SCIENTIFIC OPINION

Scientific Opinion on re-evaluation of chlorophyllins (E 140(ii)) as food additives[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Chlorophyllins (E 140(i)) are obtained by saponification of a solvent extract from sources, such as grass, lucerne, and nettle, that could not be regarded as edible plant material or food for humans. Chlorophyllins represent 90 % of the colouring matter in the food additive E 140(ii); the remaining part consists of other pigments, such as carotenoids, together with oils, fats and waxes derived from the source material. The Panel noted that the material used in many studies, identified as "chlorophyllins", was quite often, if not always, a copper complex of chlorophyllins (E 141(ii)). There are no data regarding the absorption, distribution, metabolism and excretion (ADME) and toxicity of chlorophyllins (E 140(ii)). Considering the available data on chlorophylls (E 140(i)), the Panel concluded that chlorophyllins are not metabolites of chlorophylls in humans and owing to their differences in physico-chemical properties, it was not possible to support read-across for toxicity data between these two compounds. The Panel considered that it is necessary to carefully review the definition and identity of E 140(ii) in order to adequately characterise the food additive E 140(ii)) as used in the market. This will also allow proper assessment of its safety when relevant studies of the compound to which consumers are actually exposed become available. Considering the absence of relevant ADME and toxicity data, and because chlorophyllins (E 140(ii)) are neither natural constituents of the regular diet nor metabolites of chlorophylls in humans, the Panel concluded that it was not possible to assess the safety of chlorophyllins (E 140(ii)) as food additives.

KEY WORDS

Chlorophyllins, chlorophyllin a, chlorophyllin b, chlorin e6, rhodin g7, E 140(ii), food colours

---

[1] On request from the European Commission, Question EFSA-Q-2011-00371 adopted on 15 April 2015.

[2] Panel members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Younes Maged. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the former Working Group "A" Food Additives and Nutrient Sources (2011-2014) and the members of the Standing Working Group on the re-evaluation of food colours: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Maria Jose Frutos, Pierre Galtier, David Gott, Claude Lambré, Jean-Charles Leblanc, Agneta Oskarsson, Jeanne Stadler, Paul Tobback, Ine Waalkens-Berendsen and Rudolf Antonius Woutersen, for the preparatory work on this scientific opinion and EFSA staff members: Federica Lodi, Ana Maria Rincon and Alexandra Tard for the support provided to this scientific opinion. EFSA acknowledges those European competent authorities, food industry and other stakeholders that provided occurrence data (usage and analytical data) on *food additive* (E 140(ii)) in food and beverages, and supported the data collection for the Comprehensive European Food Consumption Database.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2015. Scientific Opinion on the re-evaluation of chlorophyllins (E 140(ii)) as food additives. EFSA Journal 2015;13(5):4085, 42 pp. doi:10.2903/j.efsa.2015.4085

Available online: www.efsa.europa.eu/efsajournal

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 chlorophyllins (E 140(ii)) when used as food additives.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and additional literature that has become available since then. No new toxicological or biological information was submitted to the Panel for the re-evaluation of chlorophyllins (E 140(ii)) following European Food Safety Authority (EFSA) public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation. To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of chlorophyllins (E 140(ii)), which covered the period up to the end of 2014.

Chlorophyllins (E 140(ii)) 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. Chlorophyllins (E 140(ii)) do not appear to have been evaluated previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF).

Alkali salts of chlorophyllins are obtained by saponification of a solvent extract from sources, such as grass, lucerne and nettle, that could not be regarded as regular edible plant material or food for humans. Chlorophyllins represent 90 % of the colouring matter in the food additive E 140(ii); the remaining part consists of other pigments, such as carotenoids, together with oils, fats and waxes derived from the source material. According to the Natural Food Colours Association (NATCOL, 2011d): "*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*". The Panel also considered that the raw material should fulfil the conditions of current regulation (Commission Regulation (EU) No 231/2012) as regards maximum levels for possible contaminants, including pesticides residues applied during cultivation and mycotoxins.

The Panel noted that, according to NATCOL (2011c) "*magnesium chlorophyllin is not an article of commerce and the main constituents of the material of commerce are chlorin e6 and rhodin g7*". This is in contradiction with the main constituents of E 140(ii) as defined in Commission Regulation (EU) No 231/2012.

The Panel noted that the material used in many studies, identified as "chlorophyllin", was quite often, if not always, a copper complex of chlorophyllins (E 141(ii)). In a review, Scotter (2011) stated that: "*It is important to consider that despite a joint initiative introduced by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry, a substantial body of long-established trivial names for chlorophyll and its analogues remains in popular use by both the food colour industry and scientific researchers. The term 'chlorophyllin' covers a range of compounds identical to, or structurally related to the porphyrins*".

There are no data regarding the absorption, distribution, metabolism and excretion (ADME) and toxicity of chlorophyllins (E 140(ii)).

From the available data on chlorophylls and their related derivatives, the Panel noted that, in dogs and in humans, the major metabolites of chlorophylls are phaeophytins a and b, with only traces of

dephytylated metabolites being observed in faeces. Based on these data, the Panel considered that the cleavage of the phytol chain during digestion is unlikely in humans and consequently that chlorophyllins are not metabolites of chlorophylls in humans. Enzymatic hydrolysis of the phytol chain from chlorophylls to yield chlorophyllins can occur only through a chlorophyllase, which is present only in plants and ruminants. Therefore, the Panel concluded that the available data do not support that chlorophyllins are regular metabolites of chlorophylls in humans and it was not possible to support read-across for systemic toxicity data between these two compounds.

Some usage data provided to the Panel did not distinguish between chlorophylls (E 140(i)) and chlorophyllins (E 140 (ii)) and therefore, there was uncertainty whether some usage data refer to chlorophylls (E 140(i)) or chlorophyllins (E 140(ii)). Therefore, the exposure to chlorophyllins (E 140(ii)) may possibly be overestimated.

Using the "*maximum level exposure assessment scenario*", mean exposure to chlorophyllins (E 140(ii)) from their use as food additives ranged from 0.5 mg/kg body weight (bw)/day in the elderly to 12.0 mg/kg bw/day in toddlers. The high exposure to chlorophyllins (E 140(ii)) using this scenario ranged from 1.3 mg/kg bw/day in the elderly to 20.4 mg/kg bw/day in toddlers.

Using the refined brand-loyal assessment exposure scenario, mean exposure to chlorophyllins (E 140(ii)) from their use as a food additive ranged from 0.3 mg/kg bw/day in the elderly to 7.2 mg/kg bw/day in toddlers. The high exposure to chlorophyllins (E 140(ii)) using this scenario ranged from 1.0 mg/kg bw/day in the elderly to 16.4 mg/kg bw/day in toddlers.

Using the refined non-brand-loyal assessment exposure scenario, mean exposure to chlorophyllins (E 140(ii)) from their use as a food additive ranged from 0.1 mg/kg bw/day in adults and the elderly to 3.1 mg/kg bw/day in toddlers. The high exposure from their use as a food additive using this scenario ranged from 0.3 mg/kg bw/day in the elderly to 5.4 mg/kg bw/day in toddlers. When considering this scenario, the main contributing food categories were flavoured fermented milk products and fine bakery wares for toddlers and children, whereas, for adolescents, adults and the elderly, the main contributing food categories were soups and broths and breakfast cereals.

Considering the absence of relevant ADME and toxicity data, and because chlorophyllins (E 140(ii)) are neither natural constituents of the regular human diet nor metabolites of chlorophylls in humans, the Panel concluded that it was not possible to assess the safety of chlorophyllins (E 140(ii)) as food additives. An adequate assessment of the safety of chlorophyllins (E 140(ii)) as food additives would require a sufficient toxicological database in line with its current guidance for submission for food additives evaluations (EFSA ANS Panel, 2012).

Recommendations

The Panel recommended that:

- it is necessary to carefully review the definition and identity of (E 140(ii)) in order to adequately characterise the food additive (E 140(ii)) as used in the market. This will also allow proper assessment of its safety when relevant studies using the compound to which consumers are actually exposed become available.

- the specifications should be revised in order to adequately cover chlorophyllins as they may be obtained from sources that could not be regarded as regular edible plant materials or foods (grass, lucerne, nettle) for humans. In addition, data for the raw material should be in line with the current specifications as regards maximum levels for possible contaminants, including mycotoxins and residues of pesticides applied during cultivation.

- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for chlorophyllins (E 140(ii)) should be revised in order to ascertain

that chlorophyllins (E 140(ii)) as food additives will not be a significant source of exposure to these toxic elements in food.

TABLE OF CONTENTS

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, 26.03.2010, p19
[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 chlorophyllins (E 140(ii)) when used as food additives. Chlorophyllins 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.

Chlorophyllins (E 140(ii)) were not mentioned in the 1969 evaluations on chlorophylls (E 140(i)) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and in 1975 by the Scientific Committee on Food (SCF) (JECFA, 1969; SCF, 1975).

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available since then, and data available following European Food Safety Authority (EFSA) public calls for data[9,10,11]. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation. To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of chlorophyllins (E 140(ii)), which covered the period up to the end of 2014.

2. Technical data

2.1. Identity of the substance

The food additive chlorophyllins (E 140(ii)) is a dark-green to blue/black-coloured substance with a colour index of 75815 (Commission Regulation (EU) No 231/2012).

Chlorophyllins are pigments with a porphyrin ring (tetrapyrrole ring) as basic structure. The term "chlorophyllins" refers to a mixture of various compounds obtained by alkali treatment of chlorophylls. The alkali treatment does not result in only de-esterification of the chlorophylls but may also result in the destruction of the iso-cyclic ring (cyclo-pentanone ring), leading to further degradation of the pigments (Mortensen and Greppel, 2007; Scotter, 2011).

Chlorophyllin a has the chemical formula $C_{34}H_{31}MgN_4O_6.3H$, the Chemical Abstracts Service (CAS) Registry number 15611-43-5 and molecular weight 618.97 g/mol. The systematic name is magnesate(3-), [(7*S*,8*S*)-3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-7,8-dihydro-2,8,12,17-tetramethyl-21*H*,23*H*-porphine-7-propanoato(5-)-kN^{21},kN^{22},kN^{23},kN^{24}]-, trihydrogen, (*SP*-4-2)- (SciFinder software).

Chlorophyllin b has the chemical formula $C_{34}H_{29}MgN_4O_7.3H$, CAS Registry number 13962-39-5 and molecular weight 632.95 g/mol. The systematic name is magnesate(3-), [(7*S*,8*S*)-3-carboxy-5-

[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.

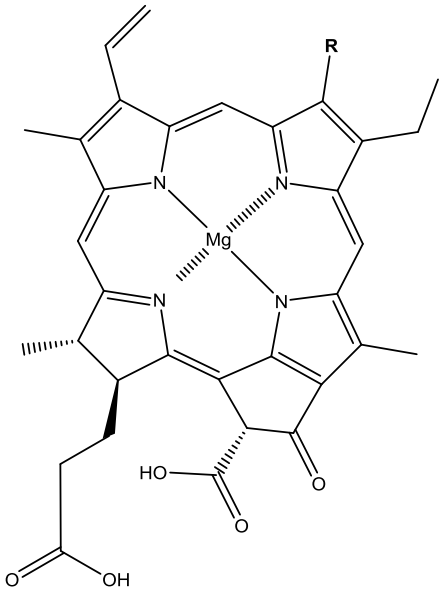
[9] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[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

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

(carboxymethyl)-13-ethenyl-18-ethyl-17-formyl-7,8-dihydro-2,8,12-trimethyl-21*H*,23*H*-porphine-7-propanoato(5-)-kN^{21},kN^{22},kN^{23},kN^{24}]-, trihydrogen, (*SP*-4-2)- (SciFinder software).

The structural formulae of chlorophyllin a and chlorophyllin b are shown in Figure 1.



R = CH_3 chlorophyllin a
R = CHO chlorophyllin b

Figure 1: Structural formulae of chlorophyllin a and chlorophyllin b

The saponification of chlorophylls also leads to the formation of chlorins (Mortensen and Greppel, 2007; Scotter, 2011). Chlorins do not contain magnesium ion (Mg^{2+}).

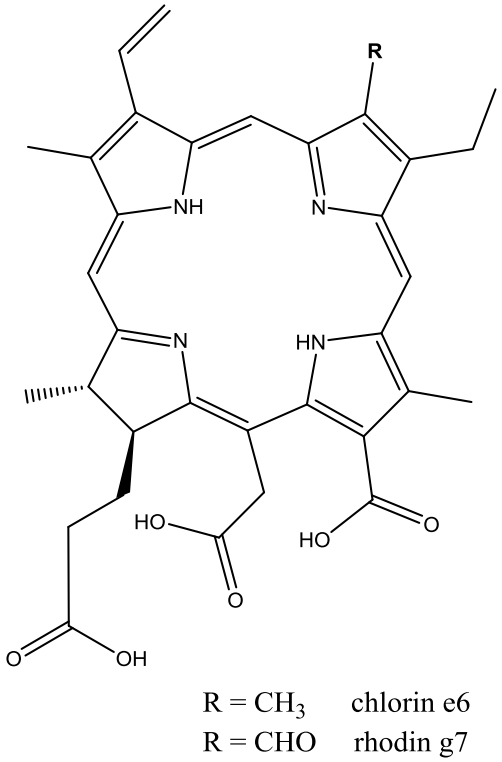
The Panel noted that the term "chlorin" in the broad sense is used to describe any (7,8-) dihydroporphin and that, by convention, four subclasses are distinguished: *rhodins*, which are chlorins of the "b" classes ("b" referring to the chlorophyll b structure); *phorbins* (or phorbides), which carry a iso-cyclic ring (cyclo-pentanone ring); *purpurins*, which have an electron-attracting group (e.g. a carbonyl group) at the γ-position; and *chlorins* (in the narrow sense), which do not belong to one of the first three classes. 7,8-Dihydroporphins, with a heterocyclic closure between the 6- and γ-position, are regarded as chlorins in this sense (Seely, 1966).

According to the Natural Food Colours Association (NATCOL, 2011b) "*On alkaline hydrolysis, chlorophyll a yields chlorin e6 and chlorophyll b yields rhodin g7. Thus a hydrolysed chlorophyll extract (E 140 (ii)) comprises: chlorin e6 as the major component together with rhodin g7 and small quantities of other derivatives of phaeophorbide. Magnesium is not present (as a chelated ion) in E 140 (ii)*". In a further communication, NATCOL reiterated that "*The two main substances are both known under the general term 'Chlorophyllins'. The previous specifications that were laid down in 1994 (based on JECFA) referred to Chlorophyllin a and Chlorophyllin b which are the first reaction products after extraction from green plant tissues. The chlorophyllins present in the food additive E 140ii are obtained by further processing: chlorin e6 is derived from Chlorophyllin a, whereas rhodin g7 is a derivative of Chlorophyllin b*" (NATCOL, 2011c).

Chlorin e_6 has the chemical formula $C_{34}H_{36}N_4O_6$, CAS Registry number 19660-77-6, European Commission (EC) number 243-209-4 and molecular weight 596.69 g/mol. The systematic name is 21*H*,23*H*-porphine-7-propanoic acid, 3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-7,8-dihydro-2,8,12,17-tretramethyl-, (7*S*,8*S*)- (SciFinder software).

Rhodin g_7 has the chemical formula $C_{34}H_{35}N4O7$, CAS Registry number 26677-12-3, EC number 247-898-2 and molecular weight 610.66 g/mol. The systematic name is 21*H*,23*H*-porphine-7-propanoic acid, 3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-17-formyl-7,8-dihydro-2,8,12-trimethyl-, (7*S*,8*S*)- (SciFinder software).

The structural formulae of the major colouring principles (chlorin e_6 and rhodin g_7) of chlorophyllins according to NATCOL (2011c) are shown in Figure 2.



R = CH_3 chlorin e6
R = CHO rhodin g7

Figure 2: Structural formulae of the main "chlorins" in the chlorophyllins (E 140(ii)) according to NATCOL (2011c)

The sodium and potassium salts of these chlorins are soluble in water (Mortensen and Greppel, 2007).

According to NATCOL (2011b), "*Na/K chlorophyllin is a water soluble colorant. Aqueous solutions containing in excess of 20 % chlorophyllin can be obtained. Na/K chlorophyllin is not soluble in non-polar solvents. Acids will precipitate the free acid from an aqueous solution*".

The most common synonyms of chlorophyllins are sodium chlorophyllin, potassium chlorophyllin and CI natural green 5. The Panel noted that Commission Regulation (EU) No 231/2012 indicates that CI natural green 5 is also a synonym for copper chlorophyllins, which is not consistent.

According to NATCOL (2011b) "*Magnesium chlorophyllin will readily lose its magnesium ion in acidic aqueous foods to yield the corresponding phaeophytin, with a consequential change in its colour shade. The magnesium being replaced by two protons. For this reason magnesium chlorophyllin is not an article of commerce*".

The Panel noted that there is a difference between the identity of the major colouring principles as described by EC specifications (Commission Regulation (EU) No 231/2012) and by NATCOL. The Panel also noted that the substances described as major colouring principles by the EC specifications correspond to the acidic form (carboxylic acid); however, according to the definition in the same EC specifications, the acid groups are neutralised to form the sodium or potassium salts.

The Panel has summarised the relevant information on the major colouring principles of chlorophyllins and considered that the EC specifications should be updated accordingly (Table 1).

Table 1: Identity data of the major colouring principles of chlorophyllins

	Name	Molecular formula	Molecular weight (g/mol)	CAS number [(a)]	EC number [(b)]	Colour index number	Chemical name [(a)]
	Chlorophyllins	N/A [(c)]	N/A [(c)]	85536-03-4	287-483-3	75815	Chlorophyllins
Major colouring principles	Chlorophyllin a	$C_{34}H_{31}MgN_4O_6.3H$ [(d)]	618.97	15611-43-5	–	–	Magnesate(3-), [(7*S*,8*S*)-3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-7,8-dihydro-2,8,12,17-tetramethyl-21*H*,23*H*-porphine-7-propanoato(5-)-kN^{21},kN^{22},kN^{23},kN^{24}]-, trihydrogen, (*SP*-4-2)-
	Chlorophyllin b	$C_{34}H_{29}MgN_4O_7.3H$ [(d)]	632.95	13962-39-5	–	–	Magnesate(3-), [(7*S*,8*S*)-3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-17-formyl-7,8-dihydro-2,8,12-trimethyl-21*H*,23*H*-porphine-7-propanoato(5-)-kN^{21},kN^{22},kN^{23},kN^{24}]-, trihydrogen, (*SP*-4-2)-
	Chlorin e_6	$C_{34}H_{36}N_4O_6$	596.69	19660-77-6	243-209-4		21*H*,23*H*-Porphine-7-propanoic acid, 3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-7,8-dihydro-2,8,12,17-tretramethyl-, (7*S*,8*S*)-
	Rhodin g_7	$C_{34}H_{35}N_4O_7$	611.66	26677-12-3	247-898-2	–	21*H*,23*H*-Porphine-7-propanoic acid, 3-carboxy-5-(carboxymethyl)-13-ethenyl-18-ethyl-17-formyl-7,8-dihydro-2,8,12-trimethyl-, (7*S*,8*S*)-
	C-13 (2) - carboxypyrophe ophorbide	$C_{34}H_{34}N_4O_5$	578.68	72939-69-6	277-114-4		3-Phorbinepropanoic acid, 21-carboxy-9-ethenyl-14-ethyl-4, 8,13,18-tetramethyl-20-oxo-, (3*S*,4*S*,21*R*)
		$C_{34}H_{32}N_4O_6$	592.66	72939-70-9	277-116-5	–	3-Phorbinepropanoic acid, 21-carboxy-9-ethenyl-14-ethyl-13-formyl-4,8,18-trimethyl-20-oxo-, (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.
(d): Molecular formula refers to the possibility that carboxyl groups in the substance can be in acid or carboxylate form.

According to Commission Regulation (EU) No 231/2012, chlorophyllins (E 140(ii)) are obtained by the saponification of a solvent extract (chlorophylls) of strains of edible plant materials, grass, lucerne and nettle. NATCOL (NATCOL, 2011c) provided information on the most commonly used raw materials for the production of chlorophylls in Europe (EFSA ANS Panel, 2015).

2.2. Specifications

Specifications for chlorophyllins (E 140(ii)) have been defined in Commission Regulation (EU) No 231/2012 (Table 2). There are no JECFA specifications for chlorophyllins.

Table 2: Specifications for chlorophyllin(s) according to Commission Regulation (EU) No 231/2012

Assay	Content of total chlorophyllins is not less than 95 % of the sample dried at ca 100 °C for 1 hour.
Solvent residues	
Acetone	≤ 50 mg/kg singly or in combination
Methanol	
Ethanol	
Propan-2-ol	
Hexane	
Methyl ethyl ketone	
Dichloromethane	≤ 10 mg/kg
Arsenic	≤ 3 mg/kg
Lead	≤ 10 mg/kg
Mercury	≤ 1 mg/kg
Cadmium	≤ 1 mg/kg

NATCOL informed EFSA that chlorophyllins (E 140(ii)) are not manufactured as aluminium lakes (NATCOL, 2011a).

The Panel noted that according to the EU specifications for chlorophyllins, impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to a concentration of 3, 10, 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, 2009; 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 chlorophyllins (E 140(ii)) should be revised in order to ascertain that chlorophyllins (E 140(ii)) as food additive will not be a significant source of exposure to these toxic elements in food.

As previously mentioned, the Panel considered that the EC specifications should be updated according to the relevant information on the major colouring principles of chlorophyllins (Table 1).

2.3. Manufacturing process

The alkali salts of chlorophyllins are obtained by saponification of chlorophylls (E 140(i)), which removes the methyl and phytol ester groups and may partially cleave the cyclopentenyl ring. The acid groups are neutralised to form the potassium and/or sodium salts. Only the following solvents may be used for the extraction: acetone, methyl ethyl ketone, dichloromethane, carbon dioxide, methanol, ethanol, propan-2-ol and hexane.

2.4. Methods of analysis in foods

Chlorophyllins may be analysed by an ultraviolet–visible rapid spectrophotometric method (Minguez-Mosquera et al., 1990). Pigment separation is carried out using thin-layer chromatography, and the

spots scraped from the plate and eluted with ether or acetone. High-performance liquid chromatography (HPLC) can be also used for the separation, identification and quantitation of chlorophyll analogues using the photodiode array and fluorimetric detectors, with the reverse-phase HPLC as the most widely used technique (Almela et al., 2000; Scotter et al., 2005). This method has higher resolution, speed, reproducibility and sensitivity than other techniques (Schoefs, 2004). The mass spectrometry methods allow isolation and structure elucidation of chlorophyll allomers and their derivatives (Schoefs, 2002). However, there are no validated methods for the analysis of chlorophyllins in foods that might be used for official purposes (Scotter, 2011).

2.5. Reaction and fate in foods

No data on the reaction and fate of chlorophyllins in food were available.

2.6. Case of need and proposed uses

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

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

Table 3 summarises foods that are permitted to contain chlorophyllins (E 140(ii)), as set by Annex II to Regulation (EC) No 1333/2008.

Table 3: MPLs of chlorophyllins (E 140(ii)) in foods according to the 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 140ii	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*

[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)
04.2.1	Dried fruit and vegetables	E 140ii	Only preserves of red fruit	*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 140ii	Only preserves of red fruit	*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 140ii	Only vegetables (excluding olives)	*Quantum satis*
04.2.3	Canned or bottled fruit and vegetables	E 140ii	Only preserves of red fruit	*Quantum satis*
04.2.4.1	Fruit and vegetable preparations excluding compote	E 140ii	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 140ii	Except chestnut purée	*Quantum satis*
04.2.5.3	Other similar fruit or vegetable spreads	Group II	Except *crème de pruneaux*	*Quantum satis*
05.2	Other confectionery including breath freshening microsweets	Group II		*Quantum satis*
05.3	Chewing gum	Group II		*Quantum satis*
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	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 140ii	Only fish paste and crustacean paste	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 140ii	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*

FCS category number	Foods	E number/group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
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 1.8	Group II		*Quantum satis*
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group II		*Quantum satis*
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group II		*Quantum satis*
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) 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 of 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*

FCS category number	Foods	E number/group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
14.2.7.3	Aromatised wine-product cocktails	Group II		*Quantum satis*
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	Group II		*Quantum satis*
15.1	Potato-, cereal-, flour- or starch-based snacks	Group II		*Quantum satis*
15.2	Processed nuts	Group II		*Quantum satis*
16	Desserts excluding products covered in categories 01, 03 and 04	Group II		*Quantum satis*
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group II		*Quantum satis*
17.2	Food supplements supplied in a liquid form	Group II		*Quantum satis*
17.3	Food supplements supplied in a syrup-type or chewable form	Group II		*Quantum satis*

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

2.7. Reported use levels of chlorophyllins (E 140(ii)) in food

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

In 2006, EFSA launched a public call[13] for scientific data on food colours, including chlorophyllins (E 140(ii)), 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, but none on chlorophyllins, 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] setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC), EFSA launched a public call[15] for food additives usage level and/or

[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: thttp://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 from: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

concentration data in food and beverages intended for human consumption in March 2013, with a deadline in September 2013. Data on chlorophyllins (E 140(ii)), including present use and use patterns (i.e. which food categories and subcategories contain the additive, proportion of foods within categories/subcategories in which it is used and actual use levels (typical and maximum)), were requested from relevant stakeholders. European food manufacturers, national food authorities, research institutions, academics, food business operators and any other interested stakeholders were invited to submit usage and/or concentration data on chlorophyllins (E 140(ii)) 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 chlorophyllins (E 140(ii)) in food have been submitted by industry. No analytical data have been provided for chlorophyllins (E 140(ii)).

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

Industry provided EFSA with data on use levels (n = 108) of chlorophyllins (E 140(ii)) in foods for 40 out of the 56 food categories in which chlorophyllins (E 140(ii)) are authorised.

Updated information on the actual use levels of chlorophyllins in foods was made available to EFSA by NATCOL, FoodDrinkEurope (FDE) and the International Chewing Gum Association (ICGA). More data were provided for the food category other confectionary (FCS 05.2).

Appendix A provides data on the use levels of chlorophyllins (E 140(ii)) in foods as reported by industry.

2.8. Information on existing authorisations and evaluations

Chlorophyllins (E 140(ii)) are authorised as food additives in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives. Chlorophylls (E 140(i)) were evaluated previously by JECFA in 1969 (JECFA, 1969) and the SCF in 1975 and 1983 (SCF, 1975, 1984), but chlorophyllins (E 140(ii)) were not mentioned in these evaluations.

2.9. Exposure assessment

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 outline in Table 4.

Table 4: Population groups considered for the exposure estimates of chlorophyllins (E 140(ii))

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Italy, the Netherlands, 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, 2011a).

Consumption records were codified according to the FoodEx food classification system (EFSA, 2011b). Nomenclature from the FoodEx food classification system has been linked to the FCS as presented in Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories and the exposure was calculated by multiplying values reported in Appendices B for each food category by their respective consumption amount per kilogram of body weight (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. 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 chlorophyllins (E 140(ii))

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

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

- 01.6.3. Other creams, only flavoured creams
- 01.7.3. Edible cheese rind
- 01.7.6. Cheese products (excluding products falling in category 16), only flavoured unripened products
- 04.2.4.1. Fruit and vegetable preparations excluding compote, only *mostarda di frutta*

- 05.4. Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4
- 06.6. Batters
- 06.7. Pre-cooked or processed cereals
- 08.3.3. Casings and coatings and decorations for meat, except edible external coating of *pasturmas*
- 14.2.4. Fruit wine and made wine
- 14.2.5. Mead

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

For the following food categories, the restrictions, which apply to the use of chlorophyllins (E 140(ii)), could not be taken into account, and therefore the whole food category was considered for the exposure estimates. This results in an overestimation of the exposure:

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

Added to these, food categories for which no reported use levels were available were not considered in the exposure assessment. This concerns 14 food categories, which are presented in Appendix C. The Panel noted that if chlorophyllins are nevertheless used in those food categories for which reported use levels were not available, the calculated refined exposure assessment might result in underestimation of exposure to chlorophyllins.

Overall, in the current exposure estimate, 11 food categories out of 56 were not taken into account in the exposure assessment because these are not referenced in the EFSA Comprehensive Database, and 14 food categories were not included in the exposure assessment because of the lack of data (two food categories belonging to both). Thus, in the current exposure estimate, 23 food categories out of 56 are not taken into account.

2.9.2. Exposure to chlorophyllins (E 140(ii)) from its use as food additives

Dietary exposure to chlorophyllins (E 140(ii)) 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 4), and with the limitations described above.

Exposure assessment to chlorophyllins (E 140(ii)) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment* scenario); and (2) reported use levels (defined as the *refined exposure assessment* scenario) as provided by industry.

2.9.2.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation No 1333/2008 and listed in Table 2. As no MPLs are set for chlorophyllins, a *maximum level exposure assessment* scenario has been performed based on the maximum levels of 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 chlorophyllins present in the food at the maximum levels.

2.9.2.2. Refined exposure assessment scenario

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

Appendix C summarises the concentration levels of chlorophyllins (E 140(ii)) used in the refined exposure assessment scenario. Based on the available dataset, the Panel calculated two estimates based on different model populations:

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

2.9.2.3. Anticipated exposure to chlorophyllins (E 140(ii))

Table 5 summarises the estimated exposure to chlorophyllins (E 140(ii)) from their use as a food additive of all five population groups (Table 4). Detailed results by population group and survey are presented in Appendix D.

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

Table 5: Summary of anticipated exposure to chlorophyllins (E 140(ii)) from their use as a food additive using the maximum level exposure assessment scenario and refined exposure scenarios, in five population groups (minimum–maximum across the dietary surveys in mg/kg bw/day)

	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario					
Mean	2.7–12.0	2.7–9.7	1.0–3.6	0.6–2.6	0.5–2.3
High level (95th percentile)	7.1–20.4	5.7–19.6	2.3–7.5	1.6–5.5	1.3–5.1
Refined estimated exposure assessment scenario					
Brand-loyal scenario					
Mean	2.1–7.2	1.6–6.0	0.7–2.2	0.4–1.6	0.3–1.5
High level (95th percentile)	5.3–16.4	3.3–13.6	1.5–4.8	1.1–3.4	1.0–3.4
Non-brand-loyal scenario					
Mean	0.6–3.1	0.6–2.6	0.3–0.9	0.1–0.7	0.1–0.7
High level (95th percentile)	1.6–5.4	1.3–5.3	0.7–2.0	0.4–1.5	0.3–1.6

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

Table 6: Main food categories contributing to exposure to chlorophyllins (E 140(ii)) using maximum usage levels (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [a]				
01.4	Flavoured fermented milk products including heat-treated products	9.2–67.2 (7)	8.8–40.3 (13)	7.3–21.2 (9)	6.8–42.8 (12)	5.7–27.8 (7)
01.7.1	Unripened cheese excluding products falling in category 16	6.0–8.4 (4)	6.6–18.5 (4)	5.5–21 (2)	5.7–27.7 (6)	7.6–25.9 (4)
01.7.5	Processed cheese	12.8–15.9 (2)				7.3 (1)
01.8	Dairy analogues, including beverage whiteners	5.1 (1)				
03	Edible ices	5.7–24.7 (4)	5.4–23.5 (14)	5.7–18.3 (11)	5.1–15.0 (11)	5.2–10.6 (4)
04.2	Processed fruit and vegetables				6.2–7.1 (2)	7.2–13.6 (5)
05.2	Other confectionery including breath freshening microsweets		5.7–8.8 (2)	5.3–10 (2)	5.7–9 (2)	
06.3	Breakfast cereals	5.5–9.9 (3)	5–23.1 (11)	5.9–26.7 (9)	5.8–23.9 (7)	5.1–33.5 (3)
07.2	Fine bakery wares	9.1–57 (6)	10.5–51.6 (13)	14.7–39.2 (11)	7.8–37.8 (14)	11.9–31.7 (6)
12.2	Herbs, spices, seasonings				5.5 (1)	5.7–5.7 (1)
12.5	Soups and broths	5.2–21.0 (3)	5.4–36.6 (6)	5.8–35.5 (5)	5.3–41.5 (7)	24.9–44.1 (2)
12.6	Sauces		5.9–8.7 (5)	6.2–14.4 (7)	5.2–14.8 (11)	6.1–10.1 (3)
12.7	Salads and savoury-based sandwich spreads		8.2–9.8 (2)	12.0 (1)	10.6–18.7 (2)	

FCS category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
14.1.4	Flavoured drinks	5.1–13.7 (4)	5.8–24.9 (11)	5.3–37 (12)	7.3–33.6 (13)	12.1–24.3 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks	7.8 (1)		8.1–9.1 (2)		
16	Desserts excluding products covered in category 1, 3 and 4	5.3–16.7 (3)	6.2–14.2 (5)	5.9–11.7 (2)	8.6–9.8 (2)	11.9 (1)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

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

Table 7: Main food categories contributing to exposure to chlorophyllins (E 140(ii)) 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 including heat-treated products	10.2–78.6 (7)	8.5–57.4 (13)	5.7–32.3 (9)	5.8–55.4 (13)	9.4–35.6 (6)
01.7.1	Unripened cheese excluding products falling in category 16	5.9–7.8 (2)	5.7–16.1 (3)	20.2 (1)	7.8–30 (3)	5.6–27.9 (4)
01.7.5	Processed cheese	8.6–20.3 (2)	5.5 (1)			
03	Edible ices	5.1–29.7 (3)	5.5–30.7 (12)	6.9–23.3 (8)	5.8–16.2 (6)	5.8–11.5 (3)
04.2	Processed fruit and vegetables					5.7–9.0 (2)
05.2	Other confectionery including breath freshening microsweets			5.3 (1)	5.5 (1)	
06.3	Breakfast cereals	5.0–8.6 (3)	6.1–38.6 (10)	6.0–31.9 (9)	5.4–39.0 (8)	5.8–38.8 (3)
07.2	Fine bakery wares	5.0–67.8 (6)	6.3–63.8 (13)	14.6–51.8 (11)	6.6–44.7 (14)	10.4–41.2 (6)
12.2	Herbs, spices, seasonings				6.7 (1)	6.5 (1)
12.5	Soups and broths	5.4–26.2 (3)	5.7–52.6 (6)	5.6–52.2 (6)	6.8–57.2 (7)	34.4–63.2 (2)
12.6	Sauces			5.0–12 (4)	5.2–11.0 (4)	5.4–5.9 (2)
12.7	Salads and savoury-based sandwich spreads		5.1–6.9 (2)	8.9 (1)	6.7–15.8 (2)	
14.1.4	Flavoured drinks	12.0 (1)	8.2–30.2 (6)	5.8–48.6 (11)	6.8–39.2 (13)	11.7–24.6 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks			5.8–7.8 (2)		

FCS category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
16	Desserts excluding products covered in category 1, 3 and 4	13.5–15.8 (2)	6.6–15.6 (3)	5.7–10.9 (2)	9.0–9.2 (2)	13.4 (1)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Table 8: Main food categories contributing to exposure to chlorophyllins (E 140(ii)) using the non-brand-loyal refined exposure scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	Foods	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]				
01.4	Flavoured fermented milk products, including heat-treated products	9.8–62.3 (7)	8.8–44.9 (13)	7.2–23.3 (9)	6.9–42.9 (12)	5.1–27.2 (7)
01.7.1	Unripened cheese excluding products falling in category 16	5.0–7.9 (4)	5.5–17.9 (4)	20.4 (1)	5.5–26.2 (5)	6.5–24.7 (4)
01.7.5	Processed cheese	8.1–9.5 (2)				
01.8	Dairy analogues, including beverage whiteners	7.3 (1)	5.2 (1)			
03	Edible ices	13.1 (1)	5.9–12.1 (8)	5.3–10 (4)	6.4–7.5 (3)	5.3–5.9 (2)
06.3	Breakfast cereals	5.2–17.9 (5)	5.2–40.3 (14)	5.8–44.2 (12)	5.8–43.1 (14)	5.2–54.7 (5)
07.2	Fine bakery wares	8.6–58.7 (6)	9.8–56 (13)	5.1–38.3 (12)	8.6–38.7 (14)	11.9–34.9 (6)
12.2	Herbs, spices, seasonings	5.3 (1)		7.2 (1)	10.8 (1)	11.2 (1)
12.5	Soups and broths	6.9–27.7 (3)	6.3–45.2 (7)	5.5–46.5 (6)	7.4–55.2 (7)	33.5–53.2 (2)
12.6	Sauces		5.2–5.3 (2)	5.1–9.9 (5)	6.0–9.1 (6)	6.1–8 (2)
12.7	Salads and savoury-based sandwich spreads				7.2 (1)	
14.1.4	Flavoured drinks	5.2–12.6 (4)	5.5–25.5 (11)	8.2–37.9 (11)	7.3–33.5 (13)	13.1–25.3 (2)
15.1	Potato-, cereal-, flour- or starch-based snacks	9.4 (1)	5.5 (1)	8.6–9.3 (2)		
16	Desserts excluding products covered in category 1, 3 and 4	5.7–18.2 (3)	6.2–15.5 (5)	6.9–12.6 (2)	9.2–11.6 (2)	14.8 (1)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

2.10. Uncertainty analysis

Uncertainties in the exposure assessment of chlorophyllins (E 140(ii)) have been discussed above. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the sources of uncertainties considered are summarised in Table 9.

Table 9: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate to chlorophyllins (E 140 (ii))

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: no data for some food categories (12 out of 56 food categories)	–
Use levels: levels considered applicable for all items within the entire food category	+
Use levels: uncertainty whether the reported use levels provided by industry refer to chlorophylls or chlorophyllins	+
Brand-loyal exposure model: exposure calculations based on the maximum reported use levels for one food category and mean reported use levels for the remaining food categories	+/–
Non-brand-loyal exposure model: exposure calculations based on the mean reported use levels	+/–

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

Considering that the uncertainties identified and that missing data correspond to food categories where chlorophyllins are authorised but not used, the Panel assumed that the exposure assessment would tend to overestimate the real exposure of chlorophyllins (E 140(ii)) as food additives in European countries.

3. Biological and toxicological data

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and additional literature that has become available since then. No new toxicological or biological information was submitted to the Panel for the re-evaluation of chlorophyllins following EFSA public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

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 chlorophyllins (E 140(ii)).

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

There are no specific data regarding the ADME of chlorophyllins (E 140(ii)).

Given that chlorophylls are compounds structurally related to chlorophyllins, the possibility of using the available chlorophylls data for read across for chlorophyllins was investigated.

The absorption of chlorophyll derivatives was investigated in dogs fed a diet containing 73 mg chlorophyll/kg diet (Fernandes et al., 2007). The apparent absorption of the chlorophyll derivatives assessed 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 seems to evidence either their low absorption or their quick metabolisation. Chlorophylls a and b were transformed into their corresponding phaeophytins during gastrointestinal passage. Beyond phaeophytinisation, no other important degradation occurred. By HPLC analysis of excreta, as identified by its retention time and spectrum, only a trace amount of dephytylated phaeophorbide a was observed. Based on these results and those of previous studies, the authors considered that the cleavage of the phytol chain during digestion in dogs was unlikely.

In humans given encapsulated crystalline chlorophyll (100 mg/day for 4 days), the decomposition of the chlorophyll in the body was quantitatively estimated in faeces by analysis of four analytically defined fractions. The major part (percentage) of the decomposed chlorophyll corresponded to faecal phaeophytin (Brugsch and Sheard, 1938).

The Panel considered that, in dog and in humans, the major metabolites of chlorophyll are the phaeophytins a and b, only traces of dephytylated metabolites being observed in faeces. Based on these data, the Panel considered that the cleavage of the phytol chain during digestion is unlikely in humans.

Overall, the Panel considered that chlorophyllins are not metabolites of chlorophylls in humans and owing to the differences in their physico-chemical properties, it was not possible to support read-across for systemic toxicity data between these two compounds.

3.2. Toxicological data

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/kg bw (Heinrichs et al., 1954). 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 either chlorophyllins (E 140(ii)) or copper complexes of chlorophyllins (E 141(ii)).

3.2.2. Short-term and sub-chronic studies

There were no short-term and subchronic studies available for chlorophyllins (E 140(ii)).

3.2.3. Genotoxicity

There are some publications which seemed to be studies of chlorophyllins (E 140(ii)); however, the Panel noted that, in these studies, either copper complexes of chlorophyllins (E 141(ii)) was used or the identity of the substance tested was not clear.

3.2.4. Chronic toxicity and carcinogenicity

No studies were available on the chronic toxicity and carcinogenicity of chlorophyllins (E 140(ii)).

3.2.5. Reproductive and developmental toxicity

No data on reproductive and developmental toxicity studies were available for chlorophyllins (E 140(ii)).

3.2.6. Hypersensitivity, allergenicity and intolerance

No cases of allergy to chlorophyllins (E 140(ii)) have been identified in the literature.

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

Chlorophyllins (E 140(ii)) 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. Chlorophyllins (E 140(ii)) do not appear to have been evaluated previously by JECFA and the SCF.

Alkali salts of chlorophyllins are obtained by saponification of a solvent extract from sources such as grass, lucerne and nettle. The Panel noted that not all sources used for the production of chlorophylls are part of the human regular diet. Chlorophyllins represent 90 % of the colouring matter in the food additive (E 140(ii)), the remaining part being other pigments, such as carotenoids, together with oils, fats and waxes derived from the source material. According to NATCOL (2011d): "*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*". Therefore, the Panel noted that these revisions should be considered in the current EC specifications. 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 pesticides applied during cultivation and mycotoxins.

The Panel noted that, according to NATCOL (2011c), "*magnesium chlorophyllin is not an article of commerce and the main constituents of the material of commerce are chlorin e_6 and rhodin g_7*". This is in contradiction with the main constituents of E 140(ii) as defined in Commission Regulation (EU) No 231/2012.

The Panel noted that the material used in many studies, and identified as "chlorophyllin", was often, if not always, a copper complex of chlorophyllins (E 141(ii)). In a review, Scotter (2011) stated that: "*It is important to consider that despite a joint initiative introduced by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry, a substantial body of long-established trivial names for chlorophyll and its analogues remains in popular use by both the food colour industry and scientific researchers. The term 'chlorophyllin' covers a range of compounds identical to, or structurally related to the porphyrins*".

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

There are no data regarding the ADME and toxicity of chlorophyllins (E 140(ii)).

From the available data on chlorophylls and their related derivatives, the Panel noted that, in dogs and in humans, the major metabolites of chlorophylls are phaeophytins a and b, and only traces of dephytylated metabolites being observed in faeces. Based on these data, the Panel considered that the cleavage of the phytol chain during digestion was unlikely in humans. Enzymatic hydrolysis of the phytol chain from chlorophylls to yield chlorophyllins can occur only through a chlorophyllase, which is present only in plants and ruminants. Therefore, the Panel concluded that the available data do not support that chlorophyllins are regular metabolites of chlorophylls in humans and, owing to the differences in their physico-chemical properties, the Panel considered that it was not possible to

support read-across of systemic toxicity data between chlorophyllins (E 140(ii)) and chlorophylls (E 140(i)).

In the refined exposure assessment scenario the ANS Panel 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 calls, 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 chlorophyllins (E 140(ii)), 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" and a "non-brand-loyal scenario".

The Panel noted that the refined exposure estimates will not cover future changes in the level of use of chlorophyllins (E 140(ii)). Only use levels reported by industry were made available to EFSA; no analytical data were provided. These data covered the majority of the food categories in which chlorophyllins (E 140(ii)) are authorised. 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 refer to chlorophylls (E 140(i)) or chlorophyllins (E 140(ii)). The present exposure assessment to chlorophyllins (E 140(ii)) could be an overestimation if the data reported are used for chlorophylls (E 140(i)).

The Panel considered that chlorophyllins (E 140(ii)) are not regular constituents of the regular human diet and that, given that they are not metabolites of chlorophylls in humans and that they have different physical and chemical properties from chlorophylls, read across was not possible. Therefore, in the absence of biological and toxicological data, it was not possible to assess the safety of chlorophyllins (E 140(ii)).

CONCLUSION

Considering the absence of relevant ADME and toxicity data, and because chlorophyllins (E 140(ii)) are neither natural constituents of the regular human diet nor metabolites of chlorophylls in humans, the Panel concluded that it was not possible to assess the safety of chlorophyllins (E 140(ii)) as food additives. An adequate assessment of the safety of chlorophyllins as a food additive would require a sufficient toxicological database in line with its current guidance for submission for food additives evaluations (EFSA ANS Panel, 2012).

RECOMMENDATIONS

The Panel recommended that:

- it is necessary to carefully review the definition and identity of (E 140(ii)) in order to adequately characterise the food additive (E 140(ii)) as used in the market. This will also allow proper assessment of its safety when relevant studies using the compound to which consumers are actually exposed become available.
- the specifications should be revised to adequately cover chlorophyllins as they may be obtained from sources that could not be regarded as regular edible plant materials or foods (grass, lucerne, nettle) for humans. In addition, data on the raw material should be in line with the current specifications as regards maximum levels for possible contaminants, including mycotoxins and residues of pesticides applied during cultivation.
- the maximum limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in the EC specification for chlorophyllins (E 140(ii)) should be revised in order to ascertain that chlorophyllins (E 140(ii)) 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 (December 2009). 14.12. 2009.

3. CIAA (Confederation of the Food and Drink Industries of the EU), 2011. Personal communication from CIAA on usage data of chlorophyllins (E 140(ii)). 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, uses and use levels of chlorophyllins (E 140(ii)). 28.03.2011 and 01.04.2011.

6. NATCOL (Natural Food Colours Association), 2011b. Personal communication from NATCOL on the identity of chlorophyllins (E 140(ii)). 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 specifications for chlorophyllins (E 140(ii)). 16.09.2011.

9. FDE (FoodDrinkEurope). Data on usage levels of chlorophyllins (E 140(ii)) in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 13.09.2013.

10. ICGA (International Chewing Gum Association). Data on usage levels of chlorophyllins (E 140(ii)) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 26.09.2013.

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

REFERENCES

Almela L, Fernández-López JA and Roca MJ, 2000. High-performance liquid chromatographic screening of chlorophyll derivatives produced during fruit storage. Journal of Chromatography A, 870, 483–489.

Brugsch JT and Sheard C, 1938 Chlorophyll, determination and quantitative estimation of decomposition of chlorophyll in the human body. Journal of Laboratory and Clinical Medicine (St. Louis), 24, 230–234.

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. The EFSA Journal 2006, 438, 1–54.

EFSA (European Food Safety Authority), 2009. Scientific opinion of the Panel on Contaminants in the Food Chain (CONTAM) on cadmium in food. The EFSA Journal 2009, 980, 1–139.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009. Scientific opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. doi:10.2903/j.efsa.2009.1351

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010. Scientific opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. doi:10.2903/j.efsa.2010.1570

EFSA (European Food Safety Authority), 2010. Standard sample description for food and feed. EFSA Journal 2010;8(1):1457, 54 pp. doi:10.2903/j.efsa.2010.1457

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. doi:10.2903/j.efsa.2011.1970

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. doi:10.2903/j.efsa.2012.2985

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. doi:10.2903/j.efsa.2012.2760

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2015. Scientific Opinion on the re-evaluation of chlorophylls (E 140(i)) as food additives. EFSA Journal 2015;13(5):4089, 56 pp. doi:10.2903/j.efsa.2015.4089

Fernandez TM, Gomes BB and Lanfer-Marquez UM, 2007. Apparent absorption of chlorophyll from spinach in an assay with dogs. Innovative Food Science and Emerging Technologies, 8, 426–432.

Heinrichs D, Rummel W and Schunk R, 1954. Zur Pharmakologic des chlorophylls. Arzneimittel Forschung, 4, 19–20.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1969. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anti-caking agents and certain other substances. FAO Nutrition Meetings Report Series No. 46A WHO/FOOD ADD/70.36. Available online: http://www.inchem.org/documents/jecfa/jecmono/v46aje03.htm

Minguez-Mosquera MI, Garrido-Fernández J and Gandul-Rojas B, 1990. Quantification of pigments in fermented manzanilla and hojiblanca olives. Journal of Agricultural and Food Chemistry, 36, 1662–1666.

Mortensen A and Greppel A, 2007. HPLC-MS analysis of the green food colorant sodium copper chlorophyllin. Innovative Food Science and Emerging Technologies, 8, 419–425.

SCF (Scientific Committee for Food), 1975. Reports of the Scientific Committee for Food (First series). Opinion expressed on 27 June 1975. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_01.pdf

Schoefs B, 2002. Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. Trends in Food Science and Technology, 13, 361–371.

Schoefs B, 2004. Determination of pigments in vegetables. Journal of Chromatography A, 1054, 217–226.

Scotter MJ, 2011. Methods for the determination of European Union-permitted added natural colours in foods: a review. Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment, 28, 527–596.

Scotter MJ, Castle L and Roberts D, 2005. Method development and HPLC analysis of retail foods and beverages for copper chlorophyll (E141 (i)) and chlorophyllins (E 141 (ii)) food colouring materials. Food Additives and Contaminants, 22, 1163–1175.

Seely GR, 1966. The structure and chemistry of functional groups. In: The Chlorophylls. Physical Chemical, and Biophysical properties. Eds Vernon LP and Seele GR. Academic Press, NY, USA and London, UK, 76–103.

APPENDICES

Appendix A. Summary of the reported use levels (mg/kg or mg/L as appropriate) of chlorophyllins (E 140(ii)) 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	128.0	500.0	NATCOL
01.6.3	Other creams	*QS*	Only flavoured creams	1	25.0	40.0	NATCOL
01.7.1	Unripened cheese excluding products falling in category 16	*QS*	Only flavoured unripened cheese	2	110.0	500.0	NATCOL
01.7.2	Ripened cheese	*QS*	Only *Sage derby cheese*	1	200.0	500.0	NATCOL
01.7.4	Whey cheese	*QS*		1	50.0	300.0	NATCOL
01.7.5	Processed cheese	*QS*	Only flavoured processed cheese	3	76.7	500.0	NATCOL
01.7.6	Cheese products (excluding products falling in category 16)	*QS*	Only flavoured unripened products	2	22.5	40.0	NATCOL
01.8	Dairy analogues, including beverage whiteners	*QS*		1	200.0	500.0	NATCOL
03	Edible ices	*QS*		6	92.5	750.0	NATCOL/FDE
04.2.2	Fruit and vegetables in vinegar, oil, or brine	*QS*	Only vegetables (excluding olives)	1	100.0	400.0	NATCOL
04.2.4.1	Fruit and vegetable preparations excluding compote	*QS*		1	100.0	300.0	NATCOL
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EEC	*QS*	Except chestnut purée	5	36.0	500.0	NATCOL
04.2.5.3	Other similar fruit or vegetable spreads	*QS*	Except *crème de pruneaux*	2	25.0	50.0	NATCOL
05.2	Other confectionery including breath freshening microsweets	*QS*		14	51.4	500.0	NATCOL
05.3	Chewing gum	*QS*		5	114.6	500.0	NATCOL/ICGA
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	*QS*		5	82.0	800.0	NATCOL

FCS category number	FCS food category	MPL	Restrictions/exceptions	Reported use levels			Information provided by
				Number of data	Typical mean	Highest maximum level	
06.3	Breakfast cereals	*QS*	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	1	400.0	800.0	NATCOL
06.5	Noodles	*QS*		1	80.0	104.0	NATCOL
06.6	Batters	*QS*		1	40.0	80.0	NATCOL
07.2	Fine bakery wares	*QS*		3	123.3	500.0	NATCOL
08.3.3	Casings and coatings and decorations for meat	*QS*	Except edible external coating of *pasturmas*	5	250.0	750.0	NATCOL
12.2.2	Seasonings and condiments	*QS*	Only seasonings, for example curry powder, tandoori	3	316.7	700.0	NATCOL/FDE
12.4	Mustard	*QS*		1	25.0	60.0	NATCOL
12.5	Soups and broths	*QS*		3	171.7	500.0	NATCOL
12.6	Sauces	*QS*	Excluding tomato-based sauces	4	87.5	500.0	NATCOL
12.7	Salads and savoury-based sandwich spreads	*QS*		3	50.0	500.0	NATCOL
14.1.4	Flavoured drinks	*QS*	Excluding chocolate milk; malt products	3	23.7	100.0	NATCOL
14.2.3	Cider and perry	*QS*	Excluding *cidre bouché*	1	30.0	100.0	NATCOL
14.2.4	Fruit wine and made wine	*QS*	Excluding *wino owocowe markowe*	1	30.0	100.0	NATCOL
14.2.5	Mead	*QS*		1	30.0	100.0	NATCOL
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	*QS*	Except: spirit drinks as defined in article 5(1) and sales denominations listed in Annex II, paragraphs 1–14, to Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	1	30.0	100.0	NATCOL
14.2.7.1	Aromatised wines	*QS*	Except *americano, bitter vino*	1	30.0	100.0	NATCOL
14.2.7.2	Aromatised wine-based drinks	*QS*	Except *bitter soda, sangria, claria, zurra*	1	30.0	100.0	NATCOL
14.2.7.3	Aromatised wine-product cocktails	*QS*		1	30.0	100.0	NATCOL

FCS category number	FCS food category	MPL	Restrictions/exceptions	Reported use levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
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	30.0	100.0	NATCOL
15.1	Potato-, cereal-, flour- or starch-based snacks	*QS*		4	143.8	500.0	NATCOL
15.2	Processed nuts	*QS*		1	100.0	300.0	NATCOL
16	Desserts excluding products covered in categories 1, 3 and 4	*QS*		5	168.0	600.0	NATCOL/FDE
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	*QS*		2	95.0	500.0	NATCOL
17.2	Food supplements supplied in a liquid form	*QS*		2	85.0	500.0	NATCOL
17.3	Food supplements supplied in a syrup-type or chewable form	*QS*		3	70.0	500.0	NATCOL

Appendix B. Concentration levels of chlorophyllins (E 140(ii)) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

FCS category number	FCS food category	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment		Data sources/comments
				Mean	Maximum	
01.4	Flavoured fermented milk products including heat-treated products		*QS*	128	500	Reported use levels
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Except unflavoured products	*QS*	–	–	Not taken into account (no data available)
01.6.3	Other creams	Only flavoured creams	*QS*	–	–	Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16	Only flavoured unripened cheese	*QS*	110	500	Reported use levels
01.7.2	Ripened cheese	Only *sage Derby cheese*	*QS*	200	500	Reported use levels
01.7.3	Edible cheese rind		*QS*	–	–	Not taken into account (no corresponding FoodEx code/no data available)
01.7.4	Whey cheese		*QS*	50	300	Reported use levels
01.7.5	Processed cheese	Only flavoured processed cheese	*QS*	77	500	Reported use levels
01.7.6	Cheese products (excluding products falling in category 16)	Only flavoured unripened products	*QS*	–	–	Not taken into account (no corresponding FoodEx code)
01.8	Dairy analogues, including beverage whiteners		*QS*	200	500	Reported use levels
03	Edible ices		*QS*	93	750	Reported use levels
04.2.1	Dried fruit and vegetables	Only preserves of red fruit	*QS*	–	–	Not taken into account (no data available)

FCS category number	FCS food category	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment		Data sources/comments
				Mean	Maximum	
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only preserves of red fruit	*QS*	–	–	Not taken into account (no data available)
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only vegetables (excluding olives)	*QS*	100	400	Reported use levels
04.2.3	Canned or bottled fruit and vegetables	Only preserves of red fruit	*QS*	–	–	Not taken into account (no data available)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only preserves of red fruit	*QS*	–	–	Not taken into account (no corresponding FoodEx code)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only *mostarda di frutta*	*QS*	–	–	Not taken into account (no corresponding FoodEx code)
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	Except chestnut purée	*QS*	36	500	Reported use levels
04.2.5.3	Other similar fruit or vegetable spreads	Except *crème de pruneaux*	*QS*	25	50	Reported use levels
05.2	Other confectionery including breath freshening microsweets		*QS*	51	500	Reported use levels
05.3	Chewing gum		*QS*	115	500	Reported use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4		*QS*	–	–	Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	*QS*	400	800	Reported use levels
06.5	Noodles		*QS*	80	104	Reported use levels

FCS category number	FCS food category	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment		Data sources/comments
				Mean	Maximum	
06.6	Batters		*QS*	–	–	Not taken into account (no corresponding FoodEx code)
06.7	Pre-cooked or processed cereals		*QS*	–	–	Not taken into account (no corresponding FoodEx code/no data available)
07.2	Fine bakery wares		*QS*	123	500	Reported use levels
08.3.3	Casings and coatings and decorations for meat	Except edible external coating of *pasturmas*	*QS*	–	–	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only fish paste and crustacean paste	*QS*	–	–	Not taken into account (no data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only precooked crustacean	*QS*	–	–	Not taken into account (no data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only surimi and similar products and salmon substitutes	*QS*	–	–	Not taken into account (no data available)
09.3	Fish roe	Except sturgeons' eggs (Caviar)	*QS*	–	–	Not taken into account (no data available)
12.2.2	Seasonings and condiments	Only seasonings, for example curry powder, tandoori	*QS*	317	700	Reported use levels
12.4	Mustard		*QS*	25	60	Reported use levels
12.5	Soups and broths		*QS*	172	500	Reported use levels
12.6	Sauces	Excluding tomato-based sauces	*QS*	88	500	Reported use levels
12.7	Salads and savoury-based sandwich spreads		*QS*	50	500	Reported use levels

FCS category number	FCS food category	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment		Data sources/comments
				Mean	Maximum	
12.9	Protein products, excluding products covered in category 1.8		*QS*	–	–	Not taken into account (no data available)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		*QS*	–	–	Not taken into account (no data available)
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		*QS*	–	–	Not taken into account (no data available)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Including dry pasta	*QS*	–	–	Not taken into account (no data available)
14.1.4	Flavoured drinks	Excluding chocolate milk and malt products	*QS*	24	100	Reported use levels
14.2.3	Cider and perry	Excluding *cidre bouché*	*QS*	30	100	Reported use levels
14.2.4	Fruit wine and made wine	Excluding *wino owocowe markowe*	*QS*	–	–	Not taken into account (no corresponding FoodEx code)
14.2.5	Mead		*QS*	–	–	Not taken into account (no corresponding FoodEx code)

FCS category number	FCS food category	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment		Data sources/comments
				Mean	Maximum	
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1–14 of Regulation (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	*QS*	30	100	Reported use levels
14.2.7.1	Aromatised wines	Except *americano, bitter vino*	*QS*	30	100	Reported use levels
14.2.7.2	Aromatised wine-based drinks	Except *bitter soda, sangria, claria, zurra*	*QS*	30	100	Reported use levels
14.2.7.3	Aromatised wine-product cocktails		*QS*	30	100	Reported use levels
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol		*QS*	30	100	Reported use levels
15.1	Potato-, cereal-, flour- or starch-based snacks		*QS*	144	500	Reported use levels
15.2	Processed nuts		*QS*	100	300	Reported use levels
16	Desserts excluding products covered in categories 01, 03 and 04		*QS*	168	600	Reported use levels

FCS category number	FCS food category	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment		Data sources/comments
				Mean	Maximum	
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		*QS*	81	500	Reported use levels
17.2	Food supplements supplied in a liquid form		*QS*			
17.3	Food supplements supplied in a syrup-type or chewable form		*QS*			

Appendix C. Summary of total estimated exposure of chlorophyllins (E 140(ii)) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment 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	12.0		7.2		3.1	
Bulgaria (NUTRICHILD)	428	2.7	7.1	2.1	5.3	0.6	1.6
Germany (DONALD 2006 2008)	261	3.6	9.5	2.5	7.0	0.8	2.2
Spain (enKid)	17	5.8		4.5		1.5	
Finland (DIPP 2003 2006)	497	3.3	10.4	2.8	9.3	0.9	2.8
Italy (INRAN SCAI 2005 06)	36	3.5		2.4		0.9	
The Netherlands (VCP kids)	322	9.7	20.4	6.5	16.4	2.5	5.4
Children							
Belgium (Regional Flanders)	625	9.7	19.6	6.0	13.2	2.6	5.3
Bulgaria (NUTRICHILD)	433	3.4	7.4	2.4	5.5	0.8	1.6
Czech Republic (SISP04)	389	4.8	10.4	2.9	6.0	1.1	2.5
Germany (DONALD 2006 2008)	660	4.8	10.0	2.8	6.5	1.1	2.5
Denmark (Danish Dietary Survey)	490	3.0	5.7	1.6	3.3	0.7	1.4
Spain (enKid)	156	4.4	10.3	3.0	7.6	1.1	2.8
Spain (NUT INK05)	399	4.4	9.9	2.7	6.2	1.1	2.7
Finland (DIPP 2003 2006)	933	3.8	8.4	2.5	6.0	0.9	2.0
Finland (STRIP)	250	7.2	12.6	3.9	7.4	2.1	4.1
France (INCA2)	482	4.8	8.7	2.8	5.1	1.2	2.3
Greece (Regional Crete)	839	4.0	8.5	2.5	5.6	1.0	2.3
Italy (INRAN SCAI 2005 06)	193	2.7	5.8	1.8	3.7	0.6	1.3
Latvia (EFSA TEST)	189	5.7	11.6	3.5	7.1	1.6	3.4
The Netherlands (VCP kids)	957	8.6	18.5	5.6	13.6	2.2	4.8
Sweden (NFA)	1 473	7.0	13.4	3.7	7.6	1.7	3.5
Adolescents							
Belgium (Diet National 2004)	584	2.9	5.8	1.8	3.4	0.8	1.6
Cyprus (Childhealth)	303	1.0	2.3	0.7	1.5	0.3	0.7
Czech Republic (SISP04)	298	2.9	6.7	1.8	3.8	0.7	1.6
Germany (National Nutrition Survey II)	1011	2.2	5.3	1.4	3.4	0.6	1.4
Denmark (Danish Dietary Survey)	479	1.9	4.1	1.0	2.4	0.4	0.9
Spain (AESAN FIAB)	86	1.3	2.8	0.9	2.2	0.3	0.7
Spain (enKid)	209	2.2	5.2	1.4	3.3	0.5	1.3
Spain (NUT INK05)	651	2.2	4.8	1.3	2.9	0.6	1.3
France (INCA2)	973	2.3	4.7	1.3	2.9	0.6	1.2
Italy (INRAN SCAI 2005 06)	247	1.7	4.0	1.1	2.5	0.4	0.9
Latvia (EFSA TEST)	470	3.6	7.5	2.2	4.8	0.9	2.0
Sweden (NFA)	1 018	3.6	7.4	2.0	4.3	0.9	1.9
Adults							
Belgium (Diet National 2004)	1304	2.3	5.1	1.5	3.4	0.6	1.5
Czech Republic (SISP04)	1 666	1.3	3.2	0.9	2.0	0.3	0.7
Germany (National Nutrition Survey II)	10 419	1.8	4.3	1.2	2.8	0.5	1.2
Denmark (Danish Dietary Survey)	2 822	0.9	2.0	0.5	1.3	0.2	0.5
Spain (AESAN)	410	1.1	2.9	0.8	1.9	0.3	0.7
Spain (AESAN FIAB)	981	0.9	2.4	0.6	1.5	0.2	0.6
Finland (FINDIET 2007)	1 575	0.9	2.5	0.7	1.9	0.2	0.6
France (INCA2)	2 276	1.3	2.9	0.8	1.8	0.3	0.7
United Kingdom (NDNS)	1 724	1.6	3.1	0.9	1.8	0.4	1.0
Hungary (National Repr Surv)	1 074	0.6	1.6	0.4	1.1	0.1	0.4
Ireland (NSIFCS)	958	1.3	2.7	0.7	1.6	0.3	0.8

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Italy (INRAN SCAI 2005 06)	2 313	0.9	2.3	0.6	1.5	0.2	0.5
Latvia (EFSA TEST)	1 306	2.3	4.7	1.5	3.1	0.6	1.3
The Netherlands (DNFCS 2003)	750	2.6	5.5	1.6	3.3	0.7	1.5
Sweden (Riksmaten 1997 98)	1 210	2.1	4.3	1.2	2.8	0.6	1.5
The elderly							
Belgium (Diet National 2004)	1 230	2.3	5.1	1.5	3.4	0.7	1.6
Germany (National Nutrition Survey II)	2 496	1.7	3.9	1.1	2.6	0.4	1.1
Denmark (Danish Dietary Survey)	329	0.6	1.7	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	1.0	2.3	0.6	1.5	0.2	0.5
Hungary (National Repr Surv)	286	0.5	1.3	0.3	1.0	0.1	0.3
Italy (INRAN SCAI 2005 06)	518	0.7	1.8	0.5	1.2	0.2	0.4

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
CIAA	Confederation of the Food and Drink Industries of the EU
CONTAM	Panel on Contaminants in the Food Chain
EC	European Commission
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
QS	*quantum satis*
SCF	Scientific Committee on Food