SCIENTIFIC OPINION

Scientific Opinion on the use of oregano and lemon balm extracts as a food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 30 March 2010 replaces the earlier version published on 24 February 2010.[4]

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion on the safety of oregano and lemon balm extracts when used as a food additive. The Panel notes that the petitioner has provided only limited chemical characterisation of the compounds present in the extracts and that the specifications as proposed are not in line with what would be expected for a botanical or botanical preparation. The Panel notes that oregano and lemon balm have a safe history of use as herbal food ingredients, and that oregano and lemon balm natural extractives are listed as natural extractives generally recognized as safe (GRAS). However, the Panel considers that this presumption of safety might not be applicable to the specific conditions of use and use levels as a food additive. The potential mean exposure to oregano or lemon balm extracts phenolics from the eight food category sources for which use and use levels were proposed by the petitioner amounted to respectively 2.0 mg/kg bw/day for women and 2.3 mg/kg bw/day for men. The Panel concludes that the intake of phenolics resulting from the use of oregano and lemon balm extracts as food additive at the proposed uses and use levels would be in the range of the intake resulting from the use of oregano and lemon balm leaves for preparation of herbal teas. However, the Panel also notes the inadequate specifications and characterisation of the extracts and the absence of data on genotoxicity, reproductive and developmental toxicity and long-term toxicity of oregano and lemon balm extracts. Altogether, with reference to the SCF ´Guidance on submissions for food additive evaluations, the Panel concludes that due to the lack of an appropriate dossier supporting the use of oregano and lemon balm extracts as additives, the safety of oregano and lemon balm extracts at the proposed uses and use levels cannot be assessed.

KEY WORDS

Oregano extract, lemon balm extract, food additive, antioxidant, CAS Registry Number 84012-24-8, CAS Registry Number 84082-61-1.

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1 On request from the European Commission, Question No EFSA-Q-2009-00376, adopted on 10 February 2010.

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4 Editorial changes only: page 1 and page 6 the CAS Registry Number for oregano extract was incorrectly stated 84012-248. The correct CAS Registry Number is 84012-24-8.

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SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the safety of oregano and lemon balm extracts when used as a food additive.

The extracts contain various phenolic compounds which possess antioxidant properties.

The Panel notes that the petitioner has provided only limited chemical characterisation of the compounds present in the oregano and lemon balm extracts.

The Panel notes that the specifications as proposed by the petitioner are not in line with what would be expected for a botanical or botanical preparation and that the presence of compounds of concern such as for example estragole, carvacrol and thymol are not specified.

In a literature study on the phenolic acids recovered in human urine after single ingestion of Origanum onites extract the main identified phenolic constituent in the extract was rosmarinic acid, representing 75% of the identified phenolic acids. Other phenolic acids, including protocatechuic acid, p-coumaric acid, ferulic acid, chlorogenic acid and gallic acid, were present in the extract in notably lower amounts. The extract also contained minor amounts of the flavonoids luteolin and eriodictyol.

Most phenolics in the oregano and lemon balm extracts are absorbed, quickly metabolized and excreted. Concentrations of a variety of phenolic acids have also been found in human fecal water.

The Panel notes that oregano and lemon balm have a safe history of use as herbal food ingredients, and that oregano and lemon balm natural extractives are listed as natural extractives generally recognized as safe (GRAS) in the USA. Herbs and flower tips of Origanum vulgare and herbs, flowers and flower tips of Melissa officinalis have been allocated the status N2 by the Council of Europe. The category N2 comprises admissible natural sources of flavourings, and refers to flavourings prepared from kitchen herbs, spices or seasonings frequently eaten in small quantities in an average diet, provided they are used technologically in amounts corresponding to those present in foodstuffs after traditional usage of the source materials. However, the Panel considers that this presumption of safety might not be applicable to the specific conditions of use and use levels as a food additive.

The potential mean exposure to oregano or lemon balm extracts phenolics from the eight food category sources for which use and use levels were proposed by the petitioner amounted to 2.0 mg/kg bw/day for women and 2.3 mg/kg bw/day for men, respectively.

Given that the oregano and lemon balm extracts are aqueous extracts, the Panel considered that the exposure to oregano and lemon balm extract phenolics from the use of these herbs as teas can be estimated based on the intake of total tea as consumed (both black tea and herbal tea) by UK adults. Assuming that extraction of the leaves provides 20% dry matter and that this extract contains 22% phenolics (according to the petitioner), this would imply a mean intake of phenolics by UK men of 4.3 mg/kg bw/day and by UK women of 5.3 mg/kg bw/day. The Panel notes however that using intake levels of black or herbal tea to estimate intake of oregano and lemon balm tea may result in an overestimation of intake.

If the potential exposure to phenolics from both the proposed use in foodstuffs and in teas are considered, the combined mean exposure is 6.6 mg/kg bw/day for men and 7.3 mg/kg bw/day for women.

The Panel also notes that the intake estimates provided by the petitioner do not include estimation of intake by high level consumers. However, at this stage the Panel does not consider additional exposure estimates essential.

The Panel concludes that the intake of phenolics resulting from the use of oregano and lemon balm extract as food additive at the proposed uses and use levels would be in the range of the intake resulting from the use of oregano and lemon balm leaves for preparation of herbal teas.

However, the Panel also notes the inadequate specifications and characterisation of the extracts and the absence of data on genotoxicity, reproductive and developmental toxicity and long-term toxicity of oregano and lemon balm extracts.

Altogether, with reference to the SCF ´Guidance on submissions for food additive evaluations, the Panel concludes that due to the lack of an appropriate dossier supporting the use of oregano and lemon balm extracts as additives the safety of oregano and lemon balm extracts at the proposed uses and use levels cannot be assessed.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Antioxidants are a functional class of food additives which are regulated under Directive 95/2/EC[5] of the European Parliament and the Council on food additives other than colours and sweeteners.

A manufacturer has requested the authorization of certain extracts of oregano (*Origanum vulgare*) and lemon balm (*Melissa officinalis*) under Directive 95/2/EC as a food additive antioxidant for use in a range of foods. The additive is produced as an aqueous extract of oregano and lemon balm with the antioxidant effect derived from the water soluble plant phenolics present in these herbs.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002[6], the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of extracts from lemon balm and from oregano as a food additive antioxidant in food.

[5] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1.

[6] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

ASSESSMENT

1. Introduction

The present opinion deals with the safety of oregano and lemon balm extracts when used as a food additive.

Plants, including oregano and lemon balm, are sources of various phenolic compounds which possess strong antioxidant properties (Zheng and Wang, 2001). The water soluble properties of these extracts distinguish them from the well-known rosemary extracts, which are extracted with organic solvents, contain different active compounds (carnosic acid, carnosol) and are oil soluble.

2. Technical data

2.1. Identity of the substance

The products evaluated in the present opinion are produced as aqueous extracts of *Origanum vulgare* (oregano) and *Melissa officinalis* (lemon balm) with the antioxidant effect derived from the water-soluble plant phenolics present in these herbs.

The CAS Registry Number of the oregano extract is 84012-24-8 and the CAS Registry Number of the lemon balm extract is 84082-61-1.

The petitioner indicates that oregano extract contains per 100 g ~13.5 g ash, ~40 mg sodium, less than 0.1 g fat, ~3.5 g protein, ~11.5 g total sugars, ~6.5 g glucose, ~4.0 g fructose, ~1.0 g sucrose, ~4.0 g total nutritional fibers, and ~7.0 g water. This extract contains ~24% phenolics and ~3% rosmarinic acid.

The petitioner indicates that lemon balm extract contains per 100 g ~24 g ash, less than 0.1 g fat, ~9 g protein, ~7.0 g total sugars, ~1.0 g glucose, ~3.0 g fructose, ~3.0 g sucrose, ~4.0 g total nutritional fibers and ~7.0 g water. This extract contains ~25% phenolics and ~7.5% rosmarinic acid.

Water soluble and oil soluble formulations of both extracts are available. The petitioner described the following products:

- Oregano extract: a water-soluble oregano extract which is an agglomerated powder
- Lemon balm extract: a water-soluble lemon balm extract which is an agglomerated powder
- Oregano extract liquid concentrate: a water soluble oregano extract aqueous concentrate.
- Lemon balm extract liquid concentrate: a water soluble lemon balm extract aqueous concentrate.
- Different water-in-oil emulsions containing oregano extract and/or lemon balm extract as part of the formulation. These formulations contain additional processing aids such as ascorbic acid (acidifier), food-grade emulsifiers, vegetable oil and water (as carriers) and natural tocopherols.

The extracts are aqueous extracts from edible herbal leaves, and hence not a pure substance. The preparation is not intended to be further purified, as at this stage it is intended to be used by the food industry. The active substances are the water-soluble plant phenolics, one of which has been identified as rosmarinic acid.

2.2. Specifications

The petitioner indicated that the preparations represent a complex mixture of materials extracted with water from edible plants, and hence there are variations in the composition of the aqueous extract. The petitioner also stated that an approach was developed that enabled minimization of variations in the composition of the extracts that occur due to changes in the raw material caused by botanical factors or environmental influences (e.g. weather conditions) during the growth period. This approach is based on acceptance criteria for evaluation and approval of raw materials including the following tests: 1) determination of phenolics content (colorimetric assay), 2) measurement of the antioxidant activity of the extract (linoleic acid oxidation kinetics), 3) percent yield by weight, and 4) colour of the extract (optical absorbance). Raw materials which conform to the minimum requirements are approved for purchase. In addition, the raw materials are tested for aflatoxins, microbial contaminants and heavy metals, performed by an external certified laboratory. Freshly harvested herbal leaves are also tested for pesticides residues (GC-MS/LC-MS methodology).

The petitioner proposed the following specifications for the oregano and lemon balm extracts:

Oregano extract: Aqueous concentrate of natural extract from edible herb species belonging to the Labiatae family (e.g. *Origanum vulgare*), brown liquid with a mild tea-like flavour and an antioxidant activity higher than 90 units standardized by an in-house method (measured as inhibition of linoleic acid oxidation with Trolox as standard), 16-24% dry matter, lead content maximum 10 mg/kg and arsenic content maximum 5 mg/kg. Microbiological specifications were also provided.

Lemon balm extract: Aqueous concentrate of natural extract from edible herb species belonging to the Labiatae family (e.g. *Melissa officinalis*), brown liquid with a mild tea-like flavour and an antioxidant activity higher than 110 units standardized by an in-house method (measured as inhibition of linoleic acid oxidation with Trolox as standard), 17-20% dry matter, lead content maximum 10 mg/kg and arsenic content maximum 5 mg/kg. Microbiological specifications were also provided.

The petitioner indicated that since methyl eugenol, a compound that is known to be genotoxic and carcinogenic (SCF, 2001a), has been reported to occur in lemon balm, analysis for methyl eugenol in the extract to be marketed has been undertaken. Methyl eugenol was not detected (Limit of Detection 0.5 mg/kg).

The Panel notes that lemon balm (*Melissa officinalis* L.) may also contain estragole (Mimica-Dukic *et al.*, 2004), a compound that is known to be genotoxic and carcinogenic as well (SCF, 2001b).

The Panel notes that the specifications as proposed by the petitioner are not in line with what would be expected for a botanical or botanical preparation (EFSA, 2009) and that the presence of compounds of concern such as for example estragole, carvacrol and thymol are not specified.

2.3. Manufacturing process

The manufacturing process was adequately described by the petitioner, and is performed under ISO HACCP certified conditions.

The products are aqueous extracts of *Origanum vulgare* (oregano) leaves and *Melissa officinalis* (lemon balm) leaves.

The process includes water extraction at elevated temperature, sieving, concentration, pasteurization, sedimentation and centrifugation to a solution that usually reaches a 37%-41% solutes concentration. The final extract is pasteurized, dispensed aseptically and stored under refrigeration until further work-up. Alternatively, the extract can be diluted to a lower level of 15% solutes or 20%-25% solutes, and is dispensed as a final liquid product into appropriate containers after passing a pasteurization step prior to the dispensing step.

The further work-up of the 37%-41% solutes solution includes spray-drying of the aqueous concentrate to obtain an agglomerated powder which is then delivered to the end user who prefers agglomerated powder.

2.4. Methods of analysis in foods

Since the various preparations are not a single compound, but complex mixtures, the proposed methods of analysis in food are based on detection of specific marker compounds, especially rosmarinic acid, by reverse phase high-performance liquid chromatography (HPLC) analysis of aqueous food extracts.

2.5. Reaction and fate in food

The natural water soluble phenolics of the herbs oregano and lemon balm are the free radical-scavengers found in oregano extract and lemon balm extract. The petitioner stated that the antioxidant activity of these molecular species is heat-stable and that they can be used as antioxidants even under frying temperatures. The thermal decomposition products of the phenolics have been found to be even more active compared to the parent compounds (Guillot *et al*., 1996). The phenolics were shown to interact with and bind to proteins that are abundant in foods (Bartolome *et al*., 2000).

2.6. Case of need and proposed uses

The petitioner reported that the following doses were successfully used as antioxidant in various food groups:

Meat, poultry and fish products: 400-600 mg/kg of oregano extract.

Baked products, cereals and snacks: a wide range of concentrations is used varying between 300 mg/kg of oregano extract in coated peanuts to 1200 mg/kg in breadsticks. Furthermore, 2000 mg/kg of a water-in-oil formulation of lemon balm extract were added to frying oil which is used to produce fried potato chips and 300 mg/kg of lemon balm extract were added to protect corn flakes from oxidation.

Sauces, spreads and emulsions: oregano extract was added to barbeque sauce, butter, mayonnaise etc. at a dose of 200 mg/kg, and to chick pea salad (humus), tomato ketchup or margarine at a concentration of 500 mg/kg.

Oils and fats: all edible oil types are protected from deterioration during frying at high temperatures (180 ºC - 200 ºC), by 1000 - 2000 mg/kg of a water-in-oil formulation of lemon balm extract, or even better by similar concentrations of a water-in-oil formulation of oregano and lemon balm extract, a formulation which is synergistically fortified by the inclusion of natural tocopherols.

2.7. Information on existing authorisations and evaluations

Oregano and lemon balm natural extractives are listed (as balm or lemon balm or *Melissa officinalis*, and as *Origanum* spp.) in the Code of Federal Regulations Part 182, Subpart A – general provisions, paragraph 182.20, as natural extractives that are generally recognized as safe (GRAS). The petitioner indicates that they are listed in the Council of Europe book of flavouring substances and natural sources of flavourings under the following references: *Origanum vulgare* – C.E.-Number 317 and *Melissa officinalis* – C.E.-Number 280 (Council of Europe, 1981).

Herbs and flower tips of *Origanum vulgare* and herbs, flowers and flower tips of *Melissa officinalis* have been allocated the status N2 by the Council of Europe. The category N2 comprises admissible natural sources of flavourings, and refers to flavourings prepared from kitchen herbs, spices or seasonings frequently eaten in small quantities in an average diet, provided they are used technologically in amounts corresponding to those present in foodstuffs after traditional usage of the source materials (Council of Europe, 1981).

Moreover, in an official letter of May 11, 2005 from the director of Labeling and Consumer Protection Staff, United States Department of Agriculture, Food Safety and Inspection Service, the use of oregano aqueous solution in fresh ground beef was granted. This was further extended to ground meat from other species in an official letter from the same department of November 28, 2005.

The World Health Organization (WHO) monograph on *Melissa officinalis* L. (Lamiaceae, Labiatae) (WHO, 2002) describes some medical oral uses and uses described in folk medicine, also indicating that the preparations should not be administered internally during pregnancy or lactation or be administered to children without medical supervision because of lack of information.

2.8. Exposure

The petitioner provided the following exposure estimate: The aqueous extraction of herbal leaves results in a ~20% dry matter yield per weight of the leaves, in the water-soluble fraction. In daily terms this is equivalent to extracting 1.0 g of tea leaves which will yield 200 mg of dry extract per cup. Assuming a 1000 g daily food consumption by a person and a mean concentration of 500 mg/kg oregano extract in those food products, the daily intake of the oregano extract is 500 mg or an equivalent of 2.5 tea cups/day.

For a 60 kg person, 500 mg oregano extract would amount to 8.3 mg/kg bw/day.

The petitioner also indicated that although both oregano and lemon balm are widely used as herbs and spices, the dietary exposure assessment was based only on the use of the extracts as antioxidants. To estimate the exposure resulting from the proposed use as antioxidant, food consumption data in the UK were used, obtained from the National Diet and Nutrition Survey (NDNS): adults aged 19 to 64 years (Hoare and Henderson, 2004). The estimates took into account the potential exposure to the phenolics of the oregano extract and lemon balm extract when used as antioxidants in foods from categories which potentially need antioxidant protection. In food categories in which a water-in-oil formulation of lemon balm extract should be used, the relative quantity of lemon balm extract included in that formulation was taken into account. The food categories which may potentially become sources of exposure to oregano or lemon balm extracts when used as antioxidants are:

1. Pasta, rice and other miscellaneous cereals
2. Breakfast cereals
3. Biscuits, buns, cakes, pastries and fruit pies
4. Fats and oils
5. Meat, meat dishes and meat products
6. Fish and fish dishes
7. Savoury snacks
8. Soft drinks, not low-calorie

To calculate the exposure estimates, two assumptions were made by the petitioner: a) a mean total phenolic content of 22% for both oregano and lemon balm extracts, and b) all the foods in each category use either type of oregano or lemon balm extracts for antioxidant protection. The oregano or

lemon balm extracts content of each food category was based on the recommended dose for each application. As a rule, the highest recommended dose in each application was used, i.e. the estimate presented by the petitioner provided a maximum exposure figure from all applicable food categories.

The actual calculations for each food category are presented in Tables 1 and 2 for men and women respectively. To calculate the daily theoretical average exposure per kg bw, an average of 70 kg total body weight for men, and 60 kg body weight for women was assumed. Based on these assumptions, the data in Table 1 and 2 indicate a potential exposure to phenolics in oregano and lemon balm extract of 2.3 mg/kg bw/day for men and 2.0 mg/kg bw/day for women.

Table 1: Estimated average exposure of men to oregano and lemon balm phenolics as provided by the petitioner

Food Category	Mean 7-day consumption consumers (g)	Highest Oregano/Lemon Balm recommended level (ppm)	Extract Intake (mg/7day)	Extract Intake (mg/day)	Phenolics Intake (mg/day)
Pasta, rice etc.	661	300	198.3	28.3	6.23
Breakfast cereals	347	500	173.5	24.8	5.45
Biscuits, buns, etc.	315	1000	315	45	9.9
Fats and oils	112	500*	2.8	0.4	0.09
Meat, etc.	1430	600	858	122.6	27.0
Fish etc.	314	600	188.4	26.9	5.9
Savory snacks	106	1200*	6.36	0.91	0.2
Soft drinks, not low-calorie	1680	2000	3360	480	105.6
Total	-	-	-	728.91	**160.37**

* Water-in-oil emulsion containing lemon balm extract.

Table 2: Estimated average exposure of women to oregano and lemon balm phenolics as provided by the petitioner

Food Category	Mean 7-day consumption consumers (g)	Highest Oregano/Lemon Balm recommended level (ppm)	Extract Intake (mg/7day)	Extract Intake (mg/day)	Phenolics Intake (mg/day)
Pasta, rice etc.	511	300	153.3	21.9	4.8
Breakfast cereals	267	500	133.5	19.1	4.2
Biscuits, buns, etc.	242	1000	242	34.6	7.8
Fats and oils	74	500*	1.85	0.26	0.06
Meat, etc.	943	600	565.8	81	17.8
Fish etc.	294	600	176.4	25.2	5.5
Savory snacks	80	1200*	4.8	0.7	0.15
Soft drinks, not low calorie	1254	2000	2508	358.3	79
Total	-	-	-	541.06	**119**

* Water-in-oil emulsion containing lemon balm extract.

The Panel notes that the intake estimates provided by the petitioner do not include estimation of intake by high level consumers.

Given that the oregano and lemon balm extracts are aqueous extracts, the Panel also considered that the exposure to oregano and lemon balm extract phenolics from the use of these herbs as teas can be estimated based on the intake of total tea as consumed (both black tea and herbal tea) by UK adults. Assuming that extraction of the leaves provides 20% dry matter and that this extract contains 22% phenolics (according to the petitioner), this would imply a mean intake of phenolics by UK men of 4.3 mg/kg bw/day and by UK women of 5.3 mg/kg bw/day (Table 3). The Panel notes however that using intake levels of black or herbal tea to estimate intake of oregano and lemon balm tea may result in an overestimation of intake.

If the potential exposure to phenolics from both the proposed use in foodstuffs and in teas are considered, the combined mean exposure is 6.6 mg/kg bw/day for men and 7.3 mg/kg bw/day for women.

Table 3: Intake of tea (black and herbal) in consumers only in UK men and women, and exposure to the dry extract, based on this intake.

	Mean (7 days) g/day*	**Mean g/day**	**Tea leaves** g/day**	**Dry extract (mg/day) 1g tea = 200mg extract**	**Phenolics (mg/day) (22% dry extract)**	**Per kg/bw (men = 70kg; women = 60kg)**
Men						
Tea (Black)	3735	533.57	5.3	1068	234.96	3.4
Herbal Tea	1033	147.57	1.5	296	65.12	0.9
Total tea	4768	681.14	6.8	1364	300.08	4.3
Women						
Tea (Black)	3737	533.86	5.3	1068	234.96	3.9
Herbal Tea	1348	192.57	1.9	386	84.92	1.4
Total tea	5085	726.43	7.26	1454	319.88	5.3

*Data based on intakes from the UK NDNS survey 2000-2001

**Tea leaves: 10g leaves in1000 ml water to make up a cup of tea

3. Biological and toxicological data

The petitioner argued that oregano and lemon balm leaves are food, that they are not considered to be toxic, that there is no limit set for their consumption, that the water soluble part of oregano and lemon balm leaves represents the native composition of water-soluble materials in those leaves, and that no toxicological phenomena have been reported while using the water -soluble part of oregano and lemon balm.

The petitioner also indicated that oregano and lemon balm leaves have been consumed by humans for centuries. Lemon balm is a perennial herb native to southern Europe (Adinee *et al.,* 2008), but found in many regions all over the world. It is used as a spice and as a medicinal herb mainly by intake as a tea (Blumenthal, 1998). Oregano is a perennial plant native to the Mediterranean region and Asia and is cultivated in various regions of the world. It is widely used as a spice especially in the Mediterranean countries e.g. Spain (Prado-De-Santayana *et al.,* 2005) or Greece (Stamatis *et al.,* 2003) as well as in folk medicine (Stamatis *et al.,* 2003, Blumenthal, 1998) in the form of herbal tea (Stamatis *et al.,* 2003).

The petitioner stated that the extensive culinary consumption of the above mentioned herbs has never been restricted by any authority worldwide, and that the herbs and extractives of these herbs have been listed as GRAS in the Code Of Federal Regulations of the USA, as well as in the Commission Regulation (EC) No. 508/1999[7] laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin.

3.1. Absorption, distribution, metabolism and excretion

A study with 6 human volunteers (each dosed with 3.75 g dried aqueous extract of oregano leaves calculated to be equivalent to 825 mg phenolics, and thus to 13.75 mg phenolics/kg bw for a 60 kg person), evaluated the intestinal absorption of various phenolic acids, the kinetics of their urinary excretion, and the identification of the excreted molecular species which included both parental compounds and their metabolites (Nurmi *et al.,* 2006a). In this work, the phenolic acids recovered in human urine after single ingestion of *Origanum onites* extract were analyzed. At the time of the baseline urine collection period, the volunteers had consumed a washout diet with strictly restricted intake of phenolic compounds for 1 week, and the inter-individual variation in the baseline excretion was reported to be low. The main identified phenolic constituent in the extract was rosmarinic acid, representing 75% of the identified phenolic acids. Other phenolic acids, including protocatechuic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid and gallic acid, were present in the extract in notably lower amounts. The extract also contained minor amounts of the flavonoids luteolin and eriodictyol. The estimates of the intake of dietary polyphenols and the amounts of phenolic compounds provided in the study supplements ranged from tens of milligrams to nearly 1000 mg/day, depending on the phenolic compound. The Panel notes that this study is on a different species of oregano and that there is no information on possible differences in composition of this extract as compared to the extract from *Origanum vulgare* evaluated in the present opinion.

The mean baseline excretion of the identified phenolic compounds was 95 μmol/day, and during the 2 days of follow-up after oral intake of the extract, the mean baseline excretion of the identified phenolic compounds was 403 and 172 μmol/day at 0-24 and 24-48 hours, respectively. Thus, the excretion was increased 4- and 2-fold during 0-24 and 24-48 hours of the follow-up, respectively. The mean increase in the excretion of phenolic compounds exceeded the ingested amount of identified phenolic acids. This result can be partly explained by rosmarinic acid, the main identified phenolic constituent in the extract, as well as by flavonoids present in minor amounts, presumably being metabolized into a double amount of simple phenolic metabolites. Furthermore, unidentified phenolic constituents in the extract partly contribute to the excretory increase. The main metabolite, *p*-hydroxybenzoic acid, was excreted rapidly. During the first day of the follow-up, the main compound excreted was *p*-hydroxybenzoic acid, followed by vanillic acid, *m*-hydroxyphenylacetic acid, protocatechuic acid, ferulic acid and 3,4-dihydroxyphenylacetic acid. Minor amounts of syringic acid, caffeic acid, and *p*-coumaric acid were also detected in urine. During the second day of the follow-up, *m*-hydroxyphenylacetic acid was the main compound excreted in urine, followed by vanillic acid, *p*-hydroxybenzoic acid, ferulic acid and 3,4-dihydroxyphenylacetic acid. Amounts of protocatechuic acid, caffeic acid, *p*-coumaric acid and syringic acid excreted were low. Rosmarinic acid, gallic acid, or chlorogenic acid were not detected in any urine sample before or after the supplementation. The authors indicated that the metabolism that occurred may explain why intact rosmarinic acid was not detected in urine. During the total 48-hour period not all phenolic acids were well absorbed from the intestine, thus their residues were found in human fecal water (Jenner *et al.,* 2005).

[7] Commission Regulation (EC) No 508/1999 of 4 March 1999 amending Annexes I to IV to Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 60, 9.3.1999, p. 16.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The petitioner indicated that a primary screening study was carried out. The study involved 12 mice treated with single intravenous doses of 500 and 1000 mg/kg bw oregano and lemon balm extract. There were some changes within the first hour following dosing, including decreased motor activity, prone position, laboured breathing, decreased exploratory activity and tension tremor. At 24 hours after treatment, all of the signs noted within the immediate period after administration were completely resolved and all test animals exhibited normal behaviour.

3.2.2. Short-term and subchronic toxicity

A study with Wistar rats (Lemhadri *et al.,* 2004) was performed with the objective to confirm traditional use of oregano water extract in the treatment of diabetes. Diabetes was induced by intravenous injection of streptozotocin into the tail vein at a dose of 65 mg/kg bw. After 18 hours, animals with fasting blood glucose levels greater than 16.5 mmol/l were considered diabetic and then included in this study. Normal and diabetic rats were randomly assigned to the different groups (n = 6 in each group). The control group received distilled water; treated groups received daily repeated oral doses of aqueous oregano extract at a dose of 20 mg/kg or the reference drug; sodium vanadate at a dose of 0.8 mg/kg. The effect of the vehicle, aqueous oregano extract or vanadate on blood glucose were determined in fasted rats 1, 2, 4 and 6 hours after a single oral administration and after 2 and 4 days, 1 and 2 weeks. The aqueous oregano extract demonstrated a potent hypoglycemic influence without affecting blood insulin levels, neither in normal rats, nor in the diabetic ones. Glucose blood levels of the normal rats were also not affected by the oregano extract. The Panel notes that the study was not designed to test the safety of oregano extract and that the dose levels tested were relatively low.

In an experiment 90 female turkeys were fed for 84 days with a feed which was supplemented with different quantities of dried oregano leaves (Bampidis *et al.,* 2005). The highest content of leaves was 3.75 g/kg feed (equivalent to 1.125 g/kg bw oregano extract). Various parameters were tested (body weight, carcase weight, carcase yield, relative heart, liver weight, gizzard and small intestine weight, and serum cholesterol content). No adverse effects were reported. The Panel notes that the study was not designed to test the safety of oregano extract.

3.2.3. Genotoxicity

No genotoxicity studies with the oregano or lemon balm extracts of the present opinion were provided.

3.2.4. Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity studies with the oregano or lemon balm extracts of the present opinion were provided.

3.2.5. Reproductive and developmental toxicity

No reproductive toxicity studies with the oregano or lemon balm extracts of the present opinion were provided.

An experiment which included 1801 oregano-treated sows and 1809 untreated control sows, lasted for 9 deliveries for each sow, 130 days for each reproductive cycle (Allan and Bilkei, 2005). The feed was supplemented with 1000 mg/kg oregano (dried leaf and flower of *Origanum vulgare*, enriched with 500 g/kg of cold-pressed essential oils of *Origanum vulgare*). The sows ingested daily, for the first 109 days of each cycle, at least 2 g of oregano, and at least 3 g daily for the rest of the cycle. The authors concluded that oregano enhanced sow health and productivity and may be a viable alternative to supplementation with antibiotics. In this study, no deleterious effects of oregano were observed. The Panel notes that the study was not designed to test the safety of oregano extract.

3.2.6. Human studies

3.2.6.1. Human studies with oregano

In a human study, aimed to investigate the effects of dried aqueous extract from *Origanum vulgare*, 45 non-smoking male volunteers consumed daily 300 mg or 600 mg phenolics for a period of four weeks (Nurmi *et al.,* 2006b). These doses (calculated to be equivalent to 1.4 g and 2.7 g respectively of oregano extract, being 23 and 45 mg oregano extract/kg bw/day for a 60 kg person) did not affect lipid oxidation, serum lipids or safety parameters (serum alanine aminotransferase, γ-glutamyltransferase, serum creatinine) compared to the controls who did not receive the extract. The authors concluded that the consumption of the aqueous oregano extract is safe.

3.2.6.2. Human studies with lemon balm

In a study, aimed at assessing the modulation of mood and cognitive performance by a single dose of dried *Melissa officinalis* (lemon balm) leaves, 20 healthy young participants consumed 600 mg, 1000 mg and 1600 mg of encapsulated dry leaves (Kennedy *et al.,* 2003). These amounts are equivalent to approximately 170 mg, 280 mg and 450 mg of dried aqueous extract, amounting to 2.8, 4.7 and 7.5 mg/kg bw/day for a 60 kg person. The authors concluded that the results indicated that the lemon balm can improve cognitive performance and mood, and might be beneficial in the treatment of people suffering from Alzheimer's disease. No adverse effects were reported in this study.

In a review on herbal remedies commonly used to treat psychiatric symptoms (Wong *et al.,* 1998) it was reported that use of lemon balm at dose levels ranging from 1 to 4 g daily for treatment of insomnia and anxiety did not result in adverse health effects, although drug interactions with central nervous system (CNS) depressants (potentiation) and thyroid medications were indicated. The authors also stated that safety in pregnancy and lactation has not been established.

In a behavioural study with 18 subjects, a 600 mg dose of lemon balm extract improved significantly the mood of subjects under the influence of a laboratory-induced stress (Kennedy *et al.,* 2004). No side-effects were observed.

A herbal sleeping aid formulation, involving a daily dose of 240 mg lemon balm extract was administered to a group of 66 volunteers for 30 days (Cerny and Schmid, 1999). The results revealed a significantly higher quality of sleep among the treatment group as compared to the placebo group (32 subjects). The researchers reported that the formulation which contained the lemon balm extract was well-tolerated.

A similar formulation was used in a multicenter study in which 918 children less than 12 years of age were treated (Müller and Klement, 2006). The children suffered from restlessness and nervous sleep disturbance. Each child received a daily dose which contained 280 to 320 mg of lemon balm extract for one month. There was a distinct reduction in the severity of all the symptoms, with a good tolerability of the herbal drug and with no adverse events.

The Panel notes that, although adverse side-effects were not observed, all studies on human volunteers were not designed to investigate the safety of oregano or lemon balm extracts.

4. Discussion

The present opinion deals with the safety of oregano and lemon balm extracts when used as a food additive antioxidant. The extracts contain various phenolic compounds which possess antioxidant properties.

The Panel notes that the petitioner has provided only limited chemical characterisation of the compounds present in the oregano and lemon balm extracts.

The Panel notes that the specifications as proposed by the petitioner are not in line with what would be expected for a botanical or botanical preparation (EFSA, 2009) and that the presence of compounds of concern such as for example estragole, carvacrol and thymol are not specified.

In a study on the phenolic acids recovered in human urine after single ingestion of *Origanum onites* extract (Nurmi *et al.,* 2006a), the main identified phenolic constituent in the extract was rosmarinic acid, representing 75% of the identified phenolic acids. Other phenolic acids, including protocatechuic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid and gallic acid, were present in the extract in notably lower amounts. The extract also contained minor amounts of the flavonoids luteolin and eriodictyol.

The Panel notes that oregano and lemon balm have a safe history of use as herbal food ingredients, and that oregano and lemon balm natural extractives are listed as natural extractives generally recognized as safe (GRAS) in the USA. Herbs and flower tips of *Origanum vulgare* and herbs, flowers and flower tips of *Melissa officinalis* have been allocated the status N2 by the Council of Europe. The category N2 comprises admissible natural sources of flavourings, and refers to flavourings prepared from kitchen herbs, spices or seasonings frequently eaten in small quantities in an average diet, provided they are used technologically in amounts corresponding to those present in foodstuffs after traditional usage of the source materials. In spite of this, the Panel considers that this presumption of safety might not be applicable to the specific conditions of use and use levels as a food additive.

The potential mean exposure to oregano or lemon balm extracts phenolics from the eight food category sources for which use and use levels were proposed by the petitioner amounted to respectively 2.0 mg/kg bw/day for women and 2.3 mg/kg bw/day for men.

Given that the oregano and lemon balm extracts are aqueous extracts, the Panel considered that the exposure to oregano and lemon balm extract phenolics from the use of these herbs as teas can be estimated based on the intake of total tea as consumed (both black tea and herbal tea) by UK adults. Assuming that extraction of the leaves provides 20% dry matter and that this extract contains 22% phenolics (according to the petitioner), this would imply a mean intake of phenolics by UK men of 4.3 mg/kg bw/day and by UK women of 5.3 mg/kg bw/day. The Panel notes however that using intake levels of black or herbal tea to estimate intake of oregano and lemon balm tea may result in an overestimation of intake.

If the potential exposure to phenolics from both the proposed use in foodstuffs and in teas are considered, the combined mean exposure is 6.6 mg/kg bw/day for men and 7.3 mg/kg bw/day for women.

The Panel notes that the intake estimates provided by the petitioner do not include estimation of intake by high level consumers. However, at this stage the Panel does not consider additional exposure estimates essential.

The Panel concludes that the intake of phenolics resulting from the use of oregano and lemon balm extract as food additive at the proposed uses and use levels would be in the range of intake resulting from the use of oregano and lemon balm leaves for preparation of herbal teas.

However, the Panel also notes the inadequate specifications and characterisation of the extracts and the absence of data on genotoxicity, reproductive and developmental toxicity and long-term toxicity of oregano and lemon balm extracts.

Altogether, with reference to the SCF ´Guidance on submissions for food additive evaluations, the Panel concludes that due to the lack of an appropriate dossier supporting the use of oregano and lemon balm extracts as additives the safety of oregano and lemon balm extracts at the proposed uses and use levels cannot be assessed.

CONCLUSIONS

The Panel notes the absence of data on genotoxicity, reproductive and developmental toxicity, and long-term toxicity of oregano and lemon balm extracts.

Altogether, the Panel concludes that due to the lack of an appropriate dossier supporting the use of oregano and lemon balm extracts as additives, the safety of oregano and lemon balm extracts at the proposed uses and use levels cannot be assessed.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Origanox for Submission for Food Additive Evaluation. October 15th 2008. Submitted by RAD Natural Technologies/FRUTAROM Ltd.

2. Origanox WS (RR-2) Primary screening and safety study in mice. Experimental report. April 21 1997. Report from Harlan Biotech Israel Ltd.

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GLOSSARY/ABBREVIATIONS

ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
GC-MS	Gas Chromatography-Mass Spectrometry
GRAS	generally recognized as safe
HACCP	Hazard Analysis Critical Control Point
HPLC	High Pressure Liquid Chromatography
LC-MS	Liquid chromatography-mass spectrometry
NDNS	National Diet and Nutrition Survey
SCF	Scientific Committee on Food
WHO	World Health Organization