SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of carnauba wax (E 903) as a food additive[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) delivers a scientific opinion re-evaluating the safety of carnauba wax (E 903). Carnauba wax (E 903) is authorised in the EU as food additive as glazing agent. It has been evaluated by the Scientific Committee on Food (SCF) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) who allocated an Acceptable Daily Intake (ADI) of 7 mg/kg bw/day. The SCF did not establish an ADI but considered the use of carnauba wax as a glazing agent acceptable. Carnauba wax is a complex mixture of compounds consisting mainly of aliphatic esters (wax esters), α-hydroxyl esters and cinnamic aliphatic diesters obtained from the Brazilian Mart wax palm, *Copernicia cerifera*. The Panel considered that carnauba wax would be predicted to not be significantly absorbed from the diet and that if hydrolysed its main constituents could be absorbed and incorporated into normal cellular metabolic pathways. Based on the available data and the lack of structural alerts on carnauba wax it was concluded that there is no concern for genotoxicity for carnauba wax. Subchronic and reproductive and developmental toxicity studies did not showed adverse effects related to carnauba wax intake. No chronic toxicity or carcinogenicity studies were available on carnauba wax. Overall, the Panel considered that long-term toxicity data on carnauba wax were lacking and therefore did not establish an ADI.. However, the Panel considered that the exposure estimates to carnauba wax from the proposed uses resulted in sufficient margins of safety compared to the identified No Observed Adverse Effect Levels (NOAELs) for carnauba wax, allowing the Panel to conclude that the use of carnauba wax as a food additive with the currently authorised uses would not be of safety concern.

KEY WORDS

Carnauba wax, INS No. 903, CAS Registry Number 8015-86-9, EINECS 232-399-0

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SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the re-evaluation of carnauba wax (E 903) as a food additive.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Carnauba wax is authorised in the EU as a food additive only as glazing agent (Directive 95/2/CE[4]; Regulation 2008). Carnauba wax is authorised to food supplements, small products of fine bakery wares coated with chocolate, snacks, nuts and coffee beans. Maximum permitted use is 200 mg/kg food. It is also permitted as surface treatment on fresh citrus fruits, melons, apples and pears, as well as on peaches and pineapples up to 200 mg/kg food. In confectionary it may be used up to 500 mg/kg and on chewing gum up to 1200 mg/kg.

Carnauba wax has been evaluated by the Scientific Committee on Food (SCF, 1992; 1997; 2001; 2002) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993). JECFA allocated an Acceptable Daily Intake (ADI) of 0-7 mg/kg bw/day (JECFA, 1993). The SCF did not establish an ADI but did not object the use of carnauba wax as a glazing agent (SCF, 2002).

Carnauba wax is a complex mixture of compounds consisting of aliphatic esters (wax esters), α-hydroxyl esters and cinnamic aliphatic diesters. It also contains free acids, free alcohols, hydrocarbons and resins. It is obtained from the leaves of the Brazilian Mart wax palm, *Copernicia cerifera* (EU, 2008). The average composition of the highest quality carnauba wax has been reported as consisting primarily of 40% (w/w) aliphatic esters, 21% (w/w) diesters of 4-hydroxycinnamic acid, 13% (w/w) esters of ω-hydroxycarboxylic acids and 12% (w/w) free alcohols (Wolfmeier et al., 2005).

Specifications have been defined in the Directive 2008/84/EC and new specifications according to Commission Regulation (EU) No 231/2012 will apply from 1st December 2012. No new data on absorption, distribution, metabolism and elimination were available for this revaluation. One modified 90-day toxicity feeding study carried out to investigate "bioaccumulation" of carnauba wax in Fischer F-344 rats indirectly suggested that the lipid like components from the wax are not accumulated in the tissues (Edwards, 1998). Overall, taking also into consideration the chemical composition of carnauba wax, the Panel considered, as with other natural waxes, that absorption of carnauba wax is expected to be low, if any.

The Panel noted that toxicological studies were conducted on carnauba wax itself and therefore the components of carnauba wax have been tested in those studies.

From a 13-weeks study with Wistar rats fed diets containing 0, 1, 5 or 10% carnauba wax, or 10% cellulose powder for 13 weeks corresponding to 0, 800, 4200 and 8800 mg/kg bw/day for males and 0, 900, 4600 and 10 200 mg/kg bw/day for females (Rowland et al., 1982) the Panel could derive a No Observed Adverse Effect Level (NOAEL) of 8800 mg/kg bw/day, the highest dose tested in male rats.

[4] European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1-40.

From a 90-day toxicity study with Fischer F-344 rats fed diets containing carnauba wax corresponding to daily intakes of 0, 15, 150 and 1500 mg/kg bw, respectively, continuously for 90 days (Edwards, 1998), the Panel could derive a NOAEL of 1500 mg/kg bw/day, the highest dose tested.

The Panel considered that based on the available data and the lack of structural alerts on carnauba wax it can be concluded that there is no concern for genotoxicity for carnauba wax.

No chronic toxicity or carcinogenicity studies were available on carnauba wax.

From a reproductive toxicity study of carnauba wax with Wistar rats administered 0, 0.1, 0.3, or 1% carnauba wax in the diet, the Panel could derive a NOAEL of approximately 670 mg carnauba wax/kg bw/day, the highest dose tested in female rats.

Refined estimates reported for carnauba wax, when considering Maximum Permitted Levels (MPLs), resulted in a mean dietary exposure of European toddlers (aged 12-35 months and weighing an average of 15 kg) ranged from 2.6-4.6 mg/kg bw/day, and from 3.1-8.1 mg/kg bw/day at the 95th percentile. The mean dietary exposure of European children (aged 3-9 years and weighing an average of 30 kg) ranged from 1.6-4.5 mg/kg bw/day, and from 3.2-7.6 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to carnauba wax for these populations were fruits and confectionary.

The mean dietary exposure of European adolescents (aged 10-17 years and weighing an average of 50 kg) ranged from 0.9-2.1 mg/kg bw/day, and from 1.9-3.8 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to carnauba wax for this population were fruits and confectionary. Whereas the mean dietary exposure of the European adult population give a mean dietary exposure in the range of 0.7-1.7 mg/kg bw/day and 1.5-3.0 mg/kg bw/day for high level consumers. The main contributors to the total anticipated mean exposure to carnauba wax for this population were fruits. For the elderly, mean exposure to carnauba wax was in the range of 0.8-1.5 mg/kg bw/day and in the range of 1.9-2.7 mg/kg bw/day at the 95th percentile. Main contributors for these populations were fruits. From the highest consumers of these populations (95th percentile) these exposures estimates would result in margins of safety from 83 to 447 when compared to the NOAEL of 670 mg/kg bw/day identified in a reproductive toxicity study with rats by Parent (Parent et al., 1983), from 31 to 67 when compared to the NOAEL of 250 mg/kg bw/day identified in a subchronic toxicity study with dogs by Parent (Parent et al., 1983b), from 185 to 1000 when compared to the NOAEL of 1500 mg/kg bw/day identified in a subchronic toxicity study with rats by Edwards (Edwards et al., 1998), and from 1086 to 5867 when compared to the NOAEL of 8800 mg/kg bw/day identified in a subchronic toxicity study with rats by Rowland (Rowland et al., 1982). These margins of safety are considered sufficient by the Panel taking into consideration that the NOAEL's identified are the highest dose tested not showing any effect in their respective studies, and that the exposure estimates to carnauba wax carried out in this opinion are very conservative.

Overall, the Panel concluded that long-term toxicity data on carnauba wax were lacking and therefore did not establish an ADI.

However, the Panel noted that available toxicity studies consistently reported no findings associated with carnauba wax intake. Furthermore, consideration of the conservative exposure estimates to carnauba wax from the currently authorised uses indicated sufficient margins of safety, which allowed the Panel to consider that the use of carnauba wax as a food additive with the currently authorised uses would not be of safety concern.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[5]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[6] of 2001. The report "Food additives in Europe 2000[7]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[5] OJ L 80, 26.03.2010, p19
[6] COM(2001) 542 final.
[7] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of carnauba wax (E 903) when used as a food additive.

Carnauba wax (E 903) is authorised in the EU as food additive, as glazing agent only (Directive 95/2/CE[8]; EU Regulation 1333/2008[9]). It has been evaluated by the Scientific Committee on Food (SCF, 1992; 1997; 2001; 2002) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993). JECFA allocated an Acceptable Daily Intake (ADI) of 0 - 7 mg/kg bw/day (JECFA, 1993). The SCF did not establish an ADI but did not object to the use of carnauba wax as a glazing agent (SCF, 2002). SCF concluded that the use of carnauba wax was up to 1200 mg/kg of food would yield a worst-case intake estimate of 48 mg/person/day (equivalent to 4.8 mg/kg bw/day for 10 kg child, or less for an adult) and these levels were within the ADI of 0 - 7 mg/kg bw/day set by JECFA (SCF, 2002). Carnauba wax was also reviewed by TemaNord in 2002 (TemaNord, 2002).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that became available since then and the data available following a public call for data[10]. The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substance

Carnauba wax is obtained from the leaf buds and leaves of the Brazilian Mart wax palm, *Copernicia cerifera* (Directive 2008/84/EC)[11]. It has the CAS Registry Number 8015-86-9.

Recognized classification of carnauba wax types has been adopted internationally and according to Wolfmeier et al. (2005) it goes from a highest value prime product (Type 1 or prime yellow) to a very crude product (Type 4 or fatty gray/filtered). According to these authors, chemically refined or derivatized carnauba waxes are no longer available commercially.

JECFA describes carnauba wax as a complex mixture consisting of aliphatic esters (wax esters), α-hydroxyl esters and cinnamic aliphatic diesters. It also contains free acids, free alcohols, hydrocarbons and resins (JECFA, 1998). Aliphatic esters are described by JECFA as consisting of straight-chain acids with even-numbered carbon chains from C_{24} to C_{28} and straight-chain alcohols with even-numbered carbon chains from C_{30} to C_{34}. Alpha-hydroxyl esters are described as consisting of straight-chain hydroxyl acids with even-numbered carbon chains from C_{22} to C_{28}, straight-chain acids with even-numbered carbon chains form C_{24} to C_{28}, straight-chain monohydric alcohols with even-numbered carbon chains from C_{24} to C_{34} and dihydric alcohols with even-numbered carbon chains from C_{24} to C_{34}. Cinnamic aliphatic esters are described as consisting of p-methoxycinnamic acid and dihydric alcohols with even-numbered carbon chains from C_{24} to C_{34} (JECFA, 1998).

[8] European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1-40.

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, p 16-33.

[10] Call for scientific data on miscellaneous waxes permitted as food additives in the EU (published: 23 November 2009). Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123b.htm

[11] Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253, 20.9.2008, p. 1-175.

Free-acids are described as straight-chain acids with even-numbered carbon chains from C_{24} to C_{28}, free alcohols are described as straight-chain alcohols with even-numbered carbon chains from C_{30} to C_{34} and hydrocarbons are described as straight-chain odd-numbered carbon chains from C_{27} to C_{31} (JECFA, 1998). The Panel noted that hydrocarbons would only constitute between 0.3 and 1% of the total composition of carnauba wax.

The average composition of the highest quality carnauba wax is reported in Table 1.

Table 1: Composition of yellow carnauba wax according to Vandenburg et al. (1970).

Compound	Amount (wt% of wax)
Aliphatic esters	38-40
Diesters of 4-hydroxycinnamic acid	20-23
Diesters of 4-methoxycinnamic acid	5-7
Esters of ω-hydroxycarboxylic acids	12-14
Free alcohols	10-12
Free acids	5-7
Hydrocarbons[12] (paraffins)	0.3-1
Triterpene diols	0.4

Carnauba wax is one of the hardest and highest-melting point natural waxes. At room temperature the wax has a weakly aromatic odour and a characteristic hay-like scent in the molten state (Wolfmeier et al., 2005). It is identified as being insoluble in water, sparingly soluble in alcohol, but very soluble in chloroform and ether (Commission Regulation (EU) No 231/2012[13]).

Two synonyms are palm wax and Brazilian wax.

2.2. Specifications

Specifications have been defined in the Directive 2008/84/EC and new specifications according to Commission Regulation (EU) No 231/2012 will apply from 1 December 2012. Specifications have also been defined by JECFA (JECFA, 1998) (Table 2).

Table 2: Specifications for carnauba wax according to Commission Regulation (EU) No 231/2012and JECFA (1998).

	Commission Regulation (EU) No 231/2012	JECFA, (1998)
DEFINITION	Carnauba wax is a purified wax obtained from the leaf buds and leaves of the Brazilian Mart wax palm, *Copernicia cerifera*	The refined wax obtained from the fronds of the Brazilian tropical palm tree *Copernicia cerifera (Arruda) Mart.* [syn. *C. purnifera (Muell.)*]
DESCRIPTION	Light brown to pale yellow powder or flakes or hard and brittle solid with a resinous fracture	A pale yellow to light brown, hard and brittle solid, having a clean fracture

[12] Hydrocarbons are defined as organic compounds consisting entirely of hydrogen and carbon,

[13] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1-295.

FUNCTIONAL USES	-	Glazing agent, bulking agent, acidity regulator, carrier
IDENTIFICATION		
Solubility	Insoluble in water, partly soluble in boiling ethanol, soluble in chloroform and diethyl ether	Insoluble in water, partially soluble in boiling ethanol, soluble in ether
Melting range	Between 82 °C and 86 °C	80 – 86 ºC
Specific gravity	About 0.997	-
PURITY		
Sulphated ash	Not more than 0.25%	Not more than 0.25% w/w
Acid value[14]	Not less than 2 and not more than 7	Between 2 and 7
Ester value[15]	Not less than 71 and not more than 88	Between 71 and 93
Unsaponifiable matter	Not less than 50% and not more than 55%	Between 50% and 55%
Saponification value[16]	-	Between 78 and 95
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Arsenic	Not more than 3 mg/kg	-
Mercury	Not more than 1 mg/kg	-

The Panel noted minor differences between JECFA and EU specifications for carnauba wax.

2.3. Manufacturing Process

Carnauba wax is obtained from the leaves of the palm *Copernicia cerifera.* The wax is located on the outer palm leaves. After drying the leaves in the sun or on steam-heated racks, the wax is removed (loosened) from the leaves manually or mechanically. The dried leaves are chopped and the loosened wax chips are separated by an air classification process to give a mixture of approximately 60% wax and 40% chopped leaves. The primary purification process of the mixture involves melting in water with oxalic acid which gives a crude wax paste. The latter is heated to its boiling point and pressed through a filter while hot. Centrifugation of the filtrate gives virtually anhydrous wax. The remaining wax can be extracted from the dried filter cake with solvents (e.g. heptane). The crude product is normally supplied in lumps. Additional bleaching with hydrogen peroxide can also be carried out (Wolfmeier et al., 2005).

2.4. Methods of analysis in foods

The analysis of carnauba wax itself is a difficult analytical challenge, although by means of flash heating the wax sample and simultaneous derivatisation, the high molecular weight esters, can be hydrolysed and converted to their methyl derivatives. Subsequent Gas chromatography–mass spectrometry (GC-MS) analysis results in characteristic fingerprint patterns and identification of marker compounds for carnauba wax (Asperger et al., 1999). Although in principle selective extraction of the wax from food systems might be possible, similar compounds originating from sources other than carnauba wax will undoubtedly complicate an already complex analysis. Solvent extraction of carnauba wax from rodent diets, silica gel clean-up and analysis by Fourier transform infra-red spectroscopy (FT-IR) has been successfully applied over the concentration range 0.01 to 2.5% wax in the diet (Walters, 1998). For the specific situation of wax coatings on apples, differential

[14] The Acid Value (AV) is defined as the mass of KOH (in mg) required to neutralise the acid groups contained in 1 g Dry Substance (DS).
[15] The ester value is defined as the number of mg of potassium hydroxide (KOH) required to saponify the esters in 1 g of a sample.
[16] The saponification value is defined as the number of mg of potassium hydroxide (KOH) to saponify the esters in 1 g of the sample and neutralize the free acids in 1 g of a sample.

scanning calorimetry has been applied using the characteristic peak maximum temperature of the wax for identification of the wax treatment agent (Ritter et al., 2001).

Alkaline hydrolysis, gas-liquid chromatography and thin layer chromatography have also been described as analytical methods (Anonymous, 1984).

2.5. Reaction and fate in foods, stability

No specific documentation has been submitted or found in the open literature, but as the components of carnauba wax are rather inert and stable it can be assumed that degradation or reaction with food components will not take place at significant extent.

2.6. Case of need and proposed uses

According to Directive 95/2/EC[17] carnauba wax is permitted only as a glazing agent to food supplements, small products of fine bakery wares coated with chocolate, snacks, nuts and coffee beans. Maximum permitted use is 200 mg/kg food. It is also permitted as surface treatment on fresh citrus fruits, melons, apples and pears as well as on peaches and pineapples up to 200 mg/kg food. In confectionary it may be used up to 500 mg/kg and on chewing gum up to 1200 mg/kg.

These use levels were confirmed as a response to the EFSA call on new data in connection with the present re-evaluation process.

No information on use levels or use frequency has been submitted or found in the literature search concerning the use on fruits.

Table 3 summarises those foodstuffs that are permitted to contain carnauba wax up to specified Maximum Permitted Levels (MPLs) set by Directive 95/2/EC.

Table 3: Maximum Permitted Levels of use of carnauba wax in foodstuffs according to the European Parliament and Council Directive 95/2/EC and maximum reported use levels of Carnauba wax in foodstuffs used for the refined exposure assessment

Foodstuffs	Maximum Permitted Level (mg/kg)	Maximum reported use level (mg/kg)
Confectionary (including chocolate), as glazing agent only	500	500
Chewing gum	1200	1200
Small products of fine bakery wares coated with chocolate, as glazing agent only	200	200
Snacks	200	200
Nuts	200	200
Coffee beans	200	200
Food supplements	200	200
Fresh citrus fruits, melons, apples, pears, peaches and pineapples (surface treatment only)	200	200

[17] European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1-40

2.7. Information on existing authorisations and evaluations

The SCF evaluated carnauba wax several times. In 1990 the SCF reviewed acute oral toxicity studies and dermal teratogenicity studies on carnauba wax not showing any treatment-related adverse effects (SCF, 1992). The SCF was unable to establish an ADI for carnauba wax but considered its use as glazing agent at the levels examined as temporarily acceptable until toxicological data and technical data on use levels have been provided (SCF, 1992). In 1994 SCF evaluated a 90-day rat toxicity study, a 28-week dog toxicity study, a combined reproductive and subchronic rat toxicity study, a rat teratogenicity study and *in vitro* mutagenicity studies in bacteria and yeast (SCF, 1997). The SCF was informed at that time that use of carnauba wax on confectionery was unlikely to exceed 200 mg/kg food, thus resulting in intakes unlikely to exceed 1-2 mg/kg bw/day. The SCF extended the temporary acceptance of carnauba wax as a glazing agent pending submission of *in vitro* chromosome aberrations tests in mammalian cells (SCF, 1997). Information was also requested on the readiness of carnauba wax ester components to hydrolyse.

In 2001 the SCF reviewed new genotoxicity assay and supplementary information on usage levels of carnauba wax as a glazing agent submitted following its request in 1997 (SCF, 2001). The SCF decided to withdraw the temporary acceptance status of carnauba wax and accepted its use as a glazing agent up to a maximum use level of 200 mg/kg of food (SCF, 2001). In an addendum to the latter opinion, SCF considered a new request for use levels of carnauba wax of 500 mg/kg in confectionery (hard and soft sugar coated centers) and 1200 mg/kg in chewing gum as a glazing agent (SCF, 2002). The SCF did not object the use of carnauba wax as a glazing agent at higher levels in products requiring it. The SCF noted that a use level of 1200 mg/kg of food would yield a worst-case intake estimate of 48 mg/person/day which equates to 4.8 mg/kg bw/day for a 10 kg child, or less for an adult, and is within the ADI of 0-7 mg/kg bw set by the JECFA in 1993 (SCF, 2002).

According to Directive 95/2/EC[2] carnauba wax (E 903) is authorised at defined maximum levels as a glazing agent only (Table 3).

Carnauba wax is permitted with no specific restriction in plastics in contact with food as Ref No 42720 (Directive 2002/72/EC[18]).

JECFA evaluated carnauba wax and based on short-term feeding studies in rats, a combined reproductive and developmental toxicity study in rats and *in vitro* mutagenicity studies, allocated an ADI of 0-7 mg/kg bw/day based on the highest dose tested in the combined study in rats (JECFA, 1993).

In the USA carnauba wax is classified as Generally Recognized as Safe (GRAS) and is permitted with no other limitation than good manufacturing practice (GMP) in a variety of food products (FDA, 1983). Carnauba wax is permitted in Canada (http://www.hc-sc.gc.ca/fn-an/securit/addit/diction/dict_food-alim_add-eng.php#c, accessed in June 2010), in Japan (http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/list-exst.add, accessed in June 2010), and in Australia and New Zealand.

Carnauba wax is used in the formulation of stick and solid cosmetics (e.g. lip stick balms and lotion bars), in face and eye makeup preparations, fragrance preparations, hair colouring and conditioning preparations, and skin care products (Anonymous, 1984).

[18] Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. OJ L 220, 15.8.2002, p. 18.

2.8. Exposure

2.8.1. Food consumption data used for exposure assessment

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Overall, the food consumption data gathered at EFSA were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

For calculation of chronic exposure, intake statistics have been calculated based on individual average consumption over the total survey period excluding surveys with only one day per subject. High level consumption was only calculated for those foods and population groups were the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011b). The Panel estimated chronic exposure for the following population groups: toddlers, other children, adolescents, and adults and the elderly. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries as mentioned in Table 4:

Table 4: Population groups considered for the exposure estimates of carnauba wax

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	from 12 up to and including 35 months of age	Bulgaria, Finland, Germany, Netherlands
Children[19]	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[19]	Older than 65 years	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

Consumption records were codified according to the FoodEx classification system (EFSA, 2011a). Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Commission Regulation (EU) N° 1129/2011[20], part D, to perform exposure estimates.

[19] The terms "children" and "the elderly" correspond respectively to "other children" and the merge of "elderly" and "very elderly" age groups in the EFSA Guidance on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

[20] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 12.11.2011.

2.8.2. Exposure to carnauba wax from its use as food additive

Exposure to carnauba wax from its use as a food additive has been calculated by using MPLs as listed in Table 3. Since no data different from the MPL were reported for normal use levels, no further refinement was calculated.

High level exposure (95th percentile of consumers only) was calculated by adding the 95th percentile of exposure from one food group (i.e. the one having the highest value) to the mean exposure resulting from the consumption of all other food groups.

This is based on the assumption that an individual might be a high level consumer of one food category and would be an average consumer of the others. This approach has been tested several times by the Panel in the re-evaluation of food colours and has shown reasonable correlation with high level total intakes when using the raw food individual consumption data. Therefore, this approach was preferred for the calculations based on the maximum reported use levels in order to avoid excessively conservative estimates.

However, the Panel noted that its estimates should be considered as being conservative as it is assumed that all processed foods contain carnauba wax added at the MPLs.

Table 5 summarises the estimated exposure to carnauba wax from its use as a food additive of all five population groups.

2.8.3. Main food groups contributing to exposure of carnauba wax using MPL

The main contributors to the total anticipated mean exposure to carnauba wax for toddlers, children, adolescents and adults were fruits and confectionary. For toddlers fruits and confectionary contributed to 64-96% and 12-18% exposure, respectively, for children these contributions were 47-79% and 12-18%, for adolescents 44-81% and 11-19%, and for adults 60-93% and 11%, respectively. For the elderly, the main contributors to the total anticipated mean exposure to carnauba wax were fruits (79-95%).

Table 5: Summary of anticipated exposure to carnauba wax using the tiered approach (EC, 2001) in children and the adult population

	Toddlers (12-35 months)	**Children** (3-9 years)	**Adolescents** (10-17 years)	**Adults** (>18 years)	**Elderly (> 65 years)**
	mg/kg bw/day	mg/kg bw/day	mg/kg bw/day	mg/kg bw/day	mg/kg bw/day
Estimated exposure using MPL					
• Mean exposure	2.6-4.6	1.6-4.5	0.9-2.1	0.7-1.7	0.8-1.5
• Exposure 95th	3.1-8.1	3.2-7.6	1.9-3.8	1.5-3.0	1.9-2.7

2.8.4. Other sources

Carnauba wax is used on pharmaceutical products (tablets) and in a range of technical applications like cosmetic, fragrance preparations, hair colouring and conditioning preparations, and manicuring, skin care, and suntan preparations. However, considering the chemical properties of the product it is unlikely that any significant systemic exposure will take place from these applications.

3. Biological and toxicological data

3.1. Absorption, distribution, metabolism and excretion

No new data on absorption, distribution, metabolism and excretion were available for this re-evaluation.

Fatty acid esters

The major constituents of carnauba wax are esters of acids and alcohols with average chain lengths of C_{26} to C_{32} (Table 1). Generally intact long-chain fatty acid esters are poorly absorbed.

However, the alcohols and acids generated from the hydrolysis of carnauba wax constituents in the intestinal tract can be absorbed and incorporated into normal cellular metabolic pathways.

3.2. Toxicological data

The Panel noted that toxicological studies were conducted on carnauba wax itself and therefore the components of carnauba wax have been tested in those studies.

3.2.1. Acute oral toxicity

Two unpublished acute oral toxicity studies in rats have been briefly reported (Anonymous, 1984).

Administration by gavage to 10 Sprague-Dawley rats of a 20 000 mg/kg dose of a lipstick formulation containing 5.6% carnauba wax (representing a dose of 1100 mg/kg of carnauba wax) did not show deaths or toxic effects (no further details).

In a second study, oral intubation of five rats (strain unknown) with a blush formulation containing 10% carnauba wax diluted to 33.3% in corn oil, making the wax concentration of 3.33%. None of the animals died when a 500 mg carnauba wax/kg bw was administered (Anonymous 1984).

3.2.2. Short-term and subchronic toxicity

Groups of 15 male and 15 female Wistar rats, approximately 5 weeks of age at the beginning of the study, were fed diets containing 0, 1, 5 or 10% carnauba wax, or 10% cellulose powder for 13 weeks corresponding to 0, 800, 4200 and 8800 mg/kg bw/day for males and 0, 900, 4600 and 10 200 mg/kg bw/day for females (Rowland et al., 1982). The rats fed diets containing cellulose powder acted as a control group for possible effects due to the replacement of a significant proportion of the diet by a non-nutrient test material. Additional groups of five rats of each sex were fed diets containing 0, 5, 10% carnauba wax or 10% cellulose powder for 2 and 6 weeks. Food consumption and body weights were recorded. Haematological, serum biochemistry, urinalysis, organ weights, and gross examinations were recorded at autopsy. Histopathological examinations were done on half of the tissue sections conserved from the control rats and tissues from those given 10% carnauba wax or 10% cellulose powder. Results showed a significant increase in mean food consumption in male and female rats given 10% carnauba wax or 10% cellulose powder, without statistically significant differences in body weights compared to controls. A higher erythrocyte count at week 2 in male rats fed 10% carnauba wax was noticed, but no other statistical significant differences were recorded on haematological findings in rats fed diet containing 10% carnauba wax at 2, 6 or 13 weeks. Glutamic-oxalacetic transaminase, glutamine-pyruvic transaminase and lactic dehydrogenase activities in serum were similar in treated and control animals. Few significant random differences in urinalysis (specific gravity, volume) were recorded. Organ weights did not show changes related to carnauba wax doses. Histopathological examination reported some inflammatory cell infiltration and few areas of focal

necrosis in the liver, as well as some interstitial pneumonitis with similar incidences in 10% carnauba wax and 10% cellulose powder treated animals (Rowland et al., 1982). A No Observed Adverse Effect Level (NOAEL) of 8800 mg/kg bw/day can be derived by the Panel from this study, the highest dose tested in male rats.

In a modified 90-day toxicity feeding study carried out to investigate "bioaccumulation" of carnauba wax, groups of 20 male and 20 female Fischer F-344 rats were fed diets containing carnauba wax corresponding to daily intakes of 0, 15, 150 and 1500 mg/kg bw, respectively, continuously for 90 days (main study) (Edwards, 1998). Additional two groups of 5 male and 5 female Fischer F-344 rats were assigned to a reversibility phase in which animals fed the control diet and a diet of 1500 mg/kg bw/day of carnauba wax for 90 days were reverted to control diet for additional 90 days.

Observations throughout the study included general condition and behaviour, body weight, food intake, ophthalmological observations, haematological examination, blood clinical chemistry and organs weight. Histological examination was conducted on selected organs after at least 90 days of treatment. Tissues from animals from the reversibility phase were not examined. A single female in the highest dosage group died spontaneously of brain haemorrhage on day 52 (Edwards, 1998).

The most frequent finding in the main study was that of red staining in the head region, which was observed in all groups, including controls. In the animals from the reversibility phase minor observations included red staining in the head region, areas of fur loss or unkempt fur and yellow staining in urinogenital areas, occurring in both treated and control groups. Given the similar patterns observed in both treated and control groups it was considered that no treatment-related adverse findings related to the condition and behaviour were observed (Edwards, 1998).

Among several endpoints, necropsied tissues such as heart, liver, kidney, spleen, lymph nodes and ileum, were microscopically evaluated. From these observations, the authors concluded that no evidence of lipid accumulation derived from carnauba wax was noted (Edwards, 1998).

The body weights of the male animals were not statistically significantly different from the controls. For females, no marked differences from the controls were reported except for a significantly higher mean body weight of the females in the 15 mg/kg group at days 3 to 10. This difference disappeared by study day 14 and was thus considered as not treatment-related. No differences in mean body weights of male or female rats were reported after the reversibility phase of the study. During the study the feed intake of male and female rats fed carnauba wax was significantly higher in some animals at all doses tested compared to the controls. This finding was not considered to be an adverse effect and it might reflect feed compensation from increasing proportions of non-nutrient carnauba wax containing diets. No differences in feed intake were reported after the reversibility phase of the study. No abnormalities were reported upon ophthalmological examination. Occasional statistically significant differences in haematological findings and clinical chemistry parameters were observed in male or female rats. These changes were not dose related and were thus considered as not treatment related. No marked differences in these parameters were reported in animals after the reversibility phase of the study. Serum chemistry analysis showed slight occasional changes in clinical chemistry from animals in the 15 and 150 mg/kg bw/day carnauba wax groups not observed in the 1500 mg/kg bw/day group (lower chloride or protein concentration, higher albumin/globulin ration, higher alanine aminotransferase and lactate dehydrogenase activities). Few differences were reported in animals after the reversibility phase of the study (Edwards, 1998).

The only differences found in organ weights in male rats were inconsistent increases in the mean absolute weight of the brain of the group fed 15 mg/kg bw/day carnauba wax and a reduction in the mean relative weight of the thymus in the 15 and 1500 mg/kg bw/day groups. No statistically significant differences in any organ weight were reported in females. Upon histopathological examination the only finding reported was higher incidences of individual cell necrosis in the liver of male rats in the 15 and 150 mg/kg bw groups; in the latter, a significant higher incidence of vacuolisation in the liver of male rats was also reported, but the 1500 mg/kg bw/day animal group did

not show these effects. No significant differences in histopathological findings were observed in the females. Overall, the histopathological examination of tissues taken at necropsy did not show any treatment related adverse findings (Edwards, 1998). The Panel identified a NOAEL of 1500 mg/kg bw/day from this study, the highest dose tested.

Four groups of 6 male and 6 female Beagle dogs were fed diets containing 0, 0.1, 0.3 or 1% carnauba wax for 28 weeks (equivalent to 25, 75 or 250 mg/kg bw/day) (Parent et al., 1983a). Food consumption, body weights and behavioural effects were recorded weekly. Blood and urinary samples were collected at weeks 11 and 26. Organs were weighed (brain, pituitary gland, thyroid gland, heart, liver, spleen, kidneys, and adrenal glands), and gross and microscopic examinations of tissues were performed at the end of the study. No significant differences in body weights or food consumption were noted between treatment and control groups of animals. Data from serum biochemistry, urinalysis and organ weights did not show any treatment-related effects in dogs consuming carnauba wax. The only significant ($p < 0.05$) clinical observation at 26 weeks were the higher free fatty acid levels only in male dogs at all dietary levels of carnauba wax as compared to controls. Observations made after 11 weeks were comparable between groups. The authors noted that serum free fatty acid levels in this study were increased in male dogs at all doses of carnauba wax tested as compared to controls. However, the authors noticed that serum free fatty acid levels in treated male dogs remained within the normal historical range for Beagle dogs from the laboratory (200-800 µM/l), whereas the values in the controls in this study were comparatively lower (138 ± 38 µM/l) than the historical values. Ophthalmologic and gross examinations revealed no significant treatment-related effects for up to 1% carnauba wax in the diet. Histopathological findings were reported comparable between groups including controls (Parent et al., 1983a). A NOAEL of 250 mg/kg bw/day could be derived by the Panel from this study, the highest dose tested.

3.2.3. Genotoxicity

Genotoxicity of carnauba wax was considered by JECFA during its evaluation (JECFA, 1993). The results from the studies available at that time were reported as summary only and the study reports were not available for this re-evaluation. Available information from the JECFA report is reproduced below in Table 5.

Table 5: JECFA summary of the results of mutagenic studies on carnauba wax (from JECFA, 1993)

End-point	Test system	Concentration of Carnauba wax	Results	References
Reverse mutation[1]	*S. typhimurium* TA1537, TA1538, TA98	3.3-1000 µg in plate tests	Negative	(Mortelmans and Griffin, 1981)
Reverse mutation[1]	*S. typhimurium* TA1537, TA1538, TA98	0.01-0.5% in suspension tests	Negative	(Mortelmans and Griffin, 1981)
Reverse mutation[1]	*S. typhimurium* TA1537, TA1538, TA98	0.1-2.5% in suspension tests	Negative	(Mortelmans and Griffin, 1981)
Reverse mutation[2]	*S. typhimurium* TA1535, TA1537, TA1538	0.01% in plate tests	Negative	(Litton Bionetics inc, 1975)
Reverse mutation[2]	*S. typhimurium* TA1535, TA1537, TA1538	0.005 and 0.01% in suspension tests	Inconsistent changes[3]	(Litton Bionetics inc, 1975)
Gene conversion[2]	*S. cerevisiae* D4	0.3 and 1.75% in suspension tests	Negative	(Litton Bionetics inc, 1975)

[1] The Ames/*Salmonella* assays in the presence and absence of an Aroclor 1254-stimulated, rat-liver homogenate metabolic activation system, were used in this study.

[2] A series of *in vitro* microbial assays with and without metabolic activation were used. In the activation assays, the tissue homogenate of liver, lung and testes were prepared from either mouse, rat or monkey.

[3] The results from non-activation suspension tests were negative. The results from activation suspension tests showed scattered increased mutation responses in the presence of rat-liver or testes homogenate with strain TA1537, and in the presence of monkey-lung homogenate with TA1538.

Additional data considered in the SCF evaluation consisted of an *in vitro* chromosomal aberration tests with carnauba wax were performed using human lymphocytes without S-9 mix for 20 hours treatment, and with S-9 mix (10% v/v and Aroclor 1254 induced) for 3 hours treatment (Edwards, 1996; 1997). According to the authors, since the test article was not soluble in culture medium or in other tested vehicles commonly used, preliminary studies were carried out on two vehicles: soybean oil and chloroform/DMSO mixtures. Human lymphocytes cultures were treated with those vehicles to determine the maximum concentrations not causing toxicity or chromosome damage. The results showed that the suspension of carnauba wax in 10% soybean oil and emulsification in culture medium was the method of choice. The test article was then suspended in soybean oil and was emulsified in culture medium. Initially, two chromosome aberration tests were conducted, each employing cultures treated in duplicate with a range of 5 concentrations of carnauba wax together with untreated, vehicle and positive controls. Cells were treated 3 hours with metabolic activation and harvested 20 hours after culture initiation. Without metabolic activation, a continuous treatment of 20 hours was applied. The experiment was repeated with an extra 44 hours harvest time for the highest test concentration without S-9 mix. Concentrations of 0.031, 0.063, 0.125, 0.25 and 0.5 mg carnauba wax/ml were tested in those experiments (Edwards, 1996; 1997).

In the first chromosome aberration test without metabolic activation, no statistically significant increases in aberrant metaphases, including or excluding gaps, were reported with the vehicle (control) or any test article concentration compared with the untreated control in the absence of S9 activation. But a statistically significant positive linear trend without gaps was reported for test article treatments and the untreated control. No significant trend was reported with gaps. A reverse pattern of responses, i.e. a significant linear trend only with gaps, was observed in the presence of S9 activation in this chromosome aberration test (Edwards, 1996).

In a second chromosome aberration test, no statistically significant increases in aberrant metaphases or linear trend, including or excluding gaps, were reported in the absence or presence of S9 activation (Edwards, 1996). However, in this trial a borderline response ($p=0.053$) was elicited by cyclophosphamide, the positive control substance in presence of metabolic activation.

To account for a low response noted in the positive control in the second chromosome aberration test, a third chromosomal aberration test was done under the same conditions as described above (Edwards, 1997). In this test the positive control (cyclophosphamide 10 µg/ml) gave statistically significant increase in aberrant metaphases, including or excluding gaps, whereas no statistically significant increases in aberrant metaphases or linear trend, including or excluding gaps, were reported with the test article in the presence and absence of S9 activation (Edwards, 1997). Overall, carnauba wax assayed at the maximum practicable concentration *in vitro*, although in the absence of sufficient cytotoxicity is not regarded to cause structural chromosome aberrations *in vitro* under the reported experimental conditions. No *in vivo* genotoxicity data were available on carnauba wax.

The Panel observed that although that there are limitations in testing insoluble compounds *in vitro*, based on the available data, the methodology used in the study and the lack of structural alerts on carnauba wax it can be concluded that there is no concern for genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

No studies on chronic toxicity or carcinogenicity were available on carnauba wax.

3.2.5. Reproductive and developmental toxicity

In a reproduction study in rats, four groups of 25 female (F0) and 25 male Wistar rats were administered 0, 0.1, 0.3 or 1% carnauba wax in the diet (Parent et al., 1983b). This administration equals 0, 80, 250, or 810 mg carnauba wax/kg bw/day for males and 0, 90, 270, or 670 mg carnauba wax/kg bw/day for females as calculated by the authors from of food consumption over the entire study (Parent et al., 1983). After 4 weeks of feeding, all F0 generation rats were paired within the groups. Test diets were continued throughout mating, gestation and lactation. F1 generation was produced from F0 females and at day 21 post-partum animals were randomly selected to form the F1 generation. F1 animals (25 of each sex/group) continued to receive the same diet as its F0 for 13 additional weeks.

Observations throughout the study included food intake, body weights, ophthalmological observations, haematological examination, blood clinical chemistry and organs weight. Complete gross necropsies were performed on all F0 animals, F1 animals that were killed after weaning, and F1 animals used for the post-weaning 13-week feeding study. Histological examination was performed on selected organs of animals in the control and high dose groups. For animals in the low and mid-dose groups, only grossly abnormal tissues were examined microscopically (Parent et al., 1983b).

Indices of fertility, gestation, viability, and lactation, or pup weights were not statistically significantly affected by the treatments. Nor were the number of born alive or born dead pups per litter although a decreased total number of pups was noticed in the treated groups (between 228 and 230 pups) compared to controls (269 pups). There were no statistically significant differences on body weight gain reported amongst the groups. Inconsistent differences in food consumption between the groups were mentioned by the authors without details (Parent et al., 1983b).

Haematology and urine analysis from F1 generation showed statistically significant increase in haematocrit of female rats fed diets containing 0.1% and 1% carnauba wax but not in the 0.3% group. Males did not show any change in haematology parameters measured. Blood clinical chemistry showed in males significantly increased nitrogen urea levels at the highest dose tested and increased chloride levels at the two highest doses tested (0.3% and 1.0%). Decreased levels of serum glutamate-pyruvate transaminase and free fatty acid levels were also reported at these doses. In female rats blood clinical chemistry only showed decreased levels in free fatty acids at the two highest doses tested, the rest of the clinical chemistry parameters measured did not differ amongst the groups.

Organ weights measured in male or female animals were not affected by the treatments and microscopic examination of tissues and organs did not reveal any treatment-related effects (Parent et al., 1983b). A NOAEL of 670 mg/kg bw/day could be derived by the Panel from this study, the highest dose tested in female rats.

This study was used by JECFA as the basis for setting the ADI of 7 mg/kg bw/day for carnauba wax (rounded up), by applying a 100 uncertainty factor to the NOAEL of approximately 670 mg/kg bw/day.

Special study on developmental toxicity

JECFA briefly reports one developmental toxicity study done with Wistar rats (FDRL, 1977; as reported by JECFA, 1993). Four groups of 25 females, were fed 0, 0.1, 0.3 or 1% carnauba wax in the diet for two weeks before mating and throughout gestation (equivalent to 0, 50, 150, or 500 mg/kg bw/day). Body weights of pregnant dams were recorded on days 0, 6, 11, 15, and 20 of gestation. On day 20 of gestation caesarean sections were performed on pregnant females, and gross pathological changes were noted. The uterine contents were examined and the number of corpora lutea, implantation sites, resorption sites, live and dead fetuses, gross malformations, and body weights of live fetus were recorded. One-half of fetuses were examined for signs of visceral pathological changes and the other half were examined for signs of skeletal abnormalities. JECFA reports that there were no

significant changes in body weights of pregnant dams during gestation, no significant differences in reproduction data among test groups, and no dose-related effects of carnauba wax on skeletal or soft tissue development in fetus (JECFA, 1993). The original study report was not available to the Panel.

3.2.6. Allergenicity, hypersensitivity and intolerance

No information relevant to carnauba was exposure via the oral route was identified by the Panel.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Carnauba wax has been evaluated by the Scientific Committee on Food (SCF, 1992; 1997; 2001; 2002) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993). JECFA allocated an Acceptable Daily Intake (ADI) of 0-7 mg/kg bw/day (JECFA, 1993). The Panel noted that toxicological studies were conducted on carnauba wax and therefore the large majority of components of carnauba wax have been tested.

Carnauba wax (E 903) is a complex mixture consisting of aliphatic esters (wax esters), α-hydroxyl esters and cinnamic aliphatic diesters. It also contains free acids, free alcohols, hydrocarbons and resins. It is obtained from the leaf buds and leaves of the Brazilian Mart wax palm, *Copernicia cerifera* (EU, 2008). It is described as consisting mainly of straight-chain acids with even-numbered carbon chains from C_{24} to C_{28} and straight-chain alcohols with even-numbered carbon chains from C_{30} to C_{34} (JECFA, 1998). The average composition of the highest quality carnauba wax has been reported as consisting primarily of 40% (w/w) aliphatic esters, 21% (w/w) diesters of 4-hydroxycinnamic acid, 13% (w/w) esters of ω-hydroxycarboxylic acids and 12% (w/w) free alcohols (Wolfmeier et al., 2005).

Specifications have been defined in the Directive 2008/84/EC and new specifications according to Commission Regulation (EU) No 231/2012 will apply from 1st December 2012.

One modified 90-day toxicity feeding study carried out to investigate “bioaccumulation” of carnauba wax in Fischer F-344 rats indirectly suggested that the lipid like components from the wax are not accumulated in the tissues (Edwards, 1998). Overall, taking into consideration also the chemical composition of carnauba wax, the Panel considered, as with other natural waxes, that absorption of carnauba wax is expected to be low, if any.

From a 13-weeks study with groups of 15 male and 15 female Wistar rats, approximately 5 weeks of age at the beginning of the study, fed diets containing 0, 1, 5 or 10% carnauba wax, or 10% cellulose powder for 13 weeks (corresponding to 0, 800, 4200 and 8800 mg/kg bw/day for males and 0, 900, 4600 and 10 200 mg/kg bw/day for females) (Rowland et al., 1982).The Panel could derive a NOAEL of 8800 mg/kg bw/day, the highest dose tested in male rats.

From a 28-week study with four groups of 6 male and 6 female Beagle dogs fed diets containing 0, 0.1, 0.3 or 1% carnauba wax (equivalent to 25, 75 or 250 mg/kg bw/day) (Parent et al., 1983a) the Panel could derive a NOAEL of 250 mg/kg bw/day, the highest dose tested.

From a 90-day toxicity study with groups of 20 male and 20 female Fischer F-344 rats fed diets containing carnauba wax corresponding to daily intakes of 0, 15, 150 and 1500 mg/kg bw, respectively, continuously for 90 days (Edwards, 1998) the Panel could derive a NOAEL of 1500 mg/kg bw/day, the highest dose tested.

The Panel considered that based on the available data and the lack of structural alerts on carnauba wax it can be concluded that there is no concern for genotoxicity for carnauba wax.

No chronic toxicity or carcinogenicity studies were available on carnauba wax, however in its 1994 opinion the SCF already noted that the structure of the main components of carnauba wax (long-chain aliphatic esters) raises the possibility that it might behave like some mineral hydrocarbons (SCF, 1994).

From a reproductive toxicity study of carnauba wax with groups of 25 female and 25 male Wistar rats administered 0, 0.1, 0.3, or 1% carnauba wax in the diet (Parent et al., 1983) the Panel could derive a NOAEL of approximately 670 mg carnauba wax/kg bw/day, the highest dose tested in female rats.

Exposure estimates reported for carnauba wax, when considering MPLs, result in a mean dietary exposure of European toddlers (aged 12-35 months and weighing an average of 15 kg) ranged from 2.6-4.6 mg/kg bw/day, and from 3.1-8.1 mg/kg bw/day at the 95th percentile. The mean dietary exposure of European children (aged 3-9 years and weighing an average of 30 kg) ranged from 1.6-4.5 mg/kg bw/day, and from 3.2-7.6 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to carnauba wax for these populations were fruits and confectionary.

The mean dietary exposure of European adolescents (aged 10-17 years and weighing an average of 50 kg) ranged from 0.9-2.1 mg/kg bw/day, and from 1.9-3.8 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to carnauba wax for this population were fruits and confectionary. Whereas the mean dietary exposure of the European adult population give a mean dietary exposure in the range of 0.7-1.7 mg/kg bw/day and 1.5-3.0 mg/kg bw/day for high level consumers. The main contributors to the total anticipated mean exposure to carnauba wax for this population were fruits and confectionary. For the elderly, mean exposure to carnauba wax was in the range of 0.8-1.5 mg/kg bw/day and in the range of 1.9-2.7 mg/kg bw/day at the 95th percentile. Main contributors for these populations groups were fruits.

From the highest consumers of these populations (95th percentile) these exposures estimates would result in margins of safety from 83 to 447 when compared to the NOAEL of 670 mg/kg bw/day identified in a reproductive toxicity study with rats by Parent (Parent et al., 1983a), from 31 to 167 when compared to the NOAEL of 250 mg/kg bw/day identified in a subchronic toxicity study with dogs by Parent (Parent et al., 1983b), from 185 to 1000 when compared to the NOAEL of 1500 mg/kg bw/day identified in a subchronic toxicity study with rats by Edwards (Edwards et al., 1998), and from 1086 to 5867 when compared to the NOAEL of 8800 mg/kg bw/day identified in a subchronic toxicity study with rats by Rowland (Rowland et al., 1982). These margins of safety are considered sufficient by the Panel taking into consideration that the NOAEL's identified are the highest dose tested not showing any effect in their respective studies, and that the exposure estimates to carnauba wax carried out in this opinion are very conservative.

Overall, the Panel considered that long-term toxicity data on carnauba wax were lacking and therefore did not establish an ADI.

However, the Panel noted that available toxicity studies consistently reported no findings associated with carnauba wax intake. Furthermore, consideration of the conservative exposure estimates to carnauba wax from the currently authorised uses indicated sufficient margins of safety, which allowed the Panel to consider that the use of carnauba wax as a food additive with the currently authorised uses would not be of safety concern.

CONCLUSION

Carnauba wax has been evaluated by the Scientific Committee on Food (SCF, 1992; 1997; 2001; 2002) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993). JECFA

allocated an Acceptable Daily Intake (ADI) of 7 mg/kg bw/day (JECFA, 1993). The SCF did not establish an ADI but did not object to use of carnauba wax as a glazing agent (SCF, 2002).

Overall, the Panel concluded that long-term toxicity data on carnauba wax were lacking and therefore did not establish an ADI.

However, the Panel noted that available toxicity studies consistently reported no findings associated with carnauba wax intake. Furthermore, consideration of the conservative exposure estimates to carnauba wax from the currently authorised uses indicated sufficient margins of safety, which allowed the Panel to conclude that the use of carnauba wax as a food additive with the currently authorised uses would not be of safety concern.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Technical University of Denmark (DTU). September 2010.

2. European Wax Federation. Data on waxes permitted as food additives. April 2010.

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GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
BIBRA	British Industrial Biological Research Association
CAS RN	Chemical Abstracts Service Registry Number
EC	European Commission
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
GRAS	Generally Recognized as Safe
JECFA	Joint Expert Committee on Food Additives
MPL	Maximum permitted use level
NOAEL	No-Observed-Adverse-Effect-Level
SCF	Scientific Committee on Food
WHO	World Health Organization