereals vary between 0.09 and 0.21 % (Wolff, 1989).

5. Stability of the botanical or botanical preparation

No further data on the stability of wheat bran preparations were available for evaluation by the Working Group. The whole production chain, from the primary step to the storage and commercialisation, of wheat bran is highly standardized by the milling industries.

6. Proposed uses and use levels

Wheat bran is consumed for its fibre content and associated beneficial effect regarding transit and laxative properties.

In general, the main nutritional content claim relates to a "source of fibres" which corresponds to a quantity of 3 g fibres/100 g of food or 1.5 g fibres/100 kcal. Wholemeal bread and cereal products have an average fibre content of 6.7 g fiber/100 g (MAFF, 1999). The claim "rich in fibres" corresponds to a quantity of 6 g fibres/100 g food or 3 g fibres/ 100 kcal.

Various recommendations for human consumption from different countries are available, but in general, an amount of 25 or 30 g fibres/day for diets comprising 2000 and 2500 kcal respectively is suggested.

The recommended dose for some products on the market is 25 g of fibres per day for women aged 31 to 50, and 38 g of fibres per day for men.

7. Information on existing assessments

EFSA has published an opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ergot as an undesirable substance in animal feed (EFSA, 2005).

8. Exposure

The per capita daily calorie intake from wheat is 521 kcal, which represents around 25 % of the daily calorie intake in the world (source FAO Statistics Global outlook – 2006[25]). No information could be found for bran itself but the exposure is considered to be important as the bran represents 17-19 % in weight of the wheat seed.

9. Toxicological data

There is an absence of reported adverse effects on human health from wheat bran consumption in the scientific literature, except for warnings where use of wheat bran is contraindicated for patients presenting the following conditions:

- Gluten-induced enteropathies (coeliac disease), because the protein fraction contains gluten (Charbonnier et al., 1980). The use of bran in children younger than 2 years of age is contraindicated (Williams, 2006).
- Wheat allergy, different from the gluten-induced disease reported for children (James et al. 1997, Pourpak et al., 2004).
- Irritable bowel syndrome, where the presence of fibres and the release of short-chain acids in the bowel can cause chemical irritation for the delicate intestinal mucosa (Lewis et al., 1998; Hebden et al. 2002; Miller et al., 2006).
- Under drug prescription: in some cases, interactions with drugs may occur because the absorption and consequently blood bioavailability of the drug could be changed and decreased by the presence of fibres.

Additional aspects concerning particular physiological or pathological human states that may be of importance are:

- Some minerals, for example, Fe, Mg, P, K and Ca may be eliminated to a higher degree, giving rise to a potential deficiency of these useful micronutrients if wheat bran fibres are given to counter chronic constipation, which may be of particular biological relevance during pregnancy and in elderly people.
- The consumption of wheat bran could be a source of digestive discomfort for some people, giving rise to flatulence.
- Finally, a major contraindication to the administration of fibres is where a stoppage of the bowel has a pathological explanation (e.g. cancer).

[25] See http://faostat.fao.org/Portals/_Faostat/documents/pdf/world.pdf

However, the consumption of wheat bran is generally recommended for pregnant women and elderly people because of their gastro-intestinal transit problem. It is also recommended for diabetics and some cardiovascular pathologies linked with a high level of (LDL) cholesterol in the blood (Liu et al., 1999).

10. Safety assessment based on available knowledge

Considering:

- the easy identification of *Triticum aestivum* and of wheat bran,
- the long history and worldwide use of wheat bran in the food chain,
- the absence of reported adverse effects in recent literature and that no threshold of toxicity has been determined,
- the mechanical effect on intestinal transit of wheat bran fibres, which are neither digested nor absorbed in the small intestine (AFSSA, 2002).

It can be concluded that there is a presumption of safety for wheat bran obtained from *Triticum aestivum* without the need for any further toxicological testing.

However, despite the absence of safety concern, consumption of wheat bran should be limited for specific groups in the population, such as small children, people allergic to gluten, and patients suffering from severe bowel pathologies.

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