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Safety of 'leaves from *Morinda citrifolia* L.' [1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2006-185)

Adopted on 10 July 2008

PANEL MEMBERS

Jean-Louis Bresson, Albert Flynn, Marina Heinonen, Karin Hulshof, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Andreu Palou, Hildegard Przyrembel, Seppo Salminen, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Henk van den Berg, Hendrik van Loveren and Hans Verhagen.

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety of dried and roasted leaves from *Morinda citrifolia L.* intended to be used for the preparation of infusions.

After cutting, the leaves of *M. citrifolia* are subjected to drying and roasting steps. The steps are standard procedures commonly applied in food production and do not give rise to concern. Compositional data on various batches of dried and roasted *M. citrifolia* leaves from French Polynesia have been provided. The information presented does not indicate detrimental nutritional effects to be expected from the consumption of tea infusions made from dried and roasted *M. citrifolia* leaves. Under the applied analytical conditions none of the anthraquinones rubiadin, alizarin and lucidin could be detected in dried and roasted *M. citrifolia* leaves (detection limits 0.25, 0.025 and 0.4 mg/kg) and in infusions (detection limits 1.04, 0.1 and 1.67 µg/l), respectively. 5,15-Dimethylmorindol, an anthraquinone previously isolated from the fruits of *M. citrifolia,* was shown to be present in dried and roasted *M. citrifolia* leaves at concentrations ranging from 11.3 to 42.6 mg/kg. In infusions from dried and roasted *M. citrifolia* leaves contents of 5,15-dimethylmorindol ranging between 5.8 and 20.9 µg/l were determined. On the basis of an average 5,15-dimethylmorindol content of 26.5 ± 11.75 mg/kg in dried and roasted leaves and an average extraction efficiency of 34 %, a total of approximately 9 ± 4 µg 5,15-dimethylmorindol is expected to be present per cup of tea (100 ml). Roasting of *M. citrifolia* leaves causes 80 and 77 % decreases of the concentrations of the glycosides rutin and kaempferol-3-*O*-α-L-rhamnopyranoysyl-(1-6)-ß-D-glucopyranoside, respectively, and 2- and 2.9-fold increases of the corresponding aglycones, quercetin and

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quercetin and kaempferol, due to degradation of their glycosidic precursors.

Intake values reported for tea in the U.K National Diet and Nutrition Surveys were used to estimate the potential intakes of infusions prepared from dried and roasted *M. citrifolia* leaves. According to the applicant, infusions are prepared by steeping roasted *M. citrifolia* leaves (1 g in a tea bag) in 240 ml of hot water at 100 ± 2° C for ten minutes. Applying the total solids content of 0.1 % to the estimated 97.5th percentile intakes of infusion reveals that the highest ingestion of *M. citrifolia* leaf material is 1.29 g/d for adult males. This corresponds to a daily intake of 18.4 mg/kg bodyweight (bw), for a 70 kg adult. Applying the same approach to children reveals that the highest ingestion (97.5th percentile) of *M. citrifolia* leaf material is 11.5 mg/kg bw for 15 to 18 year old males, assuming a body weight of 60 kg.

Acute toxicity studies in rats using aqueous and ethanolic extracts did not give indications of adverse effects. In a subchronic (90-day) feeding study in rats with Tahitian Noni® Leaf Tea the no-observed adverse effect level was 2500 mg/kg bw/day, the highest dose administered.

Tests for gene mutations in bacterial cells (Ames test) gave positive results in one tester strain (TA 98) in the presence and absence of S-9 mix, when DMSO or ethanolic extracts of roasted *M. citrifolia* leaves were used. Aqueous extracts, the most appropriate type of extract with regard to the expected route of exposure, were tested negative in this assay. Tests for gene mutations in mammalian cells (HPRT test) using aqueous and ethanolic extracts were negative in the absence of S-9 mix, whereas the aqueous extract induced a slight increase in mutant frequency at two dose levels in the presence of S-9 mix. This equivocal result was outweighed by a clearly negative result in a second HPRT assay when a more concentrated aqueous extract was used. Tests on chromosome mutations *in vitro* and *in vivo* gave negative results. The Panel was satisfied with the total information provided, which allows the conclusion that consumption of infusions prepared from *M. citrifolia* leaves is not expected to induce genotoxic effects.

The Panel noted the current limitations to assess and to predict allergenicity of foods, such as infusions from dried and roasted *M. citrifolia* leaves, and was aware of the difficulties to use data from animal models for prediction of allergenicity in humans.

The Panel concluded that, on the basis of data provided, the use of dried and roasted *M. citrifolia* leaves for the preparation of infusions at the anticipated levels of intake is safe.

Key words:

Morinda citrifolia L., leaves, tea, infusions

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BACKGROUND AS PROVIDED BY EC

On 10 November 2004, Baker & Mackenzie on behalf of Morinda Inc. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the competent authorities of Belgium for placing on the market `*Morinda citrifolia* leaf` as a novel food or novel food ingredient.

On 1 December 2005, the competent authorities of Belgium forwarded to the Commission their initial assessment report, which had reached the conclusion that an additional assessment was required.

On 21 March 2006, the Commission forwarded the initial assessment report to other Member States. Several of these Member States submitted additional comments/objections. In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) N° 258/97.

The concerns of a scientific nature raised by the Member States can be summarized as follows:

- Data on the variability of the phytochemical content of the *M. citrifolia* leaf material should be provided.
- The subchronic 90-day oral toxicity studies performed are considered to be of low sensitivity and quality. Toxicity studies should particularly focus on the risk of hepatotoxicity.
- The structure of the compound showing an absorption spectrum similar to anthraquinones should be elucidated and its potential genotoxicity and hepatotoxicity should be investigated.
- The effects of the roasting step on the composition of *M. citrifolia* leaves should be demonstrated.
- More refined intake estimations should be provided.
- A monitoring programme should accompany the marketing of the novel food.

The initial assessment report carried out by the Belgian authorities came to the conclusion that an additional assessment was required, particularly, given that EFSA had not at that time expressed its views on the concerns about noni juice possibly causing hepatotoxicity. Several Member States shared this view expressed in the initial assessment report and some Member States presented further questions.

In view of these questions and the Community interest in this matter, the European Commission has decided to seek the opinion of the European Food Safety Authority.

TERMS OF REFERENCE AS PROVIDED BY EC

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for "leaves of *Morinda citrifolia*" in the context of Regulation EC N° 258/97.

EFSA is asked to consider the elements of scientific nature in the comments raised by the other Member States.

When appropriate, EFSA is invited to take into account also the information and documents used for the `Opinion on a request from the Commission related to the safety of noni juice (juice of the fruits of *Morinda citrifolia*)` (Request N° EFSA-Q-2005-236).

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ASSESSMENT

In the initial request submitted by the applicant four products derived from the leaves of *M. citrifolia* had been included. In response to the questions raised by EFSA, the applicant limited the application to only one product: dried and roasted leaves of *M. citrifolia* L. for the preparation of infusions.

The product belongs to class 2, sub-category 2.2 (novel foods derived from sources which have not been genetically modified and which have no history of food use within the Community), as defined in the SCF recommendations concerning the assessment of novel foods (European Commission, 1997). Accordingly, information related to the structured schemes I, II, III, IX, XI, XII, XIII has been submitted. In addition, the applicant also provided data related to scheme X (previous human exposure to the novel food).

I. Specification of the novel food (NF)

The composition of raw *M. citrifolia* L. leaves as reported in the Food Composition Table for Use in East Asia (Leung et al., 1972) is shown in Table 1.

Table 1. **Compositional data on raw *M. citrifolia* L. leaves (Leung et al., 1972)**

Constituent	per 100 g
Water (g)	93.7
Protein (g)	1
Fat (g)	0.2
Carbohydrate (g)	4.4
Fiber (g)	1.1
Ash (g)	0.7
Calcium (mg)	58
Phosphorus (mg)	93
Iron (mg)	4.4
beta-Carotene (mg)	0.3
Riboflavin (mg)	0.07
Niacin (mg)	5.6
Ascorbic acid (mg)	50

For dried and roasted *M. citrifolia* L. leaves the ranges presented in Tables 2 and 3 were provided by the applicant.

Table 2. **Compositions of dried and roasted *M. citrifolia* leaves from French Polynesia for infusion**

Parameter	Mean	Standard Deviation	Minimum	Maximum	Number of batches analysed
Protein (g/100g)	19.1	1.08	17.1	20.0	8
Moisture (g/100g)	3.2	1.35	0.8	5.2	8
Fat (g/100g)	4.9	1.48	3.9	8.3	8
Ash (g/100g)	11.8	0.93	10.8	13.0	8
Carbohydrate (g/100 g)	60.7	2.2	55.5	62.7	8
Calories per 100g	363	7.64	353	376	8
Dietary Fiber (g/100 g)	45	3.10	40.7	47.5	4
β-Carotene (mg/100g)	2.9	3.17	0.47	9.70	7
Vitamin C (mg/100g)	< 1	-	< 1	1.20	7
Riboflavin (mg/100g)	1.22	0.25	0.97	1.46	3
Niacin (mg/100g)	4.35	1.08	3.17	5.30	3
Fe (mg/100g)	14.2	3.47	9.1	16.5	4
Ca (mg/100g)	2065	113	1950	2220	4
K (mg/100g)	2275	364	1770	2620	4
Na (mg/100g)	537	161	367	755	5
Mg (mg/100g)	672	78	565	742	4
Zn (mg/100g)	5.1	0.8	4.3	6.3	5
Cu (mg/100g)	0.6	0.05	0.5	0.7	4
Mn (mg/100g)	10.2	0.7	9.4	11.0	4
P (mg/100g)	348	21	327	374	4
Oxalic Acid (g/100g)	0.09	0.04	< 0.05	0.14	7
Tannic Acid (g/100g)	2.6	0.1	2.4	2.7	4

The broad ranges observed for fat and β-carotene were explained by the applicant by one outlier for each of these components. According to the applicant, rejection of these outliers would narrow the ranges for fat and β-carotene to 3.9-5.5 g/100g and 0.47-3.58 mg/100g, respectively.

Table 3. **Ranges of amino acids in dried and roasted *M. citrifolia* leaves**

Amino Acid	Range (mg/ 100 g)
Aspartic Acid	1500-1750
Threonine	550-750
Serine	550-750
Glutamic Acid	1450-1675
Proline	600-850
Glycine	700-950
Alanine	750-950
Cysteine	100-300
Valine	700-950
Methionine	100-250
Isoleucine	550-750
Leucine	1150-1350
Tyrosine	400-600
Phenylalanine	700-900
Histidine	200-375
Lysine	200-350
Arginine	500-750
Tryptophane	150-350

Data on the variability among French Polynesian *M. citrifolia* leaves have been provided for some phytochemicals, such as sterols, tannic acid, oxalic acid and tocopherols (West et al., 2007).

The contents of phytosterols are within the ranges observed for other vegetable foods. Average values obtained from seven analyses were: cholesterol < 1.0 mg/100g, campesterol 43 mg/100g, stigmasterol 50 mg/100g, β-sitosterol 84 mg/100g.

For one batch the following tocopherol contents were provided: α-tocopherol 98.0 mg/100g, γ-tocopherol 2.6 mg/100g, β-tocopherol 0.9 mg/100g.

M. citrifolia L. leaves do not contain caffeine (detection limit in dried *M. citrifolia* leaves: 0.001g/100g).

Data provided for dried *M. citrifolia* L. leaves demonstrated the absence (< 0.5 microgram/kg) of aflatoxins B1, B2, G1 and G2.

Analytical data on heavy metals and pesticides were provided. The following specifications have been given: arsenic <0.05 mg/100g, cadmium <0.02 mg/100g, lead <0.03 mg/100g, mercury <0.0025 mg/100g; organophosphates <0.050 mg/kg, organochlorinates <0.200 mg/kg, N-methylcarbamates <0.100 mg/kg.

Anthraquinones

Analyses for the presence of anthraquinones in samples of fresh and dried *M. citrifolia* leaves and in infusions made from these materials were performed according to the procedure previously applied by the manufacturer to *M. citrifolia* fruits. The method is based on extraction with ethyl acetate, separation of the compounds by reversed phase HPLC and detection using a diode array detector. Based on the characteristic UV spectra of hydroxyanthraquinones exhibiting a maximum absorption between 400 and 420 nm, the HPLC chromatograms were monitored at a wavelength of 410 nm. An overlay of the chromatograms with those obtained by HPLC analysis of a methanol extract of noni root and of authentic lucidin demonstrated the absence of lucidin and rubiadin in the leaf-derived samples. The reported limits of detection for rubiadin, alizarin and lucidin were 0.25, 0.025 and 0.4 mg/kg, respectively, in dried and roasted *M. citrifolia* leaves and 1.04, 0.1 and 1.67 µg/l, respectively, in infusions. Analyses of seven infusions from *M. citrifolia* leaves from different batches demonstrated that under the applied instrumental conditions none of the anthraquinones could be detected.

The HPLC chromatograms had shown the presence of a peak exhibiting a UV-VIS absorption spectrum similar to anthraquinones. The compound was identified as 5,15-dimethylmorindol, an anthraquinone previously isolated from the fruits of *M. citrifolia* (Kamiya et al., 2005).

The limit of quantitation for 5,15-dimethylmorindol was reported to be 0.1 mg/kg in dried and roasted *M. citrifolia* leaves and 0.42 µg/l in infusions from *M. citrifolia* leaves. After addition of 1 µg of 5,15-dimethylmorindol to 240 ml of tea infusion, a recovery rate of 92 % was determined.

In seven batches of infusions from dried and roasted *M. citrifolia* leaves contents of 5,15-dimethylmorindol ranging between 5.8 and 20.9 µg/l were determined (Table 4).

One sample of dried and roasted *M. citrifolia* leaves (batch 2007-12-26) was extracted with methanol. Analysis of the extract showed a content of 11.4 mg/kg 5,15-dimethylmorindol in dried and roasted leaves of *M. citrifolia*. The contents of lucidin, alizarin and rubiadin were below the detection limits of 0.4, 0.025 and 0.25 mg/kg.

Seven batches of dried and roasted *M. citrifolia* leaves were extracted with ethanol and the extracts were analysed by HPLC. As shown in Table 4, the contents of 5,15-dimethylmorindol in dried and roasted *M. citrifolia* leaves ranged from 11.3 to 42.6 mg/kg. The average concentration was 26.5 ± 11.75 mg/kg.

Table 4. **Contents of 5,15-dimethylmorindol in dried and roasted *M. citrifolia* leaves and in infusions**

Batch number	Dried and roasted *M. citrifolia* leaves (mg/kg)	Infusion from dried and roasted *M. citrifolia* leaves (µg/l)
2003-08-14	32.5 ± 3.0	-[a]
2003-09-09	11.3 ± 1. 7	5.8
2004-01-13	24.4 ± 4.5	12.0
2005-08-24	42.6 ± 4.3	17.0
2005-08-25	37.0 ± 4.4	19.7
2005-12-26	12.9 ± 1.0	6.9
2008-05-19	24.6 ± 5.6	6.3
2007-09-20	-[a]	20.9

[a] no data provided

Parallel extraction of six samples (0.5 g) of the same batch of dried and roasted *M. citrifolia* leaves with hot water (200 ml) demonstrated that on average 34 % of the anthraquinone 5,15-dimethylmorindol are extracted.

One tea bag contains 1g of dried and roasted *M. citrifolia* leaves. On the basis of an average 5,15-dimethylmorindol content of 26.5±11.75 mg/kg and an average extraction efficiency of 34 % , a total of approximately 9±4 µg 5,15-dimethylmorindol is expected to be present per cup of tea (100 ml).

II. Effect of the production process applied to the NF

The hand-picked leaves are inspected, sorted, cleaned, sanitised hypochlorite bath and rinsed with water. After cutting, the leaves are subjected to drying and roasting steps. The steps are standard procedures commonly applied in food production and do not give rise to concern.

To determine the influence of the roasting process, comparative HPLC fingerprint profiles were established using HPLC/UV/ESI-MS (electrospray-ionization multi-stage mass spectrometry). By comparison with reference compounds, quercetin-3-*O*-α-L-rhamnopyranosyl-(1-6)-ß-D-gluco-pyranoside (rutin), kaempferol-3-*O*-α-L-rhamnopyranoysyl-(1-6)-ß-D-glucopyranoside, quercetin, kaempferol and 5-hydroxymethylfurfural (HMF) were identified. Quantification revealed that roasting of *M. citrifolia* leaves causes 80 and 77 % decreases of the concentrations of the glycosides rutin and kaempferol-3-*O*-α-L-rhamnopyranoysyl-(1-6)-ß-D-glucopyranoside, respectively, and 2- and 2.9-fold increases of the corresponding aglycones quercetin and kaempferol, due to degradation of their glycosidic precursors. A model experiment demonstrated that roasting of rutin (175 ° C; 20 min.) resulted in a degradation of 10 %. The released aglycon, quercetin, remained stable under these conditions.

Furthermore, the Maillard reaction product HMF was shown to be formed during roasting of *M. citrifolia* leaves in amounts of 40 mg/100g.

III. History of the organism used as the source of the NF

Taxonomically, *Morinda citrifolia* L. belongs to the Rubiaceae family. Common names are “Indian Mulberry”, “Noni”, and “Nonu”. The shrub or small tree occurs from India through Southeast Asia to Eastern Polynesia. It has a long tradition as valuable dye plant, as medicinal plant and as food for the aboriginal populations.

Dye use. Due to the presence of anthraquinone derivatives in roots and bark, these parts of the plant have been used as sources for colouring agents (Thomson, 1971).

Medicinal use. Morinda citrifolia L. has long been employed throughout Polynesia as folk medicine. The use of virtually all parts of the plant (fruit, leaf, bark, root, flower and seed) has been described to treat boils, cuts, abscesses and inflammations of various types, fungal infections, constipation and diarrhoea (Hirazumi, 1997).

Food use. M. citrifolia L. leaves have been described as edible vegetable by the Department of Agricultural Research, Royal Tropical Institute of the Netherlands (Terra, 1966) and are listed in the Food Composition Tables for Use in East Asia (Leung et al., 1972) and the Pacific Islands (Dignan et al., 2004).

I. Anticipated intake and extent of use of the NF

The intake values reported for tea in the United Kingdom National Diet and Nutrition Surveys of 1997 and 2000/2001 were used to estimate the potential intakes of infusions prepared from dried and roasted *M. citrifolia* leaves. The 97.5th percentile for each group of consumers was calculated from the mean and standard deviation. The estimated mean and 97.5th percentile intakes are provided in Table 5.

Table 5. **Estimated intakes of aqueous infusions prepared from dried and roasted *M. citrifolia* leaves**

Age group (years)	Mean intake (g/d)	97.5th percentile intake (g/d)
	Males	
4-6	20	110
7-10	44	218
11-14	75	366
15-18	152	692
19-64	415	1286
	Females	
4-6	26	148
7-10	46	215
11-14	82	365
15-18	165	631
19-64	411	1278

According to the applicant, infusions were prepared by steeping roasted *M. citrifolia* leaves (1g in a tea bag) in 240 ml of hot water at 100 ± 2° C for ten minutes. The solids content of the infusions were determined by loss-on-drying at 100° C (AOAC Official Methods 925.19; 934.01). The average total solids content determined in infusions from seven samples of *M. citrifolia* leaves from different commercial batches was 0.1 % (standard deviation: ±0.02 %).

Applying this solids content to the estimated 97.5th percentile intakes of infusion reveals that the highest ingestion of *M. citrifolia* leaf material is 1.29 g/d for adult males. This corresponds to a daily intake, by weight, of 18.4 mg/kg bw, for a 70 kg adult. Applying the same approach to children reveals that highest ingestion (97.5th percentile) of *M. citrifolia* leaf material is 11.5 mg/kg bw for 15 to 18 yr. males, assuming 60 kg body weight.

II. Information from previous human exposure to the NF or its source

Morinda citrifolia leaves are listed in the food composition tables for East Asia and the Pacific Islands of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (Leung et al., 1972; Dignan et al., 2004). According to the applicant, TAHITIAN NONI® noni leaf tea, a product composed of dried and roasted *M. citrifolia* leaves, has been on

the market in Japan since January 2003. The total number of 20-serving cartons sold up to March 2004 was 127,348.

III. Nutritional information on the NF

The compositional information presented does not indicate detrimental nutritional effects to be expected from the consumption of tea infusions made from dried and roasted *M. citrifolia* leaves.

IV. Microbiological information on the NF

The applicant has conducted appropriate microbiological analyses on commercial batches of *M. citrifolia* leaves for infusion. The absence of *Salmonella* and *Staphylococcus aureus* has been demonstrated while levels of coliforms, yeasts and moulds, and *Escherichia coli* are < 10/g and aerobic organisms < 3000/g. It should be noted that none of the vegetative microorganisms would survive in an infusion at 100° C for 10 minutes.

V. Toxicological information on the NF

Acute toxicity

In studies on acute oral toxicity with mice not complying with relevant OECD guidelines but with the principles of Good Laboratory Practice (GLP), groups of 25 animals per gender received single doses of 2000 mg/kg bw of aqueous or ethanolic extracts of *M. citrifolia* leaves, which were not further specified. Mortality or gross abnormalities at autopsy were not observed. However, at the end of the 14-day observation period mean body weights were reduced in both test groups compared with the control group (aqueous extract: reduction in males 28 % , in females 18 % ; ethanolic extract: reduction in males 26 % , in females 32 %).

In acute oral toxicity studies with rats carried out according to OECD guideline 401 and complying with GLP principles, there were no indications of adverse effects when aqueous or ethanolic *M. citrifolia* leaf extracts, which were not further specified, were administered at dose levels of 2000 mg/kg bw. Body weights of treated animals were comparable to those of the historical controls.

Subchronic toxicity

In a subchronic (90-day) feeding study complying with GLP principles groups of 25 male and 25 female mice received diets containing aqueous or ethanolic extracts of *M. citrifolia* leaves. The study was not carried out in accordance with internationally agreed protocols in that only body weight data were provided. In addition, the exact dose administered to the animals cannot be calculated. According to the study report, 20 mg extract per mouse per day were mixed with the feed, which was freely available. At the end of the treatment period mean body weights in the groups receiving the test materials differed from that of the control group (body weights of animals receiving an aqueous extract were 16 % lower in males and 26 % higher in females, and body weights of animals receiving an ethanolic extract were 23 % lower in males and 26 % higher in females).

Upon request of the Competent Authorities of Belgium, the applicant provided an additional subchronic feeding study in rats carried out in accordance with OECD Guideline 408 and complying with GLP regulations. The test material Tahitian Noni® Noni Leaf Tea, which was described as an insoluble olive green powder consisting of 100 % *M. citrifolia* leaves, complied

with the revised specification provided by the applicant on request of the Panel. Daily doses of 25 mg/kg bw (low dose), 250 mg/kg bw (intermediate dose) and 2500 mg/kg bw (high dose) were administered by gavage to groups of 10 male and 10 female Sprague-Dawley derived rats. The test material was administered as a 0.25 %, 2.5 % or 25 % (w/v) solution in a 1 % (w/w) solution of carboxymethylcellulose in water, 5 consecutive days per week, for 92 or 93 days to males and females, respectively. A control group received a 1 % (w/w) solution of carboxymethylcellulose in water at a similar dose volume.

In each of the three groups receiving the test material one female was found dead during the treatment period. The histological findings in these animals indicated that gavage trauma was the likely cause of death. One male from the low-dose group killed moribund had a mouth and nose injury, which was not related to the administration of the test material.

There were no treatment-related ocular findings in eye examinations. Regular examinations of appearance, body functions and behaviour as well as a functional observation battery and a motor activity test carried out in the late phase of the treatment period revealed no relevant differences between the groups. Body weights and body weight gains were comparable in all groups. There were no relevant differences in food consumption and food efficiency.

Haematology and clinical chemistry examinations carried out at the end of the treatment period revealed statistically significant increases in mean corpuscular volume in low and high dose males. High dose males also showed an increase in mean corpuscular haemoglobin. Females of the intermediate dose group showed an increase in absolute neutrophil counts. Since the differences in males were small and not accompanied by changes in related blood parameters and the difference in females was not dose-related, the Panel does not consider these findings toxicologically relevant. In organ weight determinations a statistically significant increase in the absolute brain weight was observed in males of the intermediate dose group. Since the effect was not dose-related and not accompanied by histopathological findings in this organ it was not considered toxicologically relevant. Histopathological examinations of other organs and tissues also did not reveal relevant differences between the groups. The no-observed adverse effect level (NOAEL) in this study was 2500 mg/kg bw/day.

Chronic toxicity

Studies on chronic toxicity were not provided.

Reproductive and developmental toxicity

Studies on reproductive and developmental toxicity were not provided.

Genotoxicity

In the mammalian microsome reverse mutation assay with *Salmonella enterica* var. Typhimurium strains TA 98, TA 100 and TA 1537 (Ames test) an ethanolic extract prepared from dried *M. citrifolia* leaves was not genotoxic up to the highest tested concentration with and without metabolic activation (S-9 mix). These studies complied with GLP principles but were not conducted according to the respective OECD Guideline (471) since only three of the recommended five tester strains were used. According to additional information provided by the applicant, the test material was obtained from a non-commercial batch, which was not subjected to the same temperatures as those reached in the roasting of the commercial product.

In an *in vitro* test on chromosomal aberrations in human lymphocytes complying with OECD guideline 473 and GLP principles, an ethanolic extract from a commercial sample of dried and

roasted *M. citrifolia* leaves (0.1 g sample/ 1 ml EtOH) did not induce relevant effects up to the highest tested concentration (0.625, 1.25, 2.5, 5, & 10 µl/ml) with and without S-9 mix.

In an *in vitro* test on unscheduled DNA synthesis (UDS) using rat hepatocytes, complying with GLP principles and carried out largely in accordance with OECD guideline 482, material extracted with ethanol from powdered dried and roasted *M. citrifolia leaves* (dissolved in DMSO) did not induce DNA repair synthesis up to the highest tested concentration of 10 µl/ml. According to the study report, 10 µl of the test material were equivalent to an extract from 10 µg leaves.

On request of the Panel the applicant submitted additional information on genotoxicity.

Further tests with *Salmonella enterica* var. Typhimurium strains TA 97A, TA 98, TA 100, TA 102 and TA 1537 with aqueous and DMSO extracts of dried and roasted Noni leaves (Tahitian Noni® Noni Leaf Tea) were carried out. This study was not in accordance with OECD guideline 471 in that only one dose level was used, and a GLP certificate was not provided. The DMSO extract induced an increase in the number of revertants in strain TA 98 in the presence and absence of S-9 mix whereas the aqueous extract was tested negative. There was no increase in the number of revertants when the other tester strains were used.

Positive results were also obtained when an ethanolic extract of Tahitian Noni® Noni Leaf Tea (dissolved in DMSO) was tested using strain TA 98 only, both in the presence and absence of S-9 mix. The test material induced dose-related increases in the number of revertants at the two highest dose levels (1.6 and 5 mg/plate).

In adequately performed tests an aqueous extract prepared from roasted leaves – the most relevant type of extract with regard to the expected exposure route – was not mutagenic in strains TA 97, TA 98, TA 100, TA 102 and TA 1535 up to the highest tested concentration in the presence and absence of S-9 mix. A 20-fold concentrated aqueous extract of roasted leaves was also tested negative in strains TA 97, TA 98, TA 100, TA 102, TA1537 and TA 1538 up to the highest tested concentration.

The applicant provided tests on gene mutations in mammalian cells. The mutagenic potential of aqueous and ethanolic extracts of dried roasted Noni leaves was investigated in the HPRT (hypoxanthine phosphoribosyl-transferase) mutation test according to OECD Guideline 476 using V79 cells derived from the Chinese Hamster. In the absence of S-9 mix, there were no relevant increases in mutant frequency compared with the negative control whereas the positive control gave a clear positive result. In the presence of S-9 mix the ethanolic extract induced an increase in mutant frequency only at the lowest tested concentration, which is not considered relevant. The aqueous extract induced an increase at the lowest and highest but not at the intermediate tested concentration. Since there was no clear dose-response relationship and the mutant frequency of the negative control in the presence of S-9 mix was very low, the applicant concluded that there was no mutagenic risk. In a second HPRT test a concentrated aqueous extract of roasted leaves did not induce increases in mutation frequencies.

In an *in vivo* mouse micronucleus assay conducted according to OECD guideline 474 and complying with GLP principles, Tahitian Noni® Noni Leaf Tea at a dose of 2000 mg/kg bw did not induce an increase in the number of polychromatic erythrocytes with micronuclei compared with the negative controls whereas the positive control substance induced the expected significant increases.

Allergenicity

A test for induction of an acute systemic anaphylactic response was conducted in guinea pigs. Groups of 6 animals (of the same sex) received four subcutaneous injections, one week apart, of a freeze-dried ethanol extract of *M. citrifolia* leaves plus adjuvant or leaf extract without adjuvant. A negative control group of 6 animals was treated accordingly with vehicle (0.9 % NaCl) and adjuvant, and a positive control group of 3 animals was treated with ovalbumin (1 mg) and adjuvant. Freunds complete adjuvant was used in the first injection and Freunds incomplete adjuvant was used in the 2nd, 3rd and 4th injection.

After two weeks the animals in both test groups and the negative control group were challenged by oral gavage with the test article (10 % w/w). The animals in the positive control group were challenged by intravenous injection of ovalbumin (2 mg). The animals in both test groups showed no signs of acute systemic anaphylaxis. None of the animals in the negative control group showed signs of acute systemic anaphylaxis. The first two animals in the positive control group showed signs of acute systemic anaphylaxis, and one animal died several minutes after treatment (the last animal was not treated for animal welfare reasons).

DISCUSSION

Members of the family Rubiaceae are known to contain anthraquinones in the roots and several anthraquinones have been reported to occur in the fruits of *M. citrifolia*. In 2006 the European Commission sought the opinion of the EFSA on published case reports that might possibly have an impact on the opinion expressed by the Scientific Committee on Food on the safety of noni juice (SCF, 2002). On the basis of the available toxicological information and against the background of the data provided on consumption of noni juice without reporting of hepatotoxic effects, the Panel considered it unlikely that the consumption of noni juice, at the observed levels of intake, induces adverse liver effects. This would also apply to the anthraquinones potentially present in the commercially produced noni juice. The Panel concluded that there is no convincing evidence for a causal relationship between the acute hepatitis observed in the case studies reported and the consumption of noni juice (EFSA, 2006).

Investigations of the occurrence of anthraquinones were also a special focus of this assessment of dried and roasted leaves of *M. citrifolia*. The Panel concluded that the absence of lucidin and rubiadin in leaf-derived samples, the quantitative data provided on the presence of 5,15-dimethylmorindol in *M. citrifolia* leaves and in infusions, and the results of the toxicological studies do not indicate a concern.

In studies on acute oral toxicity in mice, which were not carried out according to internationally accepted protocols, no mortality but reduced body weights were noted after administration of extracts from *M. citrifolia* leaves. Adequately conducted acute toxicity studies in rats using aqueous and ethanolic extracts did not give indications of adverse effects. In a subchronic (90-day) feeding study in rats with Tahitian Noni® Noni Leaf Tea the no-observed adverse effect level was 2500 mg/kg bw/day, the highest dose administered.

Tests for gene mutations in bacterial cells (Ames test) gave positive results in one tester strain (TA 98) in the presence and absence of S-9 mix, when DMSO or ethanolic extracts of roasted *M. citrifolia* leaves were used. Aqueous extracts, the most appropriate type of extract with regard to the expected route of exposure, were tested negative in this assay. Tests for gene mutations in mammalian cells (HPRT test) using aqueous and ethanolic extracts were negative in the absence of S-9 mix, whereas the aqueous extract induced a slight increase in mutant frequency at two dose levels in the presence of S-9 mix. This equivocal result was outweighed by a clearly negative result in a second HPRT assay when a more concentrated aqueous extract

was used. Tests on chromosome mutations *in vitro* and *in vivo* gave negative results. The Panel was satisfied with the total information provided, which allows the conclusion that consumption of infusions prepared from *M. citrifolia* leaves is not expected to induce genotoxic effects.

The Panel noted the current limitations to assess and to predict allergenicity of foods, such as infusions from dried and roasted *M. citrifolia* leaves, and was aware of the difficulties to use data from animal models for prediction of allergenicity in humans.

CONCLUSIONS AND RECOMMENDATIONS

The Panel concluded that, on the basis of data provided, the use of dried and roasted *M. citrifolia* leaves for the preparation of infusions at the anticipated levels of intake is safe.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission to the Chairman of the European Food safety Authority with the request for an opinion on the safety of *Morinda citrifolia* leaf` (Morinda Inc.); SANCO E4/AK/ko D(2006) 540657.
2. Initial assessment report carried by Belgium. Opinion of the CSH (Public Health Board) concerning an application for authorisation to use the leaves of *Morinda citrifolia* L. (Noni) as a novel Food under Regulation EC 258/97; November 2005.
3. Letters from Member States with comments on the initial assessment report on 'fungal oil from *Mortierella alpina*' from CSH (Public Health Board) (BE).
4. Response to Member States comments on the Belgium Initial Assessmengt Report.
5. Application under regulation No 258-97 for `*Morinda citrifolia* leaf` (Morinda Inc.).

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