European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on re-evaluation of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) as food additives[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Copper complexes of chlorophylls (Cu-chlorophylls) (E 141(i)) and copper complexes of chlorophyllins (Cu-chlorophyllins) (E 141(ii)) are prepared from sources that could not be regarded as edible plant material or food (grass, lucerne, nettle) for humans. Considering their manufacturing process, these compounds cannot be regarded as natural compounds. The Panel noted that very few studies have been conducted using Cu-chlorophylls, which hampered assessment of their safety. In contrast to (non-copper) chlorophylls and chlorophyllins, the available data showed that some components of Cu-chlorophyllins can be absorbed and distributed systematically. Given the differences in purity, chemical properties, stability and manufacturing process, the Panel considered that it was not possible to use Cu-chlorophyllins (E 141(ii)) data for read-across for Cu-chlorophylls (E 141(i)). The available data were considered inadequate by the Panel to evaluate the genotoxic potential of Cu-chlorophyllins. The Panel considered that, given the discrepancies and uncertainties in the available data concerning the carcinogenic potential of Cu-chlorophyllins, further and adequate evaluation of the possible carcinogenicity of Cu-chlorophyllins was needed. Finally, the Panel concluded that reliable data on absorption, distribution, metabolism and excretion (ADME), genotoxicity, (chronic) toxicity, carcinogenicity, and reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) were lacking. Therefore, their safety of use as food additives cannot be assessed and the current Acceptable Daily Intake (ADI) should be withdrawn. In addition, the Panel considered that the specifications should be updated to include information on the non-chlorophyll components of E 141(i), which may represent up to 90 % of the extract, together with the precise identification of the various compounds that are present in the food additives E 141(i) and E 141(ii).

KEY WORDS

copper complexes of chlorophylls, E 141(i), copper complexes of chlorophyllins, E 141(ii), food colours

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SUMMARY

Following a request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of copper complexes of chlorophylls (Cu-chlorophylls) (E 141(i)) and copper complexes of chlorophyllins (Cu-chlorophyllins) (E 141(ii)) when used as food additives.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and additional literature that has become available since then. No new toxicological or biological information was submitted to the Panel for the re-evaluation of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) following European Food Safety Authority (EFSA) public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation. To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), which covered the period up to the end of 2014. A further update has been performed by the Panel.

Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008[4].

The Panel noted that the name "copper complex of chlorophylls" is meaningless on a chemical basis, and should be "copper complex of phaeophytins". Cu-chlorophylls (E 141(i) and Cu-chlorophyllins (E 141(ii)) are obtained from sources that could not be regarded as edible plant material or food (grass, lucerne, nettle) for humans. In addition, owing to their manufacturing process, the food additives Cu-chlorophylls E 141(i) and Cu-chlorophyllins E 141(ii) cannot be considered as natural compounds. The Panel considered that the raw material should fulfil the conditions of the current regulation as regards maximum levels of possible contaminants, including pesticides residues applied during cultivation and mycotoxins.

The Panel considered that the specifications do not sufficiently cover Cu-chlorophylls, which are processed from extracts from plants that do not have a long-term history of food use, and that, consequently, the specifications should be updated to include the information on the non-chlorophyll components of E 141(i), which may represent up to 90 % of the extract.

The Panel noted that, according to industry, Cu-chlorophylls (E 141(i)) is not used to produce an aluminium lake and that Cu-chlorophyllins (E 141(ii)) can be transformed into the corresponding aluminium lake.

There is great confusion in the literature. In many publications, the study material, usually named "chlorophyllin", was quite often, if not always, a sodium and/or potassium salt of Cu-chlorophyllins (E 141(ii)). Scotter (2011) stated that: "*It is important to consider that despite a joint initiative introduced by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry, a substantial body of long-established trivial names for chlorophyll and its analogues remains in popular use by both the food colour industry and scientific researchers. The term 'chlorophyllin' covers a range of compounds identical to, or structurally related to the porphyrins*".

The Panel considered that the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) should be revised to ensure that their use as food additives will not be a significant source of exposure to these toxic elements in foods.

[4] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

Cu-chlorophyllins (E 141(ii)) have been previously evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) in 1969 and 1974 (JECFA, 1970, 1975). In 1975, the Scientific Committee on Food (SCF) evaluated Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) (SCF, 1975). Based on a No Observed Adverse Effect Level (NOAEL) of 1 500 mg Cu-chlorophyllins/kg body weight (bw)/day derived from the long-term and reproduction study by Harrisson et al. (1954), JECFA (1975) set a temporary Acceptable Daily Intake (ADI) of 0–15 mg/kg bw/day to Cu-chlorophyllins. In addition, in its evaluations of 1970 and 1975, JECFA described a study by Reber and Willigan (1954) in which Cu-chlorophyllins exhibited significant adverse effects on rat survival after oral exposure to 500 mg/kg bw/day for 19 weeks. The Panel noted that the reason why JECFA did not take into account these findings for the calculation of the ADI was unclear. SCF (1975) allocated a group ADI of 15 mg/kg bw/day to the sum of both complexes and stated that "*Cu-chlorophylls and Cu-chlorophyllins are two distinct food colours and recommends separate listing of the two colours; accordingly, the Community Directive specification requires amendment*". The Panel noted that no subsequent actions appear to have been taken following this recommendation.

Based on the same NOAEL of 1 500 mg/kg bw/day identified from the Harrisson et al. (1954) study, but by applying a safety factor of 200 instead of 100 for the JECFA and the SCF evaluations, the United States Food and Drug Administration (US FDA) allocated an ADI of 7.5 mg/kg/day for sodium–copper chlorophyllins (FDA, 2002). The US FDA has recommended that sodium–copper chlorophyllins can be taken orally as a deodorant, generally at 100–200 mg/day. In some cases an additional 100 mg/day may be required, but the total daily dose should not exceed 300 mg/day (FDA, 1990).

The Panel noted that both JECFA and FDA have used the Harrisson et al. (1954) study to establish an ADI. This is an old study not carried out in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines, and which did not include the usual endpoints for reproductive and developmental toxicity and used a small number of animals. Therefore, the Panel considered that, based on current standards, this study was inadequate to identify a NOAEL from which a reliable ADI could be derived.

Most of the available toxicity data were for Cu-chlorophyllins, whereas very few studies have been conducted using Cu-chlorophylls, which hampered their safety assessment. Given the differences in purity, chemical properties, stability and manufacturing process, the Panel considered that it was not possible to use Cu-chlorophyllins (E 141(ii) data for read-across for Cu-chlorophylls (E 141(i)). The Panel noted that the amount of copper-containing material that is absorbed, as well as the full metabolic fate and bioavailability of copper, are not known. Because some reports have shown tissue distribution of copper-containing materials after ingestion of Cu-chlorophyllins, the Panel considered that this might deserve further investigations.

In a study in which the rats were fed a diet containing Cu-chlorophyllins for 19 weeks (Reber and Willigan, 1954), a NOAEL of 500 mg/kg bw/day (the only dose used) could be determined. The Panel noted that this NOAEL was not considered by either the JECFA or the SCF for their evaluations.

No genotoxicity data for Cu-chlorophylls were available, while data on Cu-chlorophyllins were considered by the Panel as inadequate to evaluate its genotoxic potential.

The Panel considered that given the discrepancies and uncertainties in the available data concerning the carcinogenic potential of Cu-chlorophyllins, further and adequate evaluation of the possible carcinogenicity of Cu-chlorophyllins is needed.

The Panel noted that the available studies for the evaluation of the reproductive and developmental toxicity of Cu-chlorophyllins (E 141(ii)) were inadequate and that no study on the reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) was available.

In the refined exposure assessment scenario, the Panel used to use only maximum concentration values (maximum reported use levels) available for each authorised food category. However, given the range of data that have been made available, the Panel considered that all data should be used in additional scenarios of the exposure assessment approach intended to provide more realistic exposure estimates. For Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), only usage levels were available for the refined exposure assessment scenario. Based on these data, and the maximum level exposure assessment scenario, the Panel calculated two refined exposure estimates based on different assumptions: a "brand-loyal scenario" and a "non-brand-loyal scenario".

The Panel noted that the refined exposure estimates will not cover future changes in the level of use of Cu-chlorophylls (E 141(i)) or Cu-chlorophyllins (E 141(ii)). Only use levels were reported by industry; no analytical data were provided to EFSA. These data covered the main food categories in which Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised. The Panel noted that some data providers did not distinguish between Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) and therefore, there was uncertainty whether some usage data refer to Cu-chlorophylls (E 141(i)) or Cu-chlorophyllins (E 141(ii)).

The Panel concluded that adequate data on absorption, distribution, metabolism and excretion (ADME), genotoxicity, (chronic) toxicity, carcinogenicity, and reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are lacking. Therefore, their safety of use as food additives cannot be assessed and the ADI should be withdrawn.

The Panel also concluded that the specifications do not adequately cover Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) and recommended that the components that are present in the commercial food additives Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) should be adequately identified and characterised. In addition, the inconsistency in the total copper content currently indicated in the specifications should be clarified.

The Panel recommended that data on the raw material should fulfil the conditions of the current regulation as regards maximum levels for possible contaminants, including residues of pesticides applied during cultivation and mycotoxins.

The Panel recommended that the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) should be revised to ensure that their use as food additives will not be a significant source of exposure to these toxic elements in foods.

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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, p. 19.
[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 the copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) when used as food additives. For reasons of readability, in this document, the general term 'copper complexes of chlorophylls' is abbreviated to Cu-chlorophylls and the term 'copper complexes of chlorophyllins' is abbreviated to Cu-chlorophyllins.

Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008.[8]

Cu-chlorophyllins (E 141(ii)) have been previously evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) in 1969 and 1974 (JECFA, 1970, 1975). In 1975, the Scientific Committee on Food (SCF) evaluated Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) (SCF, 1975).

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available since then, and data available following European Food Safety Authority (EFSA) public calls for data.[9,10,11] The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation. To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), which covered the period from the beginning of 2011 up to the end of 2014. A further update has been performed by the Panel.

2. Technical data

2.1. Identity of the substance

2.1.1. Cu-chlorophylls (E 141(i))

Cu-chlorophylls (E 141(i)) are described in Commission Regulation (EU) No 231/2012 as "*obtained by addition of a salt of copper to the substance obtained by solvent extraction of strains of edible plants material, grass, lucerna and nettle". It is also indicated: "the major principle colouring matters are the copper phaeophytins*".

Cu-chlorophylls (E 141(i)) are pigments with a porphyrin ring (tetrapyrrole ring) as basic structure with a coordinated copper ion (Cu^{2+}) and a phytol ester side chain (Figure 1).

Phaeophytins are formed when chlorophylls are depleted of magnesium. Divalent cations can replace the central magnesium cation (Mg^{2+}) of chlorophylls to form a substitutional (central) complex in the tetrapyrrole ring. "Copper complexes of chlorophylls" are in fact copper complexes of phaeophytins (Boucher and Katz, 1967; Zvezdanovic, 2007). The Panel noted that the name Cu-chlorophylls should correctly be Cu-phaeophytins. However, to avoid confusion in the different terminologies used in the EU Regulations, the Panel decided to retain in the present document the terminology used in

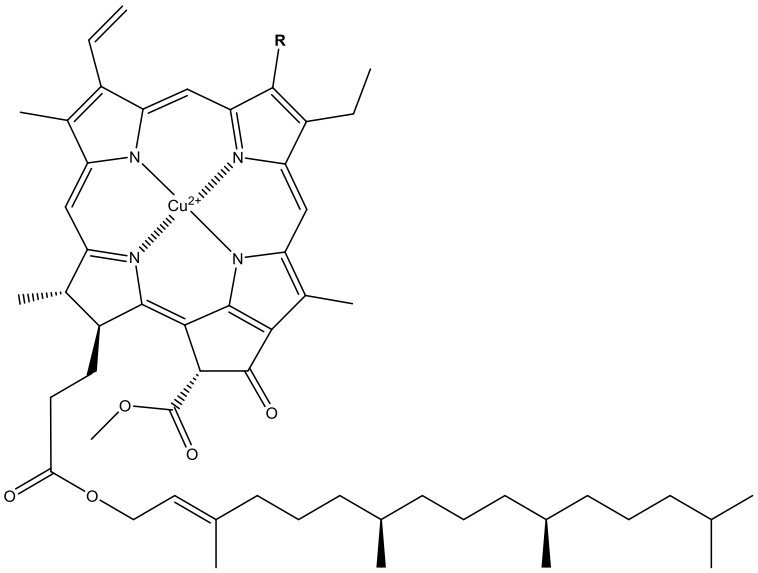
[8] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

[9] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[10] Call for food additives usages level and/or concentration data in food and beverages intended for human consumption. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

[11] Call for scientific data on selected food additives permitted in the EU. Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B). Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

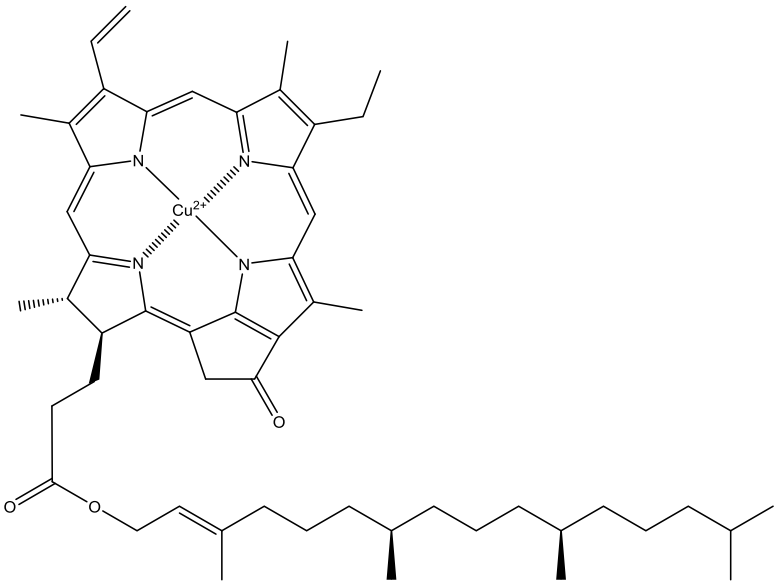
Commission Regulation (EU) No 231/2012, that is "copper complexes of chlorophylls" (abbreviated to Cu-chlorophylls), to characterise the food additive (E 141(i)).



I Cu-chlorophyll a R = CH_3
II Cu-chlorophyll b R = CHO

Figure 1: General structural formulae of the major colouring principles of Cu-chlorophylls (E 141(i))

According to Commission Regulation (EU) No 231/2012, the principal colouring principles are Cu-chlorophyll a and Cu-chlorophyll b (Figure 1). However, according to recent literature, an important component of Cu-chlorophylls is Cu-pyropheophytin a (Figure 2). According to Roca et al. (2010), who analysed different samples of E 141(i), Cu-pyropheophytin a can be the predominant component. Cu-pyropheophytin a has been used for the standard control of oil adulteration with E 141(i) (Roca et al., 2010; Perez-Galvez et al., 2015; Lian et al., 2015).



Cu-pyropheophytin a

Figure 2: Structural formula of Cu-pyropheophytin a

The colour of the food additive can vary from blue-green to dark green depending on the source material. The product contains other pigments, such as carotenoids, as well as fats and waxes derived from the source material (Commission Regulation (EU) No 231/2012). Cu-chlorophylls (E 141(i)) are insoluble in water and soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed waxes (JECFA, 2006). The Colour Index Number is 75810 (Commission Regulation (EU) No 231/2012).

Synonyms of Cu-chlorophylls are CI Natural Green 3, copper chlorophyll and copper phaeophytin (Commission Regulation (EU) No 231/2012). The Panel noted that CI Natural Green 3 is also indicated as a synonym for chlorophylls (E 140(i)) in Commission Regulation (EU) 231/2012. The Panel considered that use of the same synonyms to identify different food additives should be modified in order to prevent misidentification.

The Panel noted some inconsistencies between the names of the major colouring principles in the European Commission (EC) specifications and JECFA specifications (Table 1). The Panel also noted that the Chemical Abstracts Service (CAS) number indicated in the JECFA specifications (65963-40-8) for "chlorophylls, copper complexes" is not registered in the CAS.

Table 1: Identity of Cu-chlorophylls (E 141(i)) and the major colouring principles according to Commission Regulation (EU) No 231/2012

Name[(a)]	Molecular formula	Molecular weight (g/mol)	Structural formula (Figure 1)	CAS number[(b)]	EC number[(c)] (EINECS)	EC specifications names[(d)]	JECFA specifications names[(e)]	Chemical name[(b)]
Copper chlorophyll a	C55H72CuN4O5	932.75	I	15739-09-0	239-830-5	Copper chlorophyll a	Copper phaeophytin a	Copper, [(2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecenyl (3S,4S,21R)-9-ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-3-phorbinepropanoato(2-)-kN23,kN24,kN25,kN26)]-, (SP-4-2)-
Copper chlorophyll b	C55H70CuN4O6	946.73	II	24111-17-9	246-020-5	Copper chlorophyll b	Copper phaeophytin b	Copper, [(2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecenyl (3S,4S,21R)-9-ethenyl-14-ethyl-13-formyl-21-(methoxycarbonyl)-4,8,18-trimethyl-20-oxo-3-phorbinepropanoato(2-)-kN23,kN24,kN25,kN26)]-, (SP-4-2)-

EINECS, European Inventory of Existing Commercial chemical Substances.
(a): Names as considered by the Panel.
(b): SciFinder, software.
(c): EC inventory (online).
(d): Commission Regulation (EU) No 231/2012.
(e): JECFA (2006).

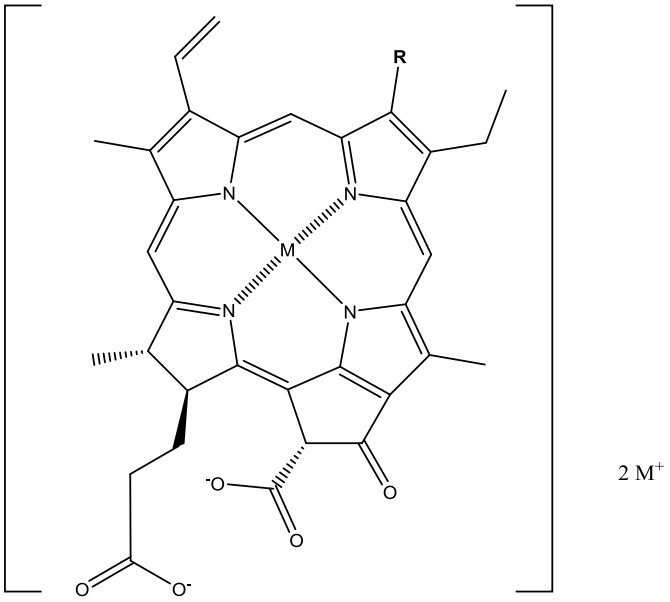
Taking into account the available information from the literature on the analysis of samples of Cu-chlorophylls (Roca et al., 2010), the Panel considered that the composition of the main components of E 141(i)) is unclear and can vary between samples. Therefore, the Panel highlights the need for the adequate identification and characterisation of the components present in the commercial Cu-chlorophylls (E 141(i)).

2.1.2. Cu-chlorophyllins (E 141(ii))

Cu-chlorophyllins (E 141(ii)) are described in Commission Regulation (EU) No 231/2012 as "the alkali salts of copper chlorophyllins are obtained by addition of copper to the product obtained by saponification of a solvent extraction of strains of edible plants material, grass, lucerna and nettle".

The saponification of chlorophylls results in the de-esterification of the methyl and phytyl ester, cleavage of the iso-cyclic ring, as well as side reactions caused by oxidation (catalysed by Cu^{2+} or determined by Cu^{2+} itself), leading to a complex mixture of components (Mortensen and Greppel, 2007).

According to Commission Regulation (EU) No 231/2012, the major colouring principles of Cu-chlorophyllins (E 141(ii)) are described as copper chlorophyllin a and copper chlorophyllin b (Figure 3). However, only copper chlorophyllin a has been identified as a minority component in some studies (Inoue and Yamshita, 1994; Ferruzzi et al., 2002; Scotter et al., 2005).

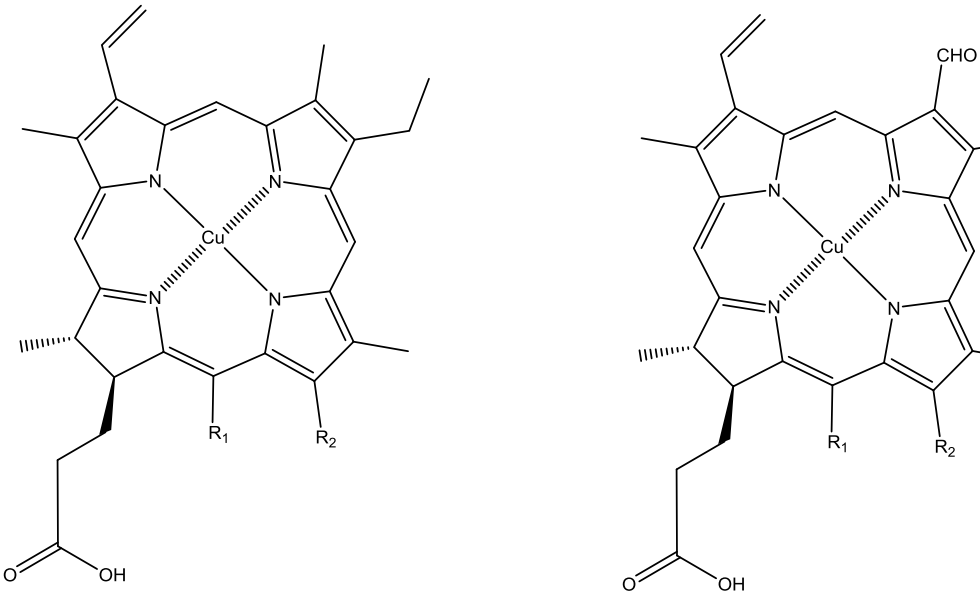


Copper complex of chlorophyllin a R = CH_3
Copper complex of chlorophyllin b R = CHO

Figure 3: Structural formulae of copper complexes of chlorophyllin a and copper complexes of chlorophyllin b (JECFA, 2006)

The Panel noted that the reporting on the main components present in the Cu-chlorophyllins differed widely among the available studies (von Dobeneck, 1953; Inoue and Yamshita, 1994; Yasuda et al., 1995; Ferruzi et al., 2002; Chernomorsky et al., 1997; Egner et al., 2000; Mortensen and Greppel, 2007). Cu-chlorin e6 has been found as a major component in all studies, except in the study by von Dobeneck (1953); all studies have also detected Cu-chlorin e4 or Cu-isochlorin e4 as major components. As reported by Mortensen and Greppel (2007), the identification of Cu-chlorin e4 can be hampered by the presence of Cu-isochlorin e4. Cu-rhodin g7 has been identified as a minor component in some studies. Mortensen and Greppel (2007) analysed five different samples of Cu-chlorophyll and

identified differences in the major components depending on the supplier company. Cu-chlorin e6, Cu-chlorin p6 and Cu-isochlorin e4 were the main identified components and Cu-rhodins were detected in only one sample. Cu-pheophorbide, referred to as Cu-chlorophyllin a in the EC specifications, has been identified as only a minority component in some studies. Besides chlorins, porphyrins have also been detected in some commercial Cu-chlorophyllins (Chernomorsky et al., 1997; Mortensen and Greppel, 2007).



Cu chlorin e_6 R_1 = -CH_2COOH R_2 =-COOH
Cu chlorin p_6 R_1 = -COOH R_2 =-COOH
Cu chlorin e_4 R_1 = -CH_3 R_2 =-COOH
Cu isochlorin e_4 R_1 = -CH2COOH R_2 =-H

Cu rhodin g_7 R_1 = -CH_2COOH R_2 =-COOH

Figure 4: Chemical structures of the copper chlorins and copper rhodin that can be found in Cu-chlorophyllins

Taking into account the available information from the literature on the analysis of samples of Cu-chlorophyllins, the Panel considered that the composition of the main components in the studies on Cu-chlorophyllins (E 141(ii)) is unclear and can vary between samples. Therefore, the Panel highlights the need for the adequate identification and characterisation of the components present in the commercial Cu-chlorophyllins (E 141(ii)).

According to Commission Regulation (EU) No 231/2012, the colour of the food additive can vary from dark green to blue/black. Sodium and potassium salts of Cu-chlorophyllins (E 141(ii)) are soluble in water, very slightly soluble in lower alcohols and ketones and diethyl ether and insoluble in chloroalkanes, hydrocarbons and fixed oils (JECFA, 2006).

2.2. Specifications

2.2.1. Cu-chlorophylls (E 141(i))

Specifications for Cu-chlorophylls (E 141(i)) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006) (Table 2).

Table 2: Specification for Cu-chlorophylls (E 141(i)) according to the Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Assay	Content of total copper chlorophylls is not less than 10 % in mass	Not less than 10 % of total copper phaeophytins
Solvent residues		
Acetone	≤ 50 mg/kg, singly or in combination	≤ 50 mg/kg, singly or in combination
Methanol		
Ethanol		
Propan-2-ol		
Hexane		
Methyl ethyl ketone		–
Dichloromethane	≤ 10 mg/kg	≤ 10 mg/kg
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 2 mg/kg	≤ 5 mg/kg
Mercury	≤ 1 mg/kg	
Cadmium	≤ 1 mg/kg	–
Copper ions	Not more than 200 mg/kg	Not more than 200 mg/kg[(a)]
Total copper	Not more than 8.0 % of the total copper phaeophytins	Not more than 8.0 % of the total copper phaeophytins

(a): As free ionisable copper.

The Panel noted that, according to the Natural Food Colours Association (NATCOL, 2011b), "*Grass and alfalfa grown for the production of E 140(i) by NATCOL members is not treated with any pesticides during the growing season. Products derived from E 140(i) have been analysed for pesticide residues and none were detected (i.e. below the level of determination of 0.02 mg/kg). Spinach may be treated with pesticides and material used for extraction is purchased as food grade with residual pesticide limits in accordance with current regulations. Nettles are not treated with pesticides*".

The Panel noted that, according to the EC specifications for Cu-chlorophylls, impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to concentrations of 3, 2, 1 and 1 mg/kg, respectively. Contamination at these levels would have a significant impact on the intake of these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA, 2009; EFSA CONTAM Panel, 2009, 2010, 2012). The Panel considered that the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for Cu-chlorophylls (E 141(i)) should be revised to ensure that Cu-chlorophylls (E 141(i)) as food additives will not be a significant source of exposure to these toxic elements in foods.

In addition, the Panel noted that in the EC specifications for Cu-chlorophylls there are limits for copper ions (not more than 200 mg/kg) and for total copper (not more than 8 % of the total copper phaeophytins). Considering the molecular weight of Cu-phaeophytin a and Cu-phaeophytin b coordinated with copper, on a stoichiometric basis, total copper should represent up to 6.7–6.8 % of the molecular weight. The Panel noted that the difference on the excess of copper content of 1.2–1.3 % would theoretically amount to 12–13 g copper/kg, which seems to be inconsistent with the maximum level of presumably free copper ions (200 mg/kg) allowed by the EC specification.

Based on the origin of the food additive E 141(i), the Panel noted that data on pesticides, mycotoxins and other components with biological activity (e.g. phyto-oestrogens, phytotoxins and allergens), possibly present in the food additive as used, are relevant for the specifications. The Panel noted that the specifications should be updated to include the information on the non-chlorophyll components of

E 141(i), which may represent up to 90 % of the extract. In addition, based on the available information from the literature on the analysis of samples of Cu-chlorophylls (section 2.1.1), the Panel considered that the composition of the main components in Cu-chlorophylls (E 141(i)) is unclear and an adequate identification and characterisation of the colouring principles that are present in the commercial food additive is needed in order to update the EC specifications for Cu-chlorophylls (E 141(i)).

According to NATCOL (2011b), E 141(i) is not used to produce an aluminium lake.

2.2.2. Cu-chlorophyllins (E 141(ii))

Specifications for Cu-chlorophyllins (E 141(ii)) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2008) (Table 3).

Table 3: Specifications for Cu-chlorophyllins (E 141(ii)) according to Commission Regulation (EU) No 231/2012 and JECFA (2008)

	Commission Regulation (EU) No 231/2012	**JECFA (2008)**
Assay	Content of total copper chlorophyllins is not less than 95 % of the sample dried at 100 °C for 1 hour	Not less than 95 % total copper chlorophyllins after drying (100 °C, 1 hour)
Solvent residues		
Acetone	≤ 50 mg/kg, singly or in combination	≤ 50 mg/kg, singly or in combination
Methanol		
Ethanol		
Propan-2-ol		
Hexane		
Methyl ethyl ketone		–
Dichloromethane	≤ 10 mg/kg	≤ 10 mg/kg
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 5 mg/kg	≤ 5 mg/kg
Mercury	≤ 1 mg/kg	
Cadmium	≤ 1 mg/kg	–
Copper ions	Not more than 200 mg/kg	Not more than 200 mg/kg [(a)]
Total copper	Not more than 8.0 % of the total copper chlorophyllins	Not more than 8.0 % of the total copper phaeophytins [(b)]

(a): As free ionisable copper.
(b): As reported by JECFA (2008).

According to Commission Regulation (EU) No 231/2012, the aluminium lake of Cu-chlorophyllins (E 141(ii)) may be used. NATCOL (2011b) informed the Panel that E 141(ii) can be transformed into the corresponding aluminium lake.

According to Commission Regulation (EU) No 231/2012, the above purity criteria also apply to the raw material from which the aluminium lake is produced. In addition, under neutral conditions, the aluminium lake should contain no more than 0.5 % HCl-insoluble material and no more than 0.2 % ether-extractable material. There are no additional specification requirements for the aluminium lake.

The Panel noted that, according to the EU specifications for Cu-chlorophyllins, impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to concentrations of 3, 5, 1 and 1 mg/kg, respectively. Contamination at these levels would have a significant impact on the intake of these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA, 2009; EFSA CONTAM Panel, 2009, 2010, 2012). The Panel considered that the

maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for Cu-chlorophyllins (E 141(ii)) should be revised to ensure that Cu-chlorophyllins (E 141(ii)) as food additives will not be a significant source of exposure to these toxic elements in foods.

In addition, the Panel noted that in the EC specifications for Cu-chlorophyllins there are limits for copper ions (not more than 200 mg/kg) and for total copper (not more than 8 % of the total copper chlorophyllins). Considering the molecular weight of the Cu-chlorophyllin a and Cu-chlorophyllin b coordinated with copper, on a stoichiometric basis, total copper should represent some 9.7–9.9 % of the molecular weight. If Cu-chlorins are present, the relative amount of copper would be higher, as the molecular weights of Cu-chlorins are lower than those of Cu-chlorophyllin a and Cu-chlorophyllin b. The Panel noted that, on the whole, this would be inconsistent with a total copper content of 8 % as indicated by the EC specifications.

Based on the origin of the food additive E 141(ii), the Panel noted that data on pesticides, mycotoxins and other components with biological activity (e.g. phyto-oestrogens, phytotoxins and allergens), possibly present in the food additive as used, are relevant for the specifications.

According to information available from the literature on the analysis of samples of Cu-chlorophyllins (section 2.1.2), the Panel considered that the composition of the main components in Cu-chlorophyllins (E 141(ii)) is unclear and an adequate identification and characterisation of the colouring principles that are present in the commercial food additive is needed in order to update the EC specifications for Cu-chlorophyllins (E 141(ii)).

2.3. Manufacturing process

2.3.1. Cu-chlorophylls (E 141(i))

Cu-chlorophylls are obtained by addition of a salt of copper to the substance obtained by solvent extraction of natural strains of edible plant material, grass, lucerne and nettle. Addition of copper salts at < 1 000 mg Cu/kg stabilises the chlorophylls in the extract, whereas addition of levels > 1 000 mg Cu/kg affects the (partial) replacement of magnesium originally present in the porphyrin-type complex molecule of chlorophylls.

According to NATCOL (2011b), "*The copper ion in copper chlorophyll is tightly bound and not released in acidic conditions. Thus the copper derivatives of chlorophyll are more widely used in acidic aqueous conditions where a leaf green shade is required. E 141(i) is manufactured from the same extract as is used to manufacture chlorophylls E 140(i)*".

The product, from which the solvent has been removed, contains other pigments, such as carotenoids, as well as fats and waxes derived from the source material. The principal colouring matters are the copper phaeophytins. According to Commission Regulation (EU) No 231/2012, only the following solvents may be used for extraction: acetone, methyl ethyl ketone, dichloromethane, carbon dioxide, methanol, ethanol, propan-2-ol and hexane.

2.3.2. Cu-chlorophyllins (E 141(ii))

According to Commission Regulation (EU) No 231/2012, the alkali salts of Cu-chlorophyllins are obtained by addition of inorganic copper salts to the product obtained by the saponification of a solvent extract of natural strains of edible plant material, grass, lucerne and nettle. The saponification removes the methyl and phytol ester groups and may partially cleave the cyclopentenyl ring. After addition of inorganic salts of copper to the purified chlorophyllins, the acid groups are neutralised to form the salts of potassium and/or sodium. Only the following solvents may be used for the extraction: acetone, methyl ethyl ketone, dichloromethane, carbon dioxide methanol, ethanol, propan-2-ol and hexane.

The Panel noted that the test material in the available literature relating to Cu-chlorophyllins is sometimes referred to as the sodium or potassium salt of the Cu-chlorophyllins. Considering the description of the manufacturing process (Commission Regulation (EU) No 231/2012), the commercial food additive E 141(ii) should be the corresponding potassium or sodium salt. Therefore, the Panel used the general term Cu-chlorophyllins to refer to the food additive used as a test material in the different studies reported in this document.

2.4. Methods of analysis in foods

2.4.1. Cu-chlorophylls (E 141(i))

The methods of analysis of Cu-chlorophylls and Cu-chlorophyllins in food are limited, being based mainly on chromatographic techniques and capillary electrophoresis. The existing methods to detect Cu-chlorophylls in oils, such as fluorescence and liquid chromatography–mass spectrometry (LC–MS), are time-consuming and costly. Roca et al. (2010) used high-performance liquid chromatography (HPLC)–diode array detection (DAD) for the detection of Cu-chlorophyll in adulterated olive oil.

Lian et al. (2015) reported a method for the rapid detection of copper chlorophyll in vegetable oils based on surface-enhanced Raman spectroscopy. This method presented the spectroscopic markers of Cu-chlorophylls and demonstrated a detection limit of 5 mg/kg.

A new probe for tracking the presence of E 141(i) in olive oil samples has also been developed based on the use of UHPLC (ultra-high-performance liquid chromatography)/atmospheric pressure chemical ionisation–time of flight MS for the characterisation of Cu-pyropheophytin a, which was found to be the main chlorophyllic derivative present in E 141(i). This technique enhances the possibility of detection of this compound, even at very low concentrations (Pérez-Gálvez et al., 2015).

2.4.2. Cu-chlorophyllins (E 141(ii))

Del Giovine and Fabietti (2005) developed a technique based on laser fluorescence detector capillary electrophoresis for the identification of Cu-chlorophylls in olive oils, which is comparable to HPLC methods. The extraction technique consisted of passing the oil onto a serum protein electrophoresis–LC–silicon cartridge, allowing further extraction of fat-free pigments for the electrophoretic separation. The Panel noted that the compound identified by these authors is not Cu-chlorophyll, as is stated in the paper, but Cu-chlorophyllin, which is used as a standard for identification and quantification.

The analysis of Cu-chlorophyllins can be carried out with HPLC using DAD and MS. The results of HPLC–MS analysis of five commercial samples of Cu-chlorophyllins have been reported. A C30-column was used to separate the chlorophylls derivatives, which were characterised using absorption spectroscopy and MS. MS enabled the distinction of coppered and uncoppered compounds. The three largest peaks in the samples used for the analysis were identified as Cu-chlorin e_6, Cu-chlorin p_6 and Cu-isochlorin e_4 (Mortensen and Geppel, 2007).

Gandul-Rojas et al. (2012) detected Cu-chlorophyllins (E 141(ii)) in adulterated green table olives with a method consisting of a fatty matter extraction with hexane from the homogenate of olive samples, and the fat-free pigment separation after several steps in a liquid water-free solution, concentrated, dissolved in acetone and aliquots redissolved in deionised water followed by an analysis with HPLC–DAD. The profiles of the peaks found corresponded to Cu-chlorin-type structures that have been previously reported by other authors as Cu-chlorin e_6 and Cu-isochlorin e_4, (Chernomorsky et al., 1997; Mortensen and Geppel, 2007) or Cu-chlorin e_4 (Inoue and Yamshita, 1994; Scotter et al., 2005).

According to Scotter et al. (2005), Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) can be analysed in some foods by spectrophotometry following a method modified from the reports by Amakawa et al. (1993) and Chernomorsky et al. (1997). The procedure has been partially validated

(single laboratory) for the following matrix: ice cream, mint sauce/jelly, soft drinks, fruit preserve, gelatine confectionery and sugar confectionery. Methods based on HPLC–photodiode array and HPLC–LC–MS could be considered for use as a basis for any future development and validation since they appear to offer adequate selectivity and sensitivity for the detection and quantitation of the main chlorophyll/copper chlorophyll analogues, and can also be used for the identification and measurement of degradation products. Fluorescence detection provides a very useful way to distinguish between coppered and non-coppered chlorophyll/chlorophyllin analogues (Scotter, 2011).

2.5. Reaction and fate in foods

Pentilla et al. (1996) studied the bleaching of sodium–copper chlorophyllins in aqueous solution to analyse the role of active oxygen intermediates in the bleaching process in light and dark under aerobic and anaerobic conditions, at 24 °C for 30 hours. It was found that the aerobic photobleaching and dark bleaching are peroxidative processes that do not involve singlet oxygen, superoxide or the OH radical and that both processes could be prevented by reductants such as ascorbate and cysteine.

The temperature sensitivity of a commercial-grade sodium–copper chlorophyllin with a purity of 47.8 % (which does not meet the EC specifications) based on a 4.5 % copper content was studied to assess the degradation kinetics by ultraviolet (UV)–visible spectrophotometry and HPLC, with test temperatures ranging from 25 °C to 100 °C. As Cu(II)-chlorin e_4 was the major component, the loss of this compound resulted in a linear relationship in the thermal degradation curves and hence temperature-dependent first-reaction kinetics at all temperatures. A visible olive-brown discoloration was also observed, suggesting the possible presence of degradation compounds that may include copper-free porphyrins or cleavage compounds, as these occur during degradation of natural chlorophylls. However, oxygen was not excluded from the experimental solutions prior to the thermal treatments and therefore the presence of an oxidative component in the system cannot be excluded (Ferruzzi and Schwartz, 2005).

The antioxidant activity of chlorophyll derivatives such as Cu-chlorophyllins has been investigated. Lanfer-Marquez et al. (2005) reported that the activity of commercial sodium–copper chlorophyllin was higher than that of natural chlorophylls, showing the importance of the presence of the chelated metal in the porphyrin ring. However, when excited by a red light source, Cu-chlorophyllins may exhibit pro-oxidant activity, and therefore Tumolo and Lanfer-Marquez (2012) suggested that the pro-oxidant activity of this compound should also be investigated not only for its possible *in vivo* effects, but also for its negative effects on food products, as certain compounds present in foods together with Cu-chlorophyllins, such as lipids, could be easily oxidised under certain light conditions.

According to NATCOL (2011b), Cu-chlorophyllins (E 141(ii)) are stable when stored at ambient temperature (c. 15 °C). Samples of E 141(ii) lost only 2.2 % of their colour content after 12 months of storage and 3.1 % after 24 months.

2.6. Case of needs and proposed uses

Maximum permitted levels (MPLs) of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are defined in Annex II to Regulation (EC) No 1333/2008[12] on food additives for use in foods (Table 4). Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised food additives in the EU at *quantum satis* (QS) in 58 food categories. Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141 (ii)) are included in Group II (food colours authorised at QS).

According to Annex II to Regulation (EC) No 1333/2008 (part A, Table 3), Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are colours which may be used in the form of lakes.

[12] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

Table 4: MPLs of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in foods according to Annex II to Regulation (EC) No 1333/2008

FCS category number	FCS food category	E number/ group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Group II		*Quantum satis*
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Group II	Except unflavoured products	*Quantum satis*
01.6.3	Other creams	Group II	Only flavoured creams	*Quantum satis*
01.7.1	Unripened cheese excluding products falling in category 16	Group II	Only flavoured unripened cheese	*Quantum satis*
01.7.2	Ripened cheese	E 141	Only *sage Derby cheese, green and red pesto cheese, wasabi, cheese and green marbled herb cheese*	*Quantum satis*
01.7.3	Edible cheese rind	Group II		*Quantum satis*
01.7.4	Whey cheese	Group II		*Quantum satis*
01.7.5	Processed cheese	Group II	Only flavoured processed cheese	*Quantum satis*
01.7.6	Cheese products (excluding products falling in category 16)	Group II	Only flavoured unripened products	*Quantum satis*
01.8	Dairy analogues, including beverage whiteners	Group II		*Quantum satis*
03	Edible ices	Group II		*Quantum satis*
04.2.1	Dried fruit and vegetables	E 141	Only preserves of red fruit	*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 141	Only preserves of red fruit	*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 141	Only vegetables (excluding olives)	*Quantum satis*
04.2.3	Canned or bottled fruit and vegetables	E 141	Only preserves of red fruit	*Quantum satis*
04.2.4.1	Fruit and vegetable preparations excluding compote	Group II	Only *mostarda di frutta*	*Quantum satis*
04.2.4.1	Fruit and vegetable preparations, excluding compote	E 141	Only preserves of red fruit	*Quantum satis*
04.2.4.1	Fruit and vegetable preparations, excluding compote	E 141	Only seaweed-based fish roe analogues	*Quantum satis*
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EEC	E 141	Except chestnut purée	*Quantum satis*
04.2.5.3	Other similar fruit or vegetable spreads	Group II	Except *crème de pruneaux*	*Quantum satis*
05.2	Other confectionery, including breath freshening microsweets	Group II		*Quantum satis*
05.3	Chewing gum	Group II		*Quantum satis*
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	Group II		*Quantum satis*

FCS category number	FCS food category	E number/ group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
06.3	Breakfast cereals	Group II	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	*Quantum satis*
06.5	Noodles	group II		*Quantum satis*
06.6	Batters	Group II		*Quantum satis*
06.7	Pre-cooked or processed cereals	Group II		*Quantum satis*
07.2	Fine bakery wares	Group II		*Quantum satis*
08.3.3	Casings and coatings and decorations for meat	Group II	Except edible external coating of *pasturmas*	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	Group II	Only surimi and similar products and salmon substitutes.	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 141	Only fish paste and crustacean paste	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 141	Only precooked crustacean	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 141	Only smoked fish	*Quantum satis*
09.3	Fish roe	Group II	Except sturgeons' eggs (caviar)	*Quantum satis*
12.2.2	Seasonings and condiments	Group II	Only seasonings, for example curry powder, tandoori	*Quantum satis*
12.4	Mustard	Group II		*Quantum satis*
12.5	Soups and broths	Group II		*Quantum satis*
12.6	Sauces	Group II	Excluding tomato-based sauces	*Quantum satis*
12.7	Salads and savoury-based sandwich spreads	Group II		*Quantum satis*
12.9	Protein products, excluding products covered in category 01.8	Group II		*Quantum satis*
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group II		*Quantum satis*
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group II		*Quantum satis*
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009	Group II		*Quantum satis*
14.1.4	Flavoured drinks	Group II	Excluding chocolate milk and malt products	*Quantum satis*

FCS category number	FCS food category	E number/ group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
14.2.3	Cider and perry	Group II	Excluding *cidre bouché*	*Quantum satis*
14.2.4	Fruit wine and made wine	Group II	Excluding *wino owocowe markowe*	*Quantum satis*
14.2.5	Mead	Group II		*Quantum satis*
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group II	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1–14 to Regulation No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	*Quantum satis*
14.2.7.2	Aromatised wine-based drinks	Group II	Except *bitter soda, sangria, claria, zurra*	*Quantum satis*
14.2.7.3	Aromatised wine-product cocktails	Group II		*Quantum satis*
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	Group II		*Quantum satis*
15.1	Potato-, cereal-, flour- or starch-based snacks	Group II		*Quantum satis*
15.2	Processed nuts	Group II		*Quantum satis*
16	Desserts excluding products covered in categories 01, 03 and 04	Group II		*Quantum satis*
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group II		*Quantum satis*
17.2	Food supplements supplied in a liquid form	Group II		*Quantum satis*
17.3	Food supplements supplied in a syrup-type or chewable form	Group II		*Quantum satis*

FCS, Food Categorisation System (food nomenclature) presented in the Annex II to Regulation (EC) No 1333/2008.

2.7. Reported use levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in food

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. For those additives for which no MPL is set and which are authorised as QS, information on actual use levels is required for performing an exposure assessment.

In 2006, EFSA launched a public call[13] for scientific data on food colours, including Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), to support the re-evaluation of all food colours

[13] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

authorised under the EU legislation. Among other information, the former EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) was seeking data on present use and use patterns (i.e. which food categories and subcategories, proportion of food within categories/subcategories in which it is used, actual use levels (typical and maximum use levels)), especially for those uses which are limited only by QS. In response to this public call, usage data on Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) were submitted to EFSA by The Confederation of the Food and Drink Industries of the EU (CIAA, currently FoodDrinkEurope (FDE)) (CIAA, 2009) and Tennant (2007).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010[14] setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC), EFSA launched a public call[15] for food additives usage level and/or concentration data in food and beverages. Data on Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), including present use and use patterns (i.e. which food categories and subcategories contain the additive, proportion of foods within categories/subcategories in which it is used and actual use levels (typical and maximum)), were requested from relevant stakeholders. European food manufacturers, national food authorities, research institutions, academics, food business operators and any other interested stakeholders were invited to submit usage and/or concentration data on Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in foods. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010).

In response to this public call, updated information on the actual use levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in food has been submitted by industry. No analytical data have been provided for Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)).

2.7.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 211) of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in foods for 43 out of the 58 food categories in which Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised.

Updated information on the actual use levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in foods was made available to EFSA by NATCOL, FDE, the International Chewing Gum Association (ICGA), Capsugel and the Association of the European Self-Medication Industry (AESGP).

Appendix A provides data on the use levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) in foods as reported by industry.

2.8. Information on existing authorisations and evaluations

Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised as food additives in the EU in accordance with Annex II to Regulation (EC) No 1333/2008[16]. Both are permitted at QS in all foodstuffs except those in which the use of colours is prohibited or restricted to food colours other than Cu-chlorophylls and Cu-chlorophyllins (94/36/EC).

[14] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010.

[15] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

[16] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

Cu-chlorophyllins (E 141(ii)) have been previously evaluated by the JECFA in 1969 and 1974 (JECFA, 1970, 1975). In 1975, the SCF evaluated Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) (SCF, 1975).

Based on a No Observed Adverse Effect Level (NOAEL) of 1 500 mg Cu-chlorophyllins/kg bw/day derived from the long-term and reproduction study by Harrisson et al. (1954), JECFA (1975) set a temporary Acceptable Daily Intake (ADI) of 0–15 mg/kg bw/day to Cu-chlorophyllins.

On the other hand, SCF (1975) allocated a group ADI of 15 mg/kg bw/day to the sum of both Cu-chlorophylls and Cu-chlorophyllins complexes. It is not clear which studies the ADI of Cu-chlorophylls has been based on, as virtually no data on this compound have been described or appear to be available. In addition to these issues, SCF stated that Cu-chlorophylls and Cu-chlorophyllins are two distinct food colours and recommended separate listing of the two colours.

Based on the same NOAEL of 1 500 mg/kg/day identified from the Harrisson et al. (1954) study, and applying a safety factor of 200, the US FDA allocated an ADI of 7.5 mg/kg bw/day for sodium–copper chlorophyllin (FDA, 2002). The US FDA has recommended that Cu-chlorophyllins can be taken orally as a deodorant, in doses up to 300 mg/day/person (FDA, 1990).

In 2000, TemaNord also reviewed Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) (TemaNord, 2002). They concluded that the results of the study by Nelson (1992) on the tumour-promoting effect of Cu-chlorophyllin warranted further assessment of the safety of this group of compounds. In addition, data on biotransformation, mainly tissue levels of copper, and the reproductive effects are warranted.

2.9. Exposure

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (Guidance of EFSA "Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment" (EFSA, 2011a)). The EFSA Comprehensive Database has been recently updated[17].

The food consumption data gathered by EFSA were collected using different methodologies, and thus direct country-to-country comparison should be made with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced by subjects' possible underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

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 calculated for only those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011a). Thus, for the present assessment, food consumption data were available from 36 different dietary surveys carried out in 20 European countries as outlined in Table 5.

[17] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Table 5: Population groups considered for the exposure estimates of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii))

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

(a): The terms "children" and "the elderly" correspond, respectively, to "other children" and the merge of "elderly" and "very elderly" in the Guidance of EFSA on the "Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment" (EFSA, 2011a).

Consumption records were codified according to the FoodEx food classification system (EFSA, 2011b). Nomenclature from the FoodEx food classification system has been linked to the FCS as presented in Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories and the exposure was calculated by multiplying values reported in Appendices B for each food category by their respective consumption amount per kilogram of body weight separately for each individual in the database. The exposure per food category was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of surveys days, resulting in an individual average exposure per day for the survey period. This was done for all individuals in the survey and per age group, resulting in distributions of individual average exposure per survey and population group. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per population group.

2.9.1.2. Food categories selected for the exposure assessment of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii))

The food categories in which the use of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories were not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This may have resulted in an underestimation of the exposure. The food categories that were not taken into account are described below (in ascending order of the FCS code):

- 01.6.3. Other creams, only flavoured creams
- 01.7.3. Edible cheese rind
- 01.7.2. Ripened cheese, only sage Derby cheese, green and red pesto cheese, wasabi, cheese and green marbled herb cheese
- 01.7.6. Cheese products (excluding products falling in category 16), only flavoured unripened products
- 04.2.4.1. Fruit and vegetable preparations excluding compote, only *mostarda di frutta*
- 05.4. Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4
- 06.6. Batters

- 06.7. Pre-cooked or processed cereals
- 08.3.3. Casings and coatings and decorations for meat, except edible external coating of *pasturmas*
- 14.2.4. Fruit wine and made wine
- 14.2.5. Mead

These food categories could be country-specific products (*mostarda di frutta*) or could be included in other food categories taken into account in the EFSA Comprehensive database (edible cheese rind with the ripened cheeses) or should represent minor food consumption amounts (batters, mead, etc.).

For the following food categories, the restrictions which apply to the use of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141 (ii)) could not be taken into account, and therefore the whole food category was considered for the exposure estimates. This results in an overestimation of the exposure:

- 04.2.5.3. Other similar fruit or vegetable spreads, except *crème de pruneaux*: *crème de pruneaux* is not referenced in the FoodEx classification nomenclature
- 06.3. Breakfast cereals, only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals: it was not possible within the FoodEx food classification to differentiate extruded or puffed or fruit-flavoured breakfast cereals, therefore whole food category was taken into consideration
- 09.3. Fish roe, except sturgeons' eggs (caviar): this exception could not be taken into account in the present exposure assessment, as no distinction is made in the FoodEx nomenclature between sturgeons' eggs and other fish eggs. Therefore, the whole food category was taken into account
- 14.2.3. Cider and perry, excluding *cidre bouché*: no distinction was possible between cider and cidre bouché; therefore, the entire food category was accounted for in the exposure estimates
- 17.1./17.2./17.3. Food supplements: it was not possible to differentiate solid, liquid or syrup-type, or chewable forms of food supplements within FoodEx codes

Food categories for which no reported use levels were available were not considered in the exposure assessment. This concerns 11 food categories, which are presented in Appendix C. The Panel noted that if Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are nevertheless used in those food categories for which reported use levels were not available, the calculated refined exposure assessment might result in underestimation of exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)).

Overall, in the current exposure estimate, 11 food categories out of 58 were not taken into account in the exposure assessment because these are not referenced in the EFSA Comprehensive Database, and 11 food categories were not included in the exposure assessment because of the lack of data. Thus, in the current exposure estimate, 22 food categories out of 58 are not taken into account.

2.9.2. Exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) from their use as food additives

Dietary exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) from their use as food colours was estimated using the approach adopted by the Panel at its 52nd plenary meeting[18]. This approach is to be followed to assess the exposure as part of the safety assessment of food additives under re-evaluation with the use of the food consumption data available within the EFSA Comprehensive Database, as presented in Table 4, and with the limitations described above. Exposure assessment to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the *maximum level*

[18] http://www.efsa.europa.eu/en/events/event/140701a-m.pdf

exposure assessment scenario) and (2) reported use levels (defined as the *refined exposure assessment* scenario) as provided by industry.

2.9.2.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation No 1333/2008 and listed in Table 2. As no MPLs are set for Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), a *maximum level exposure assessment* scenario has been performed based on the maximum levels as provided to EFSA.

The exposure estimates derived following this scenario should be considered as the most conservative since it assumes that the consumer will be continuously (over a lifetime) exposed to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) present in the food at the maximum levels.

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on information on reported use levels by industry. This exposure scenario can consider only food categories for which the above data were made available to the Panel.

Appendix C summarises the concentration levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) used in the refined exposure assessment scenario. Based on the available dataset, the Panel calculated two estimates based on different model populations:

- The brand-loyal consumer scenario: This assumes that a consumer is exposed long term to the food additive present at the maximum reported use for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the maximum reported use levels for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: This assumes that a consumer is exposed long-term to the food additive present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

2.9.2.3. Anticipated exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii))

Table 6 summarises the anticipated exposure estimates to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) from their use as food additives of all five population groups (Table 5). Detailed results by population group and survey are presented in Appendix C.

Table 6: Summary of anticipated exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) from their use as food additives using the maximum level exposure assessment scenario and refined exposure scenarios, in five population groups (minimum–maximum across the dietary surveys in mg/kg bw/day)

	Toddlers (12–35 months)	**Children (3–9 years)**	**Adolescents (10–17 years)**	**Adults (18–64 years)**	**The elderly (≥ 65 years)**
Maximum level exposure assessment scenario					
Mean	3.1–12.5	4.9–11.0	2.2–6.6	0.8–4.0	0.9–3.6
High level (95th percentile)	7.0–23.9	9.8–26.4	5.8–17.9	2.8–12.5	2.5–8.5
Refined estimated exposure assessment scenario					
Brand-loyal scenario					
Mean	2.3–6.5	2.6–7.2	1.5–4.3	0.6–2.9	0.6–2.6
High level (95th percentile)	5.2–16.5	5.8–24.2	3.8–17.4	2.0–9.3	1.7–6.5
Non-brand-loyal scenario					
Mean	0.6–1.6	0.6–1.4	0.3–0.8	0.1–0.7	0.1–0.9
High level (95th percentile)	1.9–3.3	1.2–3.1	0.7–1.9	0.3–2.1	0.3–2.3

2.9.3. Main food categories contributing to exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) using the maximum level exposure assessment scenario

Table 7: Main food categories contributing to exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) using maximum usage levels (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS food category	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]				
01.7.1	Unripened cheese excluding products falling in category 16	5.0–16.1 (4)	5.6–8.6 (2)	10.2 (1)	5.7–15.3 (3)	6.5–12.3 (4)
01.7.5	Processed cheese	9.4 (1)	–	–	–	
03	Edible ices	5.1–9.5 (3)	5.6–10.0 (11)	6.4–10.1 (6)	5.6–7.8 (3)	6.1–7.8 (2)
04.2	Processed fruit and vegetables	6.4 (1)	–	–	–	6.7 (1)
05.2	Other confectionery, including breath freshening microsweets	6.9–24.3 (5)	5.7–68.6 (15)	5.2–80.7 (13)	5.1–32.3 (8)	5.4–20.8 (4)
06.3	Breakfast cereals	7.2–60.0 (6)	5.0–11.6 (10)	5.1–11.8 (5)	5.8–20.9 (6)	6.1–38.1 (7)
07.2	Fine bakery wares	6.1–74.0 (10)	11.0–71.9 (17)	9.7–64.8 (16)	16.5–65.8 (17)	19.3–61.7 (14)
12.5	Soups and broths	5.0–20.1 (5)	5.2–21.6 (5)	5.4–21.5 (6)	6.6–27.6 (8)	8.7–31.6 (8)
12.6	Sauces	5.5–6.0 (2)	5.6–7.7 (3)	5.7–10.0 (6)	5.7–11.1 (9)	5.7–8.5 (8)
12.7	Salads and savoury-based sandwich spreads	–	5.8 (1)	7.5 (1)	6.0–13.3 (3)	7.9–10.6 (2)
14.1.4	Flavoured drinks	5.1–13.1 (5)	5.0–15.1 (11)	5.2–20.9 (12)	5.2–24.2 (10)	6.1–13.4 (3)
15.1	Potato-, cereal-, flour- or starch-based snacks	5.4–15.8 (5)	6.0–13.6 (11)	5.5–16.0 (12)	5.0–23.4 (10)	5.3–8.4 (2)
16	Desserts excluding products covered in category 1, 3 and 4	5.1–17.1 (6)	5.5–10.9 (6)	6.1–6.2 (2)	5.6–6.6 (2)	5.0–9.9 (4)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

2.9.4. Main food categories contributing to exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) using the refined exposure assessment scenarios

Table 8: Main food categories contributing to exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) using the brand-loyal refined exposure scenario (> 5 % to the total mean exposure) and number of surveys to which each food category is contributing

FCS category number	FCS food category	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]				
01.7.1	Unripened cheese excluding products falling in category 16	5.8–15.2 (2)	–	–	6.7–9.4 (2)	5.4–8.8 (3)
01.7.5	Processed cheese	7.7 (1)	–	–	–	–
03	Edible ices	8.2 (1)	5.3 (1)	6.1–7.2 (2)	5.1–5.2 (2)	5.1 (1)

FCS category number	FCS food category	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]				
05.2	Other confectionery including breath freshening microsweets	6.0–31.5 (4)	5.5–83.6 (12)	7.6–91.7 (10)	5.3–44.9 (9)	5.0–26.3 (5)
06.3	Breakfast cereals	8.3–78.1 (6)	6.1–14.1 (7)	8.7–11.2 (4)	5.5–25.0 (6)	5.7–46.5 (7)
07.2	Fine bakery wares	14.9–85.7 (9)	9.1–88.3 (17)	8.3–86.6 (16)	18.5–85.3 (17)	21.1–78.7 (14)
12.5	Soups and broths	6.2–19.9 (3)	5.2–18.6 (4)	7.4–20.9 (3)	5.7–28.5 (8)	6.3–36.9 (7)
12.6	Sauces	–	–	6.7–7.8 (2)	5.8–8.4 (5)	5.9 (1)
12.7	Salads and savoury-based sandwich spreads	–	–	–	8.6–12.1 (2)	5.0–7.8 (2)
14.1.4	Flavoured drinks	5.8–9.7 (2)	5.8–7.8 (4)	7.4–16.3 (5)	5.0–22.7 (7)	7.9–9.7 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks	5.6–19.3 (3)	5.4–14.2 (3)	5.5–15.5 (9)	5.1–32.5 (4)	6.2–10.4 (2)
16	Desserts excluding products covered in category 1, 3 and 4	5.3–14.3 (4)	6.1–8.9 (2)	–	–	7.2 (1)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	–	–	–	5.7 (1)	–

(a): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

Table 9: Main food categories contributing to exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) using the non-brand-loyal refined exposure scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS food category	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]				
01.4	Flavoured fermented milk products including heat-treated products	5.9–10.9 (4)	5.0–8.5 (5)	7.2 (1)	–	–
01.7.1	Unripened cheese excluding products falling in category 16	5.1–8.1 (2)	6.8 (1)	8.3 (1)	7.6–12.1 (2)	5.0–9.7 (3)
01.7.5	Processed cheese	6.6 (1)		–		–
03	Edible ices	5.9 (1)	5.4–9.4 (5)	5.2–7.2 (3)	5.2–5.2 (2)	–
04.2	Processed fruit and vegetables	–	–		5.0–6.2 (2)	6.4 (1)
05.2	Other confectionery including breath freshening microsweets	–	5.3–20.2 (3)	5.0–38.0 (4)	5.4–6.3 (2)	–
06.3	Breakfast cereals	9.8–84.4 (8)	7.9–47.1 (17)	6.4–43.9 (17)	5.1–59.3 (17)	7.8–75.0 (11)
07.2	Fine bakery wares	7.7–81.1 (9)	9.6–77.8 (17)	9.1–58.0 (16)	11.5–60.1 (17)	9.2–54.9 (14)
09.2	Processed fish and fishery products including molluscs and crustaceans	–	7.7 (1)	–	–	–

FCS category number	FCS food category	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]				
12.2.2	Herbs, spices, seasonings	5.3 (1)	–	5.4 (1)	9.1 (1)	9.2 (1)
12.5	Soups and broths	5.9–23.7 (3)	5.3–23.8 (7)	7.7–23.9 (6)	6.3–28.3 (8)	7.4–34.2 (8)
12.6	Sauces	5.1–9.6 (6)	5.4–13.0 (11)	5.5–17.8 (11)	5.2–17.1 (11)	5.2–15.1 (10)
12.7	Salads and savoury-based sandwich spreads	–	9.2–10.7 (2)	7.1–14.0 (2)	5.0–21.7 (4)	14.7–16.4 (2)
14.1.4	Flavoured drinks	7.6–9.0 (2)	5.1–11.5 (8)	6.0–16.6 (10)	5.2–17.9 (7)	10.8 (1)
15.1	Potato-, cereal-, flour- or starch-based snacks	6.3–6.6 (2)	5.4–6.3 (2)	5.3–6.9 (5)	8.4 (1)	–
15.2	Processed nuts	–	–	–	5.6 (1)	5.7 (1)
16	Desserts excluding products covered in category 1, 3 and 4	12.8–13.0 (2)	6.6–10.4 (3)	6.0 (1)	5.6 (1)	5.6–7.0 (3)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

2.10. Uncertainty analysis

Uncertainties in the exposure assessment of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) have been discussed above. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the sources of uncertainties considered are summarised in Table 10.

Table 10: Qualitative evaluation of influence of uncertainties on the exposure estimate to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii))

Sources of uncertainties	Direction[(a)]
Consumption data: different methodologies/representativeness/under reporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of few days to estimate long-term (chronic) exposure	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which precise types of food the levels refer to	+/–
Uncertainty in possible national differences in use levels of food categories, usage data not fully representative of foods on the EU market	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception	+
Use levels: no data for some food categories (11 out of 58 food categories)	–
Use levels: levels considered applicable for all items within the entire food category	+
Use levels: uncertainty whether the reported use levels provided by industry refer to Cu-chlorophylls (E 141(i)) or Cu-chlorophyllins (E 141(ii))	+
Brand-loyal exposure model: exposure calculations based on the maximum reported use levels for one food category and mean reported use levels for the remaining food categories	+/–
Non-brand-loyal exposure model: exposure calculations based on the mean reported use levels	+/–

(a): +, uncertainty with potential to cause over-estimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Considering the uncertainties identified, the Panel assumed that the exposure assessment would tend to overestimate the real exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) as food additives in European countries.

3. Biological and toxicological data

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and additional literature that has become available since then. No new toxicological or biological information was submitted to the Panel for the re-evaluation of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) following EFSA public calls for data[19,20]. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), which covered the period from the beginning of 2011 up to the end of 2014. A further update has been performed by the Panel.

The Panel noted that the material used in many studies and identified as "chlorophyllin" was often, if not always, Cu-chlorophyllins.

3.1. Absorption, distribution, metabolism and excretion

Limited data on absorption, distribution, metabolism and excretion (ADME) of Cu-chlorophyllins were examined by JECFA (JECFA, 1969). However, various *in vitro* and *in vivo* studies on Cu-chlorophyllins have been published since the JECFA evaluation, most of which have been reviewed by Ferruzzi and Blaskelee (2007).

3.1.1. *In vitro* studies

The stability of Cu-chlorophyllins was investigated in an in vitro digestion model that simulated both gastric and intestinal phases of digestion (Ferruzzi et al., 2002). The commercially available Cu-chlorophyllins used was a mixture of water-soluble Cu-chlorophyll derivatives (sodium salts of Cu-chlorin e4 (81 %), Cu-chlorin e6 (10 %), Cu-rhodin g7 (3 %) and Cu-phaeophorbide a (1 %)). When subjected to digestion for two hours, Cu-chlorin e4 was relatively stable with greater than 70 % unchanged Cu-chlorin e4 recovery in the aqueous fraction. Conversely, the majority of Cu-chlorin e6 in the preparation was lost (degradation products not identified) during in vitro digestion. Incorporation of Cu-chlorophyllins into an applesauce matrix decreased the loss of Cu-chlorin e6, suggesting that an inclusion matrix can stabilise labile Cu-chlorophyllins components during digestion.

3.1.2. Animal studies

3.1.2.1. Mice

Cu-chlorophyllin was tested in female ICR mice for its chemopreventative activity against tumorigenesis induced by benzo[a]pyrene (B[a]P) derivatives (Park and Surh, 1996). In this study, orally administered (by gavage) Cu-chlorophyllin sodium salt (15 mg/kg bw), was rapidly distributed in the skin and other tissues of mice.

[19] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[20] Call for scientific data on selected food additives permitted in the EU. Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B). Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

3.1.2.2. Rats

Reber and Willigan (1954) reported that Sprague–Dawley rats fed a diet containing 1 % Cu-chlorophyllins and born to parents that had received the same diet for more than 15 weeks showed, at autopsy (28 days of age), skeletal muscle of a greenish hue throughout the body, therefore indicating a systemic distribution.

JECFA (1975) and TemaNord (2002) describe a study by Harrisson et al. (1954) on the fate of Cu-chlorophyllins. Sprague–Dawley rats (40 animals/group; 80 in the control group) were fed diets containing 0, 0.1, 1 or 3 % (w/w) Cu-chlorophyllins (equal to 0, 50, 500 or 1 500 mg/kg bw/day) over their lifespan. Dietary Cu-chlorophyllins doses of 500 mg/kg bw/day or greater resulted in transmission across the gastrointestinal membrane and appearance in the plasma. The authors reported that copper also appeared in the plasma, but at only one-sixth of the content expected. As no storage of copper in the organs was noted, copper was assumed by the authors to be firmly complexed with the Cu-chlorophyllins and therefore innocuous. In addition to these findings, the authors stated that 5 % of the copper of Cu-chlorophyllins was in the free ionic state whereas the remaining 95 % was chelated. The complexed copper may physico-chemically block the conversion of Cu-chlorophyllins to other chlorophyll fractions. According to the authors, Cu-chlorophyllins appeared to be excreted in the faeces as an insoluble calcium salt, probably after exchanging sodium and potassium for calcium. However, the Panel noted that there was no direct evidence for this suggestion.

3.1.3. Human studies

Discoloration of urine has occasionally been reported in patients taking Cu-chlorophyllins, suggesting that they are absorbed to some extent. Several case reports have been published indicating that oral Cu-chlorophyllin (100–200 mg/day) decreased urinary and faecal odour in incontinent patients (Chernomorsky and Segelman, 1988).

Evidence that Cu-chlorophyllins components are absorbable by humans was provided by the studies of Egner et al. (2000). As part of a large trial initiated in 1997, in China, 180 subjects consumed a daily dose of 300 mg Cu-chlorophyllins (primarily composed of Cu-chlorin e4, Cu-chlorin e6 and Cu-chlorin e4 ethyl ester) for four months. The authors showed that Cu-chlorophyllins were absorbed into the bloodstream, and conferred a green colour on the sera. After four months of consumption, patients reached a steady state of ≈ 2.0 μg/mL total Cu-chlorophyllins in serum, mainly as a Cu-chlorin e4 ethyl ester, with Cu-chlorin e4 contributing a small proportion and no detectable levels of Cu-chlorin e6. Overall, these studies indicate that certain components of Cu-chlorophyllins (e.g. Cu-chlorin e4 and Cu-chlorin e4 ethyl ester) are absorbed from the gastrointestinal tract. They have been reported to occur in the serum of humans chronically administered large doses of these complexes. In their review on the digestion, absorption and chemopreventative activity of chlorophyll derivatives, Ferruzzi and Blakeslee (2007) suggested that the sensitivity of Cu-chlorin e6 to digestion might be responsible, in part, for its lack of appearance in serum in humans consuming Cu-chlorophyllins complexes.

As no data were available regarding the ADME of Cu-chlorophylls, and considering their differences in purity, chemical properties, stability and manufacturing process, the present data for Cu-chlorophyllins cannot be used for read-across with Cu-chlorophylls (E 141(i)).

3.2. Toxicological data

3.2.1. Acute toxicity

JECFA (1969) described a study by Harrisson et al. (1954) in which the acute oral toxicity of Cu-chlorophyllins was determined in mice. The oral LD_{50} value was set at 7 000 mg/kg bw.

JECFA also reported that no adverse effects were found in various animal species receiving an oral dose of Cu-chlorophyllins. Mice were given 2 500 mg/kg bw/day during over seven days, whereas

guinea pigs, rabbits, cats and a dog were given 1 000 mg/kg bw/day for seven days (Worden et al., 1955).

The Panel noted that the acute oral toxicity of the Cu-chlorophyllins may be low, but this information was considered to be of little relevance for the safety evaluation of this compound as a food additive.

No data were available regarding the acute toxicity of Cu-chlorophylls.

3.2.2. Short-term and subchronic studies

JECFA (1970, 1975) reported several short-term and subchronic studies. In rats fed a diet containing 15 % Cu-chlorophyllins (equal to 7 500 mg/kg bw/day) for 10 days, the only adverse effect reported was weight loss related to food refusal (Harrisson et al., 1954).

In the same study, guinea pigs receiving 0.5 % Cu-chlorophyllins in their drinking water (estimated to be equal to 500 mg/kg bw/day) for 11 weeks exhibited no adverse effects or pathological changes and there was no evidence of scurvy (vitamin C is known to deteriorate rapidly in the presence of copper). Similarly, rats fed a diet containing 3 % Cu-chlorophyllins (equal to 1500 mg/kg bw/day) over their lifetime also showed no evidence of scurvy (Harrisson et al., 1954).

Reber and Willigan (1954) conducted a study in female Sprague–Dawley rats fed for 19 weeks with rations containing 1 % Cu-chlorophyllins, corresponding to a daily intake of 500 mg/kg bw/day Cu-chlorophyllins. The compound did not produce any obvious adverse effects or pathological changes, but treated animals exhibited a statistically significant ($p < 0.01$) increase in average neutrophil count compared with controls, although within the range of standard values.

No adverse effects were found in rats (30 in total) receiving an oral dose of Cu-chlorophyllins (2 000 mg/kg bw/day) packed in gelatin capsules, for 18 weeks (Worden et al., 1955).

In a study performed to test the possibility of prevention of acrylamide toxicity by various dietary supplements (Woo et al., 2007), no signs of toxicity were reported in the group of Sprague–Dawley male rats (five animals, six weeks of age) fed a diet containing 1 % sodium Cu-chlorophyllins (equivalent[21] to 1 200 mg/kg bw/day) for five weeks.

There were no data available on the short-term and subchronic toxicity of Cu-chlorophylls.

3.2.3. Genotoxicity

The JECFA and SCF evaluations (1975) did not describe any genotoxicity studies. However, there are studies on the genotoxic potential of Cu-chlorophyllins that have become available since then.

3.2.3.1. *In vitro* studies

Cu-chlorophyllins did not show any clastogenicity or results in aneuploidy in human lymphocytes at concentrations up to 14 µmol/L in a study in which their effect on the induction of micronuclei by doxorubicin was investigated (Amara-Mokrane et al., 1996).

Cu-chlorophyllins induced a dose-dependent increase in DNA migration in a single-cell gel assay (Comet assay) in human leucocytes *in vitro* (up to 2.7-fold compared with controls). Cu-chlorophyllin was investigated at nine concentrations ranging from 0.1 to 200 µmol/L. The effects were statistically significant at and above 0.5 µmol/L and were observed in three experiments (Frenzilli et al., 2000). According to the authors, none of the effects was accompanied by a reduction in cell viability. The authors suggested that the mechanism of action of these genotoxic effects was probably associated with free radical formation under their experimental conditions.

[21] Calculated by the Panel according to EFSA Scientific Committee (2012).

In a micronucleus test, performed in order to investigate the effects of Cu-chlorophyllins on methyl methanesulphonate (MMS)-induced genotoxicity, V79 cells were treated with Cu-chlorophyllins at concentrations of 0.1375, 0.275 and 0.55 µmol/L for two hours. After treatment, the cells were cultured for 14 hours in the presence of cytochalasin B before collection. Cu-chlorophyllins did not increase the frequency of micronuclei in the binucleated cells and protected the cells from the DNA damage induced by MMS (Bez et al., 2001).

Cu-chlorophyllins was evaluated at concentrations of 6.25, 12.5 and 25 µg/mL with regard to their clastogenic and anti-clastogenic potential in Chinese hamster ovary (CHO) cells in the absence of metabolic activation (Negraes et al., 2004). Cells were exposed for two hours to Cu-chlorophyllins in different phases of the cell cycle (G1/S phase, S-phase and G2/S phase) in the presence or absence of ethyl methane sulphonate (EMS, 1 240 µg/mL). Cu-chlorophyllins were not clastogenic. According to the authors, a protective effect of Cu-chlorophyllins against EMS was found during the G2/S phase (70–80 %), and during the S-phase (25–48 %).

Cu-chlorophyllins was found to potentiate (up to about threefold) the mutagenicity of two tobacco-specific nitrosamines (nicotine-derived nitrosamine ketone (NNK) and *N*-nitrosonornicotine (NNN)) in an Ames mutagenicity assay in *Salmonella typhimurium* TA 100 at low concentrations (< 1 µmol/plate). However, at higher, but still non-toxic concentrations (≥ 1 µmol/plate in the NNK experiment and ≥ 3 µmol/plate in the NNN experiment), Cu-chlorophyllin decreased the mutagenicity of both compounds to a level equal to the spontaneous mutation frequency. The same type of dose–response relationship was observed in a hypoxanthine guanine phosphoribosyltransferase (HPRT) V79 point mutation assay in which Cu-chlorophyllins increased the mutagenicity of *N*-dimethylnitrosamine about 1.5-fold at low concentrations (< 0.05 mmol/L) while a decrease of mutagenicity was observed at higher concentrations (Romert et al., 1992).

In the study by Grossi et al. (2012), the protective effect of Cu-chlorophyllins (25 µM) on the clastogenic activity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and 7,12-dimethylbenz(a)anthracene (DMBA) was evaluated in human hepatoma cells (HepG2) using micronuclei as the endpoint. Cu-chlorophyllins significantly reduced MNNG-induced micronuclei when present during treatment, suggesting a direct interaction with the carcinogen, and DMBA-induced micronuclei when present before or after treatment. However, when Cu-chlorophyllins was assayed alone at dose levels of 2, 10, 25, 50 and 150 µM under the previously reported experimental conditions, no induction of micronuclei was observed. The Panel noted that the dose levels applied were adequate since sufficient and marked cytostasis were observed at dose levels of 50 and 150 µM, respectively.

3.2.3.2. *In vivo* studies

In a study in which the effect of Cu-chlorophyllins on mercuric chloride-induced clastogenicity was investigated in mouse bone marrow, one group of animals was treated with Cu-chlorophyllins without further exposure to mercuric chloride (Ghosh et al. 1991). In this group, a single dose of 1.5 mg/kg bw was administered orally by gavage to five male Swiss albino mice. Distilled water was used as negative control and cyclophosphamide as positive control. The bone marrow was sampled once 24 hours after exposure and 50 metaphases per animal were analysed for chromosomal aberrations and 1 000 cells per animal were scored for the mitotic index. Different types of aberrations were reported. Gaps were recorded separately and the total aberration frequency, excluding gaps, was reported. Cu-chlorophyllins did not increase the frequency of chromosomal aberrations. The mitotic index was reduced compared with controls (reduction was greater than 50 %); however, the same dose of Cu-chlorophyllins in combination with mercuric chloride did not result in a reduction in the mitotic index. The Panel noted that this study deviates from the OECD Guideline 475 with respect to the number of metaphases analysed per animal, the number of doses, the dose level and the sampling time. Overall, the Panel considered the relevance of the study limited.

The effect of Cu-chlorophyllins on nicotine-induced clastogenicity was investigated in mouse bone marrow (Sen et al., 1991). Three groups of male Swiss albino mice were treated with Cu-chlorophyllins without further exposure to nicotine. Each group comprised five mice which were administered a single dose of 0.77, 1.10 or 1.50 mg/kg bw Cu-chlorophyllins orally by gavage. Isotonic saline was used as a negative control and cyclophosphamide as a positive control. The bone marrow was sampled 6, 12, 18 and 24 hours after exposure and 50 metaphases were analysed per animal for chromosomal aberrations and 100 cells per animal were scored for the mitotic index. Different types of aberrations were reported. Gaps were recorded separately and the total aberration frequency was reported excluding gaps. Cu-chlorophyllins did not increase the frequency of chromosomal aberrations. The mitotic index was not reduced compared with controls. The Panel noted that this study deviates from OECD Guideline 475 (1984, 1997) with respect to the number of metaphases analysed per animal (for chromosomal aberrations and for mitotic index), the dose level and the sampling time. Thus, the Panel considered that the reliability of the negative result was limited.

In a study in which the effect of Cu-chlorophyllins on chromium(VI)oxide- and chlordane-induced clastogenicity was investigated in mouse bone marrow, two groups of animals were treated with Cu-chlorophyllins without further exposure to chromium(VI)oxide or chlordane (Sarkar et al. 1993). In these groups, single doses of 1.5 and 3 mg/kg bw were administered orally by gavage to five male Swiss albino mice per group. Distilled water was used as a negative control and mitomycin C injected intraperitoneally served as a positive control. The bone marrow was sampled once 24 hours after exposure and 50 metaphases per animal were analysed for chromosomal aberrations. The mitotic index was not reported. Different types of aberrations were reported. Gaps were recorded separately and the total aberration frequency was reported excluding gaps. While Cu-chlorophyllins did not increase the frequency of chromosomal aberrations at the dose of 1.5 mg/kg bw, a statistically significant increase compared with controls was observed at the dose of 3 mg/kg bw (1.2 ± 0.4, 1.2 ± 0.4 and 3.8 ± 0.8 % aberrant cells in control, low- and high-dose groups, respectively). In addition, all combinations of chlordane with the higher dose of chlorophyllin resulted in frequencies of aberrant cells higher than those induced by chlordane alone or by the higher dose of chlorophyllin alone. According to the authors, this indicates that the action of chlordane was enhanced, although not to a statistically significant extent. The Panel noted that this study deviates from OECD Guideline 475 (1984, 1997) with respect to the number of metaphases analysed per animal, the number of doses and the sampling time. In addition, the results were not reported separately for individual animals. Moreover, since only one of the two doses resulted in a statistically significant increase in the percentage of aberrant cells, the criterion of a potential dose–response relationship cannot be used for the interpretation of this result. The Panel also noted that no historical control data were reported, and thus the biological significance of the increased frequency of aberrations observed at the highest Cu-chlorophyllins dose cannot be evaluated.

In the light of these uncertainties, the Panel did not agree with the authors of the study and concluded that the results reported cannot be considered as a proof of clastogenic activity of Cu-chlorophyllin in mouse bone marrow.

In a study by Sarkar et al. (1996) designed to investigate the effect of Cu-chlorophyllins on induction of chromosomal aberrations by potassium dichromate in bone marrow of mice, groups of five male Swiss albino mice were given aqueous solutions of Cu-chlorophyllins at a dose of 1.5 mg/kg bw, without further exposure to potassium dichromate. Additional groups treated in the same way were further exposed to potassium dichromate. Animals of the negative control group were treated with distilled water. Mitomycin C and potassium dichromate were used as positive controls. Bone marrow was sampled once 24 hours after treatment and 500 metaphases per group were analysed for chromosomal aberrations. Different types of aberrations were reported. Gaps were recorded separately and the total aberration frequency was reported excluding gaps. No increased frequencies of chromosomal aberrations were observed in Cu-chlorophyllins group compared with the negative control group (0.02 chromosomal aberrations per cell, 1.2 % aberrant cells). Cu-chlorophyllins reduced the clastogenic effects of potassium dichromate to the control level.

In a study in which the effect of "chlorophyllin" ("a sodium or copper salt derived from chlorophyllin" and therefore likely to be Cu-chlorophyllins) on the induction of sister chromatid exchanges by benzo[a]pyrene was investigated in bone marrow cells of mice, four groups of five male NIH mice were treated with "chlorophyllin" (dissolved in distilled water) without further exposure to benzo[a]pyrene (Madrigal-Bujaidar et al., 1997). The mice received a single intraperitoneal injection of "chlorophyllin" at a dose of 1, 2, 4 or 8 mg/kg bw (30 minutes after the subcutaneous implantation of a BrdU tablet). At 21 hours after BrdU implantation, colchicine was injected. Bone marrow samples were obtained three hours after colchicine injection and 30 cells per animal were analysed for sister chromatid exchanges. No increases in the frequency of sister chromatid exchanges were observed in these groups when compared with the control group, which received mineral oil (used as solvent for benzo[a]pyrene). The mitotic index was not changed compared with the control.

In a study in which the effect of Cu-chlorophyllins on micronucleus induction by chromium trioxide was investigated in peripheral blood of mice, one group of animals was treated with Cu-chlorophyllins without further exposure to chromium trioxide (Garcia-Rodrigues et al., 2001). In this group, four female mice received a single intraperitoneal injection of Cu-chlorophyllins at a single dose of 20 mg/kg bw. Blood samples were obtained 0, 12 and 48 hours after treatment. The frequencies of micronucleated polychromatic erythrocytes (PCEs) were recorded based on 2 000 cells per mouse and PCEs in total erythrocytes was scored in 1 000 cells per mouse. No increase in the frequency of micronucleated PCEs was observed in this group when compared with the control group. The proportion of immature erythrocytes among total erythrocytes was somewhat reduced compared with the control group; however, the reduction was not statistically significant and, according to the authors, cell toxicity was not observed in any sample. The Panel noted that this study deviates from OECD Guideline 474 with respect to the number of animals, the number of doses and the dose level. Thus, the Panel considered that the reliability of the negative result was limited.

In a study in which the effect of "a water-soluble chlorophyll derivative", purchased from Sigma and therefore likely to be Cu-chlorophyllins) on the induction of micronuclei by sodium nitrite was investigated in bone marrow cells of mice, one group of animals was treated with Cu-chlorophyllins (dissolved in distilled water) without further exposure to benzo[a]pyrene (Diaz-Barriga Arcco et al. 2002). In this group, five male mice (NIH) received a single intraperitoneal injection of Cu-chlorophyllins at a dose of 4 mg/kgbw. Bone marrow samples were obtained 96 hours after treatment. The frequency of micronucleated PCEs and the ratio of polychromatic to normochromatic cells was scored in 1 000 cells per mouse. No increase in the frequency of micronucleated PCEs was observed in this group when compared with the control group. The proportion of immature erythrocytes among total erythrocytes was not changed compared with the control group. The Panel noted that this study deviates from OECD Guideline 474 with respect to the number of doses, the dose level, the number of cells analysed per mouse and the sampling time. Thus, the Panel considered that the reliability of the negative result was limited.

In another study (performed in the same laboratory) in which the effect of "chlorophyllin" (from Sigma Chemical Co. and therefore likely to be Cu-chlorophyllins), on the induction of sister chromatid exchanges by acetaldehyde was investigated in bone marrow cells of mice, three groups of animals were treated with "chlorophyllin" (dissolved in distilled water) without further exposure to acetaldehyde (Torres-Bezauri et al. 2002). In these groups, "chlorophyllin" was administered orally (probably by gavage) to five male mice (NIH) per group at doses of 2, 6 or 10 mg/kg bw. The control animals received distilled water. BrdU adsorbed to activated charcoal was inoculated intraperitoneally 30 minutes after administration of "chlorophyllin" and colchicine was injected subcutaneously 21 hours later. Bone marrow samples were obtained three hours after colchicine injection and 30 cells per animal were analysed for sister chromatid exchanges and 1 000 cells were scored to determine the mitotic index. No increases in the frequency of sister chromatid exchanges were observed in these groups when compared with the control group. The mitotic index did not change compared with the control group.

In the study by Grossi et al. (2012), already cited in the *in vitro* section above, the effect of pretreatment of mice with Cu-chlorophyllins (4 and 100 mg/kg bw, by gavage) for five days before MNNG and DMBA administration was evaluated *in vivo* in bone marrow cells. Cu-chlorophyllins alone did not increase the incidence of micronucleated PCE compared to untreated animals, and did not elicit a detectable protective effect on MNNG- and DMBA-induced micronuclei. However, the Panel noted that the dose-levels of Cu-chlorophyllins used were not adequately high since they were selected to study its protective effects against genotoxic compounds.

3.2.3.3. Conclusion on genotoxicity

Cu-chlorophyllins was tested in a range of *in vitro* and *in vivo* studies, which were, however, designed to investigate the modulating activity of Cu-chlorophyllins on genotoxic effects induced by other substances. These studies were not designed in order to test the genotoxic potential of Cu-chlorophyllins itself, accordingly, none of these studies was in line with OECD guidelines.

The Panel concluded that no adequate data were available on Cu-chlorophyllins (E 141(ii)), and therefore it was not possible to evaluate its genotoxic potential. However, the Panel noted that no consistent indication of genotoxicity appeared from the data available.

No genotoxicity data were available for Cu-chlorophylls (E 141(i)).

3.2.4. Chronic toxicity and carcinogenicity

The Panel noted that JECFA in 1975 (JECFA, 1975) reported the study by Harrison et al. (1954) in which groups of rats (20/sex/group) were fed Cu-chlorophyllins at dietary concentrations of 0, 0.1, 1.0 or 3 % (equal to 0, 50, 500 or 1 500 mg/kg bw/day) throughout life. No effects on growth rate, feed efficiency, haematology or urinalysis were observed. No gross or histopathological changes attributable to the compound were noted. The Panel noted that the authors reported that there was no evidence of copper toxicity or deposition in liver, kidney or spleen even at the highest dose tested (1 500 mg/kg bw, throughout life).

The Panel noted that there were no data available on chronic toxicity and carcinogenicity for Cu-chlorophylls.

3.2.4.1. Initiation–promotion studies

Mice

Crl:SKH1:hr-BR hairless mice (n = 20) that had received a diet containing sodium Cu-chlorophyllins (15.2 g/kg food) for two weeks were exposed (dorsal skin) for 10 weeks to incremental, suberythemal carcinogenic stimulated solar UV, while continuing to be fed a diet containing Cu-chlorophyllins (Cope et al., 2006). A control UV-exposed group was fed a regular diet. Tumour multiplicity was significantly higher ($p < 0.05$) in mice fed the chlorophyllin-enriched diet than in those fed the regular diet. This unusual study design is not capable of differentiating between possible promoting and/or initiating activities of Cu-chlorophyllins. The Panel considered that the relevance of this finding is low as regards identification of a carcinogenic potential of Cu-chlorophyllins.

The chemopreventive properties of sodium Cu-chlorophyllins have also been studied in a mouse transplacental carcinogenesis model (Castro et al., 2009). Pregnant B6129SF1 females, bred to 129S1/SvIm males, received purified diets containing either 2 000 mg Cu-chlorophyllins/kg diet or 10 % freeze-dried spinach beginning at gestation day (GD) 9. Lymphoma-dependent mortality was not significantly altered by maternal consumption of any of the diet and little effect on lung tumour burden in mice surviving to 10 months of age was observed. However, co-administration of 380 mg/kg Cu-chlorophyllins and dibenzo[a,l]pyrene (DBP) by gavage (molar ratio of 10:1 Cu-chlorophyllins to DBP) provided significant protection against DBP-initiated carcinogenesis. Offspring born to dams receiving Cu-chlorophyllins co-gavaged with DBP exhibited markedly less lymphoma-dependent

mortality ($p < 0.01$). The authors suggested that these data support a mechanism involving complex-mediated reduction of carcinogen uptake.

Rats

TemaNord (2002) reported a study by Nelson (1992) in Fischer 344 male rats (n = 90) in which a commercially available Cu-chlorophyllins was found to be a tumour promoter (statistically significant increase in incidence; $p < 0.005$) in a 1,2-dimethylhydrazine cancer model when administered (for 20 weeks) in the drinking water at a concentration of 1.5 mM.

In a study in Wistar rats, it was investigated whether water-soluble chlorophyllins (such as Cu-chlorophyllins) can also inhibit heme-induced carcinogenicity. Only chlorophyll, and not its copper or sodium chlorophyllins derivatives, prevented heme-induced effects (de Vogel et al., 2005).

The role of Cu-chlorophyllins was investigated in a rat multi-organ carcinogenesis model (Simonich et al., 2007). Male F344 rats in three gavage groups (n = 21, seven rats/group) received five daily doses of 250 µg/kg [^{3}H]-aflatoxin B(1) ([^{3}H]-AFB(1)) alone or with 250 mg/kg Cu-chlorophyllins. Cu-chlorophyllins decreased hepatic DNA adduction by 42 % ($p = 0.031$), AFB(1)–albumin adducts by 65 % ($p < 0.001$), and the major AFB-N(7)–guanine urinary adduct by 90 % ($p = 0.0047$). The results were in line with a mechanism involving complex-mediated reduction of carcinogen uptake, and did not support a role for phase II enzyme induction in the mechanism for reduction of aflatoxin. In a second study, 30 rats in three experimental groups received the same doses as in study 1, but over 10 days. At 18 weeks, Cu-chlorophyllins decreased the volume per cent of liver occupied by glutathione S-transferase placental form-positive foci by 74 % ($p < 0.001$) compared with livers in control groups. Cu-chlorophyllins decreased the mean number of aberrant crypt foci per colon by 63 % ($p = 0.0026$).

Based on the data evaluated, it is possible that Cu-chlorophyllins act as tumour promoters or as anti-carcinogens depending on the animal species, the initiating agent and the exposure protocol (Xu et al., 2001).

The Panel considered that, given the discrepancies and uncertainties in the available database concerning the chronic toxicity and carcinogenicity of Cu-chlorophyllins, no adequate data were available, and therefore no conclusion can be drawn for the evaluation of these endpoints.

3.2.5. Reproductive and developmental toxicity

In a lifetime study in rats, reproductive function was also examined (Harrisson et al., 1954). In this study, groups of rats (20/sex/group) received dietary concentrations of 0, 0.1, 1.0 or 3 % Cu-chlorophyllins (equal to 0, 50, 500 or 1 500 mg/kg bw/day) throughout life. Five males and five females from each group were mated. Reproduction showed no deviations in the number of pups and pup survival. The Panel noted that this is an old study, was not carried out in accordance with OECD guidelines and did not include the usual endpoints for reproductive and developmental toxicity assessment.

In a study by Reber and Willigan (1954), female Sprague–Dawley rats (six/group) were fed 0 or 1 % Cu-chlorophyllins in the diet (equal to 0 or 500 mg/kg bw/day) for 19 weeks. Animals were mated after 11 weeks. The compound did not affect the number of implantations and pups. However, of five litters (one female died after successful delivery and consequently the offspring were removed from the study), newborns from three litters all died within the first five days following delivery. This effect was attributed by the authors to either an interference with normal viability of the young rats or an adverse effect on lactation ability of the females as measured in terms of the weight of the newborns (average 25 % decrease in weaning weight). Further, offspring exhibited what appeared to be selective skeletal muscle degeneration without obvious pattern or order of degeneration. Apparently consequential locomotor difficulties were also noted. The authors suggested that the effects reported could be caused by the presence of copper. The Panel considered that the study design was inadequate to draw any reliable conclusions.

In a study carried out by García-Rodríguez et al. (2002), groups of 20 female CD-1 mice received a single intraperitoneal dose of 0, 20, 40, 50 or 100 mg/kg bw Cu-chlorophyllins on GD 8 and were killed on GD 18. The treatment induced a dose-dependent total litter loss, and a significant increase in the number of early resorptions. The frequency of malformation appeared no significantly increased in the frequency of cleft lip, cleft palate, exencephaly and polydactyly. The frequency of dolichocephaly was statistically significant only at 40 mg/kg bw. No statistically significant differences were reported in the frequency of ossification points in the anterior and posterior limbs, or in sternebrae. These reported effects were not dose related. No mortality was reported in the treated groups but the effects were, mostly, observed together with body weight loss of the pregnant mice. The Panel considered the effects on the resorption to be treatment related. However, the Panel noted that the results of this study, in which by Cu-chlorophyllins was administered intraperitoneally cannot be used directly for risk assessment following oral exposure.

The Panel noted that the available studies for the evaluation of the reproductive and developmental toxicity of Cu-chlorophyllins (E 141(ii)) were inadequate and that no studies on the reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) were available.

3.2.6. Hypersensitivity, allergenicity and intolerance

In a study by Böhm et al. (2001), a case of allergic reaction (relapsing angioedema, rhinoconjunctivitis and asthma-like symptoms) was reported in a 28-year-old woman after eating foods containing Cu-chlorophylls (E 141(i)). Although a skin-prick test and a cellular allergen stimulation test were negative it could not be excluded that the observed clinical reaction was IgE- mediated. The patient did not have a copper sensitisation.

The available data did not give rise to concern with respect to immunotoxicity or allergenic potential of Cu-chlorophylls and Cu-chlorophyllins, when used as food additives.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available since then, and data available following an EFSA public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised as food additives in the EU in accordance with Annex II to Regulation (EC) No 1333/2008[22].

The Panel noted that the name "copper complex of chlorophylls" is meaningless on a chemical basis, and should be "copper complex of phaeophytins". Cu-chlorophylls (E 141(i) and Cu-chlorophyllins (E 141(ii)) are obtained from sources that could not be regarded as edible plant material or food (grass, lucerne, nettle) for humans. In addition, owing to their manufacturing process, the food additives Cu-chlorophylls E 141(i) and Cu-chlorophyllins E 141(ii) cannot be considered as natural compounds. The Panel considered that the raw material should fulfil the conditions of the current regulation as regards maximum levels of possible contaminants, including residues of pesticides applied during cultivation and mycotoxins.

The Panel considered that the specifications do not sufficiently cover Cu-chlorophylls, which are processed from extracts from plants that do not have a long-term history of food use, and that consequently, the specifications should be updated to include the information on the non-chlorophyll components of E 141(i), which may represent up to 90 % of the extract.

[22] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

The Panel noted that, according to industry, Cu-chlorophylls (E 141(i)) is not used to produce an aluminium lake and that Cu-chlorophyllins (E 141(ii)) can be transformed into the corresponding aluminium lake.

There is great confusion in the literature. In many publications, the study material, usually named "chlorophyllin", was in fact quite often, if not always, a sodium and/or potassium salt of Cu-chlorophyllins (E 141(ii)). Scotter (2011) stated that: "*It is important to consider that despite a joint initiative introduced by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry, a substantial body of long-established trivial names for chlorophyll and its analogues remains in popular use by both the food colour industry and scientific researchers. The term 'chlorophyllin' covers a range of compounds identical to, or structurally related to the porphyrins*".

Cu-chlorophyllins (E 141(ii)) have been previously evaluated by JECFA in 1969 and 1974 (JECFA, 1970, 1975). In 1975, the SCF evaluated Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) (SCF, 1975). Based on a NOAEL of 1500 mg Cu-chlorophyllins/kg bw/day derived from the long-term and reproduction study by Harrisson et al. (1954), JECFA(1975) set a temporary ADI of 0–15 mg/kg bw/day to Cu-chlorophyllins. In addition, in its evaluations of 1970 and 1975 JECFA described a study by Reber and Willigan (1954) in which Cu-chlorophyllins exhibited significant adverse effects on rat survival after oral exposure to 500 mg/kg bw/day for 19 weeks. The Panel noted that the reason why JECFA did not take into account these findings for the calculation of the ADI was unclear. SCF (1975) allocated a group ADI to the sum of both complexes, and stated that "*Cu-chlorophylls and Cu-chlorophyllins are two distinct food colours and recommends separate listing of the two colours; accordingly, the Community Directive specification requires amendment"*. The Panel noted that no subsequent actions appear to have been taken following this recommendation.

Based on the same NOAEL of 1 500 mg/kg bw/day identified from the Harrisson et al. (1954) study, but by applying a safety factor of 200 instead of 100 for the JECFA and the SCF evaluations, the US FDA (2002) allocated an ADI of 7.5 mg/kg/day for sodium–copper chlorophyllins. The US FDA has recommended that Cu-chlorophyllins can be taken orally as a deodorant, generally at 100–200 mg/day. In some cases an additional 100 mg/day may be required, but the total daily dose should not exceed 300 mg/day (FDA, 1990).

The Panel noted that both JECFA and FDA used the Harrisson et al. (1954) study to establish an ADI. This is an old study not carried out in accordance with OECD guidelines, which did not include the usual endpoints for reproductive and developmental toxicity and used a small number of animals. Therefore, the Panel considered that, according to the current standards, this study was inadequate to identify a NOAEL from which a reliable ADI could be derived.

Most of the available toxicity data were for Cu-chlorophyllins, whereas very few studies have been conducted using Cu-chlorophylls, which hampered their safety assessment. Given the differences in purity, chemical properties, stability and manufacturing process, the Panel considered that it was not possible to use Cu-chlorophyllins (E 141(ii) data for read-across for Cu-chlorophylls (E 141(i)). The Panel noted that the amount of copper-containing material that is absorbed, as well as the full metabolic fate and bioavailability of copper, are not known. Because some reports have shown tissue distribution of copper-containing materials after ingestion of Cu-chlorophyllins, the Panel considered that this might deserve further investigations.

In a study in which the rats were fed a diet containing Cu-chlorophyllins for 19 weeks (Reber and Willigan, 1954), a NOAEL of 500 mg/kg bw/day (the only dose used) could be determined. The Panel noted that this NOAEL was not considered by either the JECFA or the SCF for their evaluations.

No genotoxicity data were available on Cu-chlorophylls, while data on Cu-chlorophyllins were considered by the Panel as inadequate to evaluate genotoxic potential.

The Panel considered that given the discrepancies and uncertainties in the available data concerning the carcinogenic potential of Cu-chlorophyllins, further and adequate evaluation of the possible carcinogenicity of Cu-chlorophyllins is needed.

The Panel noted that the available studies for the evaluation of the reproductive and developmental toxicity of Cu-chlorophyllins (E 141(ii)) were inadequate and that no study on the reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) was available.

In the refined exposure assessment scenario, the Panel used to use only maximum concentration values (maximum reported use levels) available for each authorised food category. However, given the range of data that have been made available, the Panel considered that all data should be used in additional scenarios of the exposure assessment approach intended to provide more realistic exposure estimates. For Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)), only usage levels were available for the refined exposure assessment scenario. Based on these data and added to the maximum level exposure assessment scenario, the Panel calculated two refined exposure estimates based on different assumptions: a "brand-loyal scenario" and a "non-brand-loyal scenario".

The Panel noted that the refined exposure estimates will not cover future changes in the level of use of Cu-chlorophylls (E 141(i)) or Cu-chlorophyllins (E 141(ii)). Only use levels reported by industry were made available to EFSA, no analytical data were provided. These data covered the main food categories in which Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) are authorised. The Panel noted that some data providers did not distinguish between Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) and therefore, for some of the usage data, there was uncertainty about which of the two food additives they refer to.

CONCLUSIONS

The Panel concluded that adequate data on ADME, genotoxicity, (chronic) toxicity, carcinogenicity, and reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) were lacking. Therefore, their safety of use as food additives cannot be assessed and the current ADI should be withdrawn.

RECOMMENDATIONS

- The Panel recommended that, given the current inconsistencies, the components that are present in the commercial food additives Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) should be adequately identified and characterised. In addition, the inconsistency in the total copper content currently indicated in the specifications should be clarified.

- The Panel noted that Cu-chlorophylls (E 141(i) and Cu-chlorophyllins (E 141(ii)) are obtained from sources that could not be regarded as edible plant material or food (grass, lucerne, nettle) for humans. The Panel recommended that data on the raw material should fulfil the conditions of the current regulation as regards maximum levels for possible contaminants, including residues of pesticides applied during cultivation and mycotoxins.

- The Panel recommended that the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) should be revised to ensure that their use as food additives will not be a significant source of exposure to these toxic elements in foods.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Netherlands National Institute for Public Health and the Environment (RIVM), Netherlands, September 2007.
2. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. Exercise on occurrence data – EFSA re-evaluation of some food colours. 14.12.2009.

3. NATCOL (Natural Food Colours Association). Reply to EFSA: Re-evaluation of food colours: call for data (7.12.06). Copper complexes of chlorophylls and chlorophyllins. E141 (E141i, E141ii). NATCOL Submission: 31.03.2007.

4. NATCOL (Natural Food Colours Association), 2011a. Personal communication from NATCOL on sodium copper chlorophyllins (E 141(ii)). 28.03.2011 and 01.04.2011.

5. NATCOL (Natural Food Colours Association), 2011b. Personal communication from NATCOL on the stability and manufacturing process of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)). 24.06.2011.

6. NATCOL (Natural Food Colours Association), 2011c. Application of the screening method for estimating potential intakes to chlorophylls and chlorophyllins (E 140) and copper complexes of chlorophylls and chlorophyllins (E 141). 24.06.2011.

7. Capsugel. Data on usage levels of copper complexes of chlorophyllins (E 141ii) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 31.07.2013.

8. AESGP (Association of the European Self-Medication Industry). Data on usage levels of copper complexes of chlorophyllins (E 141ii) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 09.09.2013.

9. ICGA (International Chewing Gum Association). Data on usage levels of copper complexes of chlorophylls and chlorophyllins. E141 (E141i, E141ii) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 26.09.2013.

10. FDE (FoodDrinkEurope). Data on usage levels of copper complexes of chlorophylls and chlorophyllins. E141 (E141i, E141ii) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 13.09.2013.

11. NATCOL (Natural Food Colours Association). Data on usage levels of copper complexes of chlorophylls and chlorophyllins. E141 (E141i, E141ii) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 11.10.2013.

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APPENDICES

Appendix A. Summary of the reported use levels (mg/kg or mg/L as appropriate) of Cu-chlorophylls (E141(i)) and Cu-chlorophyllins (E 141(ii)) provided by industry

FCS category number	FCS food category	MPL	Restrictions	Food additives	n	Reported use levels		Information provided by
						Typical mean	Highest maximum level	
01.4	Flavoured fermented milk products including heat-treated products	QS		Cu-chlorophyllins	4	15.0	35.0	NATCOL
				Cu-chlorophylls	1	15.0	25.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	15.0	35.0	NATCOL
01.6.3	Other creams	QS	Only flavoured creams	Cu-chlorophyllins	1	20.0	35.0	NATCOL
				Cu-chlorophylls	1	15.0	20.0	NATCOL
01.7.1	Unripened cheese excluding products falling in category 16	QS	Only flavoured unripened cheese	Cu-chlorophyllins	1	20.0	30.0	NATCOL
				Cu-chlorophylls	1	20.0	30.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	100.0	500.0	NATCOL
01.7.2	Ripened cheese	QS	Only *sage Derby cheese, green and red pesto cheese, wasabi, cheese and green marbled herb cheese*	Cu-chlorophylls and chlorophyllins	2	52.0	500.0	NATCOL
01.7.4	Whey cheese	QS		Cu-chlorophylls and chlorophyllins	1	50.0	300.0	NATCOL
01.7.5	Processed cheese	QS	Only flavoured processed cheese	Cu-chlorophyllins	1	15.0	25.0	NATCOL
				Cu-chlorophylls	1	15.0	25.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	100.0	500.0	NATCOL
01.7.6	Cheese products (excluding products falling in category 16)	QS	Only flavoured unripened products	Cu-chlorophyllins	1	20.0	30.0	NATCOL
				Cu-chlorophylls	1	20.0	30.0	NATCOL

FCS category number	FCS food category	MPL	Restrictions	Food additives	n	Reported use levels		Information provided by
						Typical mean	Highest maximum level	
01.8	Dairy analogues, including beverage whiteners	QS		Cu-chlorophyllins	1	15.0	35.0	NATCOL
03	Edible ices	QS		Cu-chlorophyllins	11	73.6	750.0	NATCOL/FDE
				Cu-chlorophylls	7	40.7	125.0	NATCOL/FDE
04.2.2	Fruit and vegetables in vinegar, oil, or brine	QS	Only vegetables (excluding olives)	Cu-chlorophyllins	1	80.0	300.0	NATCOL
04.2.4.1	Fruit and vegetable preparations excluding compote	QS		Cu-chlorophylls and chlorophyllins	1	100.0	300.0	NATCOL
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EEC	QS	Except chestnut purée	Cu-chlorophyllins	3	36.7	400.0	NATCOL
				Cu-chlorophylls	1	25.0	45.0	NATCOL
04.2.5.3	Other similar fruit or vegetable spreads	QS	Except *crème de pruneaux*	Cu-chlorophyllins	2	50.0	400.0	NATCOL
				Cu-chlorophylls	1	25.0	45.0	NATCOL
05.2	Other confectionery, including breath freshening microsweets	QS		Cu-chlorophyllins	20	92.5	500.0	NATCOL
				Cu-chlorophylls	6	67.5	3 700.0	NATCOL/FDE
05.3	Chewing gum	QS		Cu-chlorophyllins	7	107.4	1 350.0	NATCOL/FDE/ICGA
				Cu-chlorophylls	3	81.5	1 350.0	NATCOL/ICGA
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS		Cu-chlorophyllins	5	72.0	800.0	NATCOL
				Cu-chlorophylls	10	162.3	1 900.0	NATCOL/FDE
06.3	Breakfast cereals	QS		Cu-chlorophylls and chlorophyllins	1	400.0	800.0	NATCOL
06.5	Noodles	QS		Cu-chlorophylls and chlorophyllins	1	80.0	104.0	NATCOL

FCS category number	FCS food category	MPL	Restrictions	Food additives	n	Reported use levels		Information provided by
						Typical mean	Highest maximum level	
07.2	Fine bakery wares	QS		Cu-chlorophyllins	4	69.3	500.0	NATCOL/FDE
				Cu-chlorophylls	2	400.0	1 900.0	NATCOL/FDE
				Cu-chlorophylls and chlorophyllins	1	150.0	300.0	NATCOL
08.3.3	Casings and coatings and decorations for meat	QS		Cu-chlorophyllins	5	300.0	1 000.0	NATCOL
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only surimi and similar products and salmon substitutes	Cu-chlorophyllins	1	150.0	250.0	NATCOL
				Cu-chlorophylls	1	150.0	250.0	NATCOL
09.3	Fish roe	QS	Except sturgeons' eggs (caviar)	Cu-chlorophyllins	1	150.0	200.0	NATCOL
12.2.2	Seasonings and condiments	QS	Only seasonings, for example curry powder, tandoori	Cu-chlorophyllins	1	200.0	300.0	NATCOL
				Cu-chlorophylls	1	150.0	250.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	300.0	700.0	NATCOL
12.4	Mustard	QS		Cu-chlorophyllins	2	80.0	440.0	NATCOL/FDE
				Cu-chlorophylls	2	80.0	440.0	NATCOL/FDE
12.5	Soups and broths	QS		Cu-chlorophyllins	4	49.3	500.0	NATCOL/FDE
				Cu-chlorophylls	2	19.5	50.0	NATCOL/FDE
				Cu-chlorophylls and chlorophyllins	1	300.0	500.0	NATCOL
12.6	Sauces	QS	Excluding tomato-based sauces	Cu-chlorophyllins	4	240.0	780.0	NATCOL/FDE
				Cu-chlorophylls	5	124.8	780.0	NATCOL/FDE
12.7	Salads and savoury-based sandwich spreads	QS		Cu-chlorophyllins	1	60.0	80.0	NATCOL
				Cu-chlorophylls	2	105.0	500.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	250.0	500.0	NATCOL
14.1.4	Flavoured drinks	QS	Excluding chocolate milk; malt products	Cu-chlorophyllins	17	5.9	20.0	NATCOL/FDE
				Cu-chlorophylls	1	20.0	30.0	NATCOL

FCS category number	FCS food category	MPL	Restrictions	Food additives	n	Reported use levels		Information provided by
						Typical mean	Highest maximum level	
				Cu-chlorophylls and chlorophyllins	2	28.0	100.0	NATCOL
14.2.3	Cider and perry	QS	Excluding *cidre bouché*	Cu-chlorophyllins	1	15.0	20.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	25.0	50.0	NATCOL
14.2.4	Fruit wine and made wine	QS	Excluding *wino owocowe markowe*	Cu-chlorophyllins	1	15.0	20.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	25.0	50.0	NATCOL
14.2.5	Mead	QS		Cu-chlorophyllins	1	15.0	20.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	25.0	50.0	NATCOL
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	QS	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1–14 of Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	Cu-chlorophyllins	1	15.0	20.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	30.0	100.0	NATCOL
14.2.7.2	Aromatised wine-based drinks	QS	Except bitter soda, sangria, claria, zurra	Cu-chlorophyllins	1	15.0	20.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	30.0	100.0	NATCOL
14.2.7.3	Aromatised wine-product cocktails	QS		Cu-chlorophyllins	2	47.5	500.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	30.0	100.0	NATCOL

FCS category number	FCS food category	MPL	Restrictions	Food additives	n	Reported use levels		Information provided by
						Typical mean	Highest maximum level	
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	QS		Cu-chlorophyllins	1	15.0	20.0	NATCOL
				Cu-chlorophylls and chlorophyllins	1	30.0	100.0	NATCOL
15.1	Potato-, cereal-, flour- or starch-based snacks	QS		Cu-chlorophyllins	5	86.0	500.0	NATCOL
				Cu-chlorophylls	4	95.0	2 000.0	NATCOL/FDE
				Cu-chlorophylls and chlorophyllins	1	350.0	500.0	NATCOL
15.2	Processed nuts	QS		Cu-chlorophyllins	3	95.0	250.0	NATCOL/FDE
				Cu-chlorophylls	1	60.0	100.0	NATCOL
16	Desserts excluding products covered in categories 01, 03 and 04	QS		Cu-chlorophyllins	7	84.6	600.0	NATCOL/FDE
				Cu-chlorophylls	2	13.0	30.0	NATCOL/FDE
				Cu-chlorophylls and chlorophyllins	1	15.0	30.0	NATCOL
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS		Cu-chlorophyllins	3	1 366.7	4 000.0	NATCOL/AESGP/Capsugel
				Cu-chlorophylls	1	100.0	150.0	NATCOL
17.2	Food supplements supplied in a liquid form	QS		Cu-chlorophyllins	1	30.0	50.0	NATCOL
				Cu-chlorophylls	1	30.0	50.0	NATCOL
17.3	Food supplements supplied in a syrup-type or chewable form	QS		Cu-chlorophyllins	2	65.0	150.0	NATCOL
				Cu-chlorophylls	2	65.0	150.0	NATCOL

Appendix B. Concentration levels of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) used in the refined exposure scenarios (mg/kg or ml/kg as appropriate)

FCS category number	Foods	Restrictions	MPL	Concentration levels used in the refined exposure assessment		Data sources/ comments
				Mean	Maximum	
01.4	Flavoured fermented milk products including heat-treated products		QS	15	35	Reported use levels
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Except unflavoured products	QS	–	–	Not taken into account (no data available)
01.6.3	Other creams	Only flavoured creams	QS	–	–	Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16	Only flavoured unripened cheese	QS	47	500	Reported use levels
01.7.2	Ripened cheese	Only *sage Derby cheese, green and red pesto cheese, wasabi, cheese and green marbled herb cheese*	QS	52	500	Reported use levels
01.7.3	Edible cheese rind		QS	–	–	Not taken into account (no corresponding FoodEx code)
01.7.4	Whey cheese		QS	50	300	Reported use levels
01.7.5	Processed cheese	Only flavoured processed cheese	QS	43	500	Reported use levels
01.7.6	Cheese products (excluding products falling in category 16)	Only flavoured unripened products	QS	–	–	Not taken into account (no corresponding FoodEx code)
01.8	Dairy analogues, including beverage whiteners		QS	15	35	Reported use levels
03	Edible ices		QS	61	750	Reported use levels
04.2.1	Dried fruit and vegetables	Only preserves of red fruit	QS	–	–	Not taken into account (no data available)
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only preserves of red fruit	QS	–	–	Not taken into account (no data available)
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only vegetables (excluding olives)	QS	80	300	Reported use levels
04.2.3	Canned or bottled fruit and vegetables	Only preserves of red fruit	QS	–	–	Not taken into account (no data available)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only *mostarda di frutta*	QS	–	–	Not taken into account (no corresponding FoodEx code)

FCS category number	Foods	Restrictions	MPL	Concentration levels used in the refined exposure assessment		Data sources/ comments
				Mean	Maximum	
04.2.4.1	Fruit and vegetable preparations, excluding compote	Only preserves of red fruit	QS	100	300	Reported use levels
04.2.4.1	Fruit and vegetable preparations, excluding compote	Only seaweed-based fish roe analogues	QS	–	–	Not taken into account (no corresponding FoodEx code)
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EEC	Except chestnut purée	QS	34	400	Reported use levels
04.2.5.3	Other similar fruit or vegetable spreads	Except *crème de pruneaux*	QS	42	400	Reported use levels
05.2	Other confectionery including breath refreshening microsweets		QS	89	3 700	Reported use levels
05.3	Chewing gum		QS	92	1 350	Reported use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4		QS	–	–	Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	QS	400	800	Reported use levels
06.5	Noodles		QS	80	104	Reported use levels
06.6	Batters		QS	–	–	Not taken into account (no corresponding FoodEx code)
06.7	Pre-cooked or processed cereals		QS	–	–	Not taken into account (no corresponding FoodEx code)
07.2	Fine bakery wares		QS	202	1900	Reported use levels
08.3.3	Casings and coatings and decorations for meat	Except edible external coating of pasturmas	QS	–	–	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only surimi and similar products and salmon substitutes	QS	150	250	Reported use levels

FCS category number	Foods	Restrictions	MPL	Concentration levels used in the refined exposure assessment		Data sources/ comments
				Mean	Maximum	
09.2	Processed fish and fishery products including molluscs and crustaceans	Only fish paste and crustacean paste	QS	–	–	Not taken into account (no data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only precooked crustacean	QS	–	–	Not taken into account (no data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only smoked fish	QS	–	–	Not taken into account (no data available)
09.3	Fish roe	Except sturgeons' eggs (caviar)	QS	150	200	Reported use levels
12.2	Seasonings and condiments	Only seasonings, for example curry powder, tandoori	QS	217	700	Reported use levels
12.4	Mustard		QS	80	440	Reported use levels
12.5	Soups and broths		QS	77	500	Reported use levels
12.6	Sauces	Excluding tomato-based sauces	QS	176	780	Reported use levels
12.7	Salads and savoury-based sandwich spreads		QS	130	500	Reported use levels
12.9	Protein products, excluding products covered in category 1.8		QS	–	–	Not taken into account (no data available)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	–	–	Not taken into account (no data available)
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		QS	–	–	Not taken into account (no data available)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009		QS	–	–	Not taken into account (no data available)
14.1.4	Flavoured drinks	Excluding chocolate milk; malt products	QS	9	100	Reported use levels
14.2.3	Cider and perry	Excluding *cidre bouché*	QS	20	50	Reported use levels

FCS category number	Foods	Restrictions	MPL	Concentration levels used in the refined exposure assessment		Data sources/ comments
				Mean	Maximum	
14.2.4	Fruit wine and made wine	Excluding *wino owocowe markowe*	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.5	Mead		QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1–14 of Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	QS	23	100	Reported use levels
14.2.7.2	Aromatised wine-based drinks	Except *bitter soda, sangria, claria, zurra*	QS	36	500	Reported use levels
14.2.7.3	Aromatised wine-product cocktails		QS			
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol		QS	23	100	Reported use levels
15.1	Potato-, cereal-, flour- or starch-based snacks		QS	116	2 000	Reported use levels
15.2	Processed nuts		QS	86	250	Reported use levels
16	Desserts, excluding products covered in categories 01, 03 and 04		QS	63	600	Reported use levels

FCS category number	Foods	Restrictions	MPL	Concentration levels used in the refined exposure assessment		Data sources/ comments
				Mean	Maximum	
17.1	Food supplements supplied in a solid form, including capsules and tablets and similar forms, excluding chewable forms		QS	500	4 000	Reported use levels
17.2	Food supplements supplied in a liquid form		QS			
17.3	Food supplements supplied in a syrup-type or chewable form		QS			

Appendix C. Summary of total estimated exposure of chlorophyllins (E 140(ii)) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and high level (mg/kg bw per day)

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Toddlers							
Belgium (Regional Flanders)	36	12.5		6.4		1.6	
Bulgaria (NUTRICHILD)	428	7.9	19.5	6.5	16.5	0.8	1.9
Germany (VELS)	348	10.8	21.4	5.9	12.4	1.4	3.3
Denmark (IAT 2006 07)	917	4.7	9.6	2.6	5.9	0.9	1.9
Spain (enKid)	17	5.1		3.6		0.7	
Finland (DIPP 2001 2009)	500	3.1	7.0	2.3	5.2	1.1	2.6
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	9.6	18.7	5.1	10.2	1.6	3.3
United Kingdom (DNSIYC 2011)	1314	7.9	16.7	4.5	10.1	1.5	3.2
Italy (INRAN SCAI 2005 06)	36	4.8		3.6		0.6	
Netherlands (VCP kids)	322	11.4	23.9	6.4	14.1	1.3	2.6
Children							
Austria (ASNS Children)	128	8.6	16.6	5.4	11.8	1.1	2.5
Belgium (Regional Flanders)	625	11.0	21.6	6.3	12.1	1.4	2.7
Bulgaria (NUTRICHILD)	433	9.2	21.0	7.2	17.6	0.9	2.2
Czech Republic (SISP04)	389	9.1	22.6	6.1	14.2	0.9	2.1
Germany (EsKiMo)	835	6.4	13.8	3.7	9.0	0.8	1.8
Germany (VELS)	293	10.9	20.0	5.8	12.6	1.4	2.8
Denmark (DANSDA 2005-08)	298	4.9	9.8	2.6	5.8	0.6	1.2
Spain (enKid)	156	6.0	16.6	4.2	12.0	0.8	1.8
Spain (NUT INK05)	399	6.6	13.3	4.1	9.0	0.9	1.7
Finland (DIPP 2001 2009)	750	7.6	26.4	6.2	24.2	0.6	1.3
France (INCA2)	482	9.7	18.4	6.7	13.4	1.2	2.3
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	8.6	16.3	4.6	9.7	1.3	2.4
Greece (Regional Crete)	838	7.6	16.5	5.6	12.2	0.9	2.0
Italy (INRAN SCAI 2005 06)	193	5.2	12.5	4.0	9.7	0.6	1.4
Latvia (EFSA TEST)	187	9.7	23.3	6.2	12.7	1.3	3.1
Netherlands (VCP kids)	957	10.7	21.5	5.9	13.7	1.2	2.4
Netherlands (VCPBasis AVL2007 2010)	447	10.2	19.8	5.2	11.9	1.1	1.9
Sweden (NFA)	1 473	10.7	21.2	5.8	12.5	1.4	2.9
Adolescents							
Austria (ASNS Children)	237	4.4	10.4	2.9	8.1	0.6	1.3
Belgium (Diet National 2004)	576	5.0	11.4	3.0	7.2	0.7	1.4
Cyprus (Childhealth)	303	2.2	5.8	1.6	4.1	0.3	0.7
Czech Republic (SISP04)	298	5.9	15.5	4.2	10.4	0.6	1.3
Germany (National Nutrition Survey II)	1 011	4.0	12.3	2.7	8.8	0.5	1.3
Germany (EsKiMo)	393	4.7	10.0	2.8	6.9	0.6	1.2
Denmark (DANSDA 2005-08)	377	2.7	6.1	1.5	3.8	0.3	0.8
Spain (AESAN FIAB)	86	4.0	10.5	3.0	7.1	0.4	0.9
Spain (enKid)	209	4.1	10.9	2.8	7.5	0.5	1.1
Spain (NUT INK05)	651	4.0	9.3	2.6	6.2	0.5	1.1
Finland (NWSSP07 08)	306	4.9	17.9	4.3	17.4	0.3	0.7
France (INCA2)	973	4.9	11.3	3.5	8.7	0.6	1.3
United Kingdom (NDNS-	666	4.4	9.5	2.4	5.6	0.6	1.4

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
RollingProgrammeYears1-3)							
Italy (INRAN SCAI 2005 06)	247	3.0	8.3	2.2	6.2	0.3	0.9
Latvia (EFSA TEST)	453	6.0	14.1	3.7	8.8	0.8	1.9
Netherlands (VCPBasis AVL2007 2010)	1 142	6.6	14.3	3.5	8.4	0.7	1.5
Sweden (NFA)	1 018	6.2	13.8	3.6	8.7	0.7	1.5
Adults							
Austria (ASNS Adults)	308	3.9	9.2	2.7	7.4	0.5	1.3
Belgium (Diet National 2004)	1 292	3.5	8.4	2.3	5.6	0.5	1.1
Czech Republic (SISP04)	1 666	2.4	7.1	1.9	5.6	0.3	0.8
Germany (National Nutrition Survey II)	10 419	3.4	9.1	2.4	6.7	0.4	1.0
Denmark (DANSDA 2005-08)	1 739	1.5	3.7	0.9	2.3	0.2	0.4
Spain (AESAN)	410	2.1	5.9	1.5	4.6	0.3	0.7
Spain (AESAN FIAB)	981	2.5	6.2	2.0	4.8	0.2	0.6
Finland (FINDIET2012)	1 295	4.0	12.5	2.9	9.3	0.7	2.1
France (INCA2)	2 276	2.7	6.1	1.9	4.8	0.3	0.7
United Kingdom (NDNS-RollingProgrammeYears1-3)	1 266	2.4	5.2	1.5	3.4	0.4	0.9
Hungary (National Repr Surv)	1 074	0.8	2.8	0.6	2.0	0.1	0.3
Ireland (NANS 2012)	1 274	2.9	6.5	1.9	4.6	0.6	1.5
Italy (INRAN SCAI 2005 06)	2 313	1.5	3.9	1.1	3.0	0.2	0.5
Latvia (EFSA TEST)	1 271	3.5	8.0	2.3	5.4	0.5	1.1
Netherlands (VCPBasis AVL2007 2010)	2 057	3.5	7.8	2.0	4.7	0.4	1.0
Romania (Dieta Pilot Adults)	1 254	1.3	3.4	0.8	2.4	0.2	0.6
Sweden (Riksmaten 2010)	1 430	3.3	7.6	2.0	4.8	0.5	1.1
The elderly							
Austria (ASNS Adults)	92	3.6	7.7	2.5	6.5	0.5	1.1
Belgium (Diet National 2004)	1 215	3.2	7.3	2.1	4.6	0.5	1.1
Germany (National Nutrition Survey II)	2 496	2.9	7.3	2.1	5.8	0.4	0.9
Denmark (DANSDA 2005-08)	286	1.0	2.5	0.6	1.7	0.2	0.4
Finland (FINDIET2012)	413	3.6	8.5	2.6	6.4	0.9	2.3
France (INCA2)	348	2.0	5.3	1.5	4.2	0.2	0.6
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	2.8	5.9	1.8	4.1	0.5	1.2
Hungary (National Repr Surv)	286	0.9	2.8	0.7	2.5	0.1	0.3
Ireland (NANS 2012)	226	3.0	6.8	2.1	4.9	0.7	1.9
Italy (INRAN SCAI 2005 06)	518	1.1	2.8	0.8	2.4	0.1	0.4
Netherlands (VCPBasis AVL2007 2010)	173	3.0	6.4	1.9	4.4	0.4	0.9
Netherlands (VCP-Elderly)	739	3.0	5.9	1.8	3.6	0.4	1.0
Romania (Dieta Pilot Adults)	128	1.2	3.3	0.8	2.1	0.3	0.8
Sweden (Riksmaten 2010)	367	3.2	6.7	2.1	4.8	0.5	1.1

GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	absorption, distribution, metabolism and excretion
AESGP	Association of the European Self-Medication Industry
AFB	aflatoxin B
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
CIAA	Confederation of the Food and Drink Industries of the EU, now Food Drink Europe
CHO	Chinese hamster ovary
CONTAM	Panel on Contaminants in the Food Chain
DAD	diode array detector
DBP	dibenzo[a,l]pyrene
DMBA	7,12-dimethylbenz(a)anthracene
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
EMS	ethyl methane sulphonate
FAO	Food and Agriculture Organization of the United Nations
FCS	Food Categorisation System
FDE	FoodDrinkEurope
GD	gestation day
HPLC	high-performance liquid chromatography
ICGA	International Chewing Gum Association
JECFA	Joint Expert Committee on Food Additives
LC	liquid chromatography
MMS	methyl methanesulphonate
MNNG	*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine
MS	mass spectrometry
MPL	maximum permitted use level
NATCOL	Natural Food Colours Association
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes
QS	quantum satis
SCF	Scientific Committee on Food
US FDA	The United States Food and Drug Administration
UV	ultraviolet

WHO	World Health Organization