European Food Safety Authority

EFSA Journal 2015; 13(11):4288

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of cochineal, carminic acid, carmines (E 120) as a food additive[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Cochineal, carminic acid, carmines (E 120) have been previously evaluated by JECFA and by the SCF. Both committees established an ADI of 5 mg/kg bw/day. The Panel noted that the title of the EC specifications for E 120 does not adequately correspond to the specified food additive and therefore, proposes to modify it to "E 120 cochineal extract, carminic acid and carmines", which would more accurately reflect the material used. The Panel also noted that the specifications need to be updated with regard to the maximum limits for certain toxic elements present as impurities, to ensure that E 120 will not be a significant source of exposure to these toxic elements in food. No ADME studies on cochineal extract, carminic acid or carmines were available for evaluation, but indirect evidence suggests that carmines are absorbed and distributed in the body. Acute, short-term, subchronic, carcinogenicity, reproduction and developmental toxicity studies conducted in rats or mice did not show toxicological potential. Consideration of the available information regarding genotoxicity indicated that carminic acid is not genotoxic. The Panel concluded that the present dataset does not give reason to revise the ADI of 5 mg carmine (containing approximately 50 % carminic acid)/kg bw, allocated by the SCF in 1983. The Panel concluded that this ADI should be expressed as carminic acid content, which would correspond to 2.5 mg carminic acid/kg bw/day. The Panel considered that, since no threshold dose can be established for allergic reactions, it is advisable that exposure to the eliciting allergens, such as proteinaceous compounds, in E 120 is avoided by introducing appropriate purification steps in the manufacturing process. Refined exposure estimates show that exposure to E 120 for the non-brand-loyal scenario, is below the ADI of 2.5 mg carminic acid/kg bw/day for all population groups.

KEY WORDS

Cochineal extract, carminic acid, carmines, E 120, CI Natural Red 4, INS No 120, 7-β-D-glucopyranosyl-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxoanthracene-2-carboxylic acid

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[1] On request from the European Commission, Question No EFSA-Q-2011-00360, adopted on 27 October 2015.

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Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2015. Scientific Opinion on the re-evaluation of cochineal, carminic acid, carmines (E 120) as a food additive. EFSA Journal 2015;13(11):4288, 66 pp. doi:10.2903/j.efsa.2015.4288

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 cochineal, carminic acid, carmines (E 120) as a food additive.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the information available following public calls for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel. 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 cochineal extract, carminic acid and carmines (E 120), which covered the period from the beginning of 2013 up to the end of 2014. Further updates have been performed by the Panel.

Cochineal, carminic acid and carmines (E 120) are red anthraquinone dyes authorised as food additives in the European Union (EU), in accordance with Annex II to Regulation (EC) No 1333/2008.

Cochineal, carminic acid and carmine (E 120) have most recently been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2000, which set new specifications, and by the Scientific Committee for Food (SCF) in 1983. Both committees established an Acceptable Daily Intake (ADI) of 5 mg/kg body weight (bw)/day. The JECFA ADI, established in 1982 for carmines (formerly cochineal, carmines and carminic acid), includes ammonium carmine or the equivalent calcium, potassium or sodium salts. For the SCF, the ADI applies to cochineal (carmines), without further details being specified. The 1981 JECFA evaluation specifically excluded the lithium salt, considering it as not acceptable for food additive use.

Specifications have been defined in Commission Regulation (EU) No 231/2012 and by JECFA in 2000. In the EC specifications, cochineal, carminic acid and carmine colours are defined as having not less than 2.0 % carminic acid in the extracts and not less than 50 % carminic acid in the chelates. The remaining material (50 to 80 %) is not precisely specified, being only described as cations that may be present in excess in the colour and also maybe containing proteinaceous material derived from the source insect, together with free carminate or a small residue of unbound aluminium cations. The Panel noted thus that the specifications of carmines need to be updated with respect to the percentage of material not accounted for. The Panel noted that the title of the EC specifications, "E 120, cochineal, carminic acid, carmines", does not adequately correspond to the specified food colour. The Panel also noted that the actual EC specifications for cochineal extract, carminic acid, carmines do not include limits for the protein content, total ash, residual solvents, or insoluble matter. The Panel considered that further indication on the proportions or percentages of these components, particularly the protein content and the molecular weight of the key allergenic proteins, in the commercial product should be required. Furthermore, the Panel considered that the maximum limits for toxic elements (arsenic, lead, mercury and cadmium) present as impurities in the EC specifications for E 120 should be revised in order to ensure that E 120 used as a food additive will not be a significant source of exposure to these toxic elements in food.

The Panel noted that the term Cochineal *per se* is a description of the ground bodies of the female insect *Dactylopius coccus* Costa before extraction, and to the knowledge of the Panel, this material is not used as a food colour. Furthermore, the composition of cochineal extracts is not well defined, and, as described further, the established ADI was based on studies using carmine with a defined amount of carminic acid as test material. Therefore, the Panel proposes that the current title of the food additive ("E 120 cochineal, carminic acid, carmines") should be revised to "E 120 cochineal extract, carminic acid and carmines" which would more accurately reflect the material used. Carmines should meet existing carmines EC specifications including those concerning the content of ≥ 50 % carminic acid.

No studies on absorption, distribution, metabolism or excretion of cochineal extract, carminic acid or carmines were available for this evaluation. However, both the ionisation properties of carminic acid and indirect evidence from toxicological studies suggest that these compounds can be absorbed to some extent as suggested by the accumulation of colour in tissues and the red colouring of urine reported in rats treated with ammonium carmine.

Short-term and subchronic studies conducted in rats and mice did not show toxicological potential.

Two long-term studies in rats and mice investigated the carcinogenic potential of carmine and cochineal extract, respectively. The rat study on carmine reported significantly higher incidences of acinar hyperplasia and duct ectasia of the mammary tissue in female rats given carmines, at all doses, compared with controls. The mammary hyperplasia seen in the rat study was not reported in the mouse study performed with cochineal extract, and the general pattern of tumour incidence in the mouse study was not significantly different from that of the controls. After considering all the available information, the Panel considered that the incidences of mammary hyperplasia reported in the rat study were not treatment related. Overall, the Panel concluded that carmine is not carcinogenic.

No adverse effects were reported in reproductive and developmental toxicity studies in rats and mice when tested at doses of up to 1 000 mg carmine/kg bw/day or 3 000 mg cochineal extract/kg bw/day. Overall, the Panel considered that the available data suggest that cochineal extract and carmine do not show reproductive or developmental toxicity.

The available information regarding genotoxicity indicates that carminic acid is not genotoxic, and, by read-across, carmine is also considered non-genotoxic.

The Panel concluded that the present dataset does not give reason to revise the ADI of 5 mg carmine (containing approximately 50 % carminic acid)/kg bw allocated by the SCF in 1983, but considered that for clarification this ADI should only apply to cochineal extract and to carmine. The Panel concluded that this ADI should be expressed as carminic acid content, and this would correspond to 2.5 mg carminic acid/kg bw/day.

The Panel noted that the composition of cochineal tested in the toxicological studies available is not well defined and that, to the knowledge of the Panel, Cochineal (the ground bodies of the female insect *D. coccus* Costa before extraction) is not used as a food colour in the EU. Furthermore, taking into account that the ADI was derived from toxicological studies using carmine as test material with defined amounts of carminic acid (46 to 56 % carminic acid), which match those specified in the EU specifications, the Panel concluded that based on available information, the ADI of 5 mg/kg bw/day does not apply to Cochineal (the ground bodies of the female insect). Lithium salts of carminic acid are not covered by this ADI.

Using the "maximum level exposure assessment scenario", the mean exposure to E 120 from its use as a food additive ranged from 0.1 mg/kg bw/day in infants to 3.9 mg/kg bw/day in toddlers, while the high exposure using this scenario ranged from 0.3 mg/kg bw/day in infants to 6.7 mg/kg bw/day in toddlers.

Using the refined brand-loyal exposure assessment scenario, the mean exposure to E 120 from its use as a food additive ranged from 0.1 mg/kg bw/day in infants, adolescents, adults and the elderly to 2.1 mg/kg bw/day in toddlers. The high exposure to E 120 using this scenario ranged from 0.2 mg/kg bw/day in the elderly to 4.7 mg/kg bw/day in toddlers.

Using the refined non-brand-loyal exposure assessment scenario, the mean exposure to E 120 from its use as a food additive ranged from 0.02 mg/kg bw/day in infants to 0.6 mg/kg bw/day in toddlers. The high exposure to carminic acid, carmines (E 120) from its use as food additive using this scenario

ranged from 0.1 mg/kg bw/day in infants, adolescents, adults and the elderly to 1.1 mg/kg bw/day in toddlers.

Overall, refined exposure estimates for the non-brand-loyal scenario for infants, toddlers, children adolescents, adults and the elderly show that exposure to E 120 is below the ADI of 2.5 mg carminic acid/kg bw/day for all population groups.

The Panel considered that the ADI of 5 mg/kg bw/day does not cover minimum sensitising or eliciting doses for susceptible individuals. Allergic reactions have been associated with exposure to cochineal extract and carmines. Both substances are able to trigger acute hypersensitivity reactions, such as Quincke's oedema, dyspnoea and bronchospasm, in sensitised individuals, and can cause severe anaphylactic reactions. In addition, chronic hypersensitivity symptoms, such as rhinoconjuctivitis and asthma, have also been associated with occupational exposure to carmine. The reported effects are likely to be the consequence of allergic reactions involving an immunoglobulin E (IgE)-mediated mechanism, elicited by proteinaceous compounds in the food colour E 120.

The Panel noted that cases of severe allergic reactions, occurring after the consumption of carmine-containing foodstuffs, have been reported, and indicated that the information provided to alert individuals allergic to these colours is not sufficiently acted upon. The Panel considered that, since no threshold dose can be established for allergic reactions, it is advisable that exposure to the eliciting allergens, such as proteinaceous compounds, is avoided as much as possible. Therefore, the Panel considered that it may be advisable to reduce the presence of these allergens as much as possible by introducing appropriate purification steps to the manufacturing process.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The 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 the 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 the adoption of Regulation (EU) 257/2010, the 2003 Terms of Reference 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, procedure 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, p. 19.
[5] COM(2001) 542 final.
[6] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of cochineal, carminic acid, carmines (E 120) as a food additive.

Cochineal, carminic acid, carmines (E 120) are red anthraquinone dyes authorised as food additives in the EU and have been most recently evaluated by the Joint Expert Committee on Food Additives (JECFA) in 2000 (JECFA, 2001) and the Scientific Committee for Food (SCF) in 1983. Both committees established an Acceptable Daily Intake (ADI) of 5 mg/kg body weight (bw)/day. The SCF ADI applies to cochineal (carmines), without other limitations. The ADI for carmines (formerly cochineal, carmines and carminic acid) established by JECFA includes ammonium carmine or the equivalent calcium, potassium or sodium salts. Cochineal, carminic acid and carmines were also reviewed by TemaNord in 2002. The present opinion briefly reports the major studies evaluated in these opinions and describes any additional data in more detail.

The European Food Safety Authority (EFSA) 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 became available since then and information available following public calls for data.[7,8,9] The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel. To assist in identifying any emerging issue or any information relevant to the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of cochineal extract, carminic acid and carmines (E 120), which covered the period from the beginning of 2013 up to the end of 2014. Further updates have been performed by the Panel.

2. Technical data

2.1. Identity of the substances

Carmines and carminic acid are defined, according to Commission Regulation (EU) No 231/2012[10], as being "obtained from aqueous, aqueous alcoholic or alcoholic extracts from Cochineal, which consist of the dried bodies of the female insect *Dactylopius coccus* Costa". Carmines and carminic acid are described as red to dark red friable solid or powder, while cochineal extract is generally a dark red liquid, but can also be dried as a powder (Commission Regulation (EU) No 231/2012).

According to Commission Regulation (EU) No 231/2012 and to JECFA (2006), carmines are hydrated aluminium chelates (lakes) of carminic acid, in which the molar ratio of aluminium to carminic acid is thought to be 1:2. The Panel noted that this definition is based on a study by Meloan et al. (1971).

However, the Panel noted that, more recently, Harris et al. (2009) found that, based on mass spectrometry, carmine may have a tetrameric structure, and that both aluminium and calcium are involved in the chelation/salt formation.

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

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

[9] Call for scientific data on selected food additives permitted in the EU. Published: 24 March 2014. http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

[10] 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.

In another study (Schmidt-Jacobson and Sakstrup Frandsen, 2011), carmine was described as being a carminic acid lake, termed carminic acid calcium–aluminium lake, and that it is composed essentially of carminic acid, aluminium and calcium in more or less defined combinations.

Carminic acid has the systematic name 7-β-D-glucopyranosyl-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxoanthracene-2-carboxylic acid, the molecular formula $C_{22}H_{20}O_{13}$ and a molecular weight of 492.39 g/mol. Carminic acid may be present in association with ammonium, calcium, potassium or sodium cations, singly or in combination (Commission Regulation (EU) No 231/2012).

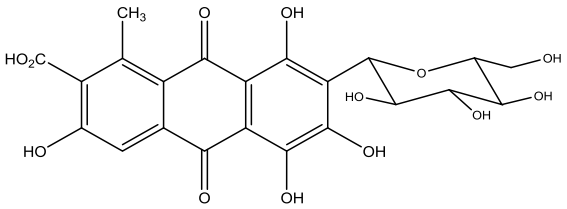
Carminic acid is the colouring principle of cochineal extracts.

The Chemical Abstracts Service (CAS), European Inventory of Existing Commercial chemical Substances (EINECS) (EC) and Colour Index (CI) numbers for cochineal, carminic acid and carmines are shown in Table 1.

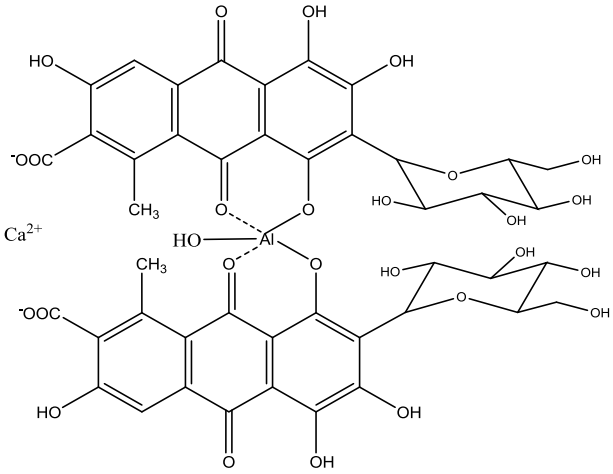
Table 1: CAS, EINECS (EC) and CI numbers for cochineal, carminic acid and carmines

	Cochineal	Carminic acid (colouring principle)	Carmines
CAS Registry number	1343-78-8	1260-17-9	1390-65-4
EINECS number	215-680-6	215-023-3	215-724-4
CI number	75470	75470	75470

Figure 1 shows the structural formulae for carminic acid and its aluminium chelate (lakes; carmine). Other dimer and tetramer structures for carmine and carminic acids have been proposed (Harris, 2009).



(a) Carminic acid



(b) Carmine

Figure 1: Structural formulae, according to Harris (2009), for (a) carminic acid and (b) carmine

The solubility of carmine preparations varies depending on the nature of the cations. Ammonium carmines exhibit solubility over a wide range of pH values (pH 3.0 to 8.5), while calcium carmines are only slightly soluble in water at pH 3.0, but freely soluble at pH 8.5 (JECFA, 2006). Commercial products also contain proteinaceous material derived from the source insect, and may contain free carminate anions or small excesses of aluminium cations (Lloyd, 1980).

Carminic acid is freely soluble in water, alcohol and ether (USP, 2009); the log $P_{o/w}$ is 0.97 (ChemID*plus* advanced, online).

The pK_a values of carminic acid have been determined as 2.81, 5.43 and 8.10 (Rasimas et al., 1996). The Panel considered that, because of similarities in acidic groups, these pK_a values would be similar for carmine.

At least 11 synonyms for cochineal, 22 synonyms for carminic acid and 10 synonyms for carmines are in use (ChemID*plus* advanced, online). The most commonly used synonyms are cochineal carmine, carmine, CI Natural Red 4 and INS (International Numbering System) No 120.

2.2. Specifications

Specifications for E 120 (cochineal, carminic acid, carmines) have been defined in EU legislation (Commission Regulation (EU) No 231/2012), while separate specifications for cochineal extract and for carmines have been established by JECFA (2006). These specifications are detailed in Table 2.

The EC specifications state that in the "commercial product the colouring principle is present in association with ammonium, calcium, potassium or sodium cations, singly or in combination, and that these cations may also be present in excess. Although not precisely specified, "commercial products may also contain proteinaceous material derived from the insect source, and may also contain free carminate or a small residue of unbound aluminium cations" (Commission Regulation (EU) No 231/2012).

Table 2: Specifications for cochineal extract and carmines according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

Specifications	Commission Regulation (EU) No 231/2012	JECFA (2006)	
		Cochineal extract	Carmines
Carminic acid	≥ 2 % for cochineal extract and ≥ 50 % for carmines	≥ 2 %	≥ 50 %
Protein	–	≤ 2.2 %	≤ 25 %
Ethanol	–	≤ 150 mg/kg	
Methanol	–	≤ 150 mg/kg	
Total ash	–	–	≤ 12 %
Total solids	–	–	
Matter insoluble in dilute ammonia	–	–	≤ 1 %
Loss on drying (at 135 °C, for 3 hours)	–	–	≤ 20 %
Arsenic	≤ 3 mg/kg		–
Lead	≤ 5 mg/kg	≤ 2 mg/kg	≤ 5 mg/kg
Mercury	≤ 1 mg/kg		–
Cadmium	≤ 1 mg/kg		–
Microbiological criteria	–	*Salmonella*: negative per test	*Salmonella*: negative per test

The Panel noted that the presence of "4-aminocarminic acid"[11] (4-ACA), a primary aromatic amine, has been reported in some beverages labelled as containing the E 120 colour (Sabatino et al., 2012), and that specifications for E 120 do not include 4-aminocarminic acid. According to the literature (Stathopoulou et al., 2013; Lech et al., 2015), 4-aminocarminic acid is naturally present in the cochineal insect and could be formed spontaneously during the production process of carmine from free carminic acid, in the presence of ammonium sources such as proteins from the cochineal insect.

The Panel noted that the title of the EC specifications "E 120 cochineal, carminic acid, carmines", does not adequately correspond to the specified food additive. The Panel noted that the term Cochineal per se is a description of the ground bodies of the female insect *D. coccus* Costa before extraction, and that, to the knowledge of the Panel, this material is not used as a food colour. Therefore, the Panel proposes that the current title of the food additive "E 120 cochineal, carminic acid, carmines" be revised to "E 120 cochineal extract, carminic acid and carmines", which would more accurately reflect the material used.

The Panel also noted that the actual EC specifications for E 120 do not include limits for the protein content, total ash, residual solvents or insoluble matter, or microbiological criteria. The Panel considered that further indication on the proportions/percentages of these components, particularly the protein content and the molecular weight of the key allergenic proteins in the commercial product, should be required. Furthermore, the monograph for carmine in the US Food Chemicals Codex (USP, 2009) requires that "before use in food, carmine must be pasteurized or otherwise treated to destroy all viable *Salmonella* microorganisms according to the pertinent US color additive regulation" (21 CFR (Code of Federal Regulations) 73.100(b)(2)).

The Panel noted that, according to the EC specifications for E 120, certain toxic elements present as impurities (arsenic, lead, mercury and cadmium) are accepted up to a concentration of 3, 5, 1 and 1 mg/kg, respectively. Contamination at these levels would have a significant impact on the exposure to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA, 2009; EFSA CONTAM Panel, 2009, 2010, 2012). The Panel considered that the maximum limits for these toxic elements (arsenic, lead, mercury and cadmium) present as impurities in the EC specifications for E 120 (cochineal, carminic acid, carmines) should be revised in order to ensure that E 120 used as food additive will not be a significant source of exposure to these toxic elements in food.

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

The Panel noted that the aluminium lakes of carminic acid (carmines), where aluminium and carminic acid are thought to be present in the molar ratio 1:2, together with the non-specified residue of unbound aluminium cations, could add to the daily intake of aluminium.

2.3. Manufacturing process

Commission Regulation (EU) No 231/2012 states that carmines and carminic acid are obtained from aqueous, aqueous alcoholic or alcoholic extract from Cochineal.

Optimised solvent extraction parameters (e.g. temperature, time, solvent concentration and the number of extractions) have been described by González et al. (2002).

[11] The Panel noted that the substance named as "4-aminocarminic acid" corresponds to 7-β-D-glucopyranosyl-9,10-dihydro-5-amino-3,6,8-trihydroxy-1-methyl-9,10-dioxoanthracene-2-carboxylic acid.

Aluminium lakes (carmines) are prepared by precipitating a carminic acid solution on a sub-stratum of hydrated aluminium oxide using aluminium and calcium cations as precipitants giving rise to the formation of aluminium or calcium–aluminium lakes. Hydrated aluminium oxide is usually freshly prepared by reacting aluminium sulphate or aluminium chloride with sodium carbonate, sodium bicarbonate or aqueous ammonia. After lake formation, the product is filtered, washed with water and dried (Commission Regulation (EU) No 231/2012; JECFA, 2001).

Two recent patents describe techniques to reduce the protein content of carmine. Ichi et al. (2002) used a two-step procedure in which, first, an enzymatic proteolysis was performed, followed by one or several of the following purification steps: adsorption and desorption to a resin; ion exchange treatment; acid precipitation; extraction by supercritical CO_2; and/or membrane filtration. According to the manufacturing process used by industry (NATCOL, 2012), in the production of carminic acid, a purification step is included to remove proteins through the use of a cationic resin to bind the carminic acid that is further released with ethanol.

Schmidt-Jacobson and Sakstrup-Frandsen (2010) described a method for the preparation of a carminic acid lake in which the presence of protein is required for product stabilisation. The authors claim the method to remove all original protein from *D. coccus* and to add protein from a source which is not known to give rise to allergic responses.

Borges et al. (2012) studied two different methods for the extraction of carminic acid from cochineal: pressurised liquid extraction (PLE) and supercritical fluid extraction (SFE), and the effect of several operation variables: extraction temperature, pH and solvent type. PLE was performed at a pressure of up to 10.5 MPa and at three different temperatures (100, 150 and 200 °C), using three different solvents (methanol:water, ethanol:water and ethanol), and a 30 minute extraction time. SFE was carried out using supercritical CO_2 in a supercritical fluid extractor at pressures of between 15 and 30 MPa and at a temperature of 40 °C. The extraction methods were shown to be highly selective with short extraction times. The authors also investigated the impact of solvent composition and acids on the protein concentration in extracts. The addition of citric or tartaric acid at levels of more than 0.25 g/L effectively removed all proteins.

The Panel noted that there are differences in the polarity of the extraction solvents described to obtain carmine and carminic acid from cochineal, which could result in different compositions of the extracts produced.

2.4. Methods of analysis in foods

Several methods for the determination of carminic acid and carmines in foods are described in the literature; in general, these methods employ variations of high performance liquid chromatography (HPLC) and spectrophotometry (González et al., 2002; Samari et al., 2010). The official method for the determination of carminic acid levels in commercial E 120 preparations is spectrophotometry, as described by JECFA (JECFA, 2001). Most modern published methods are able to achieve limits of quantification (LOQs) of below 1 mg/kg using HPLC with ultraviolet–visible (UV–VIS) or fluorescence detection. A few available HPLC-based methods for the analysis of E 120 in foods are well established and have been validated for a number of different sample types (Scotter, 2011). The determination of carmine levels in ice cream and soft drinks using stripping voltammetry has also been reported (Alghamdi et al., 2009). This electrochemical procedure can be influenced by factors such as temperature, pH and organic substances, and it is not widely used. Another electroanalytical method involving differential pulse polarography has been applied successfully to the analysis of carmine in spiked commercial milk, with an LOQ of 0.55 µM. This method is accurate, precise and requires only a short time for analysis, as extraction is not needed (Yilmaz et al., 2014). However, these methods are not suitable for the simultaneous screening of a number of dyes with similar structures, including carminic acid. A technique of ultra-high performance liquid chromatography (UHPLC) with electrospray ionisation (ESI) quadrupole orbitrap mass spectrometry (MS) can be also used. This

method was also validated according to Commission Decision 2002/657/EC.[12] It is five times more sensitive and accurate than other methods (Jia et al., 2014). An HPLC method that uses a photodiode array detector to distinguish carmine from carminic acid has also been reported; this method uses sodium hydroxide solution for the extraction of carmine from food samples and has an LOQ of 1 µg/mL (Lim et al., 2014). Recently, another method using UHPLC/tandem high-resolution MS has been developed. This method for the determination of carminic acid is also useful for the identification of unknown degradation products of carminic acid resulting from reactions induced by photo-irradiation (Gosetti et al., 2015a,b).

The extraction conditions are generally very simple and involve acid hydrolysis with or without solid phase extraction (SPE), but enzymatic digestion can be used for difficult matrices, particularly meat products. Up to now, no inter-laboratory validated method for the analysis of carminic acid and carmines in food appears to have been adopted.

The Panel noted that, given that proteinaceous compounds are a potential hazard in cochineal extracts, adequate methods for the detection of proteins, and for the determination of the protein content and the molecular weight of the key allergenic proteins in the commercial product, should be applied. Carminic acid can form strong bonds with proteins through both electrostatic and hydrophobic interactions (Nakayama et al., 2015). Traditional methods for the detection of proteins in carminic acid are colorimetric (e.g. the biuret method or the ninhydrin reaction). For analytical purposes, the separation of carminic acid from the proteins in the additive is technically difficult. The method developed by Nakayama et al. (2015) involves a suspension of cochineal extract in an acidic solution with phosphoric acid, causing protein denaturation and aggregation; urea and guanidine hydrochloride are then used to dissolve the precipitate. A further ultrafiltration method is applied to remove carminic acid from cochineal dispersed in the running buffer, for the detection of proteins and the determination of their molecular weights by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). This method allows the simple and reproducible analysis of protein contaminants in carmine.

2.5. Reaction and fate in food

In general, the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids or salts) will affect the colour (Scotter and Castle, 2004). Therefore, colourants, and particularly E 120, can undergo degradation in food and beverages. Carminic acid can be altered when it is exposed to high temperatures (above 80 °C) for more than 1 hour (González et al., 2002). Carminic acid can also be degraded by exposure to light. A stability study of a cochineal extract showed that the carminic acid solutions obtained were stable in the dark; however, the carminic acid content was sensitive to light, decreasing to 6–12 % after 84 days (Borges et al., 2012). In another study, the effect of simulated sun irradiation in 16 different beverages and in an aqueous solution containing carminic acid was investigated. It was reported that a degradation reaction occurred due to the effect of photo-irradiation, causing a decrease in the brilliant red colour intensity as a function of the irradiation time, and, eventually, leading to a complete disappearance of the red colour and thus to uncoloured beverages (Gosetti et al., 2015b). The rate of decolourisation is faster when only carminic acid is present in aqueous solution than when in more complex matrices, as beverages, because of the lack of a possible protective role of other ingredients. The decolourisation time is dependent on the beverage composition, due to the interaction with the other ingredients, with an average time required for complete decolourisation under the experimental conditions of around 13 days (Gosetti et al., 2015a, b). The most common photo-degradation products of carminic acid were identified by the same authors by UHPLC–tandem MS (MS/MS) using chemometric techniques (principal component analysis coupled with discriminant analysis–PCA-DA) (Gosetti et al., 2015b). Among the new species formed, 10 compounds were common to all of the different samples during degradation and the

[12] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ L 221, 17.8.2002, p. 8.

authors proposed the chemical structures for 16 of the 23 identified degradation compounds, based on the data obtained; 10 compounds were common to all of the different samples. The main degradation product structures maintain the original skeleton of carminic acid, whereas some structures present long linear carbon chains, often saturated and substituted, that could derive from the dye and emulsifiers (as diglycerides) that are usually used as processing aids to improve the solubility of carmine lakes in acidic media. However, because of the different compositions of beverages, it is difficult to hypothesise a common degradation pathway for carminic acid in beverages, as the degradation process passes through unpredictable cross-interactions between carminic acid and the other beverage ingredients, and different mechanisms are involved. The Panel noted that, in beverages containing carminic acid and/or carmines, after exposure to sunlight, the formation of a number of as yet unidentified degradation products occurs, and, therefore, further degradation studies on the food additive itself are required to completely characterise the degradation products of the additive.

2.6. Case of need and proposed uses

Maximum Permitted Levels (MPLs) of cochineal, carminic acid, carmines (E 120) have been defined in Annex II to Regulation (EC) No 1333/2008[13] on food additives for use in foods, and these MPLs are expressed as carminic acid, which is the colouring principle in E 120.

E 120 (cochineal, carminic acid, carmines) is an authorised food colour in the EU, with MPLs ranging from 50 to 500 mg/kg in 58 food categories and at *quantum satis* in four food categories. The additive (E 120) is included in Group III of food colours with combined maximum limit.

According to Annex II, part A, Table 3, to Regulation (EC) No 1333/2008, E 120 (cochineal, carminic acid, carmines) is a food colour which may be used in the form of lakes.

Table 3 summarises foods that are permitted to contain E 120 (cochineal, carminic acid, carmines) and the corresponding MPLs, as set in Annex II to Regulation (EC) No 1333/2008.

Table 3: MPLs of cochineal, carminic acid, carmines (E 120) in foods and beverages, according to Annex II to Regulation (EC) No 1333/2008

FCS category number	Foods	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Group III		150
01.6.3	Other creams	Group III	Only flavoured creams	150
01.7.1	Unripened cheese excluding products falling in category 16	Group III	Only flavoured unripened cheese	150
01.7.2	Ripened cheese	E 120	Only red marbled cheese and red pesto cheese	125
01.7.3	Edible cheese rind	Group III		*Quantum satis*
01.7.5	Processed cheese	E 120	Only flavoured processed cheese	100 [(a)]
01.7.6	Cheese products (excluding products falling in category 16)	E 120	Only red marbled products	125
01.7.6	Cheese products (excluding products falling in category 16)	Group III	Only flavoured unripened products	100
03	Edible ices	Group III		150

[13] 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. p. 16.

FCS category number	Foods	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
04.2.1	Dried fruit and vegetables	E 120	Only preserves of red fruit	200 [(b)]
04.2.2	Fruit and vegetables in vinegar, oil, or brine	E 120	Only preserves of red fruit	200 [(b)]
04.2.3	Canned or bottled fruit and vegetables	E 120	Only preserves of red fruit	200 [(b)]
04.2.4.1	Fruit and vegetable preparations excluding compote	E 120	Only seaweed based fish roe analogues	100
04.2.4.1	Fruit and vegetable preparations excluding compote	E 120	Only preserves of red fruit	200 [(b)]
04.2.4.1	Fruit and vegetable preparations excluding compote	Group III	Only *mostarda di frutta*	200
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	E 120	Except chestnut purée	100 [(c)]
04.2.5.3	Other similar fruit or vegetable spreads	E 120	Except *crème de pruneaux*	100 [(c)]
05.2	Other confectionery including breath freshening microsweets	Group III	Except candied fruit and vegetables	300
05.2	Other confectionery including breath freshening microsweets	Group III	Only candied fruit and vegetables	200
05.3	Chewing gum	Group III		300
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group III	Only decorations, coatings and sauces, except fillings	500
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group III	Only fillings	300
06.3	Breakfast cereals	E 120	Only fruit-flavoured breakfast cereals	200 [(d)]
06.6	Batters	Group III	Only batters for coating	500
07.2	Fine bakery wares	Group III		200

FCS category number	Foods	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	E 120	Only *breakfast sausages* with a minimum cereal content of 6 %, *burger meat* with a minimum vegetable and/or cereal content of 4 % mixed within the meat (in these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their typical appearance), merguez type products, *salsicha fresca*, *mici*, *butifarra fresca*, *longaniza fresca*, *chorizo fresco*, *cevapcici* and *pljeskavice*	100
08.3.1	Non-heat-treated meat products	E 120	Only sausages	100
08.3.1	Non-heat-treated meat products	E 120	Only *chorizo sausage/salchichon*	200
08.3.1	Non-heat-treated meat products	E 120	Only *pasturmas*	*Quantum satis*
08.3.2	Heat-treated meat products	E 120	Only sausages, patés and terrines	100
08.3.3	Casings and coatings and decorations for meat	E 120	Only edible external coating of *pasturmas*	*Quantum satis*
08.3.3	Casings and coatings and decorations for meat	Group III	Only decorations and coatings except edible external coating of *pasturmas*	500
08.3.3	Casings and coatings and decorations for meat	Group III	Only edible casings	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 120	Only fish paste and crustacean paste	100 [(e)]
09.2	Processed fish and fishery products including molluscs and crustaceans	E 120	Only precooked crustaceans	250 [(f)]
09.2	Processed fish and fishery products including molluscs and crustaceans	E 120	Only smoked fish	100 [(g)]
09.2	Processed fish and fishery products including molluscs and crustaceans	Group III	Only surimi and similar products and salmon substitutes	500
09.3	Fish roe	Group III	Except sturgeons' eggs (caviar)	300
12.2.2	Seasonings and condiments	Group III	Only seasonings, for example curry powder, tandoori	500
12.4	Mustard	Group III		300
12.5	Soups and broths	Group III		50

FCS category number	Foods	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
12.6	Sauces	Group III	Including pickles, relishes, chutney and piccalilli; excluding tomato-based sauces	500
12.9	Protein products, excluding products covered in category 1.8	Group III	Only meat and fish analogues based on vegetable proteins	100
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group III		50
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 III		50
14.1.4	Flavoured drinks	Group III	Excluding chocolate milk and malt products	100
14.2.3	Cider and perry	Group III	Excluding *cidre bouché*	200
14.2.4.	Fruit wine and made wine	Group III	Excluding *wino owocowe markowe*	200
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group III	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	200
14.2.7.1	Aromatised wines	E 120	Only *americano, bitter vino*	100 [(h),(i)]
14.2.7.2	Aromatised wine-based drinks	E 120	Only *bitter soda*	100 [(j)]
14.2.7.2	Aromatised wine-based drinks	Group III	Except *bitter soda, sangria, claria, zurra*	200
14.2.7.3	Aromatised wine-product cocktails	Group III		200

FCS category number	Foods	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	Group III	Only alcoholic drinks with less than 15 % of alcohol and *nalewka na winie owocowym, aromatyzowana nalewka na winie owocowym, nalewka na winie z soku winogronowego, aromatyzowana nalewka na winie z soku winogronowego, napój winny owocowy lub miodowy, aromatyzowany napój winny owocowy lub miodowy, wino owocowe niskoalkoholowe and aromatyzowane wino owocowe niskoalkoholowe*	200
15.1	Potato-, cereal-, flour- or starch-based snacks	Group III	Excluding extruded or expanded savoury snack products	100
15.1	Potato-, cereal-, flour- or starch-based snacks	Group III	Only extruded or expanded savoury snack products	200
15.2	Processed nuts	Group III	Only savoury-coated nuts	100
16	Desserts excluding products covered in categories 1, 3 and 4	Group III		150
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group III		300
17.2	Food supplements supplied in a liquid form	Group III		100
17.3	Food supplements supplied in a syrup-type or chewable form	Group III	Only solid food supplements	300
17.3	Food supplements supplied in a syrup-type or chewable form	Group III	Only liquid food supplements	100

(a): Maximum level individually or for the combination of E 100, E 102, E 120, E 122, E 160e and E 161b.
(b): Maximum level individually or for the combination of E 120, E 122, E 129, E 131 and E 133.
(c): Maximum level individually or in combination with E 120, E 142, E 160d and E 161b.
(d): E 120, E 162 and E 163 may be added individually or in combination.
(e): Maximum level individually or for the combination of E 100, E 102, E 120, E 122, E 142, E 151, E 160e and E 161b.
(f): Maximum level individually or for the combination of E 100, E 102, E 120, E 122, E 129, E 142, E 151, E 160e and E 161b.
(g): Maximum level individually or for the combination of E 100, E 102, E 120, E 151 and E 160e.
(h): In *americano*, E 100, E 101, E 102, E 104, E 120, E 122, E 123 and E 124 are authorised individually or in combination.
(i): In *bitter vino*, E 100, E 101, E 102, E 104, E 110, E 120, E 122, E 123, E 124 and E 129 are authorised individually or in combination.
(j): In *bitter soda*, E 100, E 101, E 102, E 104, E 110, E 120, E 122, E 123, E 124 and E 129 are authorised individually or in combination.
FCS, Food Categorisation System (food nomenclature presented in Annex II to Regulation (EC) No 1333/2008).

2.7. Reported use levels or data on analytical levels of E 120 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 *quantum satis*, information on actual use levels is required in order to perform exposure assessments.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010[14] regarding the re-evaluation of approved food additives, a call[15] for food additive usage level and/or concentration (analytical) data in food and beverages intended for human consumption was launched in March 2013, with a deadline in November 2013. Data on E 120 (cochineal, carminic acid, carmines), including present use and use patterns (i.e. which food categories and sub-categories contain the additive, the proportion of foods within categories/sub-categories in which it is used, and actual use levels (typical and maximum)), and analytical data 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 E 120 in foods. The data submission to EFSA followed the requirements of the EFSA guidance on standard sample description for food and feed (EFSA, 2010a).

In response to this public call, updated information on the actual use levels and analytical data on E 120 in foods were made available to EFSA by industry and Member States. According to Regulation (EC) No 1333/2008, the maximum levels for colours set out in Annex II shall apply to the quantities of colouring principle contained in the colouring preparation, unless otherwise stated. Therefore, the Panel considered that the concentration data provided for E 120 were also expressed as carminic acid.

2.7.1. Summarised data on reported use levels of E 120 in foods, as provided by industry

Following the call[15] for food additive usage level and/or concentration data launched in March 2013, updated information on the actual uses and use levels of E 120 (cochineal, carminic acid, carmines) was made available by FoodDrinkEurope (FDE) (n = 81), the International Chewing Gum Association (ICGA) (n = 1), the Natural Food Colours Association (NATCOL) (n = 192), the Association of the European Self-Medication Industry (AESGP) (n = 1), Specialised Nutrition Europe (SNE) (n = 4) and a private company (n = 5). The data provided cover the majority of the food categories in which this food additive is authorised; most data were provided for edible ices (FCS 03), other confectionery (FCS 05.2) and flavoured drinks (FCS 14.1.4.).

In summary, industry provided EFSA with data on use levels (n = 284) for E 120 (cochineal, carminic acid, carmines) in foods for 54 out of 62 food categories in which E 120 is authorised. One usage level, corresponding to a foodstuff for which information regarding food classification was insufficient, was not considered for the exposure assessment.

Appendix A provides data on the use levels of E 120 in foods, as reported by industry.

2.7.2. Summarised data on concentration levels of E 120 in foods from Member States

Analytical results from Member States were collected through the EFSA call[15] for concentration data. The Panel noted that complete information on the methods of analysis (e.g. validation) was made available to EFSA.

In total, 5 308 analytical results were reported to EFSA by eight countries: Austria (n = 568), Cyprus (n = 48), the Czech Republic (n = 8), Germany (n = 3 372), Hungary (n = 368), Spain (n = 304), Slovakia (n = 344) and the United Kingdom (n = 296). The data were mainly on edible ices (FCS 03), other confectionery (FCS 05.2) and flavoured drinks (FCS 14.1.4). Foods were sampled between 2004 and 2013. Out of this dataset, analytical results for E 120 were not quantified (< LOQ) in 1 442

[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, p. 19.

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

samples and not detected (< limit of detection (LOD)) in 3 068 samples, 490 were qualitative values (presence or absence) and 308 were numerical values (quantified). Only 31 samples were analysed in a non-accredited laboratory.

Analytical data expressed as qualitative data (n = 490) were not used for the exposure assessment, as these data give only binary results (i.e. an indication of the presence or absence of the food additive in the food analysed). In total, 51 out of 490 qualitative results indicated the presence of E 120; most of these results were related to other confectionery (FCS 05.2).

Analytical data (n = 33) with no information reported on the LOD or LOQ were not used in the exposure assessment. A number of analytical data (n = 304) on food categories in which the use of E 120 is not authorised, mostly on fruit juices (FCS 14.1.2) and fruit nectars (FCS 14.1.3), were not included in the exposure assessment. Out of these 304 data reported for non-authorised food categories, only 6 results, obtained for cocoa and chocolate products (FCS 05.1), bread and rolls (FCS 07.1), and fruit juices (FCS 14.1.2), were above the LOD. Other samples (n = 19) were classified either at the upper level (level 1) of the FCS (and were, therefore, lacking the information needed for their assignment under the correct FCS category) or belonged to a food sub-category in which E 120 is not authorised; these samples were, therefore, also excluded. This relates to the following food categories: processed fruit and vegetables (FCS 04.2), cereals and cereal products (FCS 06), and alcoholic beverages (FCS 14.2). In addition, two samples of herbs, spices and seasonings were also excluded, as the concentration of E 120 measured was higher than the MPL.

Overall, 4 460 out of the 5 308 analytical results reported for E 120 in foods were considered by the Panel for the exposure estimates, after discarding the data expressed as qualitative results, the data for which no information was provided on the LOD or LOQ, the results on foods in which E 120 is not authorised, the results with insufficient information regarding the food classification, and the samples exceeding the MPL.

Appendix B shows the analytical results for E 120 in foods, as reported by Member States (the whole set of analytical data reported and positive samples only).

2.8. Information on existing authorisations and evaluations

Cochineal, carminic acid, carmines (E 120) is authorised as a food additive in the EU, according to Annex II to Regulation (EC) No 1333/2008 on food additives for use in foods, at the levels of use listed in Table 3.

Cochineal and carmines have been evaluated previously by JECFA in 1974, 1977, 1981, 1982 and 2000 (JECFA, 1975, 1978, 1981, 1982, 2001) and by the SCF in 1975, 1979 and 1983 (SCF, 1975, 1979, 1983). Both committees have established an ADI of 5 mg/kg bw/day. For JECFA, this ADI for carmines (formerly cochineal, carmines and carminic acid) includes ammonium carmine or the equivalent calcium, potassium and sodium salts. The 1981 JECFA evaluation specifically excluded the lithium salt, considering it as not acceptable for food additive use. The Panel noted that a committee of the Health Council of the Netherlands recommends that lithium carbonate and lithium chloride are classified in Category 1 (substances known to cause developmental toxicity in humans) and that lithium carbonate and lithium chloride are labelled with R 61 (may cause harm to the unborn child) (Health Council of the Netherlands, 2000).

The SCF ADI applies to cochineal (carmines), without other limitations.

The safety of use of cochineal, carminic acid and carmines as food colours has been reviewed by the Nordic Council of Ministers, who concluded that findings of adverse effects with regard to teratogenicity, reproduction and allergenic potential warranted a re-evaluation of this additive (TemaNord, 2002). Furthermore, according to the TemaNord review, the limited information on the metabolism of cochineal and carmine warranted further studies. The review also considered that the

significance of results with regard to the lithium salt should be evaluated in relation to the authorised aluminium salt.

Carmines are also included in the list of colourants allowed in cosmetic products (Regulation (EC) No 1223/2009).[16]

2.9. Exposure

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (EFSA, 2011a; available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons 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 1 day per subject. High-level consumption was calculated only for those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile. The Panel estimated chronic exposure for the following population groups: infants, 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 33 different dietary surveys carried out in 19 European countries, as summarised in Table 4.

Table 4: Population groups considered for the exposure estimates of E 120

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 4 up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Netherlands, Italy, Spain
Children [(a)]	From 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	From 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	From 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly [(a)]	From 65 years of age and older	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

[16] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59.

(a): The terms "children" and "the elderly" correspond, respectively, to "other children" and both "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 exposure was calculated by multiplying the MPLs reported in Table 3 or the use/analytical levels reported in Appendix A and Appendix B for each food group with their corresponding consumption amount per kg bw separately for each individual in the database. The exposure per food category was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of surveys days, resulting in an individual average exposure per day for the survey period. This was done for all individuals in the survey and per age group, resulting in distributions of individual average exposure per survey and population group (Table 4). 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 E 120

The food categories in which the use of E 120 (cochineal, carminic acid, carmines) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at a detailed level (up to FoodEx Level 4) (EFSA, 2011b).

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

- 01.6.3. Other creams, only flavoured creams
- 01.7.2. Ripened cheese, only red marbled cheese and red pesto cheese
- 01.7.3. Edible cheese rind
- 01.7.6. Cheese products, only red marbled products
- 01.7.6. Cheese products, only flavoured unripened products
- 04.2.4.1. Fruit and vegetable preparations excluding compote, only seaweed based fish roe analogues
- 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, only decorations, coatings and sauces, except fillings
- 05.4. Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4, only fillings
- 06.6. Batters
- 08.2. Meat preparations, only *breakfast sausages* with a minimum cereal content of 6 %, *burger meat* with a minimum vegetable and/or cereal content of 4 % mixed within the meat (in these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their

typical appearance), merguez type products, *salsicha fresca, mici, butifarra fresca, longaniza fresca, chorizo fresco, cevapcici and pljeskavice*

- 08.3.1. Non-heat-treated meat products, only *pasturmas*
- 08.3.3. Casings and coatings and decorations for meat, only edible external coating of *pasturmas*
- 08.3.3. Casings and coatings and decorations for meat, only decorations and coatings, except edible external coating of *pasturmas*
- 08.3.3. Casings and coatings and decorations for meat, only edible casings
- 14.2.7.2. Aromatised wine-based drinks, only *bitter soda*
- 14.2.7.2. Aromatised wine-based drinks, except *bitter soda*, *sangria, claria, zurra*
- 14.2.7.3. Aromatised wine-product cocktails.

For the following food categories, the restrictions which apply to the use of E 120 (cochineal, carminic acid, carmines) 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.
- 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.
- 12.9. Protein products, excluding products covered in category 1.8, only meat and fish analogues based on vegetable proteins: only the food category 'meat imitates' was used in the exposure assessment. Fish analogues based on vegetable proteins are not included in the FoodEx nomenclature and therefore were not included in the exposure assessment.
- 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.
- 14.2.4. Fruit wine and made wine, excluding *wino owocowe markowe: wino owocowe markowe* is not referenced in the FoodEx classification nomenclature.
- 14.2.6. Spirit drinks, 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.
- 15.1./15.1. Potato-, cereal-, flour- or starch-based snacks: it was not possible within the FoodEx food classification to differentiate extruded or expanded savoury snack products. Using a conservative approach, all cereal-, flour- or starch-based snacks were assigned the highest MPL of 200 mg/kg, corresponding to the sub-category of "only extruded or expanded savoury snack products".

- 15.2. Processed nuts, only savoury-coated nuts: savoury-coated nuts are not referenced in the FoodEx classification nomenclature.

- 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 the FoodEx codes.

Overall, 18 food categories were not taken into account in the exposure assessment, as these are not referenced in the EFSA Comprehensive Database, and 12 food categories were included in the exposure assessment without considering the restrictions defined in Annex II to Regulation (EC) No 1333/2008.

2.9.2. Exposure to E 120 as a food additive

Dietary exposure to E 120 from its use as a food additive was estimated using the approach adopted by the Panel at its 52nd plenary meeting.[17] This approach should be followed to assess the exposure as part of the safety assessment of food additives under re-evaluation with the use of the food consumption data available within the EFSA Comprehensive Database, as presented in Table 4, and with the limitations described above.

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

2.9.2.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 3. The exposure estimates derived following this scenario should be considered as the most conservative, since it assumes that the consumer will be continuously (over a lifetime) exposed to E 120 present in food at the MPLs.

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on reported use levels from industry and analytical results submitted to EFSA by Member States. This exposure scenario can consider only food categories for which such data are available.

Appendix C summarises the concentration levels of E 120 used in the refined exposure assessment scenarios. Based on the available dataset, two estimates, based on different model populations, were calculated as follows:

1. The brand-loyal consumer scenario assumes that a consumer experiences long-term exposure to the food additive at the maximum reported use/analytical level for one food category. This exposure estimate is calculated as follows:

    a) Food consumption is combined with the maximum reported use/analytical levels for the main contributing food category at the individual level.

    b) The mean of the typical reported use levels or the mean of analytical levels is used for the remaining food categories.

2. The non-brand-loyal consumer scenario assumes that a consumer experiences long-term exposure to the food additive at the mean reported use/analytical levels in food. This exposure

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

estimate is calculated using the mean of the typical reported use levels or the mean of analytical levels for all food categories.

In both refined exposure assessment scenarios, the concentrations considered by the Panel were extracted from the dataset received. To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ), the substitution method, as recommended in the "Principles and methods for the risk assessment of chemicals in food" (WHO, 2009) and the EFSA scientific report "Management of left-censored data in dietary exposure assessment of chemical substances" (EFSA, 2010b), was used. In the present opinion, analytical data below the LOD or LOQ were assigned a value of half of the LOD or LOQ, respectively (medium-bound). Subsequently, per food category, the mean or median, as appropriate, medium-bound concentration was calculated. For the reported use levels, the mean typical reported use level per food category was calculated.

If both reported use levels and analytical results were available for the same food category, the most reliable value was used.

2.9.2.3. Anticipated exposure to E 120

Table 5 summarises the estimated exposure to E 120 from its use as a food additive for all six population groups (Table 4). Detailed results by population group and survey are presented in Appendix D.

Table 5: Summary of anticipated exposure to E 120 as a food additive using the regulatory maximum level exposure assessment scenario and the refined exposure assessment scenarios, in six population groups (minimum–maximum across the dietary surveys in mg carminic acid/kg bw/day)

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level scenario						
Mean	0.1–0.7	0.7–3.9	0.8–3.1	0.2–2.0	0.3–1.1	0.2–0.7
High level (95th percentile)	0.3–2.5	2.7–6.7	1.9–6.2	0.7–4.2	0.7–2.5	0.5–1.4
Brand-loyal scenario						
Mean	0.1–0.5	0.5–2.1	0.3–1.5	0.1–0.9	0.1–0.5	0.1–0.3
High level (95th percentile)	0.3–1.7	1.3–4.7	0.9–4.0	0.3–1.9	0.4–1.3	0.2–0.7
Non-brand-loyal scenario						
Mean	0.02–0.1	0.1–0.6	0.1–0.4	0.03–0.3	0.04–0.2	0.03–0.2
High level (95th percentile)	0.1–0.5	0.4–1.1	0.2–1.0	0.1–0.5	0.1–0.4	0.1–0.3

2.9.3. Main food categories contributing to exposure to E 120 using the regulatory maximum level exposure assessment scenario

The main food categories contributing to total mean exposure to E 120 (> 5 % of total exposure) calculated for the regulatory maximum level exposure assessment scenario, as well as the number of surveys in which each food category is a main contributor, are shown in Table 6. Flavoured drinks and fine bakery wares were the main contributors for children, adolescents, adults and the elderly, whilst in toddlers, in addition to fine bakery wares, flavoured fermented milk products were also an important contributor to the total mean exposure to E 120. In infants, processed fruit and vegetables, and soups and broths, were also important contributors; however, this was observed for only one dietary survey.

Table 6: Main food categories contributing to exposure to E 120 as a food additive using the regulatory maximum level exposure assessment scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	Foods	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
01.4	Flavoured fermented milk products	5.5–49.1 (6)	6.0–72.6 (10)	6.0–34.5 (16)	5.3–22.2 (11)	5.3–19.5 (14)	6.8–19.8 (11)
03	Edible ices	–	6.0–6.5 (2)	5.1–11.0 (11)	5.3–10.6 (6)	5.0–7.6 (3)	6.6–7.0 (2)
04.2	Processed fruit and vegetables	68.2 (1)	7.3 (1)	–	–	6.7 (1)	5.2–13.9 (5)
05.2	Other confectionery including breath freshening microsweets	–	7.2 (1)	5.6–30.5 (6)	5.1–39.5 (7)	5.4–16.6 (4)	7.1–7.2 (2)
07.2	Fine bakery wares	5.4–61.5 (4)	5.6–49.6 (9)	12.2–50.1 (16)	12.4–51.6 (15)	7.7–31.5 (17)	12.0–39.5 (14)
08.3	Processed meat	5.1–14.2 (2)	5.2–15.7 (3)	6.1–11.3 (8)	5.9–11.1 (9)	5.4–19.3 (10)	5.0–19.3 (6)
09.2	Processed fish and fishery products	8.0 (1)	7.5 (1)	–	–	5.4 (1)	6.7 (1)
12.2.2	Herbs, spices, seasonings	10.3 (1)	6.6–9.1 (2)	5.1 (1)	10.7 (1)	16.7 (1)	5.7–20.6 (2)
12.5	Soups and broths	60.4 (1)	6.4 (1)	5.5–15.0 (3)	14.1 (1)	6.7–18.9 (4)	5.2–17.5 (7)
12.6	Sauces	10.0–23.5 (3)	5.7–21.9 (6)	5.6–17.2 (10)	5.0–21.4 (11)	5.8–27.8 (14)	6.7–24.9 (12)
14.1.4	Flavoured drinks	7.4–41.0 (3)	6.8–41.6 (7)	9.1–40.8 (18)	10.5–46.2 (17)	7.7–49.4 (17)	5.7–39.0 (11)
14.2	Alcoholic beverages	–	–	–	–	5.0–16.6 (4)	5.7–6.0 (4)
15.1	Potato-, cereal-, flour- or starch-based snacks	5.1–6.4 (2)	5.5–6.7 (2)	5.3–7.6 (3)	5.2–14.5 (5)	12.1 (1)	5.9 (1)
16	Desserts excluding products covered in category 01, 03 and 04	9.8–17.0 (2)	5.7–15.0 (5)	5.2–11.3 (7)	5.0–8.4 (2)	5.4–6.3 (2)	6.4–11.3 (4)

(a): The total number of surveys may be greater than the total number of countries 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 E 120 using the refined exposure assessment scenarios

The main food categories contributing to total mean exposure to E 120 (> 5 % of total exposure) calculated for the brand-loyal and non-brand-loyal refined exposure assessment scenarios, as well as the number of surveys in which each food category is a main contributor, are shown in Tables 7 and 8, respectively.

For the brand-loyal scenario, the food categories that, at the individual level, had the highest contribution to the total individual exposure to E 120 were identified for each age group. Flavoured fermented milk products and flavoured drinks were the main contributors in toddlers, children and adolescents. In adults and the elderly, in addition to flavoured drinks, sauces were also an important contributor to the total mean exposure to E 120 (Table 7).

Table 7: Main food categories contributing to exposure to E 120 as a food additive using the brand-loyal refined exposure assessment scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	Foods	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
01.4	Flavoured fermented milk products	5.8–71.0 (6)	13.7–84.6 (10)	9.5–60.9 (16)	5.0–43.8 (14)	5.6–33.7 (15)	6.7–39.0 (12)
03	Edible ices	–	5.3–5.4 (2)	5.1–23.4 (9)	6.3–17.6 (6)	6.7–11.2 (3)	5.7–1-.1 (2)
04.2	Processed fruit and vegetables	69.3 (1)	6.3–6.5 (2)	9.0 (1)	–	5.2–6.6 (2)	6.5–20.7 (5)
05.2	Other confectionery including breath freshening microsweets	–	–	15.4 (1)	9.9–26.3 (2)	7.7–9.3 (2)	–
06.3	Breakfast cereals	–	–	–	–	–	5.8 (1)
07.2	Fine bakery wares	28.6 (1)	6.9–19.1 (2)	8.2–15.5 (5)	5.1–19.2 (7)	5.0–8.4 (7)	6.4–10.5 (6)
08.3	Processed meat	–	–	11.6 (1)	9.4–16.0 (3)	5.2–11.8 (5)	7.8–8.1 (2)
09.2	Processed fish and fishery products	–	9.3 (1)	–	–	–	5.6–7.4 (3)
09.3	Fish roe	5.7 (1)	–	–	–	–	–
12.2.2	Herbs, spices, seasonings	20.5 (1)	10.2–14.9 (2)	6.1–8.0 (2)	21.0 (1)	30.9 (1)	36.1 (1)
12.5	Soups and broths	61.3 (1)	5.2–8.0 (2)	5.3–25.2 (5)	5.6–23.7 (4)	6.9–31.2 (5)	5.9–28.6 (8)
12.6	Sauces	14.1–28.7 (4)	5.4–32.2 (6)	5.0–31.7 (11)	5.7–38.1 (14)	8.4–46.8 (14)	7.8–45.6 (13)
14.1.4	Flavoured drinks	10.8–44.3 (2)	7.6–50.7 (6)	6.5–52.7 (18)	5.8–61.0 (17)	6.8–61.6 (16)	5.7–50.4 (9)
14.2	Alcoholic beverages	–	–	–	–	–	5.3–11.7 (5)
15.1	Potato-, cereal-, flour- or starch-based snacks	9.8–11.9 (2)	7.1–14.4 (2)	5.1–16.4 (6)	5.1–31.2 (7)	5.5–22.1 (3)	11.1 (1)
16	Desserts excluding products covered in category 01, 03 and 04	6.5–20.2 (2)	6.0–16.0 (5)	5.5–24.7 (8)	5.6–16.0 (3)	6.2–9.2 (2)	6.8–16.0 (6)

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

Regarding the non-brand-loyal scenario (Table 8), the main contributors to the total mean exposure to E 120 were soups and broths in infants, adults and the elderly; flavoured fermented milk products in toddlers and children; snack and sauces in adolescents; and herbs, spices and seasonings in adults and the elderly.

Table 8: Main food categories contributing to exposure to E 120 as a food additive using the non-brand-loyal refined exposure assessment scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	Foods	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
01.4	Flavoured fermented milk products	8.9–41.9 (5)	8.8–62.6 (10)	6.9–42.2 (16)	5.5–31.5 (13)	6.2–19.2 (12)	6.1–19.4 (10)
01.7.1	Unripened cheese	–	–	5.1 (1)	–	–	–
03	Edible ices	–	–	5.4–7.3 (4)	6.6 (1)	–	–
04.2	Processed fruit and vegetables	79.7 (1)	7.4–16.7 (2)	5.5–11.6 (5)	5.3–7.8 (4)	5.2–12.8 (6)	6.3–19.5 (11)
05.2	Other confectionery including breath freshening microsweets	–	–	5.7–14.6 (2)	5.4–20.4 (3)	5.9 (1)	–
06.3	Breakfast cereals	–	–	5.1–9.0 (2)	8.3 (1)	7.9 (1)	5.5–10.1 (2)
07.2	Fine bakery wares	6.5–26.5 (2)	5.1–22.4 (5)	5.0–24.2 (14)	5.0–19.8 (15)	5.0–11.3 (11)	6.1–11.3 (9)
08.3	Processed meat	–	7.2 (1)	5.1–26.1 (8)	6.2–30.9 (6)	5.4–27.5 (6)	5.4–16.0 (3)
09.2	Processed fish and fishery products	–	15.3 (1)	–	–	5.1–7.2 (2)	7.0–13.4 (2)
09.3	Fish roe	–	8.4 (1)	–	–	–	–
12.2.2	Herbs, spices, seasonings	31.1 (1)	20.2–20.9 (2)	14.6–18.7 (3)	11.7–35.5 (2)	6.9–41.1 (4)	7.9–44.3 (4)
12.4	Mustard	–	–	–	–	5.7–11.6 (2)	9.3 (1)
12.5	Soups and broths	8.4–80.5 (2)	5.7–25.9 (6)	5.2–42.8 (9)	6.2–38.3 (7)	5.3–46.3 (9)	12.9–42.8 (8)
12.6	Sauces	10.6–30.3 (4)	5.5–33.2 (7)	5.4–35.3 (15)	7.0–41.2 (16)	6.2–40.9 (17)	6.6–36.5 (14)
14.1.4	Flavoured drinks	8.2 (1)	6.3–8.8 (2)	5.1–8.8 (9)	6.1–10.8 (9)	5.9–11.7 (5)	8.4 (1)
14.2	Alcoholic beverages	–	–	–	–	8.5–24.1 (5)	5.9–7.9 (4)
15.1	Potato-, cereal-, flour- or starch-based snacks	19.2–21.3 (2)	7.2–26.4 (5)	5.3–23.2 (15)	5.4–48.3 (15)	5.9–26.4 (10)	5.5–11.8 (2)
15.2	Processed nuts	–	–	–	–	5.8–9.9 (2)	8.3 (1)
16	Desserts excluding products covered in category 01, 03 and 04	10.2–24.3 (3)	8.4–26.1 (7)	5.4–27.8 (11)	5.7–21.9 (4)	6.2–12.8 (3)	5.3–15.5 (7)
17	Food supplements	–	–	–	–	–	5.7 (1)

(a): The total number of surveys may be greater than the total number of countries 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 E 120 have been discussed above. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the sources of uncertainties considered are summarised below (Table 9).

Table 9: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimates

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 Database: uncertainties as to which precise types of food the levels refer to	+/–
Uncertainty in possible national differences in the use levels in food categories, usage data not fully representative of foods on the EU market	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restrictions/exceptions	+
Use/analytical levels: levels considered applicable for all items within the entire food category	+/–
Use levels: assumption that the reported use levels provided by industry refer to the colouring principle carminic acid	+
Regulatory maximum level scenario: exposure calculations based on the MPLs	+
Brand-loyal exposure model: exposure calculations based on the maximum reported use/analytical levels for one food category and mean reported use/analytical levels for the remaining food categories	+/–
Non-brand loyal exposure model: exposure calculations based on the mean reported use/analytical levels	+/–

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

The Panel considered that the uncertainties identified would tend to overestimate the real exposure to E 120 used as a food additive in European countries.

3. Biological and toxicological data

Cochineal, carmine or carminic acid have previously been evaluated by JECFA in 1974, 1977, 1981, 1982 and 2000,(JECFA, 1975, 1978, 1981, 1982, 2001) and the SCF, in 1975, 1979 and 1983 (SCF, 1975, 1979, 1983). E 120 has also been reviewed by TemaNord (2002). The present opinion briefly reports the major studies evaluated in these opinions and describes the additionally reported new literature data in some more detail.

3.1. Absorption, distribution, metabolism and excretion

No studies on absorption, distribution, metabolism or excretion (ADME) of cochineal, carminic acid or carmines were available for this evaluation. However, the Panel considered that, owing to the ionisation properties of carminic acid (pK_a values of 2.81, 5.43 and 8.10), the unionised form of carminates and carmine should be absorbed by a diffusion process in the stomach.

The toxicokinetics of the cochineal colours have not been addressed in previous evaluations. As reported by JECFA (1982), indirect evidence from toxicological studies suggests that these compounds could be absorbed to some extent. The accumulation of colour in tissues and the red

coloration of urine were reported in rats chronically treated with high doses of ammonium carmine (2.5, 5.0 and 10.0 g/kg, 5 days per week, for 13 weeks) (Battelle Memorial Institute, 1962).

3.2. Toxicological data

3.2.1. Acute oral toxicity

No new information was available. An LD_{50} value of 6 250 mg/kg bw (for carminic acid) in mice is referenced by the British Industrial Biological Research Association (BIBRA, 1982) without further details.

3.2.2. Short-term and subchronic toxicity

3.2.2.1. Studies reported by JECFA

The JECFA evaluation of 1982 (JECFA, 1982) briefly describes two short-term oral studies, of which one original publication was not accessible to the Panel, and therefore no further data can be provided.

Carmine

Groups of 25 weanling rats of each sex were fed calcium carmine in the diet at levels of 0, 50, 250, and 500 mg/kg bw/day for 90 days. Blood counts, blood glucose, blood urea nitrogen and urinalyses performed three times during the study did not reveal any treatment-related effects. Furthermore, no treatment-related effects in gross or microscopic pathology, growth, or other clinical findings (not described) were reported (FDRL, 1962; as referred to by JECFA, 1982).

Groups of Wistar rats, 20 of each sex, were given ammonium carmine in 0.4 % aqueous tragacanth gum suspension by gavage, five days per week, to provide doses of 0, 2.5, 5, and 10 g/kg bw/day for 13 weeks. Haematological parameters, behaviour, general health and physical condition were determined for every animal. Eleven males from different groups died during the study; none of these deaths was attributed to the treatment. At the end of the study, 50 % of the surviving animals in the groups given 5 and 10 g/kg bw/day, together with 25 % of those from the group given 2.5 g/kg bw/day, were sacrificed, and autopsy examinations were performed on 17 organs or tissues. No compound-related effects on haematological parameters or body weight gain were reported for animals from the two lowest dose groups. Animals in the 10 g/kg bw/day group showed a transitory decrease in body weight gain between the fifth and the seventh weeks of the study, a finding that, according to the authors, was attributed to the excessive liquid volume needed to suspend the ammonium carmine in order to administer it by gastric intubation. Animals in the highest dose group were reported to have frequent attacks of diarrhoea. Other than isolated granulomas of the liver associated with parasitic infestation, no gross or microscopic findings were reported. It was highlighted in the report that no diminution of maturation in the germinal cells of the reproductive organs was detected. At the two highest levels, deposition of the colourant, mainly in the cytoplasmic mitochondria and nuclei (discolouration) of the tissues, and in the urine and faeces of the treated rats was observed (Battelle Memorial Institute, 1962). At the highest dose, discolouration was seen in several organs of the epithelial system, but did not involve the ganglion cells of the central nervous system.

3.2.2.2. New studies and/or studies not reported by JECFA

Cochineal extracts

Mori et al. (1991) shortly reported a sub-acute toxicity test (8 weeks) performed in B6C3F1 mice, to select cochineal concentrations for the longer term study, treated with 0.75, 1.5, 3 and 6 % cochineal in the diet; this study did not show toxicity.

In a 90-day study (Kawasaki et al., 1994)[18] conducted to investigate the simultaneous administration of cochineal[19] (carminic acid content not specified) and potassium aluminium sulphate ($KAl(SO_4)_2$), 5-week-old Wistar rats (15/sex/group) were fed with diets containing either 0 %; 0.75 % cochineal plus 0.75 % $KAl(SO_4)_2$ (1.5 % dose group); 1.5 % cochineal plus 1.5 % $KAl(SO_4)_2$ (3.0 % dose group); 3.0 % cochineal alone (3.0 % cochineal dose group); or 3.0 % $KAl(SO_4)_2$ alone (3.0 % $KAl(SO_4)_2$ dose group). The cochineal doses used in this study were equivalent to 0, 375, 750 and 1 500 mg cochineal/kg bw/day, respectively. Body weight, food consumption, and complete haematological and serum biochemical examinations were performed in all animals at weeks 4 and 13. Organ weights were recorded for all animals and histopathological examinations were also conducted on all animals. No effects were reported on body weight or food consumption. At the 3.0 % cochineal dose group, significantly ($P < 0.01$) reduced serum phospholipid and triglyceride levels were reported for male rats. At this same dose, in female rats, serum triglyceride and total cholesterol levels were significantly reduced, whereas levels of gamma-glutamyl transferase were significantly increased. After 13 weeks of treatment, kidney, adrenal and spleen weights (not defined if weights were absolute or relative) were significantly reduced in female rats in the 3.0 % cochineal dose group. There were no histopathological changes that could be specifically attributed to cochineal administration.

Carmine

The effects of carmine on liver and kidney function were studied in young male albino rats (Helal et al., 2000). Ten rats were treated with 1.25 mg/kg bw/day of carmine in their diet (no further details). After 30 days of treatment (experimental period), half of each group of animals was sacrificed, while the rest continued without treatment for two additional weeks (recovery period). Body weight gain, liver to body weight and kidney to body weight ratios, serum biochemistry, and total protein content of the liver, kidney, heart, muscle and brain were recorded for all animals. The results did not show differences in the liver to body weight or the kidney to body weight ratios throughout the experimental period. Carmine treatment did not affect liver enzyme activities (aspartate amino transferase or alanine amino transferase). Serum bilirubin, creatinine, total protein and globulins were increased after 30 days of treatment, but returned to normal during the recovery period. The only parameter that remained elevated during the treatment and recovery periods was serum urea levels. No other treatment-related effect was reported. The authors suggested that the elevation in serum urea levels may indicate impairment in renal function resulting from carmine exposure. The Panel noted that only one low dose was tested.

3.2.3. Genotoxicity

3.2.3.1. Studies reported by JECFA

Carminic acid

JECFA reported a number of mutagenicity studies conducted with carminic acid, all of them showing that carminic acid has no mutagenic potential. Negative results were obtained in a *Bacillus subtilis* rec^- assay (Kada et al., 1972), in Ames tests with several strains of *Salmonella typhimurium* and in *Escherichia coli* WP2 *uvrA* strain in the presence of liver microsomal preparations or with enzymatic extracts of rat caecal microflora (Barale et al., 1978; Brown and Brown, 1976; Brown et al., 1977; Haveland-Smith and Combes, 1980). Carminic acid did not induce gene conversions in *Saccharomyces cerevisiae* D strain, nor forward mutations *in vitro* or *in vivo* in a host-mediated assay using *Schizosaccharomyces pombe* (Barale et al., 1978).

3.2.3.2. New studies and/or studies not reported by JECFA

Carminic acid

[18] The published paper was in Japanese but tables of all results were in English.
[19] Defined in the publication as "a scarlet material extracted from the powdered pregnant insect, *Dactylopius Coccus* Costa, used as a color food additive in the form of aluminium lakes".

Natural Red 4 (90 % carminic acid) was evaluated in an *in vitro* DNA repair assay (unscheduled DNA synthesis (UDS) assay) in rat hepatocyte primary cultures at 10^{-4}, 10^{-5} and 10^{-6} M, and in the *in vivo/in vitro* rat liver UDS assay with oral administration at 500 mg/kg bw, 2 and 15 hours before sacrifice (Kornbrust and Barfknecht, 1985). All tests were evaluated as negative.

A sample of carminic acid, isolated from cochineal by the study authors (purity not specified), gave negative results in an unscheduled DNA Synthesis (UDS) test with rat liver primary cells exposed to concentrations of 10^{-4}, 10^{-5} and 10^{-6} M (Mori et al., 1988).

A series of *in vitro* and *in vivo* studies was performed with two commercial samples of carminic acid with different purity (Loprieno et al., 1992). In the *Salmonella typhimurium* reverse mutation test, carminic acid (53.1 % pure) was not mutagenic in strains TA 1535, TA 1537, TA 98 or TA 100 at doses from 125 to 2 000 µg/plate, in the presence and in the absence of S9 at two concentration levels (Loprieno et al., 1992). Negative results were also obtained with a sample of higher purity (87.5 % pure) tested only in strain TA 100 at concentrations up to 2 000 µg/plate. In the same publication, both carminic acid samples (57.3 % and 87.5 % pure) were reported as negative in tests for chromosome aberration and sister chromatid exchanges in Chinese hamster ovary cells treated with 20, 63 and 200 µg/mL carminic acid, in the presence and absence of S9. *In vivo*, carminic acid (57.3 %) also tested negative in a micronucleus test in bone marrow cells of CD-1 mice (five animals/sex/group) treated orally for 24 and 48 hours with doses of carminic acid of 1 250, 2 500 or 5 000 mg/kg bw. In this latter study, normochromatic erythrocyte to polychromatic erythrocyte ratios increased markedly after carminic acid treatment, indicating that the test substance had reached the bone marrow.

Carminic acid was evaluated in the somatic mutation and recombination test (SMART) in *Drosophila*, by feeding larvae with medium containing 1, 10 or 20 mg carminic acid/mL (Sarikaya et al., 2012). No increase of spots, due to somatic mutation or recombination, was observed in wings of flies from treated larvae, and thus, carminic acid was evaluated as negative in this test.

A new set of genotoxicity studies on carminic acid (95 % pure) was submitted by NATCOL. The studies, performed under Good Laboratory Practice (GLP) conditions, consisted of a bacterial reversion assay (performed in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 471) (OECD, 1997) and an *in vitro* micronucleus test (performed in accordance with OECD TG 487) (OECD, 2014).

In the bacterial reversion assay, carminic acid was tested with and without metabolic activation by S9 at six doses, ranging from 15 to 5 000 µg/plate, using the *S. typhimurium* strains TA 1535, TA 97a, TA 98, TA 100 and TA 102. Two independent experiments were performed, using the plate incorporation and pre-incubation methods. No treatment-related increase of revertant colonies, and no evidence of bacterial toxicity, was observed in any strain, with or without S9. The positive control substances elicited a distinct positive response. Thus, carminic acid was evaluated as negative, i.e. non-mutagenic, under the conditions of this test (Andres, 2014a). The Panel agreed with this conclusion.

For the *in vitro* micronucleus test, duplicated cultures of peripheral lymphocytes from healthy male and female donors were used in different experiments. Carminic acid was dissolved in culture medium and tested at 1.23, 2.46 and 4.92 (10 mM) mg/mL in experiments with short treatment times (4 hours), with and without S9; cells were harvested after 22 hours. For the extended treatment (19 hours, only without S9), the doses tested were 0.31, 0.62, 2.46 and 4.92 mg/mL. Cytotoxicity was evaluated by the cytokinesis-block proliferation index (CBPI), scoring 1 000 cells (500 for each duplicate culture); micronuclei were scored in 2 000 binucleated cells per dose (1 000 for each culture). Treatments induced only mild inhibition of cell proliferation, with less than a 10 % decrease in the CBPI after short treatment and approximately a 30 % decrease after the extended treatment. There was no significant or dose-related increase in micronuclei, either with or without S9. Based on the results

obtained, carminic acid was considered as "not genotoxic under the conditions of the test" (Geissel, 2014a). The Panel agreed with this conclusion.

Cochineal

Cochineal (composition not specified) was negative in the *Salmonella* reversion test in strains TA 1535, TA 1537, TA 92, TA 94, TA 98 and TA 100 when tested at levels of up to 20 mg/plate with and without S9 mix (Ishidate et al., 1984). In the same study, cochineal tested positive in a chromosomal aberration assay in Chinese hamster ovary cells, performed only without metabolic activation, at a dose of 12 mg/mL; test results were equivocal at 6 mg/mL. The Panel noted that the doses applied in the chromosomal aberration test exceed the OECD maximum recommended dose, and that no information on the cytotoxicity of the treatments was provided; moreover, gaps were included in the total aberration frequency, contrary to OECD test guideline recommendations[20]; thus, this study was not considered for the safety assessment of cochineal and carminic acid.

In another study, a commercial sample of cochineal (composition not specified) tested negative in a UDS test with rat liver primary cells exposed to concentrations of 1, 10 and 100 µg/mL (Mori et al., 1988).

3.2.3.3. Conclusions on genotoxicity

Based on the results of the studies available, which include properly performed *in vitro* GLP studies recommended by the EFSA Guidance on Food Additives, the Panel concluded that carminic acid is not genotoxic. The Panel noted that the chelate carmine is structurally related to carminic acid, and not bearing additional structural alert for genotoxicity. Thus, the Panel considered that read-across from carminic acid to carmine, for genotoxicity was acceptable and, therefore, also considered carmine as non-genotoxic.

3.2.4. Chronic toxicity and carcinogenicity

3.2.4.1. Studies reported by JECFA

Carmine

JECFA briefly reported an unpublished study on carmine, in which carmine was mixed in the diets of groups of 66 male and 66 female rats (strain not identified) to provide daily intakes of 50, 150 or 500 mg/kg bw/day for 8 weeks (Ford et al., 1981; as reported by JECFA, 1982). This study is described in detail in the following section.

3.2.4.2. New studies and/or studies not reported by JECFA

Carmine

In a combined chronic toxicity/carcinogenicity study involving *in utero* exposure, groups of parental (F0 generation) Wistar rats (66/sex/group) were fed carmine containing carminic acid in the range of 46 to 57 %, at levels of 50, 150 or 500 mg/kg bw/day for 60 days (Ford et al., 1987). The control groups were composed of 114 male and 114 female rats. The treatment continued whilst each female was mated with a male from the same group, and during pregnancy and the rearing of offspring. Litters were distributed into groups of animals (54/sex/group) given the same dose as their parents until sacrifice, at around week 107 for males and week 108 for females. Groups of untreated litters (90/sex) served as controls. No differences in mortality rates were reported. Body weights were not affected by treatment, and haematological investigations, urine studies and serum analyses did not reveal any treatment-related effects. Upon complete histopathological examination, the only statistically significant effects reported were acinar cell hyperplasia and duct ectasia of the mammary tissue in females in all treated groups ($P < 0.001$). The authors described this effect as a trend to a

[20] OECD TG 473 for the testing of chemicals: *in vitro* mammalian chromosomal aberration test.

more active state in the mammary glands of all females treated with carmine. The authors mentioned that it could not be entirely ruled out that the trends observed in the study were an effect of treatment. These effects did not show a dose–response relationship and no unusual lesions were reported in the mammary tissue. There was no significant treatment-related increase reported in the incidence of tumours in any organ examined, including the mammary glands. The general pattern of observed tumour incidence in treated animals was described as typical of ageing rats of the strain, the majority being evenly distributed amongst groups of both sexes. Based on the argument that the increased incidence of hyperplasia observed in mammary tissue was not dose–related, that there was a possibility that random differences in the sampling of this diffuse organ may have resulted in the apparently low incidence in the controls, and that there were no unusual lesions in the mammary tissue, the authors concluded that these findings were of no toxicological relevance, although statistically valid (Ford et al., 1987). No dose–response relationship was identified, males were not affected, and neither significant increases nor trends in mammary tumours, such as fibromas/fibroadenomas/adenomas or adenocarcinomas were reported at termination. It was also reported that the general pattern of tumour incidence in treated animals did not differ statistically significantly from controls.

Overall, the incidence of acinar cell hyperplasia reported in the control animals of this study was low in comparison with that reported in a contemporary study on amaranth from the same institute (BIBRA, 1982). In addition, since there was no dose–response relationship in mammary hyperplasia incidences, the Panel concluded that the effects reported by Ford et al. (1987) were not treatment related. The Panel considered that the No Observed Adverse Effect Level (NOAEL) for this study is 500 mg carmine (containing a mean of 48.5 % of carminic acid)/kg bw/day, the highest dose tested. The Panel noted that this NOAEL corresponds to a mean of approximately 242 mg carminic acid/kg bw/day.

Cochineal extract

In a 2-year study, 317 B6C3F1 mice (50–55/sex/group) were fed diets containing cochineal extract (containing 29.8 % carminic acid) at levels of 0, 3 and 6 %, equivalent to 0, 4 500 and 9 000 mg cochineal extract/kg bw/day (Mori et al., 1991). The Panel noted that the cochineal extract tested in this study contained carminic acid at a significantly higher concentration (≈ 30 %) than the minimum specified in the EC specifications for the same compound (≥ 2 %). Complete histopathological examinations were performed on major organs, such as the liver, spleen, kidney, pancreas, heart and lung. Clinical biochemistry was performed at the end of the study on 10 animals of each sex from each group. Cochineal extract administration was associated with a dose-related decrease in weight gain in both sexes, particularly marked in females towards the end of the experiment. Food consumption was decreased in both sexes in a dose-related manner. No significant differences in organ weights were reported. Clinical biochemistry and haematology results were not affected by treatment. No dose-related differences were reported on mortality rates amongst the groups. It is reported that all mice survived more than 1 year, and less than 25 % of the animals died of pneumonia or other causes. The general pattern of tumour incidences in treated animals was not significantly different from that of the controls. Sporadic increases in tumour incidence were not dose-related. A NOAEL of 9 000 mg cochineal extract/kg bw/day (the highest dose tested) was derived by the Panel from this study (Mori et al., 1991), which would correspond to approximately 2 700 mg carminic acid/kg bw/day.

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Carmine

Four groups of 30 mated female Wistar-derived rats were given 0, 200, 500 or 1 000 mg carmine/kg bw/day (containing > 50 % carminic acid) as an aqueous solution by gavage (5 mL/kg bw/day) during pregnancy for 19 days (Grant et al., 1987). An additional control group (17 female rats) received a solution of chlorides providing sodium, potassium and ammonium ions under the same conditions (salt group). This treatment was intended to provide a cation intake equivalent to

that of the highest dose carmine group. Investigations and autopsies were conducted on all females and full teratological investigations were conducted on fetuses. No significant dose-related effects on body weight gain during pregnancy or pregnancy rate were reported. No adverse macroscopic abnormalities were seen in females at autopsy. The high-dose group and the salt group had significantly greater mean numbers of corpora lutea than the control group. Treated groups showed greater numbers of implantations than controls, although the difference was statistically significant for only the salt group. Pre-implantation losses were similar in all groups, although post-implantation losses were statistically significant higher in the high-dose group and the salt group. In almost all cases of post-implantation losses, the losses were in the form of early resorptions. These were attributed, by the authors, to the increased average numbers of corpora lutea in the high-dose group, since the females may not have been able to maintain the larger numbers of implantations to term. In support of this, the authors remark that the numbers of live fetuses were not adversely affected by the increases in post-implantation losses. No statistically significant differences among treated groups and controls were reported with regard to litter sizes, average litter weights and fetus weights. Fetopathological examinations did not reveal any treatment-related effects, apart from a general trend towards a more advanced degree of ossification in the treated groups, but the significance of this finding remains uncertain (Grant et al., 1987). The Panel noted that these changes were also observed in the salt group; however, the trend to more advanced ossification was also reported in the study by Grant and Gaunt (1987), described below. A NOAEL of 1 000 mg carmine/kg bw/day, the highest dose tested, can be derived from this study.

In a three-generation reproduction study, groups of Wistar-derived rats (36/sex/treated group and 60 animals/control group) were given diets containing carmine (batches containing from 46 to 56 % carminic acid) at levels that provided doses of 0, 50, 150 or 500 mg carmine/kg bw/day for 60 days before mating and throughout all phases of the study (Grant and Gaunt, 1987). Full investigations and autopsies were conducted on all three generations of adults, and one male and one female pup from each litter. No significant dose-related effects on body weight, food intake, water intake, fertility or organ weights were reported for any of the groups or any of the generations, apart from an increase in absolute and relative spleen and kidney weights in the high-dose group male pups of generation F3 (also observed in female pups of the high-dose group of the F2 generation). Post-mortem examinations did not reveal any effect attributable to the treatment apart from consistent red-coloured stomachs and caecal contents in treated adult animals. Histopathological examinations of the F3 generation animals revealed no treatment-related effects. Survival, growth and development of pups in all treated groups were similar to controls, apart from a reportedly lower, but not significant, average weight of pups from the intermediate-dose group on day 14, and from the intermediate- and high-dose groups on day 21. The timing of various stages of development was similar in all groups apart from a slight delay in tooth eruption in the two highest doses tested in animals from generations F1b and F2. A similar effect was not reported for the F3 generation (Grant and Gaunt, 1987).

Post-mortem examinations of most females in all groups did not reveal significant differences, between treated animals and controls, in rates of ovulation (as indicated by the numbers of corpora lutea) or numbers of implantations or live fetuses. A single increase in post-implantation losses and number of corpora lutea was reported for the intermediate-dose group (150 mg/kg bw/day) of the F1a generation. This finding was attributed, by the authors, to the inability of females to maintain the larger numbers of implantations resulting from the increased numbers of corpora lutea in this dose group. Fetopathological examinations of the F3 generation showed consistent significantly increased incidences, compared with controls, of cranial ossification (supra-occipital, interparietal and parietal bones), 14th rib bases (kinked ribs), sternebrae (fourth, fifth, xiphisternum), fourth sacral processes present and caudal vertebrae with neural arches; however, these differences showed no evidence of a dose–response relationship (Grant and Gaunt, 1987). These findings indicate a more advanced degree of ossification in treated groups than in controls, which is consistent with other findings by the same authors (Grant et al., 1987). As reported by the authors, examination of the Bouin's-fixed fetuses revealed no differences in the incidence of soft-part findings between treated and control groups.

Overall, the Panel noted that the reported findings were slight, and none demonstrated a dose–response relationship, despite the relatively broad dose range tested. The relatively similar findings in the dosed groups, compared with the untreated groups, could indicate that control groups may show somewhat later ossification than normal, thus leading to the reported findings in all dosed groups. Based on these arguments, the Panel considered that the ossification findings were insufficient to identify a treatment-related effect of carmine.

A NOAEL of 500 mg carmine/kg bw/day (containing a mean of 51 % carminic acid), the highest dose tested, was derived by the authors. The Panel agreed with this NOAEL and noted that this NOAEL would correspond to a mean of 255 mg carminic acid/kg bw/day.

3.2.5.2. Cochineal extract

In a reproductive and neurobehavioral study, male and female CD-1 mice (10/sex/group) were given a cochineal extract (containing approximately 10 % carminic acid, not further characterised) in the diet at levels of 0, 0.5, 1 and 2 % (Tanaka, 1995). Treatment was given to the F0 generation from preconception (from 5 weeks of age to mating) until the weaning of their progeny, and to the F1 generation from 4 to 9 weeks of age. The authors calculated the cochineal extract intake from the determined food intake for each treatment period and each dose level. Cochineal extract intakes were in the range of 718–985, 1 321–1 820 and 2 818–4 043 mg/kg bw/day in groups fed 0.5, 1.0 and 2.0 % cochineal extract in the diet, respectively, except during lactation, when the mean intake was much higher: 3 074, 5 883 and 11 361 mg/kg bw/day in the 0.5, 1.0 and 2.0 % dose groups, respectively. Five functional and behavioural developmental parameters were measured during the lactation period in the F1 generation, and exploratory behaviour was measured at 8 weeks of age in the F0 generation, and at 3 and 8 weeks of age in the F1 generation.

Cochineal extract had no significant effect on average food intake and body weights in the dams. There were single cases of dams with underdeveloped glands and losses of litters within all treated groups. The average body weight of offspring during the lactation period was significantly decreased in the mid- and high-dose groups. The survival index was significantly reduced in all treated groups, although this effect was not dose−related. Effects were observed on the behavioural and functional parameters, although without any clear dose–response relationships.

In conclusion, the Panel observed that there was an effect on pup weight gain in the mid- and high-dose groups; however, no dose–response could be identified for any of the behavioural or functional parameters measured under the experimental conditions of this study. Therefore, the Panel considered that the NOAEL for pup weight gain was 3 074 mg cochineal extract/kg bw/day, corresponding to 307 mg carminic acid/kg bw/day. The Panel noted that this study has limitations (e.g. not adequately characterised cochineal extract and a low number of dams) and, therefore, cannot be used for risk assessment.

3.2.6. Allergenicity, hypersensitivity and intolerance

JECFA, at its 55th meeting (2001), re-evaluated the allergenic potential of cochineal colours, taking into account several case reports associating hypersensitivity reactions with carmine exposure from foods. Reports published since 2001 confirm the hypersensitivity potential of carmine.

3.2.6.1. Hypersensitivity reactions to carmine

A woman suffered a severe anaphylactic reaction after drinking Campari. After initial symptoms (sneezing, rhinitis and conjunctivitis), pruritus, urticaria, Quincke's oedema, dyspnoea, bronchospasm, chills, nausea, vomiting and diarrhoea developed, which required emergency treatment. Allergen testing gave a positive response to carmine (Kägi et al., 1994).

Five cases of anaphylactic reactions to carmine (cochineal, E 120), occurring after patients had consumed alcoholic beverages, have been reported (Wüthrich et al., 1997). Two patients experienced

acute urticaria and angioedema, and two other patients, who experienced an anaphylactic reaction requiring emergency treatment, were found to have reacted to carmine in a drink. Immunoglobulin E (IgE)-mediated sensitisation was documented by means of positive skin prick tests and positive radioallergosorbent test (RAST) to carmine. One non-atopic patient also had high levels of serum IgE antibodies to the carminic acid–albumin conjugate.

An allergic reaction to carmine in food was reported in a female patient who experienced an anaphylactic reaction requiring emergency treatment after eating ice-cream containing carmine. There was an immediate onset of nausea, pruritus, urticaria and hypotension, and tachycardia developed within 3 hours of hospital admission (Baldwin et al., 1997).

Two cases of non-atopic women who experienced anaphylactic reactions requiring emergency treatment after ingestion of carmine-containing yoghurt and Campari have been described (DiCello et al., 1999). Similarly, a female patient with generalised urticaria, angioedema and asthma had eaten a yoghurt containing an estimated amount of 1.3 mg carmine dye 2 hours before the onset of these symptoms. The patient reported experiencing similar episodes after eating other red-coloured foods (Moneret-Vautrin, 2000).

A female patient experienced irritation of the larynx and oedema of the eyelids 1 hour after drinking Campari, followed by generalised urticaria, severe stomach ache and diarrhoea. The patient reported experiencing similar symptoms in the past after the ingestion of strawberry-flavoured milk or red-coloured cocktails (Kume et al., 1997).

A 35-year-old non-atopic man, who reported suffering from asthma and rhinoconjuctivitis for 5 months, had worked for 4 years in a spice warehouse in which he handled carmine. Two weeks earlier, the man had experienced a similar episode of asthma and rhinoconjuctivitis after consuming a red-coloured sweet containing carmine. Prick and bronchial challenge tests gave positive results with cochineal and carmine (Acero et al., 1998).

A prevalence study addressing sensitisation and occupational asthma caused by carmine in a small sample of workers (24 subjects) from a factory processing carmine, curcuma and annatto, showed a prevalence of sensitisation to carmine of around 42 %, and a prevalence of occupational asthma caused by carmine of around 8 %, amongst active workers (Tabar-Purroy et al., 2003). The workers studied included those who worked in the production plant, in administration, in the laboratory and as cleaners. Skin test responses to carmine, cochineal and carminic acid extracts were positive in six (26 %), seven (29 %) and one (4.2 %) of the workers, respectively. Skin test responses with curcuma, annatto and chlorophyll extracts were reported to be negative.

A 42-year-old non-atopic man with a 5-year history of rhinoconjuctivitis and asthma, related to occupational exposure in the manufacture of sausages, showed positive results for carmine in a skin prick test, in a bronchial provocation test and for *in vitro* IgE measurements (Ferrer et al., 2005). A 32-year-old woman with no prior history of allergies developed generalised urticaria, associated with eyelid oedema and rhinitis, after consuming a protein–vitamin supplement containing carmine (Voltolini et al., 2014). IgE specific antibodies were detected.

3.2.6.2. Immunotoxicity studies

Carminic acid was tested *in vitro* in an antigen stimulation test with lymphocytes from 34 patients with urticaria or chronic Quincke's oedema of unknown cause, and patients with 21 non-allergic clinical conditions as controls. A skin prick test was also conducted. Five subjects from the test group showed positive reactions in the lymphocyte stimulation test, whereas 10 out of 34 patients were positive in skin prick tests with carminic acid. Subjects with positive responses in both tests were also challenged sublingually with carminic acid, with no major effects reported, apart from a pruritic reaction (Fernandes et al., 1977).

Twenty-four patients diagnosed with irritable bowel syndrome of no demonstrable organic origin (12 atopic and 12 non-atopic) adhered to an allergen exclusion diet. Serum IgE concentrations were measured and skin prick tests were performed. One atopic individual had a positive reaction in a skin prick test with carminic acid and one non-atopic patient responded to exclusion of carminic acid from the diet. In the latter case, skin prick test results with carmine were negative (Petitpierre et al., 1985).

Elevated serum IgE concentration was reported in a man who developed severe asthma after a 3-month occupational exposure to carmine (Lenz et al., 1983). Specific IgE and IgG antibodies to cochineal and carmine were found in three individuals with work-related asthma (Quirce et al., 1994).

Specific IgE antibodies from the sera of three carmine dye factory workers (without personal or family history of atopic diseases), diagnosed with occupational asthma, recognised two major intact proteins with apparent molecular masses of 17 and 28–30 kD from raw cochineal and carmine extracts, respectively (Lizaso et al., 2000). These proteins were not recognised by sera pooled from seven, non-atopic, non-exposed subjects.

Based on gel-electrophoresis studies, Chung et al. (2000) suggested that insect-derived proteins, possibly complexed with carminic acid, are responsible for the allergic reactions to carmine.

A study using the mouse popliteal lymph node assay (PLNA) implicated cochineal extract components, rather than carminic acid itself, as the potential antigen responsible for the increased popliteal lymph node cellularity index in this assay (Nagaoka et al., 2007).

Four proteins with apparent molecular weights of between 40 and 50 kD were recognised by IgE antibodies from three patients with allergic reactions to cochineal extract (Ohgiya et al., 2009). By molecular cloning, the authors demonstrated that IgE antibodies specifically recognised CC38K, a 38 kD protein, as a major allergen in cochineal extract. Analysis of CC38K amino acid sequence showed homology with an insect phospholipase A_1 (PLA_1). Subsequent analysis suggested that CC38K was the precursor for the four proteins identified as major allergens in cochineal extracts.

Overall, numerous studies have demonstrated that cochineal extracts (E 120) (mostly carmine and to some extent carminic acid) showed a marked allergenic potential, mostly related to insect proteins (Pecquet, 2013). Therefore, the Panel considered that it is advisable that the protein content of the carmine colour food additive is reduced as much as possible.

4. Discussion

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

Cochineal, carminic acid, carmines (E 120) are red anthraquinone dyes authorised as food additives in the EU and have been most recently evaluated by JECFA in 2000, and the SCF in 1983. Both committees established an ADI of 5 mg/kg bw/day. The JECFA ADI covers carmines including ammonium carmine or the equivalent calcium, potassium or sodium salts. For SCF, the ADI applies to cochineal (carmines) without further details being specified. The 1981 JECFA evaluation specifically excluded the lithium salt, considering it as not acceptable for food additive use.

Specifications have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006). In the EC specifications, cochineal, carminic acid and carmines (E 120) are defined as having not less than 2.0 % carminic acid in the extracts, and not less than 50 % carminic acid in the chelates. The remaining material (50 to 80 %) is not precisely specified, being only described as cations that may be present in excess in the colours and also maybe containing proteinaceous material derived from the source insect, together with free carminate or a small residue of unbound aluminium cations. The

Panel noted that the specifications of carmines need to be updated with respect to the percentage of material not accounted for.

The Panel considered that the maximum limits for toxic elements (arsenic, lead, mercury and cadmium) present as impurities in the EC specification for E 120 (cochineal, carminic acid, carmines) should be revised in order to ensure that E 120 used as food additive will not be a significant source of exposure to these toxic elements in food.

No studies on ADME of cochineal extract, carminic acid or carmines were available for this evaluation. However, both the ionisation properties of carminic acid (pK_a values) and indirect evidence from toxicological studies suggest that these compounds can be absorbed to some extent, as suggested by the accumulation of colour in tissues and the red colouring of urine reported in rats treated with ammonium carmine.

Short-term and subchronic studies have been conducted in rats and mice, essentially with cochineal extracts and carmine (Battelle Memorial Institute, 1962; Kawasaki et al., 1994; Helal, 2000). No toxicological studies were available on carminic acid alone, except two old studies conducted with the lithium salt of carminic acid administered intraperitoneally to mice and by intravenous injections to rabbits (Harada, 1931 as referred by JECFA, 1982). Cochineal did not produce histopathological changes in Wistar rats at a dose of 1 500 mg/kg bw/day, although a slight reduction in some organ weights was reported in male rats and decreased levels of phospholipids, triglycerides and cholesterol were reported in female rats. Haematological or gross and microscopic effects were not reported in rats exposed up to 10 g/kg bw ammonium carmine by gavage (Kawasaki et al., 1994).

Available genotoxicity studies on carminic acid report negative results in different *in vitro* systems, either in the presence or in the absence of S9. Negative results were also reported in an oral *in vivo* micronucleus test in mouse bone marrow, with evidence of exposure of the target tissue. Negative results have also been reported in recent, GLP-compliant, bacterial reversion assays and *in vitro* micronucleus tests with carminic acid. Based on the results of the studies available, the Panel concluded that carminic acid is not genotoxic. The Panel noted that the chelate, carmine, is structurally related to carminic acid, and not bearing additional structural alert for genotoxicity. Thus the Panel considered that read-across from carminic acid to carmine for genotoxicity was acceptable, and evaluated carmine as non-genotoxic either.

Two long-term studies in rats and mice investigated the carcinogenic potential of carmine and cochineal extract (Ford et al., 1987; Mori et al., 1991). The rat study on carmine reported significantly higher incidences of acinar cell hyperplasia and duct ectasia of the mammary gland tissue in female rats given carmine, at all doses, compared with controls (Ford et al., 1987). The mammary hyperplasia seen in the rat study was not reported in the mouse study (Mori et al., 1991). Ford et al. (1987) described this effect as a trend to a more active state in the mammary glands of all female rats treated with carmine. However, these effects did not show a dose–response relationship and no unusual lesions were reported in the mammary tissue, only a difference in the distribution of common findings was noted. Moreover, the acinar cell hyperplasia in the mammary glands in Wistar rats was not reported as being accompanied by an increase in the incidence of mammary tumours at the end of the 108-week study. The tumour incidences in treated animals did not differ statistically significantly from those in controls. The incidence of acinar cell hyperplasia reported in the control animals of the Ford et al. (1987) study was low in comparison with that reported in a contemporary report on amaranth from the same institute (BIBRA, 1982). This unpublished report of a long-term study with amaranth showed acinar cell hyperplasia incidences in control female Wistar rats in the order of 84 % (73/87), whereas among the three treated groups the incidences were 36/53 (68 %), 40/54 (74 %) and 38/53 (72 %). These incidences were very similar to those reported by Ford et al. (1987) for carmine: 39/53 (73 %), 35/52 (67 %) and 36/54 (67 %). However, the reported incidences in controls were much lower: 23/86 (27 %) than those reported in the amaranth study from the same institute.

Based on the aforementioned observations, the Panel concluded that the incidence of mammary hyperplasias observed in the controls of the study by Ford et al. (1987) was low, whereas the incidence of mammary hyperplasias observed in the groups treated with carmine were within historical control incidences of Wistar rats used in that institution. Moreover, since there was no dose–response relationship in mammary hyperplasia incidences, the authors concluded that the effects are not treatment related. The Panel agreed with this conclusion.

Overall, the Panel concluded that carmine is not carcinogenic.

The Panel considered that the NOAEL of the Ford et al. (1987) study is 500 mg carmine (containing approximately to 50 % of carminic acid)/kg bw/day, the highest dose tested. The Panel noted that this NOAEL would correspond to approximately 250 mg carminic acid/kg bw/day. The Panel concluded that this ADI should be expressed as carminic acid content, and this would correspond to 2.5 mg carminic acid/kg bw/day.

The Panel noted that no developmental and reproductive effects of carmine were observed at the highest doses tested (1 000 and 500 mg/kg bw/day) in rats (Grant et al., 1987; Grant and Gaunt, 1987). In a reproductive and neurobehavioural study (Tanaka, 1995) with cochineal extract in the diet in mice, a NOAEL of 3 074 mg cochineal extract/kg bw/day (corresponding to 307 mg carminic acid/kg bw/day) was observed based on a decrease in pup weight gain during lactation. The Panel noted that this study had limitations (e.g. not adequately characterised cochineal extract and a low number of dams) and, therefore, it cannot be used for risk assessment.

The Panel concluded that the present dataset does not give reason to revise the numerical value of the ADI of 5 mg/kg bw/day allocated by the SCF in 1983, but considered that for clarification, this ADI should only apply to cochineal extract and to carmine. The Panel noted that the composition of cochineal tested in the toxicological studies available is not well defined and, moreover, it was reported that Cochineal is not being used as food colour in EU. Furthermore, taking into account that the ADI was derived from toxicological studies using carmine as test material with defined amounts of carminic acid (46 % to 56 % carminic acid), which match those specified in the EU specifications, the Panel concluded that based on the available information, the ADI of 5 mg/kg bw/day does not apply to Cochineal (the ground bodies of the insect).

The Panel considered that the ADI of 5 mg/kg bw/day does not cover minimum sensitising or eliciting doses for susceptible individuals. Allergic reactions are hazards associated with exposure to cochineal extract and carmines. These substances are able to trigger acute hypersensitivity reactions, such as Quincke's oedema, dyspnoea and bronchospasm, and can cause severe anaphylactic reactions. In addition, chronic hypersensitivity symptoms, such as rhinoconjuctivitis and asthma, have also been associated with occupational exposure to carmines. The reported effects are likely to be the consequence of allergic reactions involving an IgE-mediated mechanism. In addition to several protein bands isolated (of, for example, 28–30 kD and 40–50 kD), a 38 kD insect protein recognised by IgE antibodies from cochineal-allergic individuals has also been identified as a major allergen in these colours (Lizaso et al., 2000; Ohgiya et al., 2009).

No estimate was available on the prevalence rates of sensitisation to cochineal colours in the general population. A small study conducted in workers in a carmine factory reported an occupational prevalence of 42 % for sensitisation and of 8 % for asthma in active workers (Tabar-Purroy et al., 2003).

The Panel noted that case reports of severe allergic reactions following consumption of carmine-containing foodstuffs indicate that the information provided to alert individuals allergic to these colours is not sufficiently acted upon. Therefore, the Panel considered that, since no threshold dose can be established for allergic reactions, it is advisable that exposure to cochineal proteinaceous

compounds is avoided as much as possible by introducing appropriate purification steps in the manufacturing process to reduce the presence of proteinaceous compounds in the food colour E 120.

The exposure assessment for food additives under re-evaluation is carried out by the ANS Panel based on (1) the MPLs set down in EU legislation (defined as the "regulatory maximum level exposure assessment scenario") and (2) usage or analytical data (defined as the "refined exposure assessment scenario").

To date, for the refined exposure assessment scenario, the ANS Panel has used only maximum concentration values (maximum reported use levels or maximum values from analytical results) available for each authorised food category. However, given the range of data that have been made available through the most recent call, the ANS Panel considered that all data should be used in additional scenarios of the exposure assessment approach to provide more realistic exposure estimates. Based on these data, the Panel calculated two refined exposure estimates based on different assumptions: a "brand-loyal scenario", in which it was assumed that a consumer is exposed long term to E 120 present at the maximum reported usage/analytical levels for one food category and to a mean reported usage/analytical level for the remaining food categories; and a "non-brand-loyal scenario", in which it was assumed that a consumer is exposed long term to E 120 present at the mean reported usage/analytical levels in all relevant food categories.

The refined exposure assessment scenario is considered a more realistic approach than the regulatory maximum level exposure assessment scenario. Exposure estimates derived using this latter scenario should be considered most conservative, as this scenario assumes that the consumer will be continuously (over a lifetime) exposed to a food additive present in food at the MPL. The Panel noted that the refined exposure estimates will not cover future changes in the level of use of E 120.

Using the regulatory maximum level exposure assessment scenario, mean exposure to E 120 from its use as a food additive ranged from 0.1 mg/kg bw/day in infants to 3.9 mg/kg bw/day in toddlers, while the high exposure using this scenario ranged from 0.3 mg/kg bw/day in infants to 6.7 mg/kg bw/day in toddlers. Using the refined brand-loyal exposure assessment scenario, mean exposure to E 120 from its use as a food additive ranged from 0.1 mg/kg bw/day in infants, adolescents, adults and the elderly to 2.1 mg/kg bw/day in toddlers. The high exposure to E 120 using this scenario ranged from 0.2 mg/kg bw/day in the elderly to 4.7 mg/kg bw/day in toddlers. Using the refined non-brand-loyal exposure assessment scenario, mean exposure to E 120 from its use as a food additive ranged from 0.02 mg/kg bw/day in infants to 0.6 mg/kg bw/day in toddlers. The high exposure to E 120 from its use as a food additive using this scenario ranged from 0.1 mg/kg bw/day in infants, adolescents, adults and the elderly to 1.1 mg/kg bw/day in toddlers. The lowest exposure to E 120 was estimated for infants, whilst the highest exposure to E 120 was calculated for toddlers in all scenarios. The food categories that, at the individual level, had the highest contribution to the total individual exposure to E 120 were flavoured fermented milk products and flavoured drinks.

Overall, refined exposure estimates for the non-brand-loyal scenario for infants, toddlers, children adolescents, adults and the elderly show that exposure to E 120 is below the ADI of 2.5 mg carminic acid/kg bw/day for all population groups.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The Panel concluded that the present dataset does not give reason to revise the ADI of 5 mg carmine (containing approximately 50 % carminic acid)/kg bw, allocated by the SCF in 1983, but considered that, for clarification, this ADI should only apply to cochineal extract and to carmines. The Panel concluded that this ADI should be expressed as carminic acid content, and this would correspond to 2.5 mg carminic acid/kg bw/day. The Panel noted that the composition of cochineal tested in the

toxicological studies available is in general not well defined, and that Cochineal as such (the ground bodies of the insect) is not reported being used as a food colour in the EU. Therefore, the Panel concluded that this ADI does not apply to Cochineal as such.

Refined exposure estimates for the non-brand loyal scenario for infants, toddlers, children, adolescents, adults and the elderly show that exposure to E 120 is below the ADI of 2.5 mg carminic acid/kg bw/day for all population groups.

RECOMMENDATIONS

- The Panel proposed that the current title of the food additive ("E 120 cochineal, carminic acid, carmines") should be revised to "E 120 cochineal extract, carminic acid and carmines", which would more accurately reflect the material used.

- The Panel noted that the specifications for carmines need to be updated with respect to the percentage of material not accounted for, including 4-aminocarminic acid.

- The Panel considered that the maximum limits for toxic elements (arsenic, lead, mercury and cadmium) present as impurities in the EC specifications for E 120, should be revised in order to ensure that E 120 used as a food additive will not be a significant source of exposure to these toxic elements in food.

- The Panel considered that no threshold dose can be established for allergic reactions. Therefore, it is advisable that exposure to the eliciting allergens, such as proteinacous compounds, is avoided as much as possible. The Panel considered that it may be advisable to reduce the presence of such compounds in E 120 by introducing appropriate purification steps in the manufacturing process.

- The Panel supports an appropriate labelling of products containing E 120 to provide information regarding its presence and the potential risk of allergic reactions in susceptible individuals.

DOCUMENTATION PROVIDED TO EFSA

1. AESGP (Association of the European Self-Medication Industry). Data on usage levels of cochineal extract, carminic acid, carmines (E 120) in foods in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 29 November 2013.

2. Analytical data provided by Members States in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013 and 2014).

3. Andres I, 2014a. Determination of the mutagenic potential of Carminic acid with the Bacterial Reverse Mutation Test following OECD 471 and EU B.13/14. Submitted by NATCOL, January 2015.

4. Battelle Memorial Institute, 1962. Unpublished report provided by NATCOL, April 2011.

5. 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). Submitted on 14 December 2009.

6. FoodDrinkEurope (FDE). Data on use levels of cochineal extract, carminic acid, carmines (E 120) in foods in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 29 November 2013.

7. Geissel B, 2014a. Determination of the genotoxic potential of Carminic acid with the "*in Vitro* Cell Micronucleus Test" following OECD 487 and EU B.49. Submitted y NATCOL, January 2015.

8. ICGA (International Chewing Gum Association). Data on usage levels of cochineal extract, carminic acid, carmines (E 120) in foods in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 29 November 2013.

9. NATCOL (Natural Food Colours Association), 2007. Reply to EFSA: Re-evaluation of food colours: call for data (7 December 2006). Cochineal, Carminic Acid, Carmines. E 120. NATCOL Submission, 31 March 2007.

10. NATCOL (Natural Food Colours Association), 2012. Reply to EFSA: Cochineal, Carminic acid, Carmine (E 120). Submitted in April 2012.

11. NATCOL (Natural Food Colours Association). Data on usage levels of cochineal extract, carminic acid, carmines (E 120) in foods in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 30 January 2014.

12. NATCOL (Natural Food Colours Association), 2014. Additional data under the on-going E120 re-evaluation. Submitted in March 2014.

13. NATCOL, 2015. Data submitted in response to the EFSA call for scientific data on selected food additives permitted in the EU. Submitted on 19 January 2015.

14. Specialised Nutrition Europe (SNE). Data on usage levels of cochineal extract, carminic acid, carmines (E 120) in foods in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 29 November 2013.

15. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

16. Private company. Data on usage levels of cochineal extract, carminic acid, carmines (E 120) in response to the EFSA call for food additive usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted on 4 July 2014.

17. Tennant D, 2007. Screening potential intakes of natural food colours. Report provided for the Natural Food Colours Association (NATCOL), July 2007.

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APPENDICES

Appendix A. Summary of the reported use levels (mg/kg or mg/L as appropriate) of E 120 provided by industry

FCS category No	FCS food category	MPL	Restrictions/exceptions	Reported usage levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
01.4	Flavoured fermented milk products including heat–treated products	150		8	24.5	150	FDE, NATCOL
01.6.3	Other creams	150	Only flavoured creams	2	40	100	NATCOL
01.7.1	Unripened cheese excluding products falling in category 16	150	Only flavoured unripened cheese	2	25	100	NATCOL
01.7.2	Ripened cheese	125	Only red marbled cheese and red pesto cheese	2	65	125	NATCOL
01.7.3	Edible cheese rind	qs		1	30	100	NATCOL
01.7.5	Processed cheese	100	Only flavoured processed cheese	2	20	100	NATCOL
01.7.6	Cheese products (excluding products falling in category 16)	125	Only red marbled products	–	–	–	–
01.7.6	Cheese products (excluding products falling in category 16)	100	Only flavoured unripened products	2	30	100	NATCOL
03	Edible ices	150		32	51.9	150	FDE, NATCOL, private company
04.2.1	Dried fruit and vegetables	200	Only preserves of red fruit	1	20	200	NATCOL
04.2.2	Fruit and vegetables in vinegar, oil, or brine	200	Only preserves of red fruit	1	50	200	NATCOL
04.2.3	Canned or bottled fruit and vegetables	200	Only preserves of red fruit	1	50	200	NATCOL
04.2.4.1	Fruit and vegetable preparations excluding compote	100/200	Only seaweed based fish roe analogues/only preserves of red fruit/only *mostarda di frutta*	1	180	200	NATCOL
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	100	Except chestnut purée	5	48	100	NATCOL
04.2.5.3	Other similar fruit or vegetable spreads	100	Except *crème de pruneaux*	2	35	100	NATCOL
05.2	Other confectionery including breath freshening microsweets	300	Except candied fruit and vegetables	30	90.9	300	FDE, NATCOL
05.2	Other confectionery including breath freshening microsweets	200	Only candied fruit and vegetables	4	169	200	FDE, NATCOL

FCS category No	FCS food category	MPL	Restrictions/exceptions	Reported usage levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
05.3	Chewing gum	300		6	128	300	FDE, ICGA, NATCOL
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	300/500	Only fillings/only decorations, coatings and sauces, except fillings	16	174	500	FDE, NATCOL, private company
06.3	Breakfast cereals	200	Only fruit-flavoured breakfast cereals	3	100	200	NATCOL
06.6	Batters	500	Only batters for coating	1	100	500	NATCOL
07.2	Fine bakery wares	200		9	86.5	200	FDE, NATCOL, private company
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	100	Only *breakfast sausages* with a minimum cereal content of 6 %, *burger meat* with a minimum vegetable and/or cereal content of 4 % mixed within the meat (in these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their typical appearance), merguez type products, *salsicha fresca*, *mici*, *butifarra fresca*, *longaniza fresca*, *chorizo fresco*, *cevapcici* and *pljeskavice*	–	–	–	–
08.3.1	Non-heat–treated meat products	100	Only sausages	11	85.5	200	FDE, NATCOL
08.3.1	Non-heat–treated meat products	200	Only *chorizo*/*sausage*/*salchichon*	7	129	200	FDE, NATCOL
08.3.1	Non-heat–treated meat products	qs	Only *pasturmas*	1	200	200	NATCOL
08.3.2	Heat–treated meat products	100	Only sausages, patés and terrines	7	48.6	100	NATCOL
08.3.3	Casings and coatings and decorations for meat	qs/ 500/ qs	Except edible external coating of *pasturmas*/only decorations and coatings, except edible external coating of *pasturmas*/only edible casings	6	133	500	FDE, NATCOL
09.2	Processed fish and fishery products including molluscs and crustaceans	100	Only fish paste and crustacean paste	1	30	50	NATCOL
09.2	Processed fish and fishery products including molluscs and crustaceans	250	Only precooked crustacean	1	15	20	NATCOL

FCS category No	FCS food category	MPL	Restrictions/exceptions	Reported usage levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
09.2	Processed fish and fishery products including molluscs and crustaceans	100	Only smoked fish	1	100	100	NATCOL
09.2	Processed fish and fishery products including molluscs and crustaceans	500	Only surimi and similar products and salmon substitutes	7	153	500	NATCOL
09.3	Fish roe	300	Except sturgeons' eggs (caviar)	2	175	300	NATCOL
12.2.2	Seasonings and condiments	500	Only seasonings, for example curry powder, tandoori	4	164	500	FDE, NATCOL
12.4	Mustard	300		2	125	300	NATCOL
12.5	Soups and broths	50		4	28.6	50	FDE, NATCOL
12.6	Sauces	500	Including pickles, relishes, chutney and piccalilli; excluding tomato-based sauces	8	140	500	FDE, NATCOL
12.9	Protein products, excluding products covered in category 1.8	100	Only meat and fish analogues based on vegetable proteins	1	30	50	NATCOL
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	50		5	44.8	50	NATCOL, SNE
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)	50		2	50	50	NATCOL
14.1.4	Flavoured drinks	100	Excluding chocolate milk and malt products	27	25.4	100	FDE, NATCOL
14.2.3	Cider and perry	200	Excluding *cidre bouché*	6	62.5	200	FDE, NATCOL
14.2.4	Fruit wine and made wine	200	Excluding *wino owocowe markowe*	3	40	100	
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	200	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	3	48.3	200	NATCOL

FCS category No	FCS food category	MPL	Restrictions/exceptions	Reported usage levels			
				Number of data	Typical mean	Highest maximum level	Information provided by
			Maraskino and Mistrà				
14.2.7.1	Aromatised wines	100	Only *americano, bitter vino*	4	42.5	100	NATCOL
14.2.7.2	Aromatised wine-based drinks	100/200	Only *bitter soda*/except *bitter soda, sangria, claria, zurra*	4	42.5	100	NATCOL
14.2.7.3	Aromatised wine-product cocktails	200		3	33.3	100	NATCOL
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	200	Only alcoholic drinks with less than 15 % of alcohol and *nalewka na winie owocowym, aromatyzowana nalewka na winie owocowym, nalewka na winie z soku winogronowego, aromatyzowana nalewka na winie z soku winogronowego, napój winny owocowy lub miodowy, aromatyzowany napój winny owocowy lub miodowy, wino owocowe niskoalkoholowe* and *aromatyzowane wino owocowe niskoalkoholowe*	3	41.7	200	NATCOL
15.1	Potato-, cereal-, flour- or starch-based snacks	100/200	Excluding extruded or expanded savoury snack products/only extruded or expanded savoury snack products	3	86.7	200	NATCOL
15.2	Processed nuts	100	Only savoury-coated nuts	3	66.7	100	NATCOL
16	Desserts excluding products covered in categories 01, 03 and 04	150		15	46.4	150	FDE, NATCOL
17.1/17.2/17.3	Food supplements	100/300		8	119	300	AESGP, NATCOL

qs, quantum satis.

Appendix B. Summary of analytical results (middle bound mg/kg or mg/L as appropriate) for E 120 provided by Member States

FCS category No	FCS food category	MPL	n	LC, %	Range		All data					Positive values					
					LOD	LOQ	Min	Median	Mean	P95 [(a)]	Max	n	Min	Median	Mean	P95[(a)]	Max
01.4	Flavoured fermented milk products including heat-treated products	150	17	59	0.04–20.0	0.1–60.0	0.0	3.3	8.8	–	56.1	7	1.1	18.0	18.5	–	56.1
01.6.3	Other creams	150	2	100	0.04–0.5	0.1–1.5	0.1	0.4	0.4	–	0.8	–	–	–	–	–	–
01.7.1	Unripened cheese excluding products falling in category 16	150	2	100	0.1–0.5	0.3–1.5	0.2	0.5	0.5	–	0.8	–	–	–	–	–	–
01.7.2	Ripened cheese	125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
01.7.3	Edible cheese rind	qs	5	100	0.5–0.5	1.5–1.5	0.8	0.8	0.8	–	0.8	–	–	–	–	–	–
01.7.5	Processed cheese	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
01.7.6	Cheese products (excluding products falling in category 16)	125	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
01.7.6	Cheese products (excluding products falling in category 16)	100	1	100	0.004	0.1	–	–	0.1	–	0.1	–	–	–	–	–	–
03	Edible ices	150	284	83	0.01–20.0	0.02–60.0	0.01	0.7	9.8	65.7	148.0	47	2.2	44.4	53.3	–	148.0
04.2.1	Dried fruit and vegetables	200	17	94	0.5–3.0	1.5–10.0	0.8	0.8	3.3	–	39.0	1	–	–	39.0	–	39.0
04.2.2	Fruit and vegetables in vinegar, oil, or brine	200	1	0	1.7	5.0	–	–	26.0	–	26.0	1	–	–	26.0	–	26.0
04.2.3	Canned or bottled fruit and vegetables	200	50	100	0.5–20.0	0.6–60.0	0.3	10.0	6.7	–	10.0	–	–	–	–	–	–
04.2.4.1	Fruit and vegetable preparations excluding compote	100/ 200	33	82	0.04–20.0	0.1–60.0	0.05	10.0	14.0	–	71.2	6	6.6	43.1	39.7	–	71.2
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	100	43	91	0.5–20.0	1.5–60.0	0.8	10.0	8.2	10.0	66.3	4	1.6	2.8	18.4	–	66.3
04.2.5.3	Other similar fruit or vegetable spreads	100	15	80	0.5–20.0	1.5–60.0	0.8	10.0	17.5	80.0	80.0	3	10.6	70.2	53.6	–	80.0
05.2	Other confectionery including breath freshening microsweets except candied fruit and vegetables	300	678	90	0.03–50.0	0.1–150.0	0.1	10.0	17.8	37.5	123.2	68	0.1	17.1	24.2	83.0	123.2
05.2	Other confectionery	200	13	100	0.5–20.0	1.5–60.0	0.8	10.0	6.6	–	10.0	–	–	–	–	–	–

FCS category No	FCS food category	MPL	n	LC, %	Range		All data					Positive values					
					LOD	LOQ	Min	Median	Mean	P95 [(a)]	Max	n	Min	Median	Mean	P95[(a)]	Max
	including breath freshening microsweets, only candied fruit and vegetables																
05.3	Chewing gum	300	16	100	0.5–20.0	1.5–60.0	0.8	10.0	7.2	–	10.0	–	–	–	–	–	–
05.4	Decorations, coatings and fillings, except fruit–based fillings covered by category 4.2.4	300/ 500	29	100	2.0–20.0	6.0–60.0	1.0	10.0	6.2	–	10.0	–	–	–	–	–	–
06.3	Breakfast cereals	200	3	67	0.5–20.0	1.5–60.0	0.8	10.0	8.6	–	15.1	1	–	–	15.1	–	15.1
06.6	Batters	500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
07.2	Fine bakery wares	200	836	98	0.04–20.0	0.1–60.0	0.7	10.0	9.8	10.0	47.2	15	0.7	16.7	17.0	–	47.2
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-
08.3.1	Non-heat-treated meat products, only sausages	100	110	47	0.03–20.0	0.1–60.0	0.01	10.5	11.0	32.3	39.9	63	2.1	18.2	18.9	35.4	39.9
08.3.1	Non-heat–treated meat products, only *chorizo/sausage/salchichon*	200	1	100	0.03	0.05	–	–	0.02	–	0.02	–	–	–	–	–	–
08.3.1	Non-heat–treated meat products, only *pasturmas*	qs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
08.3.2	Heat-treated meat products	100	112	76	0.03–20.0	0.1–60.0	0.01	0.01	1.9	10.0	18.0	27	0.7	3.5	6.0	-	18.0
08.3.3	Casings and coatings and decorations for meat	*qs*/500 /qs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
09.2	Processed fish and fishery products including molluscs and crustaceans only fish paste and crustacean paste	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
09.2	Processed fish and fishery products including molluscs and crustaceans, only precooked crustaceans	250	1	100	–	5.0	–	–	2.5	–	2.5	–	–	–	–	–	–
09.2	Processed fish and fishery products including molluscs and crustaceans only smoked fish	100	11	100	0.3–0.5	0.5–1.5	0.2	0.3	0.5	–	0.8	–	–	–	–	–	–
09.2	Processed fish and fishery products including molluscs	500	4	100	0.03–0.5	0.05–1.0	0.01	0.5	0.4	–	0.5	–	–	–	–	–	–

FCS category No	FCS food category	MPL	n	LC, %	Range		All data					Positive values					
					LOD	LOQ	Min	Median	Mean	P95 [(a)]	Max	n	Min	Median	Mean	P95[(a)]	Max
	and crustaceans only surimi and similar products and salmon substitutes																
09.3	Fish roe	300	2	0	0.03–0.5	0.1–1.5	7.4	106.5	106.5	–	205.5	2	7.4	106.5	106.5	–	205.5
12.2.2	Seasonings and condiments	500	34	94	0.5–20.0	1.5–60.0	0.8	1.5	22.8	–	495.0	2	148.9	322.0	322.0	–	495.0
12.4	Mustard	300	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12.5	Soups and broths	50	11	82	0.5–20.0	1.5–60.0	1.5	10.0	8.1	–	12.6	2	10.5	11.6	11.6	–	12.6
12.6	Sauces	500	32	100	0.01–20.0	0.02–60.0	0.01	10.0	7.4	–	10.0	–	–	–	–	–	–
12.9	Protein products, excluding products covered in category 1.8	100	1	100	0.6	0.6	–	–	0.3	–	0.3	–	–	–	–	–	–
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	50	37	100	0.01–20.0	0.02–60.0	0.01	10.0	7.5	–	10.0	–	–	–	–	–	–
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)	50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14.1.4	Flavoured drinks	100	1300	99	0.01–20.0	0.02–60.0	0.01	1.5	2.6	10.0	63.7	10	0.0	10.2	15.1	–	63.7
14.2.3	Cider and perry	200	11	100	0.1–3.0	0.1–10.0	0.03	0.0	0.3	–	1.5	–	–	–	–	–	–
14.2.4	Fruit wine and made wine	200	41	100	0.5–20.0	1.0–60.0	0.3	1.5	3.1	–	10.0	–	–	–	–	–	–
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	200	38	100	0.1–20.0	0.1–60.0	0.03	0.3	2.8	–	10.0	–	–	–	–	–	–
14.2.7.1	Aromatised wines	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14.2.7.2	Aromatised wine-based drinks	100/ 200	5	100	0.1–20.0	0.1–60.0	0.03	0.03	2.1	–	10.0	–	–	–	–	–	–
14.2.7.3	Aromatised wine-product cocktails	200	22	100	0.01–20.0	0.1–60.0	0.03	0.03	2.0	–	10.0	–	–	–	–	–	–
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of	200	59	95	0.01–20.0	0.1–60.0	0.03	1.5	1.9	–	10.0	3	2.9	3.4	3.5	–	4.2

FCS category No	FCS food category	MPL	n	LC, %	Range		All data					Positive values					
					LOD	LOQ	Min	Median	Mean	P95 [(a)]	Max	n	Min	Median	Mean	P95[(a)]	Max
	alcohol																
15.1	Potato-, cereal-, flour- or starch-based snacks	100/ 200	504	100	0.5–25.0	1.5–75.0	0.8	37.5	37.3	37.5	37.5	–	–	–	–	–	–
15.2	Processed nuts	100	7	100	0.6–5.0	1.0–20.0	0.3	0.3	0.7	–	2.5	–	–	–	–	–	–
16	Desserts excluding products covered in categories 01, 03 and 04	150	42	90	0.1–131	0.1–131	0.03	10.0	10.4	–	65.7	4	11.5	19.0	18.8		25.7
17.1/17.2 /17.3	Food supplements	100/300	30	100	0.8–20.0	2.4–60.0	0.4	10.0	7.4	–	10.0	–	–	–	–	–	–

(a): The 95th percentile obtained on occurrence data with fewer than 60 analytical results may not be statistically robust (EFSA, 2011a) and therefore not reported in the table.
LC, left-censored data; Max, maximum; Min, minimum; n, number of data; P95, 95th percentile; qs, *quantum satis*.

Appendix C. Concentration levels of E 120 used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

FCS category No	FCS food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Maximum	
01.4	Flavoured fermented milk products including heat-treated products	150	24.5	150	UL (higher than AL)
01.6.3	Other creams	150			Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16	150	25	100	UL (AL only two values, both LC)
01.7.2	Ripened cheese	125			Not taken into account (no corresponding FoodEx code)
01.7.3	Edible cheese rind	qs			Not taken into account (no corresponding FoodEx code)
01.7.5	Processed cheese	100	20	100	UL
01.7.6	Cheese products (excluding products falling in category 16), only red marbled products	125			Not taken into account (no corresponding FoodEx code)
01.7.6	Cheese products (excluding products falling in category 16), only flavoured unripened products	100			Not taken into account (no corresponding FoodEx code)
03	Edible ices	150	9.8	148	AL (many ALs available)
04.2.1	Dried fruit and vegetables	200	3.3	39.0	AL (only one UL available)
04.2.2	Fruit and vegetables in vinegar, oil, or brine	200	50	200	UL (higher than AL)
04.2.3	Canned or bottled fruit and vegetables	200	50	200	UL (all ALs LC)
04.2.4.1	Fruit and vegetable preparations excluding compote, only seaweed based fish roe analogues	100			Not taken into account (no corresponding FoodEx code)
04.2.4.1	Fruit and vegetable preparations excluding compote, only preserves of red fruit	200	14.0	71.2	AL (only one UL available)
04.2.4.1	Fruit and vegetable preparations excluding compote, only *mostarda di frutta*	200			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	100	48	100	UL (higher than AL)
04.2.5.3	Other similar fruit or vegetable spreads	100	17.5	80.0	AL (only two ULs available)
05.2	Other confectionery including breath freshening microsweets, except candied fruit and vegetables	300	17.8	123.3	AL (many ALs available)
05.2	Other confectionery including breath freshening microsweets, only candied fruit and vegetables	200	169	200	UL (all ALs LC)
05.3	Chewing gum	300	128	300	UL (all ALs LC)

FCS category No	FCS food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Maximum	
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4, only decorations, coatings and sauces, except fillings	500			Not taken into account (no corresponding FoodEx code)
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4, only fillings	300			Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	200	100	200	UL (higher than AL)
06.6	Batters	500			Not taken into account (no corresponding FoodEx code)
07.2	Fine bakery wares	200	10.0	47.2	AL (many ALs available)
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	100			Not taken into account (no corresponding FoodEx code)
08.3.1	Non-heat–treated meat products, only sausages	100	11.0	39.9	AL (more ALs than ULs available)
08.3.1	Non-heat–treated meat products, only *chorizo/sausage/salchichon*	200	129	200	UL
08.3.1	Non-heat–treated meat products, only *pasturmas*	qs			Not taken into account (no corresponding FoodEx code)
08.3.2	Heat–treated meat products	100	1.9	18	AL (more ALs than ULs available)
08.3.3	Casings and coatings and decorations for meat only decorations and coatings, except edible external coating of *pasturmas*	500			Not taken into account (no corresponding FoodEx code)
08.3.3	Casings and coatings and decorations for meat, only edible casings	qs			Not taken into account (no corresponding FoodEx code)
08.3.3	Casings and coatings and decorations for meat, only 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	100	30	50	UL
09.2	Processed fish and fishery products including molluscs and crustaceans, only precooked crustaceans	250	15	20	UL
09.2	Processed fish and fishery products including molluscs and crustaceans, only smoked fish	100	100	100	UL (all ALs LC)
09.2	Processed fish and fishery products including molluscs and crustaceans, only surimi and similar products and salmon substitutes	500	153	500	UL (all ALs LC)

FCS category No	FCS food category	MPL	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	Maximum	
09.3	Fish roe	300	175	300	UL (higher than AL)
12.2.2	Seasonings and condiments	500	22.8	495	AL (more AL data than UL data available)
12.4	Mustard	300	125	300	UL
12.5	Soups and broths	50	28.6	50	UL (higher than AL)
12.6	Sauces	500	140	500	UL (all ALs LC)
12.9	Protein products, excluding products covered in category 1.8	100	30	50	UL
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	50	44.8	50	UL (all ALs LC)
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)	50	50	50	UL
14.1.4	Flavoured drinks	100	2.6	63.7	AL (many ALs available)
14.2.3	Cider and perry	200	62.5	200	UL (all ALs LC)
14.2.4	Fruit wine and made wine	200	40	100	UL (all ALs LC)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	200	48.3	200	UL (all ALs LC)
14.2.7.1	Aromatised wines	100	42.5	100	UL
14.2.7.2	Aromatised wine-based drinks except *bitter soda*, *sangria*, *claria*, *zurra*	200			Not taken into account (no corresponding FoodEx code)
14.2.7.2	Aromatised wine-based drinks only *bitter soda*	100			Not taken into account (no corresponding FoodEx code)
14.2.7.3	Aromatised wine-product cocktails	200			Not taken into account (no corresponding FoodEx code)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	200	1.9	10.0	AL (more ALs than ULs)
15.1	Potato-, cereal-, flour- or starch-based snacks	100/ 200	86.7	200	UL (all ALs LC)
15.2	Processed nuts	100	66.7	100	UL (all ALs LC)
16	Desserts excluding products covered in categories 01, 03 and 04	150	46.4	150	UL (higher than AL)
17.1/17.2/17.3	Food supplements	100/ 300	119	300	UL (all ALs LC)

UL, use level; AL, analytical level; LC, left-censored data; qs, *quantum satis*.

Appendix D. Summary of total estimated exposure to E 120 as a food additive for the regulatory maximum level exposure assessment scenario and the refined exposure assessment scenarios per population group and survey: mean and high level (mg/kg bw/day)

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level	Mean	High level	Mean	High level
Infants								
Bulgaria	NUTRICHILD	859	0.3	1.3	0.1	0.4	0.03	0.2
Denmark	IAT 2006_2007	826	0.6	2.1	0.4	1.7	0.1	0.4
Finland	DIPP_2001_2009	496	0.1	0.3	0.1	0.3	0.02	0.1
Germany	VELS	159	0.7	2.5	0.3	1.4	0.1	0.4
Italy	INRAN_SCAI_2005_06	16	0.2	–	0.2	–	0.1	–
United Kingdom	DNSIYC_2011	1 362	0.6	2.3	0.5	1.7	0.1	0.5
Toddlers								
Belgium	Regional_Flanders	36	3.9	–	2.1	–	0.6	–
Bulgaria	NUTRICHILD	428	1.2	2.9	0.5	1.3	0.1	0.4
Denmark	IAT 2006_07	917	1.3	3.0	0.7	2.0	0.2	0.5
Finland	DIPP_2001_2009	500	0.7	2.7	0.6	2.4	0.1	0.5
Germany	VELS	348	2.9	6.6	1.3	3.3	0.4	0.7
Italy	INRAN_SCAI_2005_06	36	1.0	–	0.5	–	0.2	–
Spain	enKid	17	1.4	–	1.0	–	0.3	–
The Netherlands	VCP_kids	322	3.1	6.7	1.8	4.7	0.5	1.1
UK	NDNS-RollingProgrammeYears1–3	185	1.8	3.7	0.8	1.8	0.3	0.6
UK	DNSIYC_2011	1 314	1.4	3.5	0.8	2.0	0.3	0.7
Children								
Austria	ASNS_Children	128	1.3	2.8	0.5	1.1	0.2	0.4
Belgium	Regional_Flanders	625	3.0	6.2	1.5	3.7	0.4	1.0
Bulgaria	NUTRICHILD	433	1.6	3.7	0.6	1.5	0.1	0.3
Czech Republic	SISP04	389	2.0	4.2	0.8	1.8	0.2	0.5
Denmark	DANSDA 2005–08	298	1.1	2.4	0.5	1.1	0.1	0.3
Finland	DIPP_2001_2009	750	1.4	3.4	0.7	1.7	0.2	0.4
France	INCA2	482	1.7	3.4	0.6	1.3	0.2	0.4
Germany	EsKiMo	835	1.4	3.1	0.6	1.5	0.2	0.4
Germany	VELS	293	2.9	5.5	1.2	2.6	0.3	0.7
Greece	Regional_Crete	838	1.0	2.2	0.4	1.0	0.1	0.4
Italy	INRAN_SCAI_2005_06	193	0.8	1.9	0.3	0.9	0.1	0.2
Latvia	EFSA_TEST	187	1.4	3.3	0.7	1.7	0.3	0.6
Spain	enKid	156	1.4	3.3	0.7	1.9	0.2	0.5

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level	Mean	High level	Mean	High level
Spain	NUT_INK05	399	1.3	2.8	0.6	1.6	0.2	0.5
Sweden	NFA	1 473	2.9	5.6	1.2	2.4	0.3	0.7
The Netherlands	VCP_kids	957	2.9	6.0	1.5	4.0	0.4	1.0
The Netherlands	VCPBasis_AVL2007_2010	447	3.1	5.7	1.4	3.0	0.4	0.7
UK	NDNS-RollingProgrammeYears1–3	651	1.8	3.6	0.8	1.7	0.2	0.5
Adolescents								
Austria	ASNS_Children	237	0.7	1.8	0.3	0.8	0.1	0.3
Belgium	Diet_National_2004	576	1.2	2.6	0.6	1.4	0.2	0.4
Cyprus	Childhealth	303	0.2	0.7	0.1	0.3	0.03	0.1
Czech Republic	SISP04	298	1.4	3.3	0.5	1.4	0.1	0.3
Denmark	DANSDA 2005–08	377	0.6	1.3	0.3	0.6	0.1	0.2
Finland	NWSSP07_08	306	0.8	2.3	0.4	0.8	0.1	0.2
France	INCA2	973	0.9	2.0	0.3	0.7	0.1	0.2
Germany	National_Nutrition_Survey_II	1 011	0.9	2.5	0.5	1.2	0.1	0.4
Germany	EsKiMo	393	1.1	2.8	0.5	1.4	0.1	0.3
Italy	INRAN_SCAI_2005_06	247	0.6	1.6	0.2	0.7	0.1	0.2
Latvia	EFSA_TEST	453	0.9	2.1	0.4	1.0	0.2	0.5
Spain	AESAN_FIAB	86	0.7	1.5	0.2	0.7	0.1	0.2
Spain	enKid	209	0.8	1.8	0.3	0.9	0.1	0.3
Spain	NUT_INK05	651	0.8	1.7	0.4	0.8	0.1	0.3
Sweden	NFA	1 018	1.7	3.5	0.7	1.4	0.2	0.4
The Netherlands	VCPBasis_AVL2007_2010	1 142	2.0	4.2	0.9	1.9	0.3	0.5
UK	NDNS-RollingProgrammeYears1–3	666	1.2	2.4	0.5	1.1	0.1	0.4
Adults								
Austria	ASNS_Adults	308	0.7	1.5	0.3	0.9	0.1	0.3
Belgium	Diet_National_2004	1 292	0.9	2.3	0.4	1.2	0.1	0.3
Czech Republic	SISP04	1 666	0.6	1.4	0.2	0.6	0.1	0.2
Denmark	DANSDA 2005–08	1 739	0.3	0.8	0.1	0.4	0.05	0.1
Finland	FINDIET2012	1 295	0.6	1.7	0.3	0.9	0.1	0.3
France	INCA2	2 276	0.6	1.3	0.2	0.6	0.1	0.2
Germany	National_Nutrition_Survey_II	10 419	0.8	1.9	0.4	1.0	0.1	0.3
Hungary	National_Repr_Surv	1 074	0.4	1.0	0.2	0.5	0.04	0.1
Ireland	NANS_2012	1 274	0.7	1.7	0.4	1.0	0.1	0.3
Italy	INRAN_SCAI_2005_06	2 313	0.3	0.8	0.1	0.4	0.04	0.1

Country	Survey	Number of subjects	MPL scenario		Refined scenario			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	High level	Mean	High level	Mean	High level
Latvia	EFSA_TEST	1 271	0.5	1.2	0.3	0.7	0.1	0.3
Romania	Dieta_Pilot_Adults	1 254	0.3	0.7	0.1	0.4	0.1	0.1
Spain	AESAN	410	0.5	1.5	0.2	0.8	0.1	0.2
Spain	AESAN_FIAB	981	0.5	1.2	0.2	0.5	0.1	0.2
Sweden	Riksmaten 2010	1 430	0.8	1.7	0.4	0.9	0.2	0.4
The Netherlands	VCPBasis_AVL2007_2010	2 057	1.1	2.5	0.5	1.3	0.2	0.4
UK	NDNS-RollingProgrammeYears1–3	1 266	0.7	1.7	0.4	1.0	0.1	0.3
The elderly and very elderly								
Austria	ASNS_Adults	92	0.5	1.1	0.3	0.7	0.1	0.2
Belgium	Diet_National_2004	1 215	0.6	1.4	0.3	0.7	0.1	0.3
Denmark	DANSDA 2005–08	286	0.2	0.6	0.1	0.3	0.04	0.1
Finland	FINDIET2012	413	0.4	1.0	0.2	0.5	0.1	0.2
France	INCA2	348	0.4	0.9	0.2	0.5	0.1	0.2
Germany	National_Nutrition_Survey_II	2 496	0.5	1.2	0.3	0.6	0.1	0.3
Hungary	National_Repr_Surv	286	0.3	0.8	0.1	0.4	0.0	0.1
Ireland	NANS_2012	226	0.4	0.9	0.2	0.5	0.1	0.2
Italy	INRAN_SCAI_2005_06	518	0.2	0.5	0.1	0.4	0.03	0.1
Romania	Dieta_Pilot_Adults	128	0.2	0.5	0.1	0.2	0.04	0.1
Sweden	Riksmaten 2010	367	0.6	1.3	0.3	0.7	0.1	0.3
The Netherlands	VCPBasis_AVL2007_2010	173	0.7	1.4	0.3	0.7	0.1	0.3
The Netherlands	VCP-Elderly	739	0.7	1.3	0.3	0.7	0.1	0.3
UK	NDNS-RollingProgrammeYears1–3	305	0.5	1.2	0.3	0.7	0.1	0.3

ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	absorption, distribution, metabolism, excretion
AESGP	Association of the European Self-medication Industry
ANS	Panel on Food Additives and Nutrient Sources added to Food
BIBRA	British Industrial Biological Research Association
bw	body weight
CAS	Chemical Abstracts Service
CBPI	cytokinesis-block proliferation index
CI	Colour Index
EC	European Commission
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial chemical Substances
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCS	Food Categorisation System
FDE	FoodDrinkEurope
GLP	Good Laboratory Practice
ICGA	International Chewing Gum Association
IgE	immunoglobulin E
INS	International Numbering System
JECFA	Joint FAO/WHO Expert Committee on Food Additives
HPLC	high performance liquid chromatography
LD_{50}	Lethal dose, 50 %, i.e. dose that causes death among 50 % of treated animals
LOD	limit of detection
LOQ	limit of quantification
MPL	Maximum Permitted Level

MS	mass spectrometry
MS/MS	tandem mass spectrometry
NATCOL	Natural Food Colours Association
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PLE	pressurised liquid extraction
SCF	Scientific Committee on Food
SFE	supercritical fluid extraction
SNE	Specialised Nutrition Europe
TG	Test Guideline
UDS	unscheduled DNA synthesis
UHPLC	ultra high performance liquid chromatography
WHO	World Health Organization