European Food Safety Authority

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Use of rosemary extracts as a food additive [1]

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2003-140)

Adopted on 7 March 2008

PANEL MEMBERS

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SUMMARY

Following a request from the Commission, the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) was asked to deliver a scientific opinion on the safety in use of rosemary extracts when used as an antioxidant.

Rosemary extracts are derived from *Rosmarinus officinalis* L. and contain several compounds which have been proven to exert antioxidative functions. These compounds belong mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes.

The present opinion refers to rosemary extracts prepared using several solvent extraction techniques. These will be named by the acronyms provided by the petitioner and are as follows:

- F62: rosemary extract produced from dried rosemary leaves by acetone extraction,
- D74: rosemary extract prepared by extraction of dried rosemary leaves by means of supercritical carbon dioxide,

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- AR: rosemary extract prepared from a partially deodorised ethanolic extract of rosemary,
- ARD: extract prepared from a deodorised ethanolic extract of rosemary,
- RES: extract which is a decolourised and deodorised rosemary extract obtained by a two-step extraction using hexane and ethanol.

The principal antioxidative components of the extracts are the phenolic diterpenes carnosol and carnosic acid.

The Panel suggests to modify the specifications proposed by the petitioner to reflect the specifications of the extracts that were used in the safety testing, with respect to carnosol and carnosic acid and the antioxidant/volatile ratio.

Four of the five rosemary extracts considered in the present opinion, (D74, AR, ARD, and RES) were tested for genotoxicity. Several in vitro genotoxicity studies were performed in both prokaryotic and eukaryotic test systems and an in vivo mouse micronucleus test performed with rosemary extract RES. The Panel concluded that these do not give rise to safety concerns with respect to genotoxicity of the rosemary extracts.

Antioxidant rosemary extracts have low acute and sub-chronic toxicity in the rat. Sub-chronic studies on all five solvent extracts (D74, AR, ARD, F62, RES) reveal that the only effect at high doses of these rosemary extracts is a slight increase in relative liver weight. This effect has been shown to be reversible and may be the result of Phase I and II enzyme induction. The effect was not accompanied by increases in plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP). Considering the low magnitude, reversibility and the nature of the hepatic changes, and the absence of increases in plasma ALT, AST and AP, the Panel concludes that the minor increase in the liver weight reported, accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction, represent an adaptive response and are not of toxicological concern.

Overall, the 90-day feeding studies in rats with the different rosemary extracts tested, including AR, ARD, RES and D74, reveal NOAEL values in the range of 180 to 400 mg extract/kg body weight/day equivalent, depending on the carnosol and carnosic acid content of the respective extracts, to 20-60 mg /kg bw /day of carnosol plus carnosic acid.

The toxicological data on the rosemary extracts are insufficient to establish a numerical ADI, because the toxicity data set does not provide reproductive toxicity studies or a long term study. On the other hand, the existing data, including the absence of effects in the 90-day studies on reproductive organs and lack of genotoxicity, do not give reason for concern.

Dietary exposure to carnosol plus carnosic acid has been estimated for adults and pre-school children (aged 1.5 to 4.5 years) and amounts to mean values of respectively 0.04 and 0.11 mg carnosol plus carnosic acid/kg bw/day, 0.10 and 0.20 mg carnosol plus carnosic acid/kg bw/day

at the 95th percentiles and 97.5th percentile values of 0.12 and 0.23 mg carnosol plus carnosic acid/kg bw/day.

The Panel notes that the margin between the NOAEL range in the 90-day rat studies with all five extracts of 180 to 400 mg extract/kg bw/day equivalent to 20-60 mg/kg bw/day of carnosol plus carnosic acid, and the dietary exposure estimates for adults would amount between 500-1500 for the mean intake values, between 200-600 for the 95th percentile values and between 167-500 for the 97.5th percentile values. For pre-school children these margins would amount to respectively at least 182-546, 100-300 and 87-261. The Panel notes that these margins of safety are worst case estimates since the NOAELs from the different studies were generally the highest dose levels tested, and that the estimates of dietary exposure were conservative.

Therefore the Panel is of the opinion that the margin of safety is high enough to conclude that dietary exposure resulting from the proposed uses and use levels are not of safety concern.

The Panel notes that to achieve these levels of dietary exposure, high level consumers would need to select a diet that was entirely composed of foods containing rosemary extracts for those food categories in which it was permitted. In reality not all processed foods will contain added antioxidants and it seems unlikely that these extracts would be used at the maximum usage level in all the proposed food in each category or that some consumers would systematically always choose all foods containing rosemary extract.

Based on the margins of safety identified, the Panel concluded that the use of rosemary extracts at the proposed uses and use levels would not be of safety concern.

Key words:

Rosemary extract, food additive, carnosol, CAS No 5957-80-2, carnosic acid, CAS No 3650-09-7.

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BACKGROUND AS PROVIDED BY THE COMMISSION

The Health and Consumer Protection Directorate-General has received a request from the European Rosemary Extract Manufacturers Group to use rosemary extracts as an antioxidant in foodstuffs.

Although the entire rosemary (*Rosmarinus officinalis* L.) plant, excluding the woody portions, may be used, it is normally only the leaves, that are commonly used as a culinary herb, flavouring agent and naturally occurring antioxidant. Today, rosemary extracts are increasingly employed not only to provide flavour but also as natural alternatives to synthetic antioxidants for the stabilisation of oxygen-sensitive foods. The antioxidative function is probably caused by several components in the rosemary extracts, which belong mainly to the classes of phenolic acids, flavonoid diterpenoids and triterpenes.

As described above, extracts of the plant rosemary (*Rosmarinus officinalis* L.) can have both flavouring and antioxidative properties. In many cases both functions are utilised within a food, however, it can be the case that some extracts are sold primarily for their antioxidant properties. In such cases the processing of the rosemary extract can be optimised to enhance the antioxidative function and to reduce that of flavouring. It has been stated by the Standing Committee on Foodstuffs that in such cases these products should be considered as food additives and, as such, require authorisation under Directive 95/2/EC on food additives other than colours and sweeteners.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of rosemary extracts when used as an antioxidant in foodstuffs.

ACKNOWLEDGEMENTS

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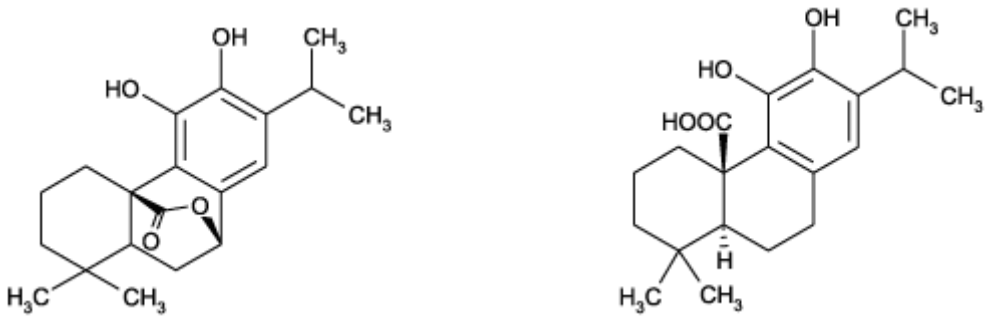
ASSESSMENT

1. Technical data

1.1. Chemistry

Rosemary extracts are derived from *Rosmarinus officinalis* L. and contain several compounds which have been shown to exert antioxidative functions. These compounds belong mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes.

The present opinion refers to rosemary extracts prepared using solvent extraction (ethanol, hexane, acetone and supercritical carbon dioxide techniques). The principal antioxidative components of the extracts are the phenolic diterpenes carnosol (CAS No 5957-80-2, molecular formula $C_{20}H_{28}O_4$) and carnosic acid (CAS No 3650-09-7, formula $C_{20}H_{28}O_4$) (Figure 1).



carnosol carnosic acid

Figure 1. **Chemical structure of the two major antioxidative compounds in rosemary extracts.**

1.2. Manufacturing Process

Rosemary extracts are prepared by extraction from dried rosemary leaves. The present opinion refers to production processes using solvent extraction by ethanol, acetone and/or hexane and supercritical carbon dioxide.

Rosemary extract F62: Rosemary extract F62 is produced from dried rosemary leaves by acetone extraction, filtration, and solvent evaporation, followed by spray-drying and sieving to obtain a fine green powder. It is standardised in food grade carriers so that its carnosol plus carnosic acid content is approximately 10%.

Rosemary extract D74 - supercritical CO_2: The dried rosemary leaves are extracted by means of supercritical carbon dioxide. During the extraction carbon dioxide-soluble substances are dissolved in the carbon dioxide. The carbon dioxide fraction obtained is precipitated. In a second process-step the raw extract is deodorised by supercritical CO_2 to define the content of essential oil as well as to ensure a defined concentration of the antioxidative compounds, carnosol and carnosic acid. Finally the deodorised extract is homogenised and mixed with appropriate food-grade carriers to convert the extract into a powder or liquid-oil form. The

petitioner indicates that extract D74 represents an optimally selectively extracted antioxidant rosemary extract product with minimal (but still significant) aroma. Its carnosol plus carnosic acid content is approximately 30%.

AR Spice extract powder – ethanol: AR Spice extract powder is prepared from a partially deodorised ethanolic extract of rosemary. This extract contains between 7 to 10% carnosol plus carnosic acid.

ARD Spice extract – ethanol/deodorised: ARD Spice extract is prepared from a deodorised ethanolic extract of rosemary dissolved in suitable carriers and semi-purified by molecular distillation. This extract contains approximately 5 to 7% carnosol plus carnosic acid.

RES Rosemary extract – hexane and ethanol: This product is a decolourised and deodorised rosemary extract obtained by a two-step extraction using hexane and ethanol followed by treatment with active carbon and finally spray drying. The carnosol plus carnosic acid content in this extract is 14.9%.

Rosemary extracts are commercially available in both liquid form (solubilised in appropriate food-grade carriers) and powder form (dried by conventional food drying methods).

The petitioner provided data on the analysis of the composition of the rosemary extracts, including those used in the safety studies. A detailed analysis of single samples of the various extracts has been conducted to show the various components present. Secondly, consecutive batches have been analysed to show reproducibility.

To provide a direct comparison between the extracts and dried rosemary, Table 1 provides the analytical profile for all samples adjusted to 10% carnosol plus carnosic acid content. The results show the comparability of the solvent extracts when characterised on the basis of the key active principals carnosol plus carnosic acid. Reference key volatiles are very low compared to dried rosemary. Similar analytical results for consecutive batches of D74 and F62 indicate reproducibility of these two extraction procedures. HPLC fingerprints for rosemary dried leaves, and extracts D74, F62, ARD, AR and RES reveal reproducibility of consecutive batches and also indicate the patterns of the different extracts to be comparable with carnosic acid and carnosol being major components (Table 2).

Table 1. **Profiles of Extracts Tested vs. Dried Rosemary Leaves – Standardised to 10% Carnosic Acid + Carnosol Content**

Parameter	Unit	Dried Leaves	Extract D74	Extract F62	Extract AR	Extract ARD	Extract RES
Phenolic diterpenes							
Carnosic acid	%w/w	9.7	8.7	9.16	7.49	7.6	6.9
Carnosol	%w/w	0.3	1.3	0.84	2.51	2.4	3.1
Carnosol + carnosic acid	%w/w	10	10	10	10	10	10
Triterpenes							
Betulin	mg/g	<4.76	6.0	5.6	8.45	9.46	6.79
Amyrin	mg/g	<0.5	0.034	0.2	0.16	0.23	0.36
Triterpenic acids							
Betulinic acid	mg/g	65.2	48.0	46.9	56.0	64.5	35.1
Sum oleanic + ursolic acid	mg/g	148.1	48.5	100.5	119.8	164.5	60
Organic acids							
Citric acid	mg/g	<0.5	<0.034	0.2	<0.1	<0.11	<0.05
Malic acid	mg/g	<0.5	<0.034	0.2	<0.1	<0.11	<0.05
Volatiles							
1.8-Cineole	mg/g	56.1	0.08	1.70	1.32	0.053	0.03
Camphor	mg/g	25.2	0.22	2.39	2.08	0.12	0.02
Borneol	mg/g	10.0	0.09	0.96	0.84	0.04	0.01
Verbenone	mg/g	2.24	0.63	0.27	0.34	0.39	0.02
Bonyl acetate	mg/g	1.00	0.07	0.29	0.27	0.32	0.01
Antioxidant/Volatiles Ratio:	mg/g	0.1	10	1.8	_	11	111
Flavonoids							
Genkwanin	mg/kg	2.9	0.65	1.60	2.30	3.66	2.1
Tannins							
Expressed as gallotannin	mg/g	177.6	<0.5	<0.5	70.7	99.0	<0.5
Polyphenols							
Expressed as gallic acid	mg/g	262.9	0.65	1.12	99.5	115.5	1.6
Polysaccharides							
Expressed as starch	mg/g	104.8	<0.7	<2	9.3	8.1	<1
Protein							
Total nitrogen x 6.25	%	23.3	<0.03	<0.1	0.57	0.86	<0.05
Lipophilic substances							
Hexane-extractable matter	%	43.3	16.3	24.7	20.84	24.6	21.0
Anions							
Fluoride	mg/kg	<47	<3.4	<5	<10	<12	<5
Chloride	mg/kg	3000	<6.8	<10	2904	3612	<10
Bromide	mg/kg	<50	<3.4	<5	<10	<12	<5
Nitrate	mg/kg	<50	<3.4	<250	<250	269	<5
Phosphate	mg/kg	1809	<6.8	21755*	107	140	<10
Sulfate	mg/kg	3571	<3.4	<50	451	613	<5
Cations							
Cadmium	mg/kg	<0.23	<0.02	<0.03	<0.05	<0.05	<0.03
Chromium	mg/kg	4.76	0.10	0.49	0.57	0.74	0.33
Copper	mg/kg	22.4	<0.03	0.15	1.07	1.2	1.33
Nickel	mg/kg	5.2	<0.03	0.13	0.37	0.48	0.14
Lead	mg/kg	2.90	0.09	0.03	0.13	0.15	0.18
Mercury	mg/kg	<0.24	<0.02	<0.02	<0.05	<0.05	<0.03
Zinc	mg/kg	90	2.93	1.01	6.64	8.39	1.84
Arsenic	mg/kg	1.14	<0.034	0.05	0.25	0.25	0.32

* H_3PO_4 is used in the process to precipitate the carnosic acid.

Table 2. **HPLC fingerprints for rosemary dried leaves, and extracts D74, F62, ARD, AR and RES**

Parameter	Unit	Dried Leaves	Extract D74	Extract F62	Extract AR*	Extract ARD	Extract RES*
Phenolic diterpenes							
Carnosic acid	mg/g	15-25	240-260	155-175	30-50	60-80	110-150
Carnosol	mg/g	1-2	35-45	15-17	20-30	18-22	30-50
Triterpenic acids							
Betulinic acid	mg/g	10-15	130-145	85-110	15-30	55-65	60-80
Sum oleanic + ursolic acid	mg/g	20-35	130-150	185-220	35-45	130-170	90-120

The mentioned figures of extracts AR and RES are estimated under consideration of:

- Used solvent (polarity) and mentioned total content of CA & C in II.4.1
- Correlation to extract ARD when considering the processing of the extracts mentioned in II.4.1
- Influence of the mentioned clean up steps

1.3. Specifications

Extraction of rosemary leaves yields an almost colourless, volatile oil. The chemical composition of the oil is dependent upon the region of growth (Chalchat *et al*., 1993). Phenolic diterpenes, flavones and rosmarinic acid distribution may also vary during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis* (del Baño *et al*., 2003).

The antioxidant activity of rosemary extract can be attributed mainly to two components, carnosic acid and carnosol (Addis and Warner, 1991; Richeimer *et al*., 1996). Antioxidant rosemary extracts are characterised principally by their carnosol plus carnosic acid content in relation to key volatile (flavouring) components.

The petitioner provided detailed analytical data on the various solvent-based extracts (Table 1).

The extraction solvents used, ethanol, hexane and acetone are food approved solvent systems (EEC, 1988).

The petitioner also proposed specifications (Table 3). However, the proposed specifications do not accurately reflect the content of the assessed extracts. The content of reference antioxidant compounds carnosic acid and carnosol in the five samples used for the safety testing amounted to approximately 10% for the acetone extract F62, approximately 30% for the supercritical carbon dioxide extract D74, 7 to 10 % for the ethanol extract AR, 5 to 7% for the ethanol extract ARD and 14.9 % for the hexane-ethanol extract RES, being significantly higher than the not less than 3.5% w/w, expressed as the total of carnosic acid and carnosol suggested in the proposed specifications.

This also holds for the antioxidant/volatile ratio proposed in the specifications provided by the petitioner to be at a level of > 0.1, which is the level observed for dried leaves, whereas the level in the samples used for the safety testing amounted to 5.61 for the acetone extract F62, 10 for the supercritical carbon dioxide extract D74, 11 for the ethanol extract ARD, and 111 for the hexane-ethanol extract RES.

Table 3. **Proposed specifications for antioxidant rosemary extracts provided by the petitioner**

Synonym:	Rosemary extract (antioxidant)
Description:	Rosemary extract antioxidant is prepared by extraction of the leaves of *Rosmarinus officinalis* using a food approved solvent system. Extracts may then be deodorised and decolourised. Extracts may be standardised using permitted excipients, diluents and carriers.
Composition	
Reference antioxidative compounds: (phenolic diterpenes)	Carnosic acid and Carnosol (which comprise not less than 90% of the total phenolic diterpenes)
Content of reference antioxidative compounds:	Not less than 3.5% w/w, expressed as the total of carnosic acid and carnosol
Reference key volatiles:	Borneol, Bornyl Acetate, Camphor, 1,8-Cineol, Verbenone
Antioxidant / Volatiles – Ratio:	(Total % of carnosic acid / carnosol) > 0.1 (% of reference key volatiles) *
Purity	
Residual solvents:	Not more than 25 mg/kg
Arsenic:	Not more than 3 mg/kg
Lead:	Not more than 5 mg/kg

* as a percentage of total volatiles in the extract

1.4. Methods of analysis in foods

The petitioner indicates that carnosol and carnosic acid can be accurately measured in food systems. To determine the concentration of individual phenolic diterpenes in pure extracts of rosemary and fats, an HPLC method with electrochemical detection has been developed (Schwarz and Ternes, 1992a, b; Schwarz *et al.*, 1992; Ternes and Schwarz, 1995).

1.5. Reaction and fate in foods, stability

Schwarz *et al.* (1992) have also shown that when the antioxidant activity of extracts under simultaneous storage and thermal stress was assessed, it was found to depend directly on the concentration of phenolic diterpenes. Differences in rates of degradation of individual phenolic diterpenes at different temperatures were obtained.

1.6. Case of need and proposed uses

Extracts of rosemary can have both flavouring and antioxidative properties. In many cases both functions are utilised, but some extracts are to be used primarily for their antioxidant properties. In such cases the processing of the rosemary can be optimised to enhance the antioxidative function and to reduce that of the flavouring.

Antioxidants are required in foods to prevent oxidation of oils and production of off -flavours. The level required is dependent on the fat content of the food. Data on the levels of use proposed by the petitioner for different foods (expressed as total carnosol plus carnosic acid) are provided in Table 4. The petitioner indicates that the use levels in Table 4 reflect maximum levels necessary to achieve the desired antioxidant effect in all circumstances, but that in

practice, for individual products within categories, usage levels could be lower than the figures provided.

The present opinion does not evaluate the efficacy of rosemary extracts for antioxidant use.

The use as flavourings is not evaluated in the present opinion.

Table 4. **Uses and use levels for antioxidant rosemary extract proposed by the petitioner**.

Foodstuff	Level of carnosol and carnosic acid mg/kg		Notes
	Expressed on fat basis	Expressed on finished product	
Non-emulsified oils and fats of animal or vegetable origin	50	-	Domestic cooking oils, salad oils, etc.
Olive oil	50	-	Olive oil as such and as an ingredient
Fats and oils for the professional manufacture of heat-treated foodstuffs	50	-	Oils used in the manufacture of commercial food products.
Frying oil and frying fat	50	-	Cooking oils for catering outlets
Fine bakery wares	-	30	Biscuits and cookies, cakes and pastries, crisp breads and crackers uncooked
Meat, poultry and fish/seafood products (non-processed)	100	-	Meat/poultry/fish-based products; sausages, burgers, etc.
Meat, poultry and fish/seafood products (processed)	150	-	Ham, cooked meat, fish and poultry products
Dehydrated granulated potatoes	-	200	In powder before reconstitution
Sauces	100	-	Manufactured "cook-in" sauces
Snack foods	50	-	Savoury snacks based on cereals, potatoes or starch
Milk powder for vending machines	200	-	In powder before reconstitution
Dehydrated soups and broths	-	50	In powder before reconstitution
Seasoning and condiments	200	-	Ketchup, mayonnaise, etc.
Pasta	-	25	As ingredients in manufactured foods
Processed nuts	200	-	Snacks, etc.
Egg products	-	200	As ingredients in manufactured foods
Chewing gum	-	200	As is
Dietary supplements	-	400	As is
Confectionery products	200	-	Fillings, compounds, fondant.
Dried milk for ice-cream	-	30	As ingredients in manufactured foods.
Dehydrated meat	-	150	Freeze-dried products
Flavourings*		1000	e.g. citrus oils

* use in flavourings not taken into account in intake estimates

1.7. Dietary exposure

Rosemary, usually in the dried form, has been used for centuries in food dishes, predominantly as a seasoning, but also as an antioxidant. The petitioner provided some examples of the culinary use of dried rosemary (typically containing *e.g.* 2% carnosol plus carnosic acid) from UK cookery books. From this overview it seems that as much as 7.5 g of dried rosemary can be contained in one serving. Assuming a mean carnosol plus carnosic acid concentration of 2% this would correspond to 150 mg of carnosol plus carnosic acid which equals 2.5 mg/kg bw for a 60 kg person. The Panel noted, however, that in nearly all recipes the use of dried rosemary is lower (ranging from 0.4-2.5 g dried rosemary per serving and corresponding to 0.1-0.8 mg/kg bw carnosol plus carnosic acid).

The petitioner also indicates that rosemary extract is used as flavouring in processed foods, with use levels varying from 10 to 2000 mg/kg (examples: soft drinks: 20-200 mg/kg; meat: 200-400 mg/kg; salad dressings: 200-500 mg/kg; flavours: 200-2000 mg/kg).

The petitioner performed an assessment of dietary exposure based on the proposed uses of rosemary extract as an antioxidant.

The dietary exposure assessment was based on food consumption in the UK in order to obtain conservative estimates, since UK consumers tend to have a higher consumption of processed foods than consumers in other EU Member States. Individual dietary records (7 and 4 days, respectively) from the National Dietary and Nutrition Surveys (NDNS) of adults (Henderson *et al.*, 2002) and of pre-school children (Gregory *et al*., 1995) were used to estimate the potential dietary exposure to carnosol plus carnosic acid. The NDNS data comprised records of the amounts of more than 2000 different food items. In addition to information about the amounts of food consumed on each eating occasion, the NDNS data include information about the fat content of foods consumed in the surveys so that estimates of dietary exposure can be based on whole food or just the fat consumed. Individuals were considered to be consumers if they consumed one or more food products in which the antioxidant rosemary extract is proposed for use in at least one of the survey days. The NDNS food codes were matched to the usage categories in Table 4 (assuming maximum usage levels and presence in all foods where it could be used) and potential dietary exposure was calculated for each individual. For those uses of the antioxidant rosemary extract that relate to the use of oils and fats in cooking and processing, the consumption figure was based on the fat content of the finished product. In cases where the antioxidant rosemary extract was used in foods and not the entire food was treated, a correction factor was applied (for instance meat mixture in a meat dish) based on the household recipes. Total dietary exposure of each individual was estimated by summing up exposure from all the food groups. Individual body weights were available to calculate individual's exposure per kg bw/day. The average and high percentile exposures were then calculated for consumers only.

These exposure estimates do not take into account potential exposures to carnosol and carnosic acid from use of rosemary as a flavouring or from its use as a herb.

A use level of 1000 mg/kg is proposed for flavouring essences such as citrus oils which can be used at household level in some recipes. However, no consumption data are available for such products. Flavouring essences are not likely to be used on a regular basis and will probably not lead to a significant exposure to carnosol and carnosic acid.

The main potential sources of exposure to rosemary extracts used as antioxidant were 'fine bakery wares', 'dehydrated soups and broths' and 'seasonings and condiments' in UK adults and 'fine bakery wares' and 'meat, poultry and fish/seafood products (non-processed)' in pre-school children. Potential mean exposure to carnosol plus carnosic acid from all proposed uses was respectively in adults and pre-school children: 0.04 and 0.11 mg/kg bw/day. At the 90th, 95th and 97.5th percentile the potential exposure to carnosol plus carnosic acid was 0.08, 0.10

and 0.12 mg/kg bw/day for adults and 0.18, 0.20 and 0.23 mg/kg bw/day for pre-school children.

In the present assessments, background exposures from culinary and flavouring uses were not taken into account. However, the Panel considered that the exposure assessments in the present opinion provide a conservative estimate of dietary exposure to carnosol plus carnosic acid because it was assumed that the extracts would be used at the maximum usage level in all the proposed foods in each category. For individual food categories this might be realistic since consumer loyalty and individual preferences might cause a person to always choose a particular food containing rosemary extract. However, when potential exposure from all foods are combined the scenario becomes less likely and so exposure from all sources at the maximum usage level becomes less probable.

1.8. Existing authorisations and evaluations

Rosemary oil was notified for Generally Recognised as Safe (GRAS) status by the Fragrance and Essence Manufacturers Association of the USA (FEMA) in 1965 and has been listed by the U.S. Food and Drug Administration (FDA) for food use (GRAS). In 1970 the Council of Europe included rosemary oil in the list of substances, spices and seasonings deemed admissible for use, with a possible limitation of the active principles in the final product (Opdyke, 1974). The Panel notes that the chemical characteristics of rosemary oil are different from those of the rosemary extracts considered in the present opinion.

2. Toxicological data

2.1 Absorption, Distribution, Metabolism and Excretion

Rosemary extracts are by their nature complex mixtures of a varied chemical nature. No specific studies on the absorption, distribution, metabolism and excretion of rosemary extracts have been provided by the petitioner. The petitioner indicates that it has been shown that the principal target organ in the body is the liver where the induction of biotransformation enzymes is promoted. This is discussed in more detail below.

2.2. Acute Oral Toxicity

According to the petitioner, acute oral administration by gavage of ethanol (AR) extract at dose levels of 8.5 and 10 g/kg bw to male and female mice respectively induced no mortality. Similarly, the daily administration by gavage for 5 days of AR extract at dose levels of 4.3 and 5 g/kg bw to male and female mice respectively induced no mortality. Body weight was slightly increased in males, whereas in females the body weights remained within the limits of the corresponding controls. Liver weight was increased in both sexes. A marked increase in fatty liver was observed after repeated administration of AR extract to males.

According to the petitioner, neither an acute (single dose) oral administration of ARD extract by gavage at dose levels of 24 and 28.5 g/kg bw to male and female mice respectively nor the daily administration by gavage during 5 days of ARD extract at dose levels of 11.8 and 14.1 g/kg body weight to male and female animals respectively induced mortality. Body and organ

weights remained within the limits of the corresponding controls, except for the liver weight of females which was slightly increased with repeated administration of ARD extract.

No gross macroscopic lesions were observed at autopsy other than fatty liver in mice subjected to repeated administration of ARD.

Thus the two products can be considered to be of low acute toxicity.

2.3. Short-term and sub-chronic toxicity

The core studies cited in this section have been conducted, where applicable, according to published, internationally acceptable regulatory protocols. In most cases the studies were conducted under Good Laboratory Practice. They were submitted by the petitioner.

The petitioner reports that two rosemary extracts, the acetone extract F62 and the supercritical CO_2 extract D74, were assessed for toxicity during oral (dietary) administration to the rat in a 14-day range-finding study. Parameters tested included clinical parameters (signs of ill health or overt toxicity), morbidity and mortality, bodyweight and food consumption. Clinical chemistry including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) was not evaluated. No treatment-related signs were observed throughout the study for either extract. The high-dose concentrations tested were 3800 mg/kg diet for F62 and 2400 mg/kg diet for D74 (flavouring sensory threshold prevented testing of a higher concentration).

In a subsequent 13-week oral toxicity study groups of male and female rats (20 animals/group) were given rosemary extracts F62 or D74 orally in the diet at doses of 300, 600 or 2400 mg/kg diet (extract D74) or 3800 mg/kg diet (extract F62). Parameters tested included clinical signs, morbidity and mortality, body weight, food consumption, ophthalmoscopy, clinical pathology, organ weights and histopathology, haematology, bone marrow smears, clinical chemistry including amongst others aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP), and urine analysis.

In the clinical chemistry there was no consistent pattern of variation in the data at week 13 to indicate an effect of treatment. A number of inter-group differences from control did achieve statistical significance on occasion, however, these differences were not dose-related or slight or inconsistent between the sexes and are indicated by the petitioner to represent normal biological variation. AST and ALT activities were not significantly affected for any dose group and AP was significantly reduced (not increased) by 20% ($p<0.01$) in the 3800 mg/kg diet extract F62 group only.

In the absence of any significant effects, the study authors considered 3800 mg/kg diet to be the No-Observed-Adverse-Effect Level (NOAEL) for the acetone extract F62.

Marginal reduction in body weight and food consumption was observed at the dose level of 2400 mg/kg of rosemary extract D74 in the diet. In the absence of any overtly toxic histopathological changes associated with this dose, this was not considered indicative of compound-related toxicity in this study. The effect was rather ascribed to palatability problems. The NOAEL for extract D74 was therefore considered by the petitioner to be 2400 mg/kg of diet. The NOAEL, when expressed as mean test article consumption averaged over 13 weeks, was approximately 180 mg/kg bw/day for males and 200 mg/kg bw/day for females. This is equivalent to approximately 70 mg/kg bw/day carnosol plus carnosic acid consumption.

In a subsequent study, rosemary supercritical CO_2 extract D74 was tested in a 91-day dietary toxicity study in the female rat at a dose of 2400 mg/kg diet (equivalent to approximately 195 mg/kg bw/day), followed by a 28-day treatment-free period. This study was set out to

reproduce the effects seen in the 13-week study and to show that they were reversible after a 4-week treatment-free period. Female rats were used because they had shown the most marked effects in the 13 week study. Parameters tested included clinical parameters (signs of ill health or overt toxicity), morbidity and mortality, body weight and food consumption, organ weights and histopathology. Clinical chemistry including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) was not evaluated.

At 91 days, the liver weight of the dosed animals was slightly increased compared to control (group mean-adjusted increase was approximately 8%) as seen in the original study. This difference was no longer apparent after the treatment-free period. There were no treatment-related macroscopic or microscopic effects noted.

Samples of liver were taken for analysis of levels of hepatic microsomal P450 enzymes and the activities of selected enzymes, including CYP1A (7-ethoxyresorufin O-deethylase), CYP3A (testosterone 6β-hydroxylase), CYP2B (testosterone 16β-hydroxylase), CYP2E (lauric acid 11-hydroxylase), and CYP4A (lauric acid 12-hydroxylase), both at 91 days and following the 28-day treatment-free period. In the testosterone assay, the activities of other enzymes (including testosterone 7α- and 16α-hydroxylase, markers for respectively CYP2A, CYP2C11) were also determined. At 91 days there was some evidence of an increase in microsomal protein concentration. Total cytochrome P450 content was increased (*ca* 1.5-fold over corresponding control activities), as were activities associated with CYP2A (1.4-fold), CYP2C11 (2-fold), CYP2E1 (1.4-fold) and CYP4A (1.5-fold). These mild increases (expressed in activities per g of liver) were shown to be reversible on suspension of treatment. There were no notable effects on activities associated with CYP1A, CYP2B, or CYP3A.

The petitioner concluded that the minor increase in the liver weight reported, accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction, may represent an adaptive response and may not indicate hepatotoxicity, and also that this is supported by the fact that the effects are reversible.

The petitioner also reported a 90-day feeding trial on the AR and ARD ethanolic rosemary extracts. In this study rosemary extracts AR and ARD were added to the diet of Sprague-Dawley rats at levels of 0, 500, 1500 and 5000 mg/kg (equivalent to approximately 0, 40, 120 and 400 mg/kg bw/day of test article) for each extract for 90 days in a standard sub-chronic study. There were few differences between the control and treatment groups. The body weights developed normally in all dose groups with the exception of the top dose extract AR (5000 mg/kg diet) which showed a very slight reduction in body weight gain which probably resulted from the 10% lower food intake by this group compared to control throughout the study. This effect, which was not apparent in the top-dose group with the deodorised extract ARD, was probably due to palatability problems. The only parameter which showed a clear dose-response relationship was the relative liver weight (this effect was not seen in absolute liver weight). The relative liver weights were slightly but significantly increased due to the presence of AR or ARD in the diets. No pathological modifications of this organ were observed and no liver-related changes of other parameters were measured. The study authors concluded that the test products provoked no adverse effects in the experimental animals in the described experiment except for a slight increase in relative liver weight that can be explained by an increase in metabolic activity of this organ. Cytochrome P450 results, although not statistically different, showed a slightly higher trend in the top-dose groups of AR and ARD. There were no treatment related differences in AST and ALT activities in plasma.

The petitioner also provided results from a subchronic feeding study on the hexane plus ethanol rosemary extract (RES) in Sprague-Dawley rats. This study was performed according to OECD guidelines (OECD 408). Rosemary extract RES (approximately 15% carnosol plus carnosic

acid) was fed to male Sprague-Dawley rats at dietary levels of 0, 1000, 2500 and 5000 mg/kg diet, equivalent to approximately 0, 65, 164, and 320 mg/kg bw/day test product, for 3 months, followed by a 1-month dose-free recovery period (for high dose and control only). Terminal studies were conducted at the end of each experimental period at 28, 91, or 119 days.

No mortality was observed during the study and the few clinical observations, such as slight ocular discharge, hair loss or tail injuries were considered minor and not treatment-related. Body weight was not influenced by the treatment. Food consumption was slightly influenced during the first week of the study only. Increased food intake by male animals of the lowest RES group was observed, while females showed a decreased intake in the highest RES group. The presence of the test product in the diet slightly influenced its flavour and palatability issues have been reported in other studies. The changes in food intake were not biologically significant and did not influence body weight. There were no treatment-related macroscopic observations at necropsy.

Liver weights (absolute and relative) increased in a dose-dependent manner reaching significance only in the highest dose group. The hepatic DNA content suggested a hypertrophic process although no morphological equivalence could be detected and the effect was reversible. The histopathological findings of bile duct hyperplasia observed at the first sacrifice (1 month), were treatment-related and the petitioner indicated that they are probably due to the extremely high level of RES uptake relative to body weight during the acute growing phase of the animals. This effect decreased on continued treatment and disappeared after the 1-month recovery indicating an adaptive mechanism. The tinctorial features of hepatocellular cytoplasm indicated a metabolic process resulting in liver hypertrophy. This is further suggested by the slight treatment-related decrease of bilirubin and of plasma proteins.

The decrease in pancreas weight observed in female animals after 1-month treatment (only), may be related to the high dose of RES relative to body weight during the early phase of the study, but remained an isolated observation not associated with morphological alterations or clinical chemistry disorders affecting this organ.

All the haematological values recorded in this study were within the normal range. There were no dose-related changes observed. There were similarly no pathological values observed. There were no changes in AST and AP, and ALT was only increased at the second interim kill in males from the 1000 mg/kg diet group ($p<0.05$) but not at the higher dose levels or in the females.

Decreased bilirubin, observed at all the different sacrifices may have been related to the bile duct hyperplasia. The petitioner indicated that the slightly reduced hepatic DNA content (mg/g tissue) in highest dose male animals, at the second interim sacrifice (3 months), and its subsequent increase in female animals (relative to proteins) after the recovery period, when related to the total liver mass, may suggest that the liver weight gain was caused by hypertrophy rather than hyperplasia.

The study authors concluded that no toxic effects were induced upon repeated oral exposure to rosemary extract RES at any of the levels treated. This implies that the NOAEL for the hexane-ethanol rosemary extract RES amounts to at least 320 mg/kg bw/day.

Table 5 provides an overview of the toxicity studies performed and the NOAEL values derived.

Table 5. **A Summary of Toxicology Tests and End Point Phenolic Diterpene Doses**

Toxicity Test	Test Extract	Marker phenolic diterpenes Carnosol + carnosic acid content of extract % w/w	Levels of Total Extracts Tested	Equivalent dose of Carnosol + carnosic acids tested
Acute mouse	AR- Ethanolic	7-10	8.5 g/kg bw male single dose 10 g/kg bw female single dose 4.5 g/kg bw male 5 day 5 g/kg bw female 5 day	595-850 mg/kg bw 700-1000 mg/kg bw 300-430 mg/kg bw 350-500 mg/kg bw
Acute mouse	ARD Ethanolic	5-7	24.0 g/kg bw male single dose 28.5 g/kg bw female single dose 11.8 g/kg bw male 5 day 14.1 g/kg bw female 5 day	1200-1680 mg/kg bw 1425-1995 mg/kg bw 590-826 mg/kg bw 705-987 mg/kg bw
14-day oral – rat	F62 Acetone	10	3800 mg/kg diet equals 190 mg/kg bw/day	380 mg/kg diet equals 19 mg/kg bw/day
14-day oral – rat	D74 CO_2	31	2400 mg/kg diet equals 120 mg/kg bw/day	840 mg/kg diet equals 42 mg/kg bw/day
90-day sub-chronic – rat	AR Ethanolic	7-10	500, 1500, 5000 mg/kg diet the latter being the NOAEL and equal to 400 mg/kg bw/day	NOAEL 28-40 mg/kg bw/day
90-day sub-chronic - rat	ARD Ethanolic (Deodorised)	5-7	500, 1500, 5000 mg/kg diet the latter being the NOAEL and equal to 400 mg/kg bw/day	NOAEL 20-28 mg/kg bw/day
90-day sub-chronic with reversibility	RE-S Hexane plus ethanol	15	1000, 2500, 5000 mg/kg diet approximately 65, 164 and 320 mg/kg bw/day test product the latter being the NOAEL	9.8, 24.6 and NOAEL 48 mg/kg bw/day
13-week sub-chronic – rat	F62 Acetone	10	3800 mg/kg diet	NOAEL 380 mg/kg diet equivalent to 19 mg/kg bw/day
13-week sub-chronic – rat	D74 CO_2	31	300, 600 & 2400 mg/kg diet NOAEL 2400 mg/kg diet equivalent to 180-200 mg/kg bw/day	105, 210 & 840 mg/kg diet NOAEL 840 mg/kg diet equivalent to 56- 62 mg/kg bw/day
Reversibility study rat 90-days + 28-day treatment-free	D74 CO_2	31	2400 mg/kg diet 180-200 mg/kg bw/day	105, 210 & 840 mg/kg diet NOAEL 840 mg/kg diet equivalent to 56-62 mg/kg bw/day
Enzyme induction *In vitro* cyt. P450	D74 CO_2	31	2400 mg/kg diet Weak enzyme induction – peer review concluded adaptive response	As above

Overall the Panel considers that the 90-day studies with the different rosemary extracts tested, including AR, ARD, RES, F62 and D74, reveal NOAEL values in the range of 180 to 400 mg extract/kg bw/day, equivalent to approximately 20-60 mg /kg bw /day of carnosol plus carnosic acid, depending on the carnosol and carnosic acid content of the respective extracts.

The petitioner also reviewed the literature available regarding the potential beneficial effects of rosemary extracts. These studies are almost all dealing with the protection by rosemary extracts against CCl_4 liver toxicity (Debersac *et al*., 2001a, b; Singletary, 1996; Singletary and Rokusek, 1997; Sotelo-Felix *et al.*, 2002a; Sotelo-Felix *et al.*, 2002b; Fahim *et al.*, 1999; Hoefler *et al.*, 1987). Although these studies were not designed to investigate the safety of rosemary extracts no adverse effects of rosemary, carnosol or carnosic acid were reported.

2.4. Reproductive and developmental toxicity

No reproductive toxicity studies were conducted with the rosemary extracts considered in the present opinion. The petitioner indicates that all of the subchronic studies reported conducted histopathology on the reproductive organs of male and female rats. No significant differences were observed between dose groups and controls.

In order to evaluate if rosemary extract induces abortion and/or interferes with the normal development of the fetus, very high doses of 26 mg of a 30% (w/v) *R. officinalis* aqueous extract (13 mg solids/ml) from leaves, flowers and stem were administered daily by gavage during 2 different periods of Wistar rat pregnancy (Lemonica *et al*., 1996). One group of animals (n=12) received the extract from days 1 to 6 of pregnancy (preimplantation period) and another group (n=14) received the same extract from days 6 to 15 of pregnancy (organogenic period). Control groups (n=12) received saline in the same volume and during the same periods as their respective experimental groups. The animals were sacrificed at term. The treatment of the dams during either the preimplantation or the organogenic period did not cause significant changes in the postimplantation loss or in the number of anomalies or malformations of the term fetuses, which also showed a similar degree of development when compared with the respective controls. The percent of preimplantation loss in the group treated before embryo implantation showed no significant difference compared to the control.

Although this material was an aqueous extract, and carnosol plus carnosic acid contents in such extracts are normally very low, very large doses were actually used, equivalent to 7.8 mg of rosemary per rat or about 39 mg/kg bw/day. For a 60 kg human adult this would be equivalent to 2340 mg of dried rosemary per day.

Recently Nusier *et al*. (2007) published results from a study on the effects of a 70% ethanol:30% water extract of Rosemary (*Rosmarinus officinalis*) on reproductive function in adult male Sprague Dawley rats ingesting rosemary extracts dissolved in water at levels of 250 and 500 mg/kg bw/day for 63 days. Body weight and absolute and relative testes weights were not affected, but in the highest dose group the average weight of the epididymides, ventral prostates, seminal vesicles, and preputial glands significantly decreased. A significant decline in spermatogenesis in testes due to a decrease in the number of primary and secondary spermatocytes and spermatids in the high dose group was observed and attributed to a significant decrease in testosterone. In rats of the highest dose group sperm motility and density were also significantly decreased in the cauda epididymis and in the testes. For the high dose group the treatment also markedly increased the number of fetal resorptions in female rats impregnated by the high dose males, thereby reducing their fertility. For the 250 mg/kg bw dose groups no statistically significant decreases in these parameters were observed and it can therefore be concluded that 250 mg extract/kg bw/day is the NOAEL in this study. Analytical details on the extract used in the study were not provided.

2.5. Mutagenicity

Unpublished studies provided by the petitioner described the results of tests on the mutagenicity of various rosemary extracts in several bacterial and mammalian test systems.

Rosemary extract D74 did not induce mutation in five strains of *S. typhimurium* (TA98, TA100, TA 1535, TA 1537, and TA102) when treated up to 5000 µg/plate both in the absence and in the presence of rat liver metabolic activation system (S9). In a range-finding experiment evidence of toxicity was observed at the maximum test dose of 5000 µg/plate but not at the lower test doses up to 1000 µg/plate following treatment with S9. In the absence of S9 there was no evidence of toxicity. In the first test evidence for toxicity was observed only at 1000 and 5000 µg/plate for TA102 but not for the other test strains. In a second test evidence of toxicity was observed at the higher test concentrations in all test strains in the presence of S9, but in the absence of S9 only at the highest dose level for TA102.

Rosemary extracts AR and ARD, derived from the extraction of steam-stripped rosemary leaves, were tested in the Ames test. The pre-incubation test and the standard plating test were performed with strains TA1535, TA1537, TA1538, TA98, and TA100, and doses up to 20 mg/plate. Bactericidal effects were observed for all of the test materials with each strain. For ARD a decrease in the number of revertant colonies was observed only at the highest dose levels tested (20 mg/plate), but for AR this was already observed at a dose of 0.75-1 mg/plate. These effects were reduced in the presence of metabolic activation. In the presence or absence of metabolic activation, no dose-dependent increase in numbers of revertants was observed for any of the samples tested with any of the strains. From these results it is concluded that AR and ARD are not mutagenic in the Ames test.

Rosemary extract RES, and its main active components, the diterpenes carnosic acid and carnosol, were checked for their potential mutagenicity in the bacterial Ames test using strains TA97, TA98, TA100, and TA102, both in the presence and absence of S9 mix. RES was tested at doses up to 6 mg/plate, whilst carnosic acid and carnosol were checked at doses equivalent to their concentrations in RES. In the absence of S9 the test compounds were bactericidal for all strains at the highest dose levels tested (3, 4.5 and 6 mg/plate) but for TA102 at almost all dose levels tested. When S9 mix was present, bactericidal activity was reduced and no longer observed or only observed at the highest dose levels tested (6 mg/plate). In strain TA102, which specifically detects oxidative mutagens, a significant mutagenic effect was observed in one set of experiments using RES. However, this effect could not be reproduced in subsequent experiments utilising less cytotoxic concentrations of RES. In the three other strains, RES was not found to be mutagenic. Purified carnosol and carnosic acid were not mutagenic.

Using a human lymphocyte *in vitro* assay, the ethanolic rosemary extract AR was tested for chromosome damaging properties. AR was tested up to 100 mg/ml. No sign of gaps, breaks or other chromosomal aberrations were observed with AR in the absence or presence of the metabolic activation. It was concluded that AR is not genotoxic in this test system.

The potential of the hexane-ethanol rosemary extract RES to induce gene-locus mutations in the human lymphoblastoid cell line (TK6) was also investigated. Different concentrations of rosemary extract RES were examined in the presence and absence of metabolic activation for mutagenic effects in the thymidine kinase (tk) and hgprt loci of TK6 cells. In the absence of an exogenous activation system, treatment with up to 50 µg/ml RES did not result in a dose-dependent increase in mutation frequency. Furthermore, no significant increase in mutation frequency above that of the solvent control was observed in any of the treated cultures. Similarly, a dose-dependent increase in mutant frequency was not observed in the presence of an exogenous activation system and most of the treated cultures did not show a significant increase in mutant frequency above that of the solvent control. At one dose (35 µg/ml) of RES

an increase in mutations in the tk but not in the hgprt locus was observed. Although this increase in mutations in the tk locus was significant compared to the solvent control, it was not significant compared to the untreated cells. Taking all of these findings into consideration, primarily the lack of a dose-dependent increase in mutation frequency or of a significant increase of mutation frequency over the controls, it was concluded that RES is not mutagenic under the conditions used.

The hexane-ethanol rosemary extract RES was also tested in the micronucleus mutagenicity test in OF1 mice to investigate possible chromosome damaging properties under *in vivo* conditions. Mice were administered RES daily by gavage (0.375, 0.75, or 1.5 g/kg bw) for 5 days to investigate possible dose-related effects. In addition, RES (1.5 g/kg bw) administered once by gavage 24, 48, or 72 hours prior to sacrifice was used to investigate potential time-dependent effects. The highest dose of RES (1.5 g/kg bw) that could be used for these studies was limited by the maximal amount of RES which could be suspended in sunflower seed oil, the vehicle. No significant time- or dose-related induction of micronucleated cells was observed in bone marrow cells. It was concluded that RES does not induce a significant increase in mutagenicity *in vivo* when administered orally to mice at doses up to 1.5 g/kg body weight.

Other studies have shown rosemary extract and carnosic acid to demonstrate antimutagenic activity in bacteria (Minnuni *et al.*, 1972; Santamaria *et al.*, 1987) or in *in vitro* human liver and bronchial cell models (Offord *et al.*, 1997).

2.6. Carcinogenicity

No longterm studies on rosemary extracts were provided by the petitioner.

Several studies reported that rosemary may be protective at various stages of carcinogenesis in animal models *in vivo* (Ho *et al.*, 1994; Singletary and Nelshoppen, 1991; Huang *et al.*, 1994; Amagase *et al.*, 1996; Singletary *et al.*, 1996; Huang *et al.*, 1989, 1992; Chen *et al.*, 1992; Percival, 1991; Offord *et al.*, 1995; Offord *et al.*, 1997; Singletary, 1996; Tawfiq *et al.*, 1994).

2.7. Human data

Rosemary has been used in Western and Eastern medicines since ancient times to treat various diseases (Leung and Foster, 1996; Grieve, 1979; Bown, 1995; Nadkarni, 1976; Karnick, 1994; Wichtl and Bisset, 1994; Boyle, 1991).

3. Discussion

The petitioner suggested specifications for the antioxidant rosemary extracts based on carnosic acid and carnosol as the reference antioxidative compounds. However, the proposed specifications do not accurately reflect the content of the extracts tested in the safety studies. The content of reference antioxidant compounds carnosic acid and carnosol in the five rosemary extracts used for the safety testing amounted to approximately 10% for the acetone extract F62, approximately 30% for the supercritical carbon dioxide extract D74, 7 to 10 % for the ethanol extract AR, 5 to 7% for the ethanol extract ARD and 14.9 % for the hexane-ethanol extract RES, being significantly higher than the “not less than 3.5% w/w, expressed as the total of carnosic acid and carnosol” proposed in the specifications. Therefore, the Panel suggests to modify the specifications to reflect the specifications of the extracts that were used in the safety testing. The Panel also notes that the safety testing was carried out with preparations containing

between 5 and 30% carnosol and carnosic acid and that NOAEL values were recalculated in terms of carnosol and carnosic acid content.

This also holds for the antioxidant/volatile ratio proposed by the petitioner to be specified at a level of $\geq$ 0.1 which is the level observed for dried leaves, whereas the level in the samples used for the safety testing amounted to approximately 5.61 for the acetone extract F62, 10 for the supercritical carbon dioxide extract D74, 11 for the ethanol extract ARD, and 111 for the hexane-ethanol extract RES. Therefore, the Panel suggests to modify the specifications suggested by the petitioner to reflect the specifications of the extracts that were used in the safety testing.

Four of the five rosemary extracts of the present opinion, (D74, AR, ARD, and RES) were submitted to genotoxicity tests. Several *in vitro* genotoxicity studies were performed in both prokaryotic and eukaryotic test systems and one *in vivo* genotoxicity test performed with rosemary extract RES. The Panel considers that these do not give rise to safety concerns with respect to genotoxicity of the rosemary extracts.

The Panel noted the absence of genotoxicity data for extract F62 but that the composition of the F62 extract is not markedly different from that of the other extracts and that therefore no genotoxicity data for F62 are needed.

Antioxidant rosemary extracts have low acute and sub-chronic toxicity in the rat. Sub-chronic studies on all five different solvent extracts (D74, AR, ARD F62, RES) have shown that the only effect at high doses of rosemary extracts is a slight increase in relative liver weight. This effect has been shown to be reversible and may be the result of Phase I and II enzyme induction. Liver enlargement, featured by centrilobular hepatocyte hypertrophy and accompanied by microsomal enzyme induction, is a common adaptive response of rodent liver to the exposure to xenobiotics (McGuire *et al*., 1986; Greaves, 2000). This type of change is generally not considered as a toxic response (Cattley and Popp, 2002; Lewis *et al*., 2002). The effect was not accompanied by increases in plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (AP). Considering the low magnitude, reversibility and the nature of the hepatic changes, and the absence of increases in plasma ALT, AST and AP, the Panel concludes that the minor increase in the liver weight reported, accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction, represent an adaptive response and are not of toxicological concern.

In these toxicity studies palatability problems have also been shown to slightly affect food consumption and body weight gain in extracts with less deodorisation.

Overall the 90-day feeding studies in rats with the different rosemary extracts tested, including AR, ARD, RES and D74, reveal NOAEL values in the range of 180 to 400 mg extract/kg bw/day equivalent to 20-60 mg/kg bw/day of carnosol plus carnosic acid, depending on the carnosol and carnosic acid content of the respective extracts.

No reproductive toxicity studies were conducted with the rosemary extracts of the present opinion.

Recently Nusier *et al*. (2007) published a study reporting adverse effects of a 70% ethanol:30% water extract of Rosemary (*Rosmarinus officinalis*) on reproductive function in adult male Sprague Dawley rats. Analytical details on this extract were not provided.

The petitioner indicates that this extract in the Nusier *et al.* (2007) study is of a polar nature because the initial extraction is performed using 70% ethanol:30% water, and the extract is filtered to remove unsoluble matter (including carnosol and carnosic acid) and then concentrated and dissolved in water, whereas the rosemary extracts of the present application refer to solvent-based extracts, specifically excluding water extracts. The petitioner indicates

that none of the extracts in the present application, including the ethanolic extracts, are soluble in water. Therefore the Panel concluded that the extract tested by Nusier *et al.* (2007) does not represent the extracts in the present application.

The petitioner indicates that all of the subchronic studies reported conducted histopathology on the reproductive organs of male and female rats. No significant differences were observed between dose groups and controls.

These studies included:

- A 90-day oral toxicity study with RES in Sprague Dawley rats at dose levels of 65, 164 and 320 mg/kg bw/day test product in which there were no effects on testes weight and no macroscopic changes for testes, prostate, seminal vesicles and epididymides.
- A 13-week oral toxicity study in which rats were exposed to rosemary extracts F62 or D74 orally in the diet at doses of 300, 600 or 2400 mg/kg diet (extract D74) or 3800 mg/kg diet (extract F62), in which no effects on organ weight, macroscopic an microscopic data for testes, prostate, epididymides and seminal vesicles were observed.
- A 90-day oral toxicity study in which AR and ARD were added to the diet of Sprague-Dawley rats at levels of 0, 500, 1500, and 5000 mg/kg in which testes weight was unaffected.

Thus, the Panel concludes that the rosemary extracts of the present opinion, at the dose levels tested, do not affect the male reproductive system.

The toxicological data on the rosemary extracts are insufficient to establish an ADI, because the toxicity data set does not provide reproductive and developmental toxicity studies or a long-term study. On the other hand, the existing data, including the absence of effects in the 90-day studies on reproductive organs and negative genotoxicity data, do not give reason for concern.

Considering the composition of the extracts (see Table 1) and the potential dietary exposure estimates from the proposed uses and use levels (see below), the Panel concluded that the lack of developmental toxicity studies on these extracts does not raise a concern.

Potential dietary exposure to carnosol plus carnosic acid from rosemary extracts at the proposed uses and use levels have been estimated for adults and pre-school children (aged 1.5 to 4.5 years) and amount to respectively 0.04 and 0.11 mg carnosol plus carnosic acid/kg bw/day at the mean, 0.10 and 0.20 mg carnosol plus carnosic acid/kg bw/day at the 95th percentiles and 0.12 and 0.23 mg carnosol plus carnosic acid/kg bw/day at the 97.5th percentile.

The Panel notes that the margin between the NOAEL range in the 90-day rat studies with all five extracts, of 180 to 400 mg extract/kg bw/day equivalent to 20-60 mg/kg bw/day of carnosol plus carnosic acid, and the exposure estimates for adults would range between 500-1500 for the mean intake values, between 200-600 for the 95th percentile values and between 167-500 for the 97.5th percentile values. For pre-school children these margins would amount to respectively at least 182-546, 100-300 and 88-261. The Panel notes that these margins of safety are worst case estimates since the NOAELs from the different studies were generally the highest dose levels tested, and that the estimates of dietary exposure were conservative. Therefore the Panel is of the opinion that the margin of safety is high enough to conclude that dietary exposure resulting from the proposed uses and use levels are not of safety concern.

These exposure estimates do not take into account potential exposures to carnosol and carnosic acid from use of rosemary as a flavouring or from its use as a herb.

No consumption data are available for such flavouring. However they are not likely to be used on a regular basis and are therefore not expected to lead to a significant chronic exposure to carnosol and carnosic acid.

The Panel notes that the levels of exposure considered in the present evaluation represent the dietary exposure of consumers that would select a diet that was entirely composed of foods containing rosemary extracts, for those food categories in which it was permitted. In reality not all processed foods will contain added antioxidants and it seems unlikely that these extracts would be used at the maximum usage level in all the proposed food in each category or that some consumers would systematically always choose all foods containing rosemary extracts.

CONCLUSIONS

The rosemary extracts evaluated have low acute and sub-chronic toxicity in the rat. Sub-chronic studies on all five different solvent extracts (D74, AR, ARD, F62, RES) have shown that the only effect at high doses of these rosemary extracts is a slight increase in relative liver weight. This effect has been shown to be reversible and may be the result of Phase I and II enzyme induction. Considering the low magnitude, reversibility and the nature of the hepatic changes, and the absence of increases in plasma ALT, AST and AP, the Panel concludes that the minor increase in the liver weight reported, accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction, represent an adaptive response and are not of toxicological concern.

The toxicological data on the rosemary extracts are insufficient to establish a numerical ADI, because the toxicity data set does not provide reproductive and developmental toxicity or long-term studies. On the other hand, the existing data including the absence of effects in the 90-day studies on reproductive organs and a lack of genotoxicity, do not give reason for concern.

Potential dietary exposure to carnosol plus carnosic acid from rosemary extracts at the proposed uses and use levels have been estimated for adults and pre-school children (aged 1.5 to 4.5 years) and amount to respectively 0.04 and 0.11 mg carnosol plus carnosic acid/kg bw/day at the mean, 0.10 and 0.20 mg carnosol plus carnosic acid/kg bw/day at the 95th percentiles and 0.12 and 0.23 mg carnosol plus carnosic acid/kg bw/day at the 97.5th percentile.

The Panel notes that the margin between the NOAEL range in rat studies with all five extracts, of 180 to 400 mg extract/kg bw/day equivalent to 20-60 mg/kg bw/day of carnosol plus carnosic acid, and the exposure estimates for adults would range between 500-1500 for the mean intake values, between 200-600 for the 95th percentile values and between 167-500 for the 97.5th percentile values. For pre-school children these margins would amount to respectively at least 182-546, 100-300 and 88-261. The Panel notes that these margins of safety are worst case estimates since the NOAELs from the different studies were generally the highest dose levels tested, and that the estimates of dietary exposure were conservative.

Based on the margins of safety identified the Panel concluded that the use of the rosemary extracts described in this opinion at the proposed uses and use levels would not be of safety concern.

The Panel notes that the proposed specifications should be modified to better reflect the content of the extracts tested in the safety studies.

DOCUMENTATION PROVIDED TO EFSA

1. Rosemary Extracts and Their Use as Antioxidants *(Source: Leaves from Rosmarinus officinalis L., a herb belonging to the Labiatae family).* March 2001. Submitted by the European Rosemary Extract Manufacturers Group (EREMG). REVISED December 13, 2005.

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GLOSSARY / ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Scientific Panel on Food Additive, Flavourings, Processing Aids and Materials in Contact with Food
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AP	Alkaline Phosphatase
bw	Body Weight
CAS	Chemical Abstract Service
FDA	Food and Drug Administration
GRAS	Generally Recognised As Safe
NOAEL	No Observed Adverse Effect Level
tk	Thymidine Kinase
UK NDNS	UK National Dietary and Nutrition Survey