SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of chlorophylls (E 140(i)) 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

Chlorophylls (E 140(i)) were previously evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1969 and the Scientific Committee on Food (SCF) in 1975 and 1983 and, in relation to special medical purposes, for young children in 1996. Neither of the Committees established a numerical Acceptable Daily Intake (ADI). Specifications should be updated to adequately cover chlorophylls (E 140(i)), as currently up to 90 % of the extract is unidentified and chlorophylls (E 140(i)) may be obtained from sources that could not be regarded as regular edible plant materials or foods (grass, lucerne, nettle) for humans. Based on the origin of chlorophylls (E 140(i)), the Panel also concluded that data on pesticides, mycotoxins and other components with biological activity (e.g. phytoestrogens, phytotoxins and allergens) should be included in the specification and kept as low as possible to avoid any potential adverse effects (allergenicity, endocrinal effects). The few biological data available indicate that chlorophylls are poorly absorbed by humans and are not metabolised to chlorophyllins (the dephytylated form of chlorophylls). The Panel considered that the few toxicological studies available for chlorophylls were limited and did not comply with the Organisation of Economic Co-operation and Development (OECD) guidelines or current regulatory requirements, and therefore did not allow for an ADI to be established. The Panel concluded that the available database for chlorophylls was inadequate for risk assessment. However, chlorophylls are natural dietary constituents, which are present at relatively high concentrations in a number of foods. In addition, the exposure resulting from the use of chlorophylls (E 140(i)) as food additives is lower than the exposure to chlorophylls from the regular diet. Therefore, the Panel concluded that, at the reported use levels, chlorophylls (E 140(i)) are not of safety concern as regards their current use as food additives.

KEY WORDS

chlorophylls, chlorophyll a, chlorophyll b, phaeophytin a, phaeophytin b, E 140(i), 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 chlorophylls (E 140(i)) 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 chlorophylls (E 140(i)) following a European Food Safety Authority (EFSA) public call for data. The Panel noted that not all of the original studies on which previous evaluations were based were available to the Panel. To assist in identifying any emerging issues 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 chlorophylls (E 140(i)), which covered the period up to the end of 2014.

Chlorophylls (E 140(i)) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008. The Panel noted that, in this regulation, chlorophylls and chlorophyllins are authorised with the same E number, E 140. However, according to Commission Regulation (EU) No 231/2012, separate specifications are defined for chlorophylls (E 140(i)) and chlorophyllins (E 140(ii)). The Panel decided to re-evaluate these two food additives separately, given their different physico-chemical properties. Chlorophylls (E 140(i)) were previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1969 and the Scientific Committee on Food (SCF) in 1975 and 1983 (SCF, 1975, 1984) and, in relation to special medical purposes, for young children in 1996 (SCF, 1997). Neither of the Committees established a numerical acceptable daily intake (ADI).

Chlorophylls (E 140(i)) may be obtained from sources that could not be regarded as edible plant materials or foods (grass, lucerne, nettle) for humans. According to the current specifications, chlorophylls may represent as few as 10 % of the food additive. The Panel considered that the specifications should be updated to provide more information about the remaining 90 %, which according to information provided by industry, might consist of other pigments such as carotenoids as well as proteins, oils, fats and waxes derived from the source material. Based on the origin of chlorophylls (E 140(i)), the Panel considered that data on pesticides, mycotoxins and other components with biological activity (e.g. phytoestrogens, phytotoxins and allergens) should be included in the specification.

The Panel considered that the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for chlorophylls (E 140(i)) should be revised to ensure that chlorophylls (E 140(i)) as food additives will not be a significant source of exposure to these toxic elements in food.

The few toxicological studies that are available do not specifically focus on chlorophylls as food additives, do not comply with the Organisation of Economic Co-operation and Development (OECD) guidelines and are not compliant with current regulatory requirements.

The Panel considered that the *in vivo* studies indicate that, at most, less than 5 % of ingested chlorophylls would be absorbed from the gastrointestinal tract of dogs or humans. Consequently, absorption and bioavailability of chlorophylls are likely to be low. The major metabolites of chlorophylls are phaeophytins a and b; only traces of dephytylated metabolites have been observed in faeces. Based on these data, the Panel considered the cleavage of the phytol chain during digestion of chlorophylls in humans to be unlikely.

Only few data on the genotoxic potential of chlorophylls were available. The Panel noted several inconsistencies in these studies, which were designed to investigate the modulating activity of

chlorophylls for the genotoxic effect induced by other substances and not to test the genotoxic potential of chlorophylls themselves. Accordingly, the Panel concluded that the genotoxic potential of chlorophylls cannot be assessed based on the available data.

No data on chronic toxicity/carcinogenicity or on reproductive and developmental toxicity were available and, therefore, it was not possible to conclude on these topics.

No reported cases of allergy to chlorophylls (E 140(i)) were found.

Using the "*maximum level exposure assessment scenario*", mean exposure to chlorophylls (E 140(i)) from their use as food additives ranged from 0.4 mg/kg body weight (bw)/day in the elderly to 10.7 mg/kg bw/day in toddlers. The high exposure to chlorophylls (E 140(i)) using this scenario ranged from 1.1 mg/kg bw/day in the elderly to 19.3 mg/kg bw/day in toddlers.

Using the refined brand-loyal assessment exposure scenario, mean exposure to chlorophylls (E 140(i)) from their use as food additives ranged from 0.3 mg/kg bw/day in the elderly to 6.9 mg/kg bw/day in toddlers. The high exposure to chlorophylls (E 140(i)) using this scenario ranged from 0.8 mg/kg bw/day in the elderly to 15.9 mg/kg bw/day in toddlers.

Using the refined non-brand-loyal assessment exposure scenario, mean exposure to chlorophylls (E 140(i)) from their use as food additives ranged from 0.1 mg/kg bw/day in adults and the elderly to 2.7 mg/kg bw/day in toddlers. The high exposure to chlorophylls (E 140(i)) from their use as food additives using this scenario ranged from 0.2 mg/kg bw/day in the elderly to 5.0 mg/kg bw/day in toddlers.

Considering the levels of consumption in Europe recorded in the Comprehensive Database for each vegetable, combined with concentrations from the literature, the intake of chlorophylls from the regular diet for adults ranged from 0 to 6.7 mg/kg bw/day at the mean and from 0.4 to 18.3 mg/kg bw/day for the 95th percentile. For children, the dietary exposure from the natural diet ranged from 0 to 14.7 mg/kg bw/day at the mean and from 1.4 to 34.8 mg/kg bw/day for the 95th percentile.

It is important to mention that some data providers did not distinguish between chlorophylls (E 140(i)) and chlorophyllins (E 140(ii)) and, therefore, for some of the usage data, there was uncertainty about whether they referred to chlorophylls (E 140(i)) or chlorophyllins (E 140(ii)). Therefore, the present exposure assessment to chlorophylls (E 140(i)) could be overestimated. This further supported the conclusion that exposure resulting from the use of chlorophylls (E 140(i)) as food additives is lower than the exposure to chlorophylls from the regular diet.

The Panel concluded that the available database for chlorophylls was inadequate for risk assessment and cannot support derivation of an ADI. However, chlorophylls are natural dietary constituents, which are present at relatively high concentrations in a number of foods. In addition, the exposure resulting from the use of chlorophylls (E 140(i)) as food additives is lower than the exposure to chlorophylls from the regular diet. Therefore, the Panel concluded that, at the reported use levels, chlorophylls (E 140(i)) are not of safety concern as regards their current use as food additives.

The Panel recommended that:

- the definition and identity of the food additive E 140(i), in particular the specifications, should be updated, as they do not include up to 90 % of the extract. The possible residual solvents should also be described.
- data on pesticides, mycotoxins and other components with biological activity (e.g. phytoestrogens, phytotoxins and allergens) should be included in the specification and kept as low as possible to avoid any potential adverse effects.

- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for chlorophylls (E 140(i)) should be revised in order to ascertain that chlorophylls (E 140(i)) as food additives will not be a significant source of exposure to these toxic elements in food.

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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[4]. 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[5] of 2001. The report "Food additives in Europe 2000[6]" 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.

[4] OJ L 80, 26.3.2010, p. 19.
[5] COM (2001) 542 final.
[6] 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 chlorophylls (E 140(i)) when used as food additives.

Chlorophylls (E 140(i)) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008[7]. The Panel noted that, in this regulation, chlorophylls and chlorophyllins are authorised with the same E number, E 140. However, according to Commission Regulation (EU) No 231/2012[8], separate specifications are defined for chlorophylls (E 140(i)) and chlorophyllins (E 140(ii)). The Panel decided to re-evaluate these two food additives separately, given their different physico-chemical properties.

Chlorophylls (E 140(i)) were previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1969 (JECFA, 1969) and by the Scientific Committee on Food (SCF) in 1975 and 1983 (SCF, 1975, 1984), and in 1997 in relation to special medical purposes for young children in foods (SCF, 1997).

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]. 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 issues 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 chlorophylls (E 140(i)), which covered the period up to the end of 2014.

2. Technical data

2.1. Identity of the substance

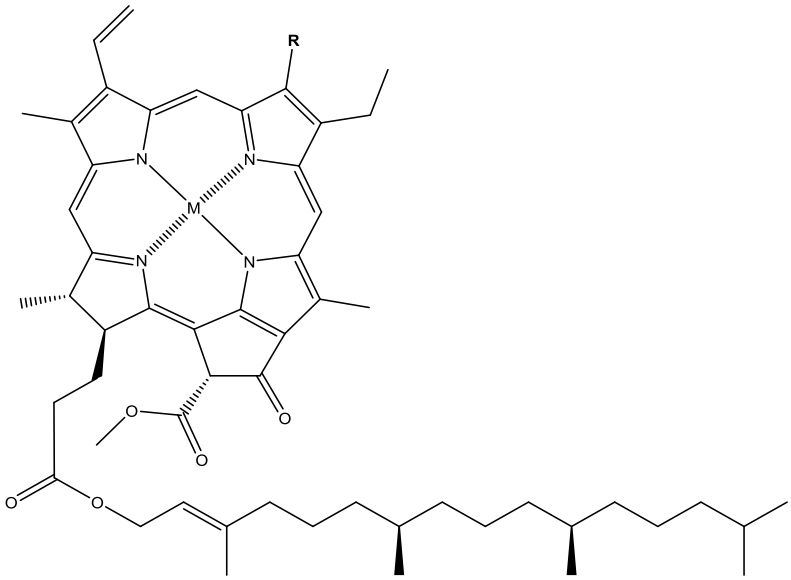
The colouring principles of the family of chlorophylls have a porphyrin ring (tetrapyrrole ring) as basic structure, with a coordinated magnesium ion (Mg^{2+}) (i.e. chlorophylls a and b) or without a coordinated magnesium ion (Mg^{2+}) (i.e. phaeophytins a and b) (Figure 1).

[7] 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.

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

[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



I	Chlorophyll a	R = Methyl (CH_3)	M = Mg^{2+}
II	Chlorophyll b	R = Formyl (CHO)	M = Mg^{2+}
III	Phaeophytin a	R = Methyl (CH_3)	M = None
IV	Phaeophytin b	R = Formyl (CHO)	M = None

Figure 1: General structural formula of the major colouring principles of chlorophylls

According to the European Commission (EC) specifications (Commission Regulation (EU) No 231/2012), chlorophylls are obtained by solvent extraction of strains of edible plant material, grass, lucerne and nettle. According to the Natural Food Colours Association (NATCOL, 2011b), the most commonly used raw materials for the production of chlorophylls in Europe are grass, lucerne[11], nettle and spinach (Tables 1 and 2). The Panel noted that not all sources used for the production of chlorophylls are part of the regular human diet.

Table 1: Plants commonly used for the extraction of chlorophylls (NATCOL, 2011b)

Source plant/ common name	Scientific Latin name	Botanical family	Varieties
Grass	*Festuca* spp.	Poaceae	Principal grass used is *F. arundinacea*
Alfalfa / Lucerne	*Medicago sativa* L.	Fabaceae	All varieties approved for and grown by European agriculture
Nettle	*Urtica* spp.	Urticaceae	Mainly *U. dioica*, but also *U. urens*
Spinach	*Spinacia oleracea*	Amaranthaceae	All varieties approved for and grown by European agriculture

The Panel noted that, according to an industry source (NATCOL, 2011b), in addition to the sources given in Table 1 for the manufacturing of food colours in the EU, other raw materials (e.g. from *Basella* spp. and *Morus* spp.) may be used for the extraction of chlorophyll products outside of the EU. Therefore, compounds extracted from these other raw materials may be present in the food additive E 140(i), and the Panel noted that these may not be included in the compounds tested in the available toxicity studies.

[11] Also known as alfalfa.

Table 2: Geographical origins, growth and harvesting conditions and the part used of source plants (NATCOL, 2011b)

Source plant/ common name	Geographical origin	Growth and harvesting conditions	Part used
Grass	UK, principally England	Cultivated; cut sequentially before flowering during the growing season, i.e. several cuts made each season	Aerial—the whole plant (not the roots)
Alfalfa / Lucerne	EU, principally eastern England and north-eastern France		
Nettle	EU, principally eastern Europe	Cultivated; harvested before flowering	
Spinach	EU; widely grown in France, Germany and Italy		

The main colouring principles are chlorophylls a and b and phaeophytins a and b. The colour of the food additive chlorophylls (E 140(i)) can vary from olive green to dark green, depending on the content of coordinated magnesium (Commission Regulation (EU) No 231/2012), that refers to the proportion of chlorophylls a and b (containing coordinated magnesium) and phaeophytins a and b (without coordinated magnesium) in the food additive.

The extracted product contains other pigments such as carotenoids, as well as oils, fats and waxes derived from the source material (Commission Regulation (EU) No 231/2012).

Chlorophylls are insoluble in water and soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and oils (JECFA, 2006).

The most common synonyms of chlorophylls are magnesium chlorophyll, magnesium phaeophytin and CI natural green 3.

Tables 3 and 4 summarise the information concerning the identity of the food additive E 140(i), including the major colouring principles.

Table 3: Identity data of chlorophylls (E 140(i)) as food additives and the major colouring principles

	Name [a]	Molecular formula	Molecular weight (g/mol)	Structural formula (Figure 1)	CAS number [b]	EC number [c] (EINECS)	Colour index number	EC specifications names [d]	JECFA specifications names [e]
	Chlorophylls	N/A [f]	N/A [f]	N/A [f]	1406-65-1	215-800-7	75810	Chlorophylls	Chlorophylls
Major colouring principles	Chlorophyll a	$C_{55}H_{72}MgN_4O_5$	893.51	I	479-61-8	207-536-6	–	Chlorophyll a (magnesium complex)	Phaeophytin a magnesium complex
	Chlorophyll b	$C_{55}H_{70}MgN_4O_6$	907.49	II	519-62-0	208-272-4	–	Chlorophyll b (magnesium complex)	Phaeophytin b magnesium complex
	Phaeophytin a	$C_{55}H_{74}N_4O_5$	871.22	III	603-17-8	210-031-3	–	Chlorophyll a	Phaeophytin a
	Phaeophytin b	$C_{55}H_{72}N_4O_6$	885.20	IV	3147-18-0	221-565-1	–	Chlorophyll b	Phaeophytin b

(a): Names as considered by the Panel.
(b): SciFinder software. SciFinder® the choice for chemistry researchTM.
(c): EC inventory (online).
(d): Commission Regulation (EU) No 231/2012.
(e): JECFA (2006).
(f): Not possible to assign a single value/data because it is mixture.

The Panel noted that there are some inconsistencies between the names and the European Inventory of Existing Commercial chemical Substances (EINECS) numbers for the major colouring principles of chlorophylls presented in the EC specifications (Commission Regulation (EU) No 231/2012) and the information on the substances from official databases (EC Inventory online; Chemical Abstracts Service (CAS)).
(Table 3). The Panel has summarised the relevant information on the major colouring principles of chlorophylls and considered that the EC specifications should be updated accordingly (Table 4).

Table 4: Identity data of the major colouring principles of chlorophylls

	Name	Molecular formula	Molecular weight (g/mol)	CAS number [(a)]	EC number [(b)]	Colour index number	Chemical name [(a)]
	Chlorophylls	N/A [(c)]	N/A [(c)]	1406-65-1	215-800-7	75810	Chlorophylls
Major colouring substances	Chlorophyll a	$C_{55}H_{72}MgN_4O_5$	893.51	479-61-8	207-536-6	–	Magnesium, [(2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl (3*S*,4*S*,21*R*)-9-ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-3-phorbinepropanoato(2-)-kN^{23},kN^{24},kN^{25},kN^{26})]-, (*SP*-4-2)-
	Chlorophyll b	$C_{55}H_{70}MgN_4O_6$	907.49	519-62-0	208-272-4	–	Magnesium, [(2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecenyl (3*S*,4*S*,21*R*)-9-ethenyl-14-ethyl-13-formyl-21-(methoxycarbonyl)-4,8,18-trimethyl-20-oxo-3-phorbinepropanoato(2-)-kN^{23},kN^{24},kN^{25},kN^{26})]-, (*SP*-4-2)-
	Phaeophytin a	$C_{55}H_{74}N_4O_5$	871.22	603-17-8	210-031-3	–	3-Phorbinepropanoic acid, 9-ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-, (2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecen-1-yl ester, (3*S*,4*S*,21*R*)-
	Phaeophytin b	$C_{55}H_{72}N_4O_6$	885.20	3147-18-0	221-565-1	–	3-Phorbinepropanoic acid, 9-ethenyl-14-ethyl-13-formyl-21-(methoxycarbonyl)-4,8,18-trimethyl-20-oxo-, (2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecen-1-yl ester, (3*S*,4*S*,21*R*)-

(a): SciFinder software. SciFinder® the choice for chemistry researchTM.
(b): EC inventory (online).
(c): Not possible to assign a single value/data because it is mixture

2.2. Specifications

Specifications for chlorophylls (E 140(i)) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006) (Table 5).

Table 5: Specifications for chlorophylls (E 140(i)) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Description	Waxy solid ranging in colour from olive green to dark green depending on the content of coordinated magnesium	Waxy solid ranging in colour from olive green to dark green depending on the content of coordinated magnesium
Assay [(a)]	Content of total combined chlorophylls and their magnesium complexes is not less than 10 % $E^{1\%}_{1\,cm}$ 700 at ca. 409 nm in chloroform	Content of total combined phaeophytins and their magnesium complexes is not less than 10 %
Purity		
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	–

(a): Method described by JECFA.

The Panel noted that the assay requirement in the EC specifications for the content of "*total combined chlorophylls and their magnesium complexes*" is not less than 10 %; this should correspond to the total of chlorophylls a and b and phaeophytins a and b. In the definition in the EC specifications, it is mentioned that other pigments such as carotenoids, as well as oils, fats and waxes derived from the source material, may be present in the food additive. It is not specified if the remaining 90 % (maximum) corresponds to the above-mentioned substances or if there are other, unidentified, additional substances.

According to an industry source (NATCOL, 2011b): "*The level of total chlorophylls present in an undiluted extract derived from grass or lucerne is typically 20 %. The actual value depends on the growing conditions and age/height of the plant. Spinach and nettle give lower chlorophyll contents (method used for the estimation of chlorophyll content is as per directive 2008/128/EC).*

"*The leaves of photosynthetic plants contain both chlorophyll a and chlorophyll b with chlorophyll a being predominant. By calculation (using molecular mass), the maximum magnesium content is approximately 0.5 % of E 140(i) product with 20 % chlorophylls. However the actual level will be significantly lower than this value since a proportion of the chlorophyll a/b will be present as the corresponding phaeophytin*".

Analytical data on the food additive chlorophylls (E 140(i)) from the different extracts were provided by NATCOL (2012, 2014a, b, 2015). However, the actual respective percentages of the individual components (chlorophylls, waxes, fatty acid, carotenoids, protein, carbohydrates, water and ash) in the food additive chlorophylls (E 140(i)) in the different extracts prepared from lucerne, nettle, spinach or grass were not available to the Panel.

According to industry (NATCOL, 2011c): "*The levels for residual solvent require two revisions to accommodate for the regular presence of ethanol and methanol at levels that are not caused by the use as solvents but result from the carryover from raw materials or reactions during extraction and processing. Although their presence is not related to their use as extraction solvents, it will make sense to consider these extra amounts together with the solvent residues providing clarification by a foot note*". From further information provided by NATCOL (2014a, b, 2015), the Panel noted that the residual solvents (methanol and ethanol) in the commercial food additive are above the maximum limit indicated in the EC specifications (Commission Regulation (EU) No 231/2012).

The Panel noted that, according to the EU specifications for chlorophylls, impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to a concentration of 3, 5, 1 and 1 mg/kg, respectively. Contamination at these levels would have a significant impact on the exposure to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA, 2009a; EFSA Panel on Contaminants in the Food Chain (CONTAM), 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 chlorophylls (E 140(i)) should be revised to ensure that chlorophylls (E 140(i)) as food additives will not be a significant source of exposure to these toxic elements in foods. Furthermore, according to industry (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 considered that the possible enrichment of pesticides from spinach during the manufacturing process should be excluded by the choice of suitable extraction methods in the production of the food colour.

Aflatoxins B1, B2, G1 and G2 were not detected (limit of detection 0.25 µg/kg, limit of quantification 0.5 µg/kg) in one tested sample of chlorophyll preparation from nettle (NATCOL, 2015).

The Panel noted that the specifications should be updated to include information on non-chlorophyll components of (E 140(i)), which may represent up to 90 % of the extract.

Based on the origin of the food additive E 140(i), the Panel noted that data on pesticides, mycotoxins and other components with biological activity (e.g. phytoestrogens, phytotoxins and allergens), possibly present in the food additive as used, are relevant for the specifications.

2.3. Manufacturing process

Chlorophylls are obtained by solvent extraction of natural strains of edible plant material, grass, lucerne and nettle and subsequent removal of solvent. Only the following solvents may be used for the extraction: acetone, methyl ethyl ketone, dichloromethane, carbon dioxide, methanol, ethanol, propan-2-ol and hexane (Commission Regulation (EU) No 231/2012).

According to industry (NATCOL, 2011b): "*During the removal of solvent, the naturally present co-ordinated magnesium may be completely or partly removed from the chlorophylls to give the corresponding phaeophytins*".

The Panel was provided with flow charts of the manufacturing process of chlorophylls (E 140(i)) extracted from lucerne, nettle, spinach and grass (NATCOL, 2014b). In all cases, the extraction was done with organic solvents that were removed in a further step. However, the Panel noted that the residual amount of ethanol in some of the extracts was above the maximum permitted level given in the EC specifications (Commission Regulation No 231/2012). In one case, the amount of methanol, although not used in the extraction process, was also above the limit indicated in the specifications for E 140(i); this was claimed to be due to "*a carry-over from the raw material and released methyl group*" (NATCOL, 2015). According to industry (NATCOL, 2014b): "*a chlorophyll-containing*

oleorosin that meets the specifications laid down in 231/2012 is in practice a very viscous pasta that is very difficult to handle and the pigments contained therein are prone to rapid deterioration". Therefore, to maintain the integrity of the chlorophyll, *some carriers and antioxidants* are added to the commercial chlorophylls (E 140(i)).

The Panel noted that chlorophylls (E 140(i)) cannot be used as aluminium lakes for colouring purposes (Commission Regulation (EU) No 231/2012).

2.4. Methods of analysis in foods

Reversed-phase high-performance liquid chromatography (HPLC)-based methods have been used for the determination of chlorophylls and their analogues in a limited range of foodstuffs using ultraviolet-visible, fluorimetric and/or mass spectrometric detection. Very few of these methods have been reported with analytical validation data. Because chlorophylls are particularly labile pigments, appropriate care is required during extraction and analysis. It is generally recommended that manipulations should be carried out rapidly in darkness or in dim light to prevent photodestruction or photoisomerisation, and at relatively low temperatures (Bertrand and Schoefs, 1996; Schoefs, 2002, 2004, 2005; Scotter, 2011). The Panel noted that methods based on photodiode array detector HPLC, fluorimetric HPLC and liquid chromatography tandem mass spectrometry could be considered a basis for any future development and validation, as they offer adequate selectivity and sensitivity for the detection and quantitation of the main chlorophyll analogues (IRMM, 2013).

2.5. Reaction and fate in foods

No specific data were available for the food additive E 140 (i); however, the fate and/or degradation of natural chlorophylls during processing has been reported in several studies.

Chlorophylls are susceptible to degradation owing to various processing treatments and storage, and degradation reactions are influenced by conditions such as weak acids, heat, light and oxygen or enzymatic activities in senescent tissues of plants (Heaton and Marangoni, 1996; Shoefs, 2005; Erge et al., 2008). Thermal processing causes structural and chemical variations in the green vegetable tissue that result in colour changes (Chen and Chen, 1993). The amount of chlorophylls retained during thermal processing depends on the temperature and duration of the treatment (Schwartz and Von Elbe, 1983; Schwartz and Lorenzo, 1991), together with the number of acid moieties formed (Gunawan and Barringer, 2000). Degradation of chlorophylls a and b by microwave cooking or blanching is greater than degradation by steaming or baking. The major chlorophyll derivatives formed during baking and blanching are phaeophytins. In spinach leaves, pyrophaeophytins a and b are formed after 30 or 5 minutes of steaming or microwave cooking, respectively, owing to the removal of the carbomethoxyl group from phaeophytins caused by the greater heat penetration of these treatments (Teng and Chen, 1999). In green peas, both chlorophyll and phaeophytin conversion can be minimised by the presence of Maillard reaction products resulting from the reaction between reducing sugars and amino groups of proteins, improving the colour stability (Kumar et al., 2013). During refrigerated storage for 15 days at 8 °C, phaeophytins a and b are the predominant chlorophyll degradation derivatives in spinach with a percentage of chlorophyll loss of 20 % (López-Ayerra et al., 1998).

Scotter and Castle (2004) have reviewed the chemical interactions and the fate of chlorophylls in foodstuffs. They found that degradation of chlorophylls can proceed by an acid-, base- or enzyme-catalysed reaction. Weak acids liberate the magnesium bound to the porphyrin ring to form phaeophytins by substitution with two hydrogen ions, and green chlorophylls are converted into the olive brown phaeophytins (Van Boekel, 2000). Chlorophylls may also undergo photo-oxidation accompanied by the loss of desirable colour in dehydrated green vegetables and the singlet oxygen increase would induce fatty acid peroxidation (Francis, 1985); this leads to the production of free radicals and the degradation of chlorophylls (Heaton and Marangoni, 1996; López-Ayerra et al., 1998). An-Erl King (2001) found that chlorophyll degradation was higher in freeze-dried products with high porosity owing to the deleterious high exposure to oxygen.

2.6. Case of need and proposed uses

Maximum permitted levels (MPLs) of chlorophylls (E 140(i)) have been defined in Annex II to Regulation (EC) No 1333/2008[12] on food additives for use in foods. Chlorophylls (E 140(i)) are authorised food additives in the EU at *quantum satis* (QS) in 56 foods categories. Chlorophylls (E 140(i)) are included in Group II (food colours authorised at QS).

According to Annex II, part A, Table 3, to Regulation (EC) No 1333/2008, chlorophylls (E 140(i)) are not colours which may be used in the form of lakes.

Table 6 summarises foods that are permitted to contain chlorophylls (E 140(i)), as set out in Annex II to Regulation (EC) No 1333/2008.

Table 6: MPLs of chlorophylls (E 140(i)) in foods and beverages according to Annex II to Regulation (EC) No 1333/2008

FCS category number	Foods	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 140(i)	Only *sage Derby 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 140(i)	Only preserves of red fruit	*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 140(i)	Only preserves of red fruit	*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 140(i)	Only vegetables (excluding olives)	*Quantum satis*
04.2.3	Canned or bottled fruit and vegetables	E 140(i)	Only preserves of red fruit	*Quantum satis*
04.2.4.1	Fruit and vegetable preparations excluding compote	E 140(i)	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*
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	E 140(i)	Except chestnut purée	*Quantum satis*

[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.

FCS category number	Foods	E number/ Group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
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*
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	E 140(i)	Only fish paste and crustacean paste	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 140(i)	Only precooked crustacean	*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*
09.3	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*

FCS category number	Foods	E number/ Group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group II	Including dry pasta	*Quantum satis*
14.1.4	Flavoured drinks	Group II	Excluding chocolate milk and malt products	*Quantum satis*
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 (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	*Quantum satis*
14.2.7.1	Aromatised wines	Group II	Except *americano, bitter vino*	*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 Annex II to Regulation (EC) No 1333/2008.

2.7. Reported use levels or data on analytical levels of chlorophylls (E 140(i)) in foods

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 chlorophylls (E 140(i)), to support the re-evaluation of all food colours 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 chlorophylls were submitted to EFSA by the Confederation of the Food and Drink Industries of the EU (CIAA, currently FoodDrinkEurope (FDE)) (CIAA, 2009) and NATCOL (NATCOL, 2011c).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010[14] regarding the re-evaluation of approved food additives, a call[15] for food additives usage level and/or concentration data in food and beverages intended for human consumption was launched in March 2013, with a deadline in September 2013. Data on chlorophylls (E 140(i)), 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 chlorophylls (E 140(i)) in foods. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010b).

In response to this public call, updated information on the actual use levels of chlorophylls (E 140(i)) in food have been submitted by industry. No analytical data have been provided for chlorophylls.

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

Following the call for food additives usage level and/or concentration data launched in March 2013, updated information on the actual uses and use levels of chlorophylls (E 140(i)) was made available by FDE (n = 18), the International Chewing Gum Association (ICGA) (n = 1), NATCOL (n = 102) and a private company (n = 1). The data provided cover the majority of the food categories in which this food additive is authorised; most data were provided for other confectionery (Food Categorisation System (FCS) 05.2), flavoured drinks (FCS 14.1.4) and food supplements (FCS 17). No data resulting from non-authorised uses has been reported to EFSA. Some data providers did not distinguish between chlorophylls (E 140(i)) and chlorophyllins (E 140(ii)) and, therefore, for some of the usage data (n = 102), there was uncertainty about whether they referred to chlorophylls (E 140(i)) or chlorophyllins (E 140(ii)). The present exposure assessment to chlorophylls (E 140(i)) could be an overestimation if the data reported are for chlorophyllins (E 140(ii)).

For the food categories for which no updated information was provided through this call for data, the usage levels of chlorophylls (E 140(i)) in foods and beverages reported previously to EFSA (NATCOL, 2011; CIAA, 2009) were considered for the present exposure assessment.

[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

[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

In total, 130 usage levels for 49 out of 56 food categories in which chlorophylls (E 140(i)) are authorised were considered for the exposure assessment.

Appendix A provides data on the use levels of chlorophylls (E 140(i)) in foods reported by industry.

2.8. Information on existing authorisations and evaluations

Chlorophylls (E 140(i)) are authorised as food additives in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives. Specific purity criteria on chlorophylls (E 140(i)) have been defined in Commission Regulation (EU) No 231/2012.

Chlorophylls (E 140(i)) were evaluated previously by JECFA in 1969 (JECFA, 1969) and the SCF in 1975 and 1983 (SCF, 1975, 1984), both Committees did not establish a numerical acceptable daily intake (ADI).

JECFA (1969) concluded that the use of chlorophylls was "*not limited except for good manufacturing practice*" (JECFA, 1969).

In 1975, the SCF did not establish an ADI but considered chlorophylls prepared from natural foods acceptable for use in food, despite no biological data being available (SCF, 1975). In 1983, the SCF continued to hold the same opinion but noted that there had been significant technological advances in the isolation, preparation and extraction of colouring principles from natural sources. Consequently, the SCF recommended that a review should be undertaken to assess the impact of these changes on the evaluation of these materials and to improve the specifications of purity (SCF, 1984).

In 1997, the SCF evaluated food additives in foods for special medical purposes for young children aged 12 months and up, and concluded that chlorophylls mixed with carotenes, beetroot red and anthocyanins are acceptable, from a safety point of view, up to the maximum level requested in the products, once diluted for consumption (chlorophylls 20 mg/L) (SCF, 1997). The SCF also noted that: "*Chlorophylls are obtained by solvent extraction of grass or lucerne. The colour requested (E 140) refers to chlorophylls (and chlorophyllins) obtained from natural sources. In 1975, the SCF noted that no biological data were available for natural chlorophylls and did not establish an AD1 but agreed that their use in food generally was acceptable. The Committee notes that in 1975 only chlorophylls obtained by physical processes from natural food sources normally consumed were discussed, whereas under the current EU Directive on colours, chlorophylls from non-human food sources (e.g. grass) are allowed*" (SCF, 1997). In the current Commission Regulation (EU) No 231/2012, grass, lucerne and nettle are considered, despite not being foods with a long history of consumption for the general population. Therefore, the Panel noted that, according to the EC specifications (Commission Regulation (EU) No 231/2012), currently used chlorophylls (E 140(i)) can be obtained from non-human food sources.

Chlorophylls were also evaluated by TemaNord in 2002, which concluded that "*although, very limited data is available on many of the toxicological aspects of chlorophyll, the available toxicity data do not suggest any overt toxicity of chlorophyll, at doses that by far exceed a normal human intake*" (TemaNord, 2002).

In 2009, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) expressed an opinion on alfalfa, an extract from lucerne (alfalfa), in which it was stated that alfalfa contained several compounds potentially of concern such as coumestrol, L-canavanine and β-carotene, and that, given that it contains proteins which have homologies with some peanut proteins, it may also have an allergenic potential (EFSA, 2009b).

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 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). The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents, adults and the elderly. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 European countries as outlined in Table 7.

Table 7: Population groups considered for the exposure estimates of chlorophylls (E 140(i))

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, Finland, Germany, Netherlands, Italy, Spain
Children[(a)]	From 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

(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, 2011b).

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 use levels reported in Appendix B for each food group by their consumption amount per kilogram body weight (bw) 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 (Table 7). 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 chlorophylls (E 140(i))

The food categories in which the use of chlorophylls (E 140(i)) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at a detailed level (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories and/or their relative restrictions/exceptions are not referenced in the EFSA Comprehensive Database and therefore could not be taken into account in the present estimate. This might result in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 01.6.3. Other creams, only flavoured creams
- 01.7.1. Unripened cheese excluding products falling in category 16, only flavoured unripened cheese
- 01.7.2. Ripened cheese, only *sage Derby cheese*
- 01.7.3. Edible cheese rind
- 01.7.4. Whey 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, excluding *wino owocowe markowe*
- 14.2.5. Mead
- 14.2.7.2. Aromatised wine-based drinks, except *bitter soda*, *sangria*, *claria*, *zurra*
- 14.2.7.3. Aromatised wine-product cocktails

For the following food categories, the restrictions, which apply to the use of chlorophylls (E 140(i)), 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:

- 01.5. Dehydrated milk as defined by Directive 2001/114/EC, except unflavoured products: unflavoured dehydrated milk is not referenced in the FoodEx classification nomenclature
- 04.2.2. Fruit and vegetables in vinegar, oil or brine, only vegetables (excluding olives): it was not possible within the FoodEx food classification to distinguish olives from the vegetables in vinegar, oil or brine

- 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 between extruded, puffed or fruit-flavoured breakfast cereals; therefore, the 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
- 14.2.7.1. Aromatised wines, except americano, bitter vino: no distinction is possible between americano and other products or between bitter soda and other products of each food category; 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 between solid, liquid or syrup-type or chewable forms of food supplements within FoodEx codes

Overall, 15 food categories were not taken into account in the exposure assessment because these are not referenced in the EFSA Comprehensive Database, and 10 food categories were included in the exposure assessment without considering the restrictions as set out in Annex II to Regulation No 1333/2008.

2.9.2. Exposure to chlorophylls (E 140(i)) from their use as food additives

Dietary exposure to chlorophylls (E 140(i)) from their use as food colours was estimated using the approach adopted by the Panel at its 52nd plenary meeting[16]. 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 (Table 7), and with the limitations described above.

Exposure assessment for food additives under re-evaluation was carried out by the ANS Panel based on (1) MPLs set out in EU legislation (defined as the *regulatory maximum level exposure assessment* scenario) and (2) reported use levels or analytical data (defined as the *refined exposure assessment* scenario).

2.9.2.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set out in Annex II to Regulation No 1333/2008. As no MPLs are set for chlorophylls (E 140(i)), a *maximum level exposure assessment* scenario has been performed based on the maximum use levels from data provided to EFSA.

The exposure estimates derived following this scenario should be considered as the most conservative, as this scenario assumes that the consumer will be continuously (over a lifetime) exposed to chlorophylls (E 140(i)) present in the food at the maximum reported use level.

Appendix B summarises the concentration levels of chlorophylls (E 140(i)) used in the maximum level exposure assessment scenario.

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

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario was based on reported use levels from industry. This exposure scenario can consider only food categories where the above data were made available to the Panel.

Appendix B summarises the concentration levels of chlorophylls (E 140(i)) used in the refined exposure assessment scenario. Based on the available dataset, two estimates based on different model populations were calculated:

1. The brand-loyal consumer scenario. This assumes that a consumer experiences long-term exposure to the food additive at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
    a. Food consumption is combined with the maximum reported use levels for the main contributing food category at the individual level.
    b. The mean of the typical reported use levels is used for the remaining food categories.
2. The non-brand-loyal consumer scenario. This assumes that a consumer experiences long-term exposure to the food additive 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.

Food categories (n = 7) for which FoodEx classification linkage was available, but for which no usage levels were reported, were not considered in the exposure assessment (Appendix B). The Panel noted that if chlorophylls (E 140(i)) are nevertheless used in those food categories for which reported use/analytical levels were not available, the calculated refined exposure assessment might result in underestimation of exposure.

2.9.2.3. Anticipated exposure to chlorophylls (E 140(i))

Table 8 summarises the estimated exposure to chlorophylls (E 140(i)) from their use as food additives of all five population groups (Table 7). Detailed results by population group and survey are presented in Appendix C.

Table 8: Summary of anticipated exposure to chlorophylls (E 140(i)) from their use as food additives using the maximum level exposure assessment scenario and refined exposure scenarios in five population groups (minimum to 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 scenario					
Mean	2.6–10.7	2.0–8.5	0.8–3.5	0.6–2.4	0.4–2.0
High level (95th percentile)	6.5–19.3	5.1–17.9	1.7–7.9	1.5–5.2	1.1–4.7
Brand-loyal scenario					
Mean	2.0–6.9	1.5–5.5	0.6–2.2	0.4–1.5	0.3–1.4
High level (95th percentile)	4.9–15.9	3.2–13.2	1.3–5.1	1.1–3.3	0.8–3.3
Non-brand-loyal scenario					
Mean	0.6–2.7	0.4–2.2	0.2–1.0	0.1–0.6	0.1–0.6
High level (95th percentile)	1.3–5.0	1.1–4.8	0.5–2.6	0.3–1.5	0.2–1.5

2.9.3. Main food categories contributing to exposure to chlorophylls (E 140(i)) using the maximum level exposure assessment scenario

The main food categories contributing to total mean exposure to chlorophylls (E 140(i)) (> 5 % of total exposure) calculated for the maximum level scenario, as well as the number of surveys in which each food category is a main contributor, are shown in Table 9. Flavoured fermented milk products and fine bakery wares were the main contributors for toddlers and children, whilst, in adolescents,

adults and the elderly, soups and broths were also significant contributors to the total mean exposure to chlorophylls (E 140(i)). In the elderly, processed fruit and vegetables were also important contributors to the total mean exposure to chlorophylls (E 140(i)).

Table 9: Main food categories contributing to exposure to chlorophylls (E 140(i)) using maximum usage levels (maximum level scenario) (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
01.4	Flavoured fermented milk products	9.6–70.2 (7)	10.9–50.7 (13)	8.4–31.9 (9)	5.3–45.7 (13)	6.4–27.6 (7)
01.8	Dairy analogues, including beverage whiteners	5.3 (1)	–	–	–	–
03	Edible ices	5.1–28.7 (5)	5.5–25.5 (14)	5.8–24.7 (12)	5.9–17.9 (11)	6.0–15.3 (4)
04.2	Processed fruit and vegetables	–	–	5.8 (1)	5.3–33.6 (6)	5.0–41.3 (7)
05.2	Other confectionery including breath freshening microsweets	–	6.3–10.0 (2)	5.7–11.4 (2)	6.1–10.1 (2)	–
06.3	Breakfast cereals	9.9 (1)	6.3–23.2 (8)	6.4–34.4 (6)	8.4–25.6 (5)	5.6–33.3 (3)
07.2	Fine bakery wares	9.1–54.5 (6)	5.2–50.2 (14)	5.6–41.8 (12)	6.0–40.2 (14)	9.7–35.3 (3)
12.2.2	Herbs, spices, seasonings	6.1–6.4 (2)	–	10.0 (1)	15.7 (1)	5.2–18.1 (2)
12.5	Soups and broths	6.0–23.5 (3)	5.3–37.7 (7)	6.2–37.0 (5)	6.4–43.5 (7)	5.1–49.6 (3)
12.6	Sauces	–	8.0 (1)	5.5–11.9 (3)	6.2–15.0 (7)	5.2–10.2 (3)
12.7	Salads and savoury-based sandwich spreads	–	10.1–10.2 (2)	12.5 (1)	11.4–19.6 (2)	–
14.1.4	Flavoured drinks	5.9–12.7 (3)	5.1–23.1 (11)	5.7–33.4 (10)	6.3–32.7 (14)	9.5–25.5 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks	8.1 (1)	–	5.3–11.7 (4)	–	–
16	Desserts excluding products covered in categories 01, 03 and 04	5.9–21.2 (3)	5.0–17.5 (6)	9.2–13.5 (2)	9.3–11.2 (2)	5.2–13.9 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7, as some countries submitted more than one survey for a specific population.

2.9.4. Main food categories contributing to exposure to chlorophylls (E 140(i)) using the refined exposure assessment scenarios

The main food categories contributing to total mean exposure to chlorophylls (E 140(i)) (> 5 % of total exposure) calculated for the brand-loyal and non-brand-loyal refined scenarios, as well as the number of surveys in which each food category is a main contributor, are shown in Tables 10 and 11, respectively.

For the brand-loyal scenario, the food categories that, at the individual level, had the highest contribution to the total individual exposure to chlorophylls (E 140(i)) were identified for each age group. Flavoured fermented milk products and fine bakery wares were the main contributors in toddlers and children. In adolescents, besides fine bakery wares, flavoured fermented milk products were also an important contributor. In adults and the elderly, soups and broths, flavoured fermented

milk products and processed fruit and vegetables made important contributions to the total mean exposure to chlorophylls (E 140(i)) (Table 10).

In the non-brand-loyal scenario (Table 11), fine bakery wares and flavoured fermented milk products were the most important contributors to the total mean exposure to chlorophylls (E 140(i)) in toddlers and children. In adolescents, adults and the elderly, the exposure was mostly from soups and broths and breakfast cereals.

Table 10: Main food categories contributing to exposure to chlorophylls (E 140(i)) using the 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	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
01.4	Flavoured fermented milk products	10.9–80.3 (7)	10.5–60.2 (13)	6.8–39.0 (9)	6.4–57.8 (13)	9.1–36.9 (6)
03	Edible ices	5.3–32.2 (4)	5.2–31.8 (13)	5.3–29.7 (10)	5.3–20.0 (7)	5.7–17.7 (3)
04.2	Processed fruit and vegetables	–	–	–	5.5–35.2 (2)	6.2–46.1 (4)
05.2	Other confectionery including breath freshening microsweets	–	–	–	5.4 (1)	–
06.3	Breakfast cereals	8.5 (1)	6.2–38.0 (8)	6.6–41.5 (6)	9.6–42.2 (5)	6.2–37.0 (3)
07.2	Fine bakery wares	5.7–63.3 (4)	5.4–58.3 (13)	12.2–55.8 (11)	6.0–50.2 (13)	6.5–45.4 (6)
12.2.2	Herbs, spices, seasonings	7.3 (1)	–	10.8 (1)	17.6 (1)	18.9 (1)
12.5	Soups and broths	5.8–29.0 (3)	6.0–52.5 (6)	6.2–51.4 (6)	7.5–57.4 (7)	6.3–68.3 (3)
12.6	Sauces	–	–	6.0–9.6 (2)	5.3–11.4 (4)	5.5–5.5 (2)
12.7	Salads and savoury-based sandwich spreads	–	5.1–9.1 (2)	8.6 (1)	8.0–15.3 (2)	–
14.1.4	Flavoured drinks	11.4 (1)	5.7–34.5 (6)	6.3–54.6 (9)	5.6–42.7 (13)	12.2–26.5 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks	5.0 (1)	–	7.5–8.1 (2)	–	–
16	Desserts excluding products covered in categories 01, 03 and 04	18.1–20.9 (2)	5.9–18.7 (4)	9.1–12.3 (2)	9.1–10.3 (2)	15.5 (1)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7, as some countries submitted more than one survey for a specific population.

Table 11: Main food categories contributing to exposure to chlorophylls (E 140(i)) following 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	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
01.4	Flavoured fermented milk products	11.3–64.2 (7)	10.0–56.5 (13)	8.2–38.0 (9)	5.1–45.0 (13)	5.8–31.8 (7)
01.8	Dairy analogues, including beverage whiteners	7.6 (1)	6.1 (1)	–	–	–
03	Edible ices	5.8–18.2 (2)	5.2–17.1 (12)	6.5–17.9 (8)	7.6–13.3 (4)	8.8–9.9 (2)
04.2	Processed fruit and vegetables	–	5.2 (1)	5.5 (1)	5.1–34.4 (3)	5.3–41.4 (3)
05.2	Other confectionery including breath freshening microsweets	–	5.1 (1)	6.0 (1)	5.5 (1)	–
06.3	Breakfast cereals	5.8–17.7 (3)	6.3–40.6 (10)	5.3–56.3 (9)	5.3–48.2 (11)	9.8–53.3 (3)
07.2	Fine bakery wares	6.7–46.9 (6)	7.7–46.1 (13)	11.6–42.4 (11)	5.1–45.0 (14)	8.3–39.5 (6)
12.2.2	Herbs, spices, seasonings	10.9–12.9 (2)	8.9–10.6 (2)	20.2 (1)	7.4–29.2 (2)	7.5–32.6 (3)
12.5	Soups and broths	8.3–32.0 (3)	7.3–45.7 (7)	7.6–46.0 (6)	9.3–54.4 (7)	7.1–59.7 (3)
12.6	Sauces	–	–	7.1–7.7 (2)	6.0–9.8 (5)	5.4–7.4 (2)
12.7	Salads and savoury-based sandwich spreads	–	–	–	7.1 (1)	–
14.1.4	Flavoured drinks	5.7–8.0 (2)	6.0–16.6 (6)	6.1–25.3 (9)	5.0–23.9 (11)	7.1–18.8 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks	5.6–10.8 (2)	5.1–6.6 (4)	6.1–12.8 (5)	5.2–7.0 (2)	–
16	Desserts excluding products covered in categories 01, 03 and 04	6.5–23.3 (3)	5.9–19.1 (6)	5.4–14.5 (3)	6.1–12.0 (3)	5.1–16.2 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7, as some countries submitted more than one survey for a specific population.

2.9.5. Exposure via the regular diet

Chlorophylls are widely distributed in fruits and vegetables. Important sources of chlorophylls include green vegetables such as asparagus, beans, broccoli, celery, Chinese mustard, collards, cucumber, kale, lettuce, okra, olive, peas and spinach. The average content of chlorophylls varies considerably between vegetables (from 70 to 10 890 µg/g of fresh vegetable tissue) (Gross, 1991; Ferruzzi and Schwartz, 2001). Some varieties of olives can contain up to 24 360 µg chlorophyll/g (Ferruzzi and Schwartz, 2001).

Consumption data for these vegetables were from the Comprehensive Database (EFSA, 2011a). The mean contents of chlorophylls (total of chlorophyll a and b) used for the calculation of the exposure from the natural diet (Ferruzzi and Schwartz, 2001) were: asparagus, 300 mg/kg; beans, 71 mg/kg; broccoli, 160 mg/kg; collards, 1 225 mg/kg; celery, 1 368 mg/kg; cucumber, 88 mg/kg; kale, 1 870 mg/kg; lettuce, 396 mg/kg; okra, 292 mg/kg; olive, 10 890 mg/kg; peas, 118 mg/kg; and spinach, 1 576 mg/kg. The mean content of chlorophylls in fruit was not available for the exposure assessment, which may have led to underestimation of the real exposure to chlorophylls via the regular diet.

Considering the levels of consumption in Europe given in the Comprehensive Database (mean minimum/maximum and 95th percentile minimum/maximum) for each green vegetable as described above combined with the mean content of chlorophylls, the mean intake of chlorophylls from the regular diet for adults was calculated to range from 0 to 6.7 mg/kg bw/day and for the 95th percentile from 0.4 to 18.3 mg/kg bw/day. For children, the mean dietary exposure from the natural diet ranged from 0 to 14.7 mg/kg bw/day and for the 95th percentile from 1.4 to 34.8 mg/kg bw/day (Table 12).

Table 12: Summary of anticipated exposure to chlorophylls in children and adults from the natural diet

	Population (< 18 years old) (mg/kg bw/day)	**Population (≥ 18 years old) (mg/kg bw/day)**
Mean exposure	0–14.7	0–6.7
Exposure 95th percentile	1.4–34.8	0.4–18.3

Considering the exposure to chlorophylls (E 140(i)) from their use as food additives (section 2.9.2.3) and the exposure to chlorophylls from the natural diet, the Panel decided to present a table summarising the exposure to chlorophylls from a combination of both sources (Table 13).

Table 13: Summary of anticipated exposure to chlorophylls from their use as food additives, from the natural diet, and from a combination of food additive use and natural diet in children and the adult population

	Population (< 18 years) (mg/kg bw/day)	**Population (≥ 18 years old) (mg/kg bw/day)**
From food additive		
Refined estimated exposure scenario – non-brand-loyal scenario		
Mean exposure	0.2–2.7	0.1–0.6
Exposure 95th percentile	0.5–5.0	0.2–1.5
From natural diet		
Mean exposure	0–14.7	0–6.7
Exposure 95th percentile	1.4–34.8	0.4–18.3
From food additive and natural diet		
Mean exposure	0.2–17.4	0.1–7.3
Exposure 95th percentile	1.9–39.8	0.6–19.8

Although the results should be interpreted with caution, as different methodologies were used for the exposure assessments, exposure to chlorophylls (E 140(i)) used as food additives appeared small compared with exposure to chlorophylls from the regular diet and it represents approximately 10–20 % of overall exposure to chlorophylls.

2.10. Uncertainty analysis

Uncertainties in the exposure assessment of chlorophylls (E 140(i)) 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 14.

Table 14: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate to chlorophylls (E 140 (i))

Sources of uncertainties	Direction [(a)]
Consumption data: different methodologies/representativeness/underreporting/ 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 owing to missing FoodEx linkage	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception	+
Use levels: uncertainty whether the reported use levels provided by industry refer to chlorophylls or chlorophyllins	+
Use levels: no data for some food categories (7 out of 56 food categories)	–
Use levels: levels considered applicable for all items within the entire food category	+/–
Maximum level scenario: exposure calculations based on the maximum value (use levels reported from industry)	+
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	+/–
Exposure via the regular diet: fruits not considered for exposure assessment	–

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

The Panel considered that the uncertainties identified would tend to an overestimation of the real exposure to chlorophylls (E 140(i)) as food additives, and would tend to an underestimation of the real exposure to chlorophylls via the regular diet 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 chlorophylls following an EFSA public call for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

A literature search was conducted on the most commonly available online databases for toxicological and biological information (PubMed, Science Direct, Toxline and Web of Knowledge) to cover recent published literature on chlorophylls (E 140(i)).

The Panel noted that the few toxicological studies that are available are not specifically for chlorophylls (E 140 (i)) as food additives, but for chlorophylls in the regular diet.

3.1. Absorption, distribution, metabolism and excretion (ADME)

There are limited ADME data for chlorophylls reported by JECFA (1969) and TemaNord (2002).

Various *in vitro*, *in vivo* and human studies on chlorophylls have been published since the JECFA evaluation. Many of them have been reviewed by Ferruzzi and Blaskelee (2007).

3.1.1. *In vitro* studies

The digestion of chlorophyll derivatives contained in green vegetables including fresh spinach purée, heat- and acid-treated spinach purée and zinc chloride-treated spinach purée was investigated by an *in vitro* digestion method, which simulated the gastric and small intestinal phases (Ferruzzi et al., 2001). Native chlorophylls were converted to magnesium-free phaeophytins during digestion. Bioaccessibility for lipophilic food constituents was defined as the amount of a compound ingested that is transferred during digestion from the food matrix to the micelles. Intestinal cell uptake of micellarised pigments was investigated using human Caco-2 cells, a human heterogeneous epithelial colorectal adenocarcinoma cell line. Caco-2 cells contained 5–10 % micellarised chlorophyll derivatives. According to the authors, these results demonstrated the uptake of some chlorophyll derivatives by human intestinal cells *in vitro*.

The digestive stability, efficiency of micellarisation and cellular accumulation of the chlorophyll pigments of different preparations of pea were investigated (Gallardo-Guerrero et al., 2008). Fresh pea (FP), cooked fresh pea (CFP), frozen pea (FZP), cooked frozen pea (CFZP) and canned pea (CP) were subjected to a simulated *in vitro* digestion procedure coupled with uptake by human Caco-2 cells. The transfer of the chlorophyll pigments from the digesta to the micellar fraction was significantly the most efficient in CFZP (57 %), and more efficient in CFP, FZP and CP (28 to 35 %) than in FP (20 %). Incubation of Caco-2 cells with micellar fractions at the same concentration prepared from each test food showed that the lowest ($p < 0.006$) pigment absorption was in cells incubated with FP. According to the authors, the preservation process of freezing or cooking had a positive effect on the bioaccessibility and bioavailability of chlorophylls.

Chlorophylls a and b, and phaeophytins a and b, dissolved in an oily matrix, were subjected to a simulated *in vitro* digestion procedure coupled with uptake by human intestinal Caco-2 cells (Gandul-Rojas et al., 2009). The native chlorophylls showed higher instability to the digestive process than the magnesium-free chlorophyll derivatives. After digestion, the degree of pigment dispersion in the colloidal system (aqueous "micellar" phase) showed significant differences among series a and b derivatives. However, when a mixture of phaeophytins a and b was digested, the dispersion degree and the accumulation rate by the Caco-2 cells were significantly higher for the dephytylated chlorophyll derivatives phaeophorbide a than for the intact chlorophylls. According to the authors, "*whereas phytylated chlorophyll derivatives showed passive absorption by simple diffusion, the dephytylated ones showed passive absorption by facilitated diffusion in the lower range of concentrations tested*". In addition, "*the structural modification of chlorophyll pigments, mainly the de-esterification of phytol, significantly increased its transfer from the food matrix to the intestinal epithelial cells, during digestion, making it more bio-accessible*".

Overall, the Panel considered these *in vitro* studies as poorly relevant to the risk assessment, as they represent specific cases that are not closely related to the *in vivo* human situation.

3.1.2. Animal studies

The absorption of chlorophyll derivatives was investigated in dogs fed a diet containing 73 mg chlorophylls/kg diet for 10 days (Fernandez et al., 2007). Absorption of the chlorophyll derivatives, assessed by the measurements in faecal samples, ranged from 2.5 to 4.0 %, with an average of 3.4 %. In a second experiment, where dogs consumed a diet containing 10 % dried spinach for 10 days, no chlorophyll derivatives could be found in the peripheral blood until 150 minutes after consumption, which suggests either their low absorption and/or rapid metabolism/biliary excretion. Chlorophylls a and b were transformed into their respective phaeophytins in the gastrointestinal tract. Beyond phaeophytins, no other degradation products were detected. By HPLC analysis of excreta, only a trace amount of dephytylated phaeophorbide a was observed. Based on these results and those from previous studies, the authors considered that the cleavage of the phytol chain during digestion of chlorophylls was unlikely in dogs. The Panel agreed with that conclusion.

3.1.3. Human studies

In humans given encapsulated crystalline chlorophyll (100 mg per day for 4 days, exact composition not given), the decomposition of chlorophyll in the body was quantitatively estimated in faeces by analysis of four analytically defined fractions. The largest percentage of decomposed chlorophyll corresponded to faecal phaeophytin (Brugsch and Sheard, 1938).

The importance of chlorophylls as a source of phytanic acid, which accumulates in Refsum disease, was investigated in humans (Baxter, 1968). Although humans cannot derive significant amounts of phytanic acid from the consumption of chlorophylls present in plants, Refsum disease is an autosomal recessive metabolic disease with neurological symptoms that results from the over-accumulation of phytanic acid in cells and tissues. Uniformly ^{14}C-labelled phaeophytin a (the magnesium-free derivative of phytylated chlorophyll a) was fed to two normal human subjects and to two patients with Refsum disease. Faeces, serum and urine were collected. In all subjects, 90–95 % of the administered radioactivity was recovered in the faeces, largely in the form of phaeophytin a (72 to 74 % of the administered radioactivity). The phytol radioactivity recovered in the faeces after nine days averaged about 95 % of that in the administered material; the author concluded that there is little absorption of the phytol moiety. In faeces, only 1–2 % of the radioactivity migrated as dephytylated materials. According to the authors, "*determination of serum radioactivity in each subject at about 6 hr after the phaeophytin-^{14}C had been ingested (at the time of peak levels as shown by previous studies) showed that less than 0.5 % of the ingested radioactivity was present in the plasma. Less than 1 % of the ingested radioactivity was found in the urine collected during the first 24 hr*". The authors concluded that no more than 5 % of the ingested chlorophyll phytol was absorbed by humans, whether healthy or with Refsum disease.

The Panel considered that *in vivo* studies suggested that very low amounts of ingested chlorophylls were absorbed from the gastrointestinal tract of dogs and humans, as 1 % was excreted in urine but 95 % was excreted in faeces. The possibility exists that chlorophylls and/or phaeophytins were absorbed to a significant extent but were rapidly excreted via the bile; however, low serum levels reported in dogs indicated a low systemic availability. In dogs and humans, the major metabolites of chlorophylls are phaeophytins a and b, but only traces of dephytylated metabolites have been observed in faeces. Based on these data, the Panel concluded that there is no evidence that a cleavage of the phytol chain resulting in the formation of chlorophyllins may occur during digestion of chlorophylls in humans.

3.2. Toxicological data

In addition to not specifically addressing the food additive E 140(i), the few toxicological studies available on chlorophylls did not comply with the Organisation for Economic Co-operation and Development (OECD) guidelines and are not compliant with current regulatory requirements (internationally accepted guidelines and Good Laboratory Practice).

3.2.1. Acute toxicity

JECFA (1969) reported several acute toxicity studies. In the mouse, no acute toxicity was noted after single oral exposure of up to 10 000 mg chlorophyll/kg bw (Heinrichs et al., 1954). Intraperitoneal and intravenous studies in the mouse and an intravenous study in the guinea pig were reported (Heinrichs et al., 1954), but, given the route of administration, these data were not considered relevant for the evaluation of the safety of use of chlorophylls as food additives. In addition, the Panel noted that the compound used in the Heinrichs et al. (1954) study was soluble in water and therefore was probably not chlorophylls (E 140(i)) but rather chlorophyllins (E 140(ii)) or a copper complex of chlorophyllins (E 141(ii)).

3.2.2. Short-term and sub-chronic studies

JECFA (1969) did not describe any short-term or sub-chronic studies. However, the JECFA evaluation included a few remarks on the toxicity of chlorophylls. JECFA stated that "*chlorophylls may lower the*

body temperature of mice, at oral doses of 500 mg/kg bw/day", but no details were provided on the studies from which these conclusions were derived.

In a 13-week sub-chronic oral toxicity study, F344 rats of both sexes (10 animals/sex/group) were fed diet containing 0, 0.18, 0.55, 1.66 and 5 % chlorophyll (containing 40 % oil) and vehicle (edible oil: Dezain 200C from Lever Brothers (Japan)) alone (Furukawa et al., 1998). The corresponding daily doses, calculated by the authors, were 0, 82, 249, 726 and 2 146 mg/kg bw/day and 0, 100, 300, 911 and 2 635 mg/kg bw/day in males and females, respectively. The main colouring principle was a mixture of chlorophylls a and b, and the chlorophyll constituent concentration was 18.5 %. No changes in body weight or food intakes were found in any of the groups. The reported changes were moderate (never above 10 %), were not dose related and did not show any specific trend but were statistically significant at $p \leq 0.05$ compared with the untreated control. In the liver, vacuolar degeneration in hepatocytes and sinusoidal wall cells was noted (not dose related) in all male and female groups except in the highest dose group. Minor granulomatous changes were observed in liver mesenteric lymph nodes and in Peyer's patches in the female high-dose groups. These changes were not seen in the control (untreated or solvent-treated) groups and the authors considered that they were not suggestive of obvious toxicity. The authors concluded that the non toxic dose can be considered to be 5 % in the diet (2146 mg/kg bw/day); however, given the granuloma formation in liver and lymph nodes, they suggested that the no effect dose was 1.66 % in the diet (726 mg/kg bw/day). The Panel agreed with this conclusion. However, the Panel noted that the chlorophyll extract was prepared from the boraginaceous plant comfrey, which is known to contain hepatotoxic pyrrolizidine alkaloids, and that the material tested did not comply with the specifications of the food additive E 140(i); therefore, the Panel considered that this study was not relevant for risk assessment.

3.2.3. Genotoxicity

3.2.3.1. *In vitro*

A micronucleus test was performed with chlorophylls a and b to investigate the effects of these substances on the methyl methanesulphonate-induced genotoxicity. V79-Cells were treated with 0.1375, 0.275 and 0.55 µmol/L of chlorophylls for two hours. After treatment, the cells remained for 14 hours in cytochalasin B before collection. Chlorophylls a and b did not increase the frequency of micronuclei in the bi-nucleated cells and protected the cells from the DNA damage induced by methyl methanesulphonate (Bez et al., 2001). The Panel noted that the above-mentioned study was not designed to investigate the genotoxic potential of chlorophylls and that the maximum concentration of 0.55 µmol/L used in this study did not meet the requirements of OECD Guideline 487 (according to which the maximum concentration should aim to produce 55 ± 5 % cytotoxicity). Consequently, the Panel considered the results of this study inconclusive for genotoxicity assessment.

3.2.3.2. *In vivo*

Commercially available purified chlorophyll and a chlorophyll-rich acetone extract of fresh spinach leaves were suspended in water and administered by gavage to male Swiss mice once or daily for seven days. Both test materials were given at a dose of 1.5 mg chlorophyll equivalent/kg bw (corresponding to 1.5 g of fresh spinach leaves in the case of the acetone extract). Mice were sacrificed 24 hours after the last administration and chromosomal aberrations were scored in 100 metaphases per animal. A significant ($p < 0.001$) increase in chromosomal aberration was observed in all treated groups compared with controls receiving distilled water (Sarkar et al., 1996a). In the same study, 1.5 mg/kg of purified chlorophyllin or an aqueous extract of spinach leaves (1.5 g fresh leaves/kg bw, corresponding to 1.5 mg chlorophyll) were completely ineffective. The Panel noted that the catalogue number reported in the paper does not correspond to chlorophyll but to another chemical (cinnamalfluorene), and that the same set of results had been presented by the same authors in other studies on the modifying effect of spinach extracts on chemically induced clastogenicity (Sarkar et al., 1995, 1996b). The Panel also noted that, although statistically significant, the effect observed was small (4.2, 4.6 and 1.2 % aberrant cells in chlorophyll, spinach extract and control group, respectively), and that in the absence of historical control data the biological relevance of the effect

reported cannot be evaluated. Overall, the Panel concluded that, owing to these numerous inconsistencies, and in the absence of experimental confirmation of these findings in well-controlled experimental conditions, the results of these studies cannot be considered for risk assessment.

3.2.3.3. Conclusion on genotoxicity

Only few data on the genotoxic potential of chlorophylls were available. The Panel noted several inconsistencies in these studies, which were designed to investigate the modulating activity of chlorophylls for the genotoxic effects induced by other substances and not to test the genotoxic potential of chlorophylls themselves. Accordingly, the Panel concluded that the genotoxic potential of chlorophylls cannot be assessed based on the available data.

3.2.4. Chronic toxicity and carcinogenicity

In the previous evaluations by JECFA (1969) and the SCF (1975), no data on chronic toxicity/carcinogenicity of chlorophylls were available and, therefore, it was not possible to conclude on this topic. Since then, no further relevant data have become available.

3.2.5. Reproductive and developmental toxicity

No data on reproductive and developmental toxicity were available.

3.2.6. Hypersensitivity, allergenicity and intolerance

No reported cases of allergy to chlorophylls (E 140(i)) have been identified.

4. Discussion

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 chlorophylls following an EFSA public call for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

Chlorophylls (E 140(i)) are authorised as food additives in the EU in accordance with Annex II to Regulation (EC) No 1333/2008. The Panel noted that, in this regulation, chlorophylls and chlorophyllins are authorised with the same E number, E 140. However, according to Commission Regulation (EU) No 231/2012, separate specifications are defined for chlorophylls (E 140(i)) and chlorophyllins (E 140(ii)). The Panel decided to re-evaluate these two food additives separately, given their different physico-chemical properties.

Chlorophylls (E 140(i)) were previously evaluated by JECFA in 1969 and the SCF in 1975 and 1983 (SCF, 1975, 1984) and, in relation to their use in foods for special medical purposes, for young children in 1996 (SCF, 1997). Neither of the Committees established a numerical ADI.

JECFA (1969) concluded that the use of chlorophylls was "*not limited except for good manufacturing practice*". The SCF did not establish an ADI but considered chlorophylls prepared from natural foods acceptable for use in food, despite no biological data being available (SCF, 1975, 1984). In 1997, the SCF concluded that chlorophylls mixed with carotenes, beetroot red and anthocyanins are acceptable, from a safety point of view, in foods for special medical purposes for young children aged 12 months and up (SCF, 1997). The SCF (1975) also noted that: "*only chlorophylls obtained by physical processes from natural food sources normally consumed were discussed, whereas under the current EU Directive on colours, chlorophylls from non-human food sources (e.g. grass) are allowed*". Finally, the Panel noted that, according to the EC specifications (Commission Regulation (EU) No 231/2012), currently, chlorophylls (E 140(i)) can be obtained from food sources that could not be regarded as edible plant material for humans.

According to the current specifications, chlorophylls may represent as little as 10 % of the food additive (E 140(i)). The Panel considered that the specifications should be updated to provide more information about the remaining 90 %, which, according to industry, may consist of other pigments such as carotenoids as well as proteins, oils, fats and waxes derived from the source material. The Panel noted that chlorophylls (E 140(i)) prepared from lucerne might be rich in compounds with allergenic and oestrogenic potential. Therefore, the specifications might require that the protein and phytoestrogen content of chlorophylls (E 140(i)) should be as low as possible. The Panel also considered that the raw material should fulfil the conditions of current regulation as regards maximum levels for possible contaminants including residues of pesticide applied during cultivation and mycotoxins. Therefore, the Panel considered that the current specifications do not sufficiently cover chlorophylls derived from plants that do not have a history of safe use in humans. In addition, no data were available on the actual amounts of residual solvents in chlorophylls (E 140(i)). According to industry (NATCOL, 2011c): "*The levels for residual solvent require two revisions to accommodate for the regular presence of ethanol and methanol at levels that are not caused by the use as solvents but result from the carryover from raw materials or reactions during extraction and processing. Although their presence is not related to their use as extraction solvents, it will make sense to consider these extra amounts together with the solvent residues providing clarification by a foot note*". From further information provided by NATCOL (2014a, b, 2015), the Panel noted that the residual solvents (methanol and ethanol) in the commercial food additive are above the maximum limit indicated in the EC specifications.

The Panel considered that the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for chlorophylls (E 140(i)) should be revised in order to ascertain that chlorophylls (E 140(i)) as food additives will not be a significant source of exposure to these toxic elements in food.

The few toxicological studies that were available are for natural chlorophylls and not specifically for the food additive E 140(i), do not comply with the OECD guidelines and are not compliant with current regulatory requirements.

The Panel considered that the *in vivo* studies indicated that, at most, less than 5 % of ingested chlorophylls would be absorbed from the gastrointestinal tract of dogs or humans. Consequently, absorption and bioavailability of chlorophylls are likely to be low. Most of the ingested chlorophylls are excreted in faeces as phaeophytins. In dogs and humans, the major metabolites of chlorophylls are phaeophytins a and b; only traces of dephytylated metabolites have been observed in faeces. Based on these data, the Panel considered the cleavage of the phytol chain, resulting in the formation of chlorophyllins, during digestion of chlorophylls in humans to be unlikely.

In the only sub-chronic toxicity study available (Furukawa et al., 1998), the authors reported some minor biochemical and histopathological changes in the treated rats, but these were not dose related. However, the Panel noted that the chlorophyll extract was prepared from the boraginaceous plant comfrey, which is known to contain hepatotoxic pyrrolizidine alkaloids and that, in addition, the material tested did not comply with the specifications for chlorophylls (E 140(i)). Therefore, the Panel concluded that this study was not relevant for the assessment of chlorophylls (E 140(i)) as food additives.

No data on chronic toxicity, carcinogenicity, reproductive and developmental toxicity, or hypersensitivity were available and, therefore, it was not possible to conclude on these effects. Similarly, based on the available data, the Panel could not conclude on the genotoxicity of chlorophylls (E 140(i)).

Exposure assessments of food additives under re-evaluation are carried out by the ANS Panel based on (1) MPLs set out in EU legislation (defined as the *regulatory maximum level exposure assessment* scenario) and (2) usage or analytical data (defined as the *refined exposure assessment* scenario). It was not possible to carry out a scenario based on the MPLs set out in EU legislation, as, for all food

categories, chlorophylls (E 140(i)) are authorised according to QS. However, maximum levels of the available data were used to provide a conservative estimate scenario (noted as the *maximum level exposure assessment* scenario). It is important to mention that some data providers did not distinguish between chlorophylls (E 140(i)) and chlorophyllins (E 140 (ii)) and, therefore, for some of the usage data, there was uncertainty about whether they referred to chlorophylls (E 140(i)) or chlorophyllins (E 140(ii)). The present exposure assessment to chlorophylls (E 140(i)) could be an overestimation if the data reported are used for chlorophyllins (E 140(ii)).

To date, in the refined exposure assessment scenario, the ANS Panel have used only maximum concentration values (maximum reported use levels or maximum values from the analytical results) available for each authorised food category. However, given the range of data that have been made available through the most recent call, the ANS Panel considered that all data should be used in additional scenarios of the exposure assessment approach intended to provide more realistic exposure estimates. For chlorophylls (E 140(i)), only usage levels were available for the refined exposure assessment scenario. Based on these data, the Panel calculated two refined exposure estimates based on different assumptions: a "brand-loyal scenario", in which it is assumed that a consumer experiences long-term exposure to chlorophylls (E 140(i)) present at the maximum reported usage levels for one food category and at the mean reported usage level for the remaining food categories, and a "non-brand-loyal scenario", in which it is assumed that a consumer experiences long-term exposure to chlorophylls (E 140(i)) present at the mean reported usage levels in all relevant food categories.

Because of the above-mentioned assumptions, and the use of reported use levels, the refined exposure scenario is considered a more realistic approach than the *maximum level exposure assessment* scenario. Exposure estimates derived following the latter scenario should be considered more conservative, as this scenario assumes that the consumer will be continuously (over a lifetime) exposed to a food additive present in the food at the maximum reported use level. The Panel noted that the refined exposure estimates will not cover future changes in the level of use of chlorophylls (E 140(i)).

Only use levels reported by industry were made available to EFSA; no analytical data were provided. The data covered the majority of the food categories in which chlorophylls (E 140(i)) are authorised.

Using the "*maximum level exposure assessment scenario*", mean exposure to chlorophylls (E 140(i)) from their use as food additives ranged from 0.4 mg/kg bw/day in the elderly to 10.7 mg/kg bw/day in toddlers. The high exposure to chlorophylls (E 140(i)) using this scenario ranged from 1.1 mg/kg bw/day in the elderly to 19.3 mg/kg bw/day in toddlers (Table 8). Using the refined brand-loyal assessment exposure scenario, mean exposure to chlorophylls (E 140(i)) from their use as food additives ranged from 0.3 mg/kg bw/day in the elderly to 6.9 mg/kg bw/day in toddlers. The high exposure to chlorophylls (E 140(i)) using this scenario ranged from 0.8 mg/kg bw/day in the elderly to 15.9 mg/kg bw/day in toddlers (Table 8). Using the refined non-brand-loyal assessment exposure scenario, mean exposure to chlorophylls (E 140(i)) from their use as food additives ranged from 0.1 mg/kg bw/day in adults and the elderly to 2.7 mg/kg bw/day in toddlers. The high exposure to chlorophylls from their use as food additives using this scenario ranged from 0.2 mg/kg bw/day in the elderly to 5.0 mg/kg bw/day in toddlers (Table 8). When considering the non-brand-loyal exposure scenario, the main contributing food categories for toddlers and children were flavoured fermented milk products and fine bakery wares, whereas, for adolescents, adults and the elderly, the main contributing food categories were breakfast cereals and soups and broths (Table 11).

Considering the levels of consumption in Europe recorded in the Comprehensive Database (average minimum/maximum and 95th percentile minimum/maximum) for each vegetable, combined with concentrations from the literature, the intake of chlorophylls from the regular diet for adults ranged from 0 to 6.7 mg/kg bw/day at the mean and from 0.4 to 18.3 mg/kg bw/day for the 95th percentile. For children, the dietary exposure from the natural diet ranged from 0 to 14.7 mg/kg bw/day at the mean and from 1.4 to 34.8 mg/kg bw/day for the 95th percentile.

The Panel noted that exposure to chlorophylls (E 140(i)) resulting from their use as food additives was likely overestimated, whereas exposure from the regular diet was likely underestimated. This further supported the conclusion that exposure resulting from the use of chlorophylls (E 140(i)) as food additives is lower than the exposure to chlorophylls from the regular diet.

CONCLUSIONS

The Panel concluded that the available database for chlorophylls was inadequate for risk assessment and cannot support derivation of an ADI. However, chlorophylls are natural dietary constituents, which are present at relatively high concentrations in a number of foods. In addition, the exposure resulting from the use of chlorophylls (E 140(i)) as food additives is lower than the exposure to chlorophylls from the regular diet.

Therefore, the Panel concluded that, at the reported use levels, chlorophylls (E 140(i)) are not of safety concern as regards their use as food additives.

RECOMMENDATIONS

The Panel recommended that:

- the definition and identity of the food additive E 140(i), in particular the specifications, should be updated, as they do not include up to 90 % of the extract. The possible residual solvents should also be described.
- data on pesticides, mycotoxins and other components with biological activity (e.g. phytoestrogens, phytotoxins and allergens) should be included in the specification and kept as low as possible to avoid any potential adverse effects.
- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for chlorophylls (E 140(i)) should be revised in order to ascertain that chlorophylls (E 140(i)) as food additives will not be a significant source of exposure to these toxic elements in food.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Netherlands National Institute for Public Health and the Environment (RIVM), Netherlands, September 2008.

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. CIAA (Confederation of the Food and Drink Industries of the EU), 2011. Personal communication from CIAA on usage data of chlorophylls (E 140(i)). 18.04.2011.

4. NATCOL (Natural Food Colours Association). Reply to EFSA: Re-evaluation of food colours: call for data (7.12.06). Chlorophylls & Chlorophyllins E 140: NATCOL Submission. 3 April 2007. Corrigendum 26.04.2007.

5. NATCOL (Natural Food Colours Association), 2011a. Personal communication from NATCOL on the identity and use levels of chlorophylls (E 140(i)). 28.03.2011 and 01.04.2011.

6. NATCOL (Natural Food Colours Association), 2011b. Personal communication from NATCOL on the identity/composition of chlorophylls (E 140(i)). 24.06.2011.

7. 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.

8. NATCOL (Natural Food Colours Association), 2011d. Personal communication from NATCOL on the extraction process of chlorophylls (E 140(i)). 11.11.2011.

9. NATCOL (Natural Food Colours Association), 2012. Personal communication from NATCOL on the identity/composition, specification and manufacturing process of chlorophylls (E 140(i)). 30.03.2012.

10. NATCOL (Natural Food Colours Association), 2014a. Personal communication from NATCOL on identity/composition, specifications and analytical data of chlorophylls (E 140(i)). 14.03.2014 and 01.04.2014.

11. NATCOL (Natural Food Colours Association), 2014b. Personal communication from NATCOL on analytical data and manufacturing process of chlorophylls (E 140(i)). 18.11.2014.

12. NATCOL (Natural Food Colours Association), 2015. Personal communication from NATCOL on analytical data of chlorophylls (E 140(i)). 22.01.2015.

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14. ICGA (International Chewing Gum Association), 2013. Data on usage levels of chlorophylls (E 140(i)) 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. Submitted on 26.09.2013.

15. NATCOL (Natural Food Colours Association), 2013. Data on usage levels of chlorophylls (E 140(i)) in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Submitted on 11.10.2013.

16. Private company, 2013. Data on usage levels of chlorophylls (E 140(i)) in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Submitted on 04.07.2013.

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APPENDICES

Appendix A. Summary of the reported use levels (mg/kg or mg/L as appropriate) of chlorophylls (E 140(i)) provided by industry

FCS category number	FCS food category	MPL	Restrictions/exceptions	Reported use levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
01.4	Flavoured fermented milk products including heat-treated products	QS		5	128	500	NATCOL
01.6.3	Other creams	QS	Only flavoured creams	1	25	40	NATCOL
01.7.1	Unripened cheese excluding products falling in category 16	QS	Only flavoured unripened cheese	2	110	500	NATCOL
01.7.2	Ripened cheese	QS	Only *sage Derby cheese*	1	200	500	NATCOL
01.7.3	Edible cheese rind	QS		1	40	50	NATCOL [(a)]
01.7.4	Whey cheese	QS		1	50	300	NATCOL
01.7.5	Processed cheese	QS	Only flavoured processed cheese	3	77	500	NATCOL
01.7.6	Cheese products (excluding products falling in category 16)	QS	Only flavoured unripened products	2	23	40	NATCOL
01.8	Dairy analogues, including beverage whiteners	QS		1	200	500	NATCOL
03	Edible ices	QS		5	118	750	NATCOL, private company
04.2.2	Fruit and vegetables in vinegar, oil or brine	QS	Only vegetables (excluding olives)	1	100	400	NATCOL
04.2.4.1	Fruit and vegetable preparations excluding compote	QS	Only preserves of red fruit	1	100	300	NATCOL
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	QS	Except chestnut purée	5	36	500	NATCOL
04.2.5.3	Other similar fruit or vegetable spreads	QS	Except *crème de pruneaux*	2	25	50	NATCOL
05.2	Other confectionery including breath freshening microsweets	QS		17	56	500	NATCOL, FDE
05.3	Chewing gum	QS		5	115	500	NATCOL, ICGA
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	QS		6	468	3 000	NATCOL, FDE

FCS category number	FCS food category	MPL	Restrictions/exceptions	Reported use levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
06.3	Breakfast cereals	QS	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	1	400	800	NATCOL
06.5	Noodles	QS		1	80	104	NATCOL
06.6	Batters	QS		1	40	80	NATCOL
07.2	Fine bakery wares	QS		4	93.3	500	NATCOL, FDE
07.2	Fine bakery wares ("cake from batter" only)	QS		1	2 400	3 000	FDE
08.3.3	Casings and coatings and decorations for meat	QS	Except edible external coating of *pasturmas*	4	250	750	NATCOL
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only fish paste and crustacean paste	1	15	15	NATCOL [(a)]
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only precooked crustacean	1	15	15	NATCOL [(a)]
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only surimi and similar products and salmon substitutes	1	15	15	NATCOL [(a)]
09.3	Fish roe	QS	Except Sturgeons' eggs (Caviar)	1	15	15	NATCOL [(a)]
12.2.2	Seasonings and condiments	QS	Only seasonings, for example curry powder, tandoori	5	440	1 000	NATCOL, FDE
12.4	Mustard	QS		1	25	60	NATCOL
12.5	Soups and broths	QS		3	172	500	NATCOL
12.6	Sauces	QS	Excluding tomato-based sauces	4	88	500	NATCOL, FDE
12.7	Salads and savoury-based sandwich spreads	QS		3	50	500	NATCOL
12.9	Protein products, excluding products covered in category 01.8	QS		1	15	15	NATCOL [(a)]
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS		1	20	20	NATCOL [(a)]
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		1	20	20	NATCOL [(a)]

FCS category number	FCS food category	MPL	Restrictions/exceptions	Reported use levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
14.1.4	Flavoured drinks	QS	Excluding chocolate milk and malt products	10	16	100	NATCOL, FDE
14.2.3	Cider and perry	QS	Excluding *cidre bouché*	1	30	100	NATCOL
14.2.4	Fruit wine and made wine	QS	Excluding *wino owocowe markowe*	1	30	100	NATCOL
14.2.5	Mead	QS		1	30	100	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, to Regulation (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	1	30	100	NATCOL
14.2.7.1	Aromatised wines	QS	Except *americano, bitter vino*	1	30	100	NATCOL
14.2.7.2	Aromatised wine-based drinks	QS	Except *bitter soda, sangria, claria, zurra*	1	30	100	NATCOL
14.2.7.3	Aromatised wine-product cocktails	QS		1	30	100	NATCOL
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	QS		1	30	100	NATCOL
15.1	Potato-, cereal-, flour- or starch-based snacks	QS		4	144	500	NATCOL
15.2	Processed nuts	QS		2	52	300	NATCOL, FDE
16	Desserts excluding products covered in categories 01, 03 and 04	QS		5	168	600	NATCOL, FDE
17.1/17.2/17.3	Food supplements	QS		7	81	500	NATCOL

(a): Use levels reported in 2011 (NATCOL, 2011c).

Appendix B. Concentration levels (only usage levels available) of chlorophylls (E 140(i)) used in the maximum level and refined exposure scenarios (mg/kg or ml/kg as appropriate)

FCS category number	FCS food category	MPL	Restrictions/exceptions	Concentration levels used in maximum level exposure scenario	Concentration levels used in the refined exposure scenarios		Data source/comments
					Mean	Maximum	
01.4	Flavoured fermented milk products including heat-treated products	QS		500	128	500	
01.5	Dehydrated milk as defined by Directive 2001/114/EC	QS	Except unflavoured products	–	–	–	No data available
01.6.3	Other creams	QS	Only flavoured creams	–	–	–	Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16	QS	Only flavoured unripened cheese	–	–	–	Not taken into account (no corresponding FoodEx code)
01.7.2	Ripened cheese	QS	Only *sage Derby cheese*	–	–	–	Not taken into account (no corresponding FoodEx code)
01.7.3	Edible cheese rind	QS		–	–	–	Not taken into account (no corresponding FoodEx code)
01.7.4	Whey cheese	QS		–	–	–	Not taken into account (no corresponding FoodEx code)
01.7.5	Processed cheese	QS	Only flavoured processed cheese	500	77	500	
01.7.6	Cheese products excluding products falling in category 16	QS	Only flavoured unripened products	–	–	–	Not taken into account (no corresponding FoodEx code)
01.8	Dairy analogues, including beverage whiteners	QS		500	200	500	
03	Edible ices	QS		750	118	750	

FCS category number	FCS food category	MPL	Restrictions/exceptions	Concentration levels used in maximum level exposure scenario	Concentration levels used in the refined exposure scenarios		Data source/comments
					Mean	Maximum	
04.2.1	Dried fruit and vegetables	QS	Only preserves of red fruit	–	–	–	No data available
04.2.2	Fruit and vegetables in vinegar, oil or brine	QS	Only preserves of red fruit	–	–	–	No data available
04.2.2	Fruit and vegetables in vinegar, oil or brine	QS	Only vegetables (excluding olives)	400	100	400	
04.2.3	Canned or bottled fruit and vegetables	QS	Only preserves of red fruit	–	–	–	No data available
04.2.4.1	Fruit and vegetable preparations excluding compote	QS	Only preserves of red fruit	300	100	300	
04.2.4.1	Fruit and vegetable preparations excluding compote	QS	Only *mostarda di frutta*	–	–	–	(No data available.) 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/EC	QS	Except chestnut purée	500	36	500	
04.2.5.3	Other similar fruit or vegetable spreads	QS	Except *crème de pruneaux*	50	25	50	
05.2	Other confectionery including breath freshening microsweets	QS		500	56	500	
05.3	Chewing gum	QS		500	115	500	

FCS category number	FCS food category	MPL	Restrictions/exceptions	Concentration levels used in maximum level exposure scenario	Concentration levels used in the refined exposure scenarios		Data source/comments
					Mean	Maximum	
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	QS		–	–	–	Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	QS	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	800	400	800	
06.5	Noodles	QS		104	80	104	
06.6	Batters	QS		–	–	–	Not taken into account (no corresponding FoodEx code)
06.7	Pre-cooked or processed cereals	QS		–	–	–	(No data available.) Not taken into account (no corresponding FoodEx code)
07.2	Fine bakery wares	QS		500	93.3	500	
07.2	Fine bakery wares	QS		2 400	300	2 400	Considered for "cakes from batter" only
08.3.3	Casings and coatings and decorations for meat	QS	Except edible external coating of *pasturmas*	–	–	–	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only fish paste and crustacean paste	15	15	15	
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only precooked crustacean	15	15	15	
09.2	Processed fish and fishery products including molluscs and crustaceans	QS	Only surimi and similar products and salmon substitutes	15	15	15	

FCS category number	FCS food category	MPL	Restrictions/exceptions	Concentration levels used in maximum level exposure scenario	Concentration levels used in the refined exposure scenarios		Data source/comments
					Mean	Maximum	
09.3	Fish roe	QS	Except Sturgeons' eggs (Caviar)	15	15	15	
12.2.2	Seasonings and condiments	QS	Only seasonings, for example curry powder, tandoori	1 000	440	1 000	
12.4	Mustard	QS		60	25	60	
12.5	Soups and broths	QS		500	172	500	
12.6	Sauces	QS	Excluding tomato-based sauces	500	88	500	
12.7	Salads and savoury-based sandwich spreads	QS		500	50	500	
12.9	Protein products, excluding products covered in category 01.8	QS		15	15	15	
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS		20	20	20	
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		20	20	20	
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	QS	Including dry pasta	–	–	–	No data available

FCS category number	FCS food category	MPL	Restrictions/exceptions	Concentration levels used in maximum level exposure scenario	Concentration levels used in the refined exposure scenarios		Data source/comments
					Mean	Maximum	
14.1.4	Flavoured drinks	QS	Excluding chocolate milk and malt products	100	16	100	
14.2.3	Cider and perry	QS	Excluding *cidre bouché*	100	30	100	
14.2.4	Fruit wine and made wine	QS	Excluding *wino owocowe markowe*	–	–	–	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	QS	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1–14, to Regulation (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	100	30	100	
14.2.7.1	Aromatised wines	QS	Except *americano, bitter vino*	100	30	100	
14.2.7.2	Aromatised wine-based drinks	QS	Except *bitter soda, sangria, claria, zurra*	–	–	–	Not taken into account (no corresponding FoodEx code)
14.2.7.3	Aromatised wine-product cocktails	QS		–	–	–	Not taken into account (no corresponding FoodEx code)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	QS		100	30	100	

FCS category number	FCS food category	MPL	Restrictions/exceptions	Concentration levels used in maximum level exposure scenario	Concentration levels used in the refined exposure scenarios		Data source/comments
					Mean	Maximum	
15.1	Potato-, cereal-, flour- or starch-based snacks	QS		500	144	500	
15.2	Processed nuts	QS		300	52	300	
16	Desserts excluding products covered in categories 01, 03 and 04	QS		600	168	600	
17.1/17.2/17.3	Food supplements	QS		500	81	500	

Appendix C. Summary of total estimated exposure of chlorophylls (E 140(i)) from their use as food additives for the maximum level scenario and refined exposure scenarios per population group and survey: mean and high level (mg/kg bw/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	10.7	–	6.9	–	2.7	–
Bulgaria (NUTRICHILD)	428	2.6	6.5	2.0	4.9	0.6	1.3
Finland (DIPP)	497	3.2	10.2	2.8	9.2	0.9	2.7
Germany (DONALD_2006_2008)	261	3.1	9.2	2.2	7.1	0.8	2.4
Italy (INRAN_SCAI_2005_06)	36	2.9	–	2.2	–	0.7	–
Spain (EnKid)	17	4.1	–	3.2	–	1.0	–
The Netherlands (VCP_kids)	322	8.3	19.3	6.1	15.9	2.1	5.0
Children							
Belgium (Regional_Flanders)	625	8.5	17.9	5.5	12.5	2.2	4.8
Bulgaria (NUTRICHILD)	433	3.1	7.0	2.3	5.3	0.6	1.4
Czech Republic (SISP04)	389	4.4	9.9	2.8	6.1	1.0	2.6
Denmark (Danish_Dietary_Survey)	490	2.6	5.1	1.5	3.2	0.6	1.3
Finland (DIPP)	933	3.5	7.8	2.4	6.0	0.8	1.9
Finland (STRIP)	250	6.5	11.8	3.6	7.4	1.9	4.2
France (INCA2)	482	3.9	7.6	2.4	4.9	1.0	2.2
Germany (DONALD_2006_2008)	660	4.4	9.5	2.7	6.4	1.1	2.5
Greece (Regional_Crete)	839	3.2	7.5	2.2	5.3	0.8	2.1
Italy (INRAN_SCAI_2005_06)	193	2.0	5.2	1.5	3.4	0.4	1.1

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Latvia (EFSA_TEST)	189	5.5	11.8	3.5	7.1	1.6	3.3
Spain (enKid)	156	2.9	8.1	2.2	6.3	0.7	2.1
Spain (NUT_INK05)	399	3.3	8.3	2.2	5.6	0.8	2.2
Sweden (NFA)	1 473	6.5	12.7	3.5	7.2	1.5	3.2
The Netherlands (VCP_kids)	957	7.6	17.6	5.3	13.2	1.9	4.6
Adolescents							
Belgium (Diet_National_2004)	584	2.4	5.2	1.5	3.2	0.6	1.4
Cyprus (Childhealth)	303	0.8	1.7	0.6	1.3	0.2	0.5
Czech Republic (SISP04)	298	2.7	6.5	1.7	3.8	0.6	1.4
Denmark (Danish_Dietary_Survey)	479	1.6	3.8	1.1	2.5	0.3	0.8
France (INCA2)	973	1.9	4.3	1.2	2.8	0.5	1.3
Germany (National_Nutrition_Survey_II)	1 011	1.9	5.1	1.3	3.3	0.5	1.6
Italy (INRAN_SCAI_2005_06)	247	1.2	3.6	0.9	2.4	0.3	0.8
Latvia (EFSA_TEST)	470	3.5	7.9	2.2	5.1	1.0	2.6
Spain (AESAN_FIAB)	86	1.1	2.7	0.8	1.8	0.2	0.6
Spain (enKid)	209	1.4	4.0	1.0	2.9	0.3	0.8
Spain (NUT_INK05)	651	1.7	3.7	1.1	2.5	0.4	1.0
Sweden (NFA)	1 018	3.4	7.1	1.9	4.2	0.7	1.6
Adults							
Belgium (Diet_National_2004)	1 304	2.0	4.5	1.3	3.1	0.5	1.3
Czech Republic (SISP04)	1 666	1.2	3.2	0.8	2.1	0.3	0.8
Denmark (Danish_Dietary_Survey)	2 822	0.8	1.9	0.5	1.3	0.2	0.4
Finland (FINDIET_2007)	1 575	0.8	2.4	0.6	1.9	0.2	0.6
France (INCA2)	2 276	1.2	2.8	0.8	1.9	0.3	0.9
Germany (National_Nutrition_Survey_II)	10 419	1.6	4.0	1.1	2.8	0.4	1.3
Hungary (National_Repr_Surv)	1 074	0.6	1.5	0.4	1.1	0.1	0.3
Ireland (NSIFCS)	958	1.1	2.6	0.7	1.5	0.3	0.7
Italy (INRAN_SCAI_2005_06)	2 313	0.6	1.9	0.5	1.4	0.1	0.5
Latvia (EFSA_TEST)	1 306	2.2	4.7	1.5	3.3	0.6	1.4
Spain (AESAN)	410	1.0	2.7	0.7	1.8	0.2	0.6
Spain (AESAN_FIAB)	981	0.8	2.0	0.5	1.4	0.2	0.4
Sweden (Riksmaten_1997_98)	1 210	2.0	4.1	1.1	2.7	0.5	1.5
The Netherlands (DNFCS_2003)	750	2.4	5.2	1.5	3.3	0.6	1.4
United Kingdom (NDNS)	1 724	1.3	2.7	0.8	1.7	0.3	0.8
The elderly and very elderly							
Belgium (Diet_National_2004)	1 230	2.0	4.7	1.4	3.3	0.6	1.5

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Denmark (Danish_Dietary_Survey)	329	0.5	1.5	0.4	1.0	0.1	0.4
Finland (FINDIET_2007)	463	0.5	1.6	0.4	1.2	0.2	0.5
France (INCA2)	348	0.9	2.0	0.6	1.4	0.2	0.6
Germany (National_Nutrition_Survey_II)	2 496	1.4	3.6	1.0	2.6	0.4	1.3
Hungary (National_Repr_Surv)	286	0.4	1.1	0.3	0.8	0.1	0.2
Italy (INRAN_SCAI_2005_06)	518	0.4	1.4	0.3	1.1	0.1	0.4

ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism and Excretion
ADI	Acceptable Daily Intake
AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	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
CONTAM	Panel on Contaminants in the Food Chain
DNA	Deoxyribonucleic acid
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
EU	European Union
FAO/WHO	Food and Agriculture Organization/World Health Organization
FCS	Food Categorisation System
FDE	FoodDrinkEurope
HPLC	high-performance liquid chromatography
ICGA	International Chewing Gum Association
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MPL	maximum permitted level
NATCOL	Natural Food Colours Association
NDA	Panel on Dietetic Products, Nutrition and Allergies
OECD	Organisation for Economic Co-operation and Development
QS	*quantum satis*
SCF	Scientific Committee on Food