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Re-evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives

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Abstract

The present opinion deals with the re-evaluation of the safety of food-grade carrageenan (E 407) and processes Eucheuma seaweed (E 407a) used as food additives. Because of the structural similarities, the Panel concluded that processed Eucheuma seaweed can be included in the evaluation of food-grade carrageenan. Poligeenan (average molecular weight 10–20 kDa) has not been authorised as a food additive and is not used in any food applications. In its evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a), the Panel noted that the ADME database was sufficient to conclude that carrageenan was not absorbed intact; in a subchronic toxicity study performed with carrageenan almost complying with the EU specification for E 407 in rats, the no-observed-adverse-effect level (NOAEL) was 3,400–3,900 mg/kg body weight (bw) per day, the highest dose tested; no adverse effects have been detected in chronic toxicity studies with carrageenan in rats up to 7,500 mg/kg bw per day, the highest dose tested; there was no concern with respect to the carcinogenicity of carrageenan; carrageenan and processed Eucheuma seaweed did not raise a concern with respect to genotoxicity; the NOAEL of sodium and calcium carrageenan for prenatal developmental dietary toxicity studies were the highest dose tested; the safety of processed Eucheuma seaweed was sufficiently covered by the toxicological evaluation of carrageenan; data were adequate for a refined exposure assessment for 41 out of 79 food categories. However, the Panel noted uncertainties as regards the chemistry, the exposure assessment and biological and toxicological data. Overall, taking into account the lack of adequate data to address these uncertainties, the Panel concluded that the existing group acceptable daily intake (ADI) for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) of 75 mg/kg bw per day should be considered temporary, while the database should be improved within 5 years after publication of this opinion.

Keywords: Carrageenan, E 407, processed Eucheuma seaweed, E 407a, degraded carrageenan, food additives

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Summary

The present opinion deals with the re-evaluation of the safety of food-grade carrageenan (E 407) and processed Eucheuma seaweed (E 407a) used as food additives.

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised as food additives in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012[1]. In the EU, these two food additives have been evaluated by the Scientific Committee for Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in different occasions. The SCF (1978), endorsed the acceptable daily intake (ADI) of 0–75 mg/kg body weight (bw) per day for carrageenan (E 407) previously established by JECFA (1974). JECFA in 1984 changed to an ADI 'not specified' (JECFA, 1984), and in 2002, a group ADI 'not specified' was allocated to the sum of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) (JECFA, 2002). The SCF maintained the original group ADI for all types of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) of 0–75 mg/kg bw because of some uncertainty 'about the general immuno-reactive potential of the various carrageenans now in use as food additives' (SCF, 1996). In 2003, the SCF had no objection to the use of carrageenan in follow-on formulae up to a maximum level of 0.3 g/L (SCF, 2003b). In its latest evaluation, JECFA in 2015, concluded that 'the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1,000 mg/L is not of concern' (JECFA, 2015).

According to the Commission Regulation (EU) No 231/2012, 'carrageenan (E 407) consists chiefly of the potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharides. The prevalent polysaccharides in carrageenan are designated as κ-, ι-, λ- depending on the number of sulphate by repeating unit (i.e. 1,2,3 sulphate)'. In the processed Eucheuma seaweed (E 407a), the main polysaccharide is κ-carrageenan.

Because of the structural similarities of processed Eucheuma seaweed and the conventionally processed food-grade carrageenan and the similarities of effects they caused in the comparative study (Documentation provided to EFSA n. 38), the Panel concluded that the re-evaluation of processed Eucheuma seaweed (E 407a) can be included in that of food-grade carrageenan (E 407).

According to one of the interested parties (Documentation provided to EFSA n. 19), carrageenan has a molecular weight distribution from 30 kDa to as high as 5,000 kDa and is defined as having a weight-average molecular weight between 200 and 800 kDa. Furthermore, it has been confirmed by industry (Documentation provided to EFSA n. 19) that commercial carrageenan (E 407) may have a weight-average molecular weight as low as 200 kDa. In view of the Panel, the molecular weight distribution of such a carrageenan product may have a considerable fraction of molecules encompassing weight-average molecular weight of degraded carrageenan.

The Panel noted that in the literature the information on the molecular weight in the individual carrageenan preparation is often unprecise mixing the terms 'molecular weight', 'weight-average molecular weight' and 'number-average molecular weight'.

The debate in literature with respect to the safety of native (undegraded) carrageenan has been related to the presence of degraded carrageenan. Degraded carrageenan is also known in forms of artificially produced products from carrageenan (e.g. poligeenan, C16) associated with adverse effects. Poligeenan is a much lower weight-average molecular weight polymer (10–20 kDa) generated by subjecting ι-carrageenan to the extreme conditions of acid hydrolysis at low pH (0.9–1.3) and high temperatures (> 80°C) for several hours (Cohen and Ito, 2006; McKim, 2014; JECFA, 2015; USDA, 2016). C16 is artificially formed from ι-carrageenan under conditions of acidic hydrolysis with 0.1 M sulfuric acid, at 60°C for 1.5 h (Glaxo Lab).

The Panel emphasised that degraded carrageenan (e.g. poligeenan or C16) has not been authorised as a food additive in the EU. Information available from the US indicated that poligeenan is not used in any food applications (USDA, 2016). The Panel further noted that the term 'poligeenan' has been used in literature including test protocols in a broader sense. However, more specifically poligeenan is a breakdown product of ι-carrageenan consisting of the same sulfated galactose and sulfated anydrogalactose units, linked by α-1,3- and β-1,4-glycosidic bonds as in all types of carrageenan. According to information provided from interested parties (Documentation provided to EFSA n. 18), poligeenan comprises polysaccharide molecules of molecular weight ranging from approximately 2 to 200 kDa, and the weight-average molecular weight of poligeenan ranges from 10 to 20 kDa. The Panel noted that based on the data provided (Documentation provided to EFSA n.

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

18) the overlapping between the low molecular weight tail of carrageenan and high molecular weight tail of poligeenan is increasing with the decrease of the weight-average molecular weight of the carrageenan preparation.

Uno et al. (2001a), in a survey of 29 samples of food-grade carrageenan representing κ-, ι- and λ-carrageenan determined a number-average molecular weight of 193–324 kDa and a weight-average molecular weight of 453–652 kDa. According to Uno et al. (2001a), 'no obvious peak of poligeenan was detected' and the 'detection limit' for poligeenan was about 5% in the sample of carrageenan.

According to information from one of the interested parties (Documentation provided to EFSA n. 19), 'in native carrageenan, this low molecular weight tail (LMT) region represents less than 10% of the total carrageenan molecule. Although there is a Commission specification of not more than 5% at less than 50 kDa, in practice there is no validated analytical method that can accurately quantify the percent of LMT present'. The Panel noted that, according to the interested parties, an interlaboratory validated analytical method to accurately measure the low molecular weight distributions of carrageenan is not fully developed or available at present.

The Panel noted that in most toxicity studies no adequate information on the molecular weight distribution of carrageenan was available. Therefore, it was not possible to assess whether the carrageenan tested complied with the EU specifications (specially the limit of low molecular weight carrageenan $<$ 5% below 50 kDa). Furthermore, the Panel noted the methodological limitation of *in vivo* studies, in which carrageenan was given in drinking water, which is not representative of the dietary exposure where food-grade carrageenan will be bound to protein. Therefore, the extent to which drinking water studies might be representative for food-grade carrageenan used as a food additive and consequently of the relevance of these studies for its safety assessment was deemed limited.

The available database on processed Eucheuma seaweed was limited to the endpoints of subchronic toxicity and genotoxicity but was sufficient for the evaluation of the similarities between processed Eucheuma seaweed and food-grade carrageenan.

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised in a wide range of foods. The Panel did identify brand loyalty for specific food categories, e.g. flavoured fermented milk products for infants and toddlers, thus the Panel considered this scenario as the most appropriate and realistic scenario for risk characterisation for all population groups.

A maximum estimated exposure assessment scenario taking into account the food for special medical purposes for infants and young children (FC 13.1.5.1. Dietary foods for infants for special medical purposes and special formulae for infants, and FC 13.1.5.2. Dietary foods for babies and young children for special medical purposes as defined by Commission Directive 1999/22/EC) was also performed using food industry data to estimate exposure for infants and toddlers who may be on a specific diet. This exposure scenario considered products belonging to food categories 13.1.2 and 13.1.4 excluding food category 13.1.1 (infant formulae) and 13.1.3 (processed cereal-based foods) where carrageenan (E 407) and processed Eucheuma seaweed (E 407a), according to EU Regulation, are not authorised. Considering that this diet is required due to specific needs, it is assumed that consumers are loyal to the food brand, therefore, the maximum reported use level for the foods for special medical purposes (FSMP) was used and the mean of the typical reported for the remaining food categories.

The refined estimates were based on 41 out of 79 food categories in which carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised. Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the real exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in European countries for both the maximum level exposure scenario and for the refined exposure assessment scenarios when considering only food additives uses for which data have been provided.

No information on the usage levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) has been reported to EFSA for 19 food categories. However, the Panel noted that, given the information from the Mintel Global New Products Database (GNPD), it may be assumed that carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are used in several food categories (n = 7) for which no data have been provided by food industry. The Panel noted that out of these 19 food categories, pasta and breakfast cereals are products highly consumed. If this would be confirmed, it would therefore result in an underestimation of the exposure.

For FSMP, the Panel noted that one industry reported a use of carrageenan as food additive at the level of the maximum permissible level (MPL) (300 mg/kg), according to the Annex II to Regulation (EC) No 1333/2008, in one niche product which is not align with information reported from the Mintel

GNPD (Appendix D), where no FSMP products seem to be labelled on the market. If this information is confirmed, the uncertainties arising from the FSMP exposure scenario would lead to an overestimation of the current exposure to carrageenan products as food additives calculated by the Panel.

Concerning uses of carrageenan in food for infants and young children the Panel concurred with the SCF (1998a, 2003a,b) 'In the absence of any further information on possible absorption of carrageenan by the immature gut in the very young infant, the Committee reaffirms its earlier view (SCF, 1998a) that it remains inadvisable to use carrageenan in infant formulae that are fed from birth, including those in the category of foods for special medical purposes. The Committee has no objection to the use of carrageenan in foods for older infants, such as follow-on milks (SCF, 1983) and weaning foods (SCF, 2003a)'.

In its evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a), the Panel noted that:

- according to industry carrageenan (E 407) is defined as having a weight-average molecular weight of 200–800 kDa;
- Uno et al. (2001a) determined in a survey on samples of food-grade carrageenan, representing κ-, ι- and λ-carrageenan, a weight-average molecular weight of 453–652 kDa and detected no obvious peak of poligeenan with a detection limit for poligeenan of about 5%;
- the ADME database was sufficient to conclude that high molecular weight carrageenan (e.g. κ/λ-carrageenan with a number-average molecular weight of approx. 800 kDa, study in rhesus monkeys) was not absorbed intact, while low molecular weight carrageenan (number-average molecular weight: 88 kDa or less) was found in tissues of rats or guinea pigs after administration of this material by gavage or diet;
- in one subchronic toxicity good laboratory practice (GLP) study in rats performed with κ-carrageenan with an average molecular weight range of 196–257 kDa (not specified if number- or weight-average molecular weight; LMT of 1.9–12% < 50 kDa (mean 7%)), almost complying with the EU specification, the no-observed-adverse-effect level (NOAEL) was equal to 3,394–3,867 mg/kg bw per day for males and females, respectively, the highest dose tested;
- no adverse effects have been detected in several chronic toxicity studies in rats with carrageenan (mostly κ/λ-type, no adequate indication of molecular weight distribution); from the available rat studies, NOAELs up to 7,500 mg/kg bw per day, the highest dose tested, were identified (Nilson and Wagner, 1959). However, in another study in rats given carrageenan preparations with a molecular weight of 244 and 252 kDa, a lowest-observed-adverse-effect level (LOAEL) of 1% in the diet (equivalent to 500 mg/kg bw per day) was identified by the Panel (Documentation provided to EFSA n. 43). The Panel noted that the characterisation of the test material in all the chronic studies was limited;
- carrageenan (different types) and processed Eucheuma seaweed did not raise a concern with respect to genotoxicity;
- there was no concern with respect to carcinogenicity for carrageenan (mostly κ/λ-type);
- the NOAEL of sodium and calcium κ/λ-carrageenan for developmental effects found in dietary prenatal developmental toxicity studies was 3,000 and 5,000 mg/kg bw per day for rats and hamsters (Documentation provided to EFSA n. 46), and 3,060 mg calcium carrageenan/kg bw per day for rats (Collins et al., 1977a), the highest doses tested;
- the Panel considered that for the safety evaluation of processed Eucheuma seaweed (E 407a) read across can be made from study results used in the toxicological evaluation of carrageenan (E 407).

In addition, the Panel observed that:

- from all the data received, data were adequate for a refined exposure assessment for 41 out of 79 food categories;
- in the general population, based on the reported use levels, a refined exposure, in the brand-loyal scenario of up to 758.6 mg/kg bw per day for toddlers (from 12 months up to and including 35 months of age) was estimated;
- for populations consuming foods for special medical purposes and special formulae, the 95th percentile of maximum exposure assessments calculated based on the maximum reported data from food industry (equal to the MPL) were up to 49.4 mg/kg bw per day for infants (from 12 weeks up to and including 11 months of age);

For the food additives E 407 and E 407a, the fraction of low molecular weight carrageenan is limited in the EC specifications by the purity criteria. The Panel was informed by one interested party that the material on the market does not necessarily comply with the EU specifications regarding the limit of the low molecular weight fraction. This fraction has been associated with potential health hazards similar to those reported for preparations of degraded carrageenan, such as poligeenan or C16, to which this fraction shows similarity in molecular structure and in weight-average molecular weight. Although, full identity of degraded carrageenan such as poligeenan or C16 with the low molecular weight fraction of carrageenan has not been specifically demonstrated.

Concerning degraded carrageenan (e.g. poligeenan, C16), the Panel therefore noted the following:

- degraded carrageenan has been described to be absorbed and to be present in various tissues, namely the liver, and the urine of animals when administered in drinking water or via the diet;
- degraded carrageenan did not raise concern with respect to genotoxicity;
- rats exposed to degraded ι-carrageenan (weight-average molecular weight of 20–40 kDa) via drinking water, diet or by gavage for up to 24 months developed in first instance colitis, secondary metaplasia and finally tumours (squamous cell carcinomas, adenocarcinomas, adenomas);
- monkeys given degraded ι-carrageenan (C16) via drinking water showed histopathological lesions in the colon which varied from slight mucosal erosions at the low dose (750 mg/kg bw per day) to ulceration associated with inflammatory infiltration of the lamina propria at the high dose (2,900 mg/kg bw per day). In this study, all monkeys on C16 lost blood frequently from the intestinal tract in a dose-related degree and developed some degree of anaemia. A LOAEL of 750 mg degraded ι-carrageenan (C16)/kg bw per day was noted;
- toxicity studies have been mainly conducted with degraded ι-carrageenan, thus, degraded κ- and λ-carrageenan being sparsely toxicologically characterised.

The following uncertainties were noted by the Panel as regards the chemistry and fate of carrageenan (E 407) and processed Eucheuma seaweed (E 407a):

- no data are available showing the molecular weight distribution for different food-grade carrageenan preparations within the defined weight-average molecular weight range of 200–800 kDa;
- no data are available showing the molecular weight distribution for individual food-grade processed Eucheuma seaweed preparations;
- the weight-average molecular weight range of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) is not defined in the EU specifications allowing for the presence of a low weight-average molecular weight fraction of carrageenan. Based on the information provided on weight-average molecular weights of commercially available carrageenans, the low molecular weight material needs to be accurately quantified;
- in most of the toxicological studies the carrageenan used is not well specified and its weight-average molecular weight and its content of low molecular weight fragments are not given;
- although it has been claimed that there is no adequate analytical method available to measure the low molecular weight fraction, the Panel noted that there is indication of the percentage of the low molecular weight fraction in the food additive carrageenan (E 407) tested in a few toxicological studies;
- only limited information on the stability of carrageenan in food was available. No data on stability of carrageenan and/or processed Eucheuma seaweed addressing the usual variation of parameters (temperature, pH) relevant for the authorised food uses were available. Information on possible degradation products under acidic conditions in relevant food products is missing;
- studies investigating the hydrolysis of the κ-, ι- and λ-carrageenan showed contradictory results.

The Panel further noted the following uncertainty as regards the exposure assessment scenario:

- the refined estimates were based on 41 out of 79 food categories in which carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised and result in an overestimation of the real exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in European countries considering only food additives uses for which data have been provided were considered.

Among the uncertainties from the biological and toxicological data, the Panel considered the following:

- the lack of reliable comparative toxicokinetic and toxicological studies between the different types of carrageenan and their corresponding low molecular weight fractions;
- the theoretical possibility that limited degradation could occur under conditions representative of the *in vivo* situation;
- no firm conclusion on the other types of carrageenan could be drawn on the observation of occult blood in faeces of rhesus monkeys dosed with a commercial carrageenan;
- the characterisation of the test material in most of the toxicological studies was limited;
- there were no adequate toxicological studies performed with low weight-average molecular weight carrageenan (around 200 kDa), apart from one 90-day study (with an average molecular weight carrageenan in the range of 196–257 kDa, not specified if it was a number-average or a weight-average);
- testing for chronic toxicity and reproductive and developmental toxicity was performed almost exclusively with κ/λ-carrageenan; almost no data on ι-carrageenan were available;
- inadequate data on the possible relevance of carrageenan exposure for existing inflammatory bowel diseases in humans;
- the relevance for humans of the observations in animal studies pointing to the induction of glucose intolerance and glucosuria by carrageenan is unclear;
- the possible role of sulfate and the interactions of the various forms of carrageenans with the gut microflora in some of the reported inflammatory effects of carrageenans.

Altogether in most toxicological studies, the molecular weight distribution of the carrageenan tested is not or only inadequately described. The low molecular weight fraction of carrageenan is associated with potential adverse effects. This is due to its similarity in molecular structure and weight-average molecular weight with those of degraded carrageenan, such as poligeenan or C16, known to cause inflammatory intestinal effects. Moreover, results suggesting that carrageenan might enhance inflammatory bowel disease in humans need clarification. The test compounds used in a large number of the toxicity tests are thus not considered to reflect adequately the diversity of preparations of the food additive on the market, particularly with respect to the broad range of weight-average molecular weights reported. The Panel noted that most of these issues were also raised and discussed in a recent review (David et al., 2018).

The Panel also considered other pending uncertainties regarding the relevance of the studies available to assess the safety of the authorised food additive carrageenan (E 407). The physicochemical properties of carrageenan depend on the chemical conformation (helical or random coil) in which it exists in the preparations and in foods (solid or liquids foods) and are influenced by the presence of cations, proteins and the pH and temperature. These conditions could affect the toxicity of carrageenan and are thus relevant for the safety assessment of the food additive in the authorised uses. Furthermore, findings in some studies suggest that carrageenan exposure could be related to the induction of glucose intolerance and glucosuria. In the view of the Panel, all this information still needs clarification.

Overall, taking into account the lack of adequate data to address the above-mentioned uncertainties, the Panel concluded that the existing group ADI for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) of 75 mg/kg bw per day should be considered temporary, while the database should be improved within 5 years after publication of this opinion. Within the given time frame, high importance should be ascribed to the establishment of an adequate interlaboratory validated analytical method to quantify, at the existing 5% limit, the low weight-average molecular weight fraction of carrageenan, and to establish whether or not this fraction is associated with health risks.

The Panel further concluded that in the refined brand-loyal exposure assessment scenario the exposure estimates exceeded, in some cases by up to approximately 10-fold, the temporary existing ADI at the high level (95th percentile) for all population groups and at the mean for all population groups except for infants and the elderly. Although the exposure may be overestimated, the extent of the exceedance of the ADI (10-fold) may be a safety concern.

The Panel recommended that the EC considers:

- in the definition for the food additives E 407 and E 407a in the Commission Regulation (EU) No 231/2012, specifying the weight-average molecular weight range in a narrow way avoiding a significant overlap with the molecular weight range of preparations of degraded carrageenan;

- with respect to the purity criteria in the Commission Regulation (EU) No 231/2012, the need of an interlaboratory validated analytical method to detect low molecular weight carrageenan in the food additives E 407 and E 407a at the limit specified in the Regulation;
- possibility of extending the molecular weight of the existing 5% limit for low molecular weight carrageenan from 50 kDa to up to 200 kDa for the food additives E 407 and E 407a in the Commission Regulation (EU) No 231/2012. This would take into account the limitation of the available analytical methods to quantify the percentage of low weight-average molecular weight carrageenan and will further make certain that the presence of low weight-average molecular weight carrageenan is at a low level; it should be indicated if the 5% limit refers to the food additive as such or on dried basis.
- the need of information on the stability of the food additives E 407 and E 407a in food. No data on stability of carrageenan (E 407) and/or processed Eucheuma seaweed (E 407a) addressing the usual variation of parameters (temperature, pH) relevant for the authorised food uses were available. Information on possible degradation products under acidic conditions in relevant food products was missing;
- revising the maximum limits for the impurities of toxic elements (lead, mercury, cadmium and arsenic) in the EC specification for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in order to ensure that the food additives will not be a significant source of exposure to these toxic elements in food;
- collecting monitoring data for the food categories contributing the most to the exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in order to allow for more refined estimates of exposure;
- establishing, as a general principle, numerical MPLs for all food authorised uses with special consideration of the main food categories contributing to the exposure (e.g. flavoured milk products, flavoured drinks, food supplements and fine bakery wares) to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in order to reduce consumers exposure.

Table of contents

1. Introduction

The present opinion deals with the re-evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) when used as food additives.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission

Regulation (EC) No 1333/2008[2] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[3]. 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[4] of 2001. The report 'Food additives in Europe 2000[5] 'submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

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

1.1.2. Terms of Reference as provided by the European Commission

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

1.1.3. Interpretation of Terms of Reference

The ANS Panel described its risk assessment paradigm in the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012a,b). This Guidance states, that 'in carrying out its risk assessments, the Panel seeks to define a health-based guidance value, e.g. an Acceptable Daily Intake (ADI) (IPCS, 2004) applicable to the general population'. According to the guidance above mentioned, the ADI as established for the general population does not apply to infants below 12 weeks of age (JECFA, 1978; SCF, 1998b). In this context, the re-evaluation of the use of food additives in food for infants below 12 weeks represents a special case for which specific recommendations were given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

[2] 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. 16–33.
[3] 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. 19–27.
[4] COM(2001) 542 final.
[5] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

(JECFA, 1972, 1978) and by the SCF (SCF, 1996, 1998a) and by EFSA (EFSA Scientific Committee, 2017). The Panel endorsed these recommendations.

In the current EU legislation (Annex II of Regulation (EC) No 1333/2008), use levels of additives in food for infants under the age of 12 weeks are included in categories 13.1.1, 13.1.5.1 and 13.1.5.2.[6] The Panel considers that these uses would require a specific risk assessment in line with the recommendations given by JECFA and the SCF and endorsed by the Panel by the Panel in its current Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012a,b). Therefore, risk assessments for the general population are not considered applicable for infants under the age of 12 weeks and will be performed separately by EFSA.[7]

In the present opinion, the term 'carrageenan' is used synonymously with the terms 'native carrageenan' or 'undegraded carrageenan' or 'conventionally processed carrageenan'.

Poligeenan (CAS No. 53973-98-1) also called 'polygeenan' is a specific type of degraded ι- carrageenan. C16 is another type of degraded ι-carrageenan. The Panel noted that poligeenan and C16 are not authorised food additives and they are not the same as the authorised food additive carrageenan (E 407). The Panel noted that in some studies degraded carrageenan derived from κ- and λ-types has been used.

1.2. Information on existing evaluations and authorisations

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised as food additives in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012[1], where in the definition is stated that: 'the wording carrageenan is reserved for the non hydrolysed or otherwise chemically degraded polymer'.

In the EU, carrageenan (E 407) and processed Eucheuma seaweed (E 407a) have been evaluated by the SCF in 1977 (SCF, 1978) and 1996, respectively. The SCF endorsed the ADI of 0–75 mg/kg body weight (bw) per day for carrageenan established by JECFA (1974) without other information. In 1994, the SCF evaluated the possible degradation of carrageenan in food, the uptake of small amounts of carrageenan in certain species, the effect on immune system in animals and the increased intestinal permeability found in very young infants. While the SCF considered that 'there is no direct evidence of harm from carrageenan in human infants and no toxicologically significant effects were seen in infant baboons fed carrageenan in commercial infant formulae', the SCF did not exclude 'the possibility that the absorbed material might affect the immune system in infant' and consequently 'did not consider carrageenan acceptable for use in infant formulae' (SCF, 1994a).

Toxicological data for processed Eucheuma seaweed (E 407a) were evaluated by the SCF in 1996 (opinion on 'alternatively refined carrageenan' produced from *Eucheuma cottonii* and *Eucheuma spinosum*). The Committee considered the 'alternatively refined carrageenan' (processed Eucheuma seaweed (E 407a)) to be an acceptable carrageenan preparation. The absence of genotoxicity and of toxic effects in the 90-day study (no references given by the SCF but presumably the same database was used as by JECFA, 1999) support this opinion of the SCF and its conclusion that further toxicological testing is not needed. The SCF maintained the original group ADI for all types of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) of 0–75 mg/kg bw because of some uncertainty 'about the general immuno-reactive potential of the various carrageenans now in use as food additives' (SCF, 1996).

In the latest opinion dated 2003, the SCF evaluated the following: 'In this review the Committee has addressed several issues. The first issue discussed by Tobacman (2001) was the possibility of exposure to degraded carrageenan. Degraded carrageenan, also called poligeenan,[8] has a weight-average molecular weight of 20–30 kDa (Weiner, 1991). It is known to cause haemorrhage and ulceration of the large intestine in some laboratory animal species (guinea pig, rabbit and monkey), whereas undegraded food-grade carrageenan, with a weight-average molecular weight of above

[6] Food of category 13.1.1: Infant formulae as defined by Directive 2006/141/EC; Food of category 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. This interpretation also applies to those food additives in food category 13.1.5.2 (Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) for which exceptional uses in food for infants under the age of 12 weeks are indicated.

[7] EFSA-Q-2018-00270 Question number of carrageenan and PES.

[8] Poligeenan (CAS No. 53973-98-1) is an artificially formed polymer produced by subjecting carrageenan to extensive acid hydrolysis at low pH (0.9–1.3) and high temperatures (> 80°C) for an extended period of time until the weight-average molecular weight (Mw) is reduced to the range 10–20 kDa. For more information see Section 3.1.1.

100 kDa, does not (SCF, 1978; JECFA, 1984). Tobacman (2001) discussed the possible sources of degraded carrageenan, i.e. contamination by lower molecular weight components in the additive as such, generation of degraded carrageenan during the processing of foods containing the additive, or digestion of the additive by acid hydrolysis or degradation by bacteria in the gut. The second issue raised by Tobacman (2001) was a possible tumour promotion effect of undegraded carrageenan in the gut. In considering these issues, the Committee took into consideration the recent review of carrageenan by JECFA (2002). The other paper of Tobacman (2001) considered in this review proposed an hypothesis that the increasing incidence of breast cancer in the USA during the twentieth century may be related to consumption of carrageenan and possibly other water-soluble polymers. The Committee also reconsidered the unresolved issue of whether carrageenan might be absorbed by the immature gut and have effects on immune function in the very young (SCF, 1994b)'.

The SCF concluded that: 'The Committee concluded that the information available since its last review of carrageenan as an additive for general food use did not provide any reason to alter the ADI of 0–75 mg/kg bw established previously. The Committee notes that intakes are considerably below the ADI. The Committee does however consider that, if feasible, a molecular weight limit of not > 5% below 50 kDa should be introduced into the specification, in order to ensure that the presence of any degraded carrageenan is kept to a minimum. In the absence of any further information on possible absorption of carrageenan by the immature gut in the very young infant, the Committee reaffirms its earlier view (SCF, 1998a) that it remains inadvisable to use carrageenan in infant formulae that are fed from birth, including those in the category of foods for special medical purposes. The Committee has no objection to the use of carrageenan in foods for older infants, such as follow-on milks (SCF, 1983) and weaning foods (SCF, 2003a)'.

In 2003, the SCF also re-evaluated carrageenan in the revision of the essential requirements of infant formulae and follow-on formulae intended for the feeding of infants and young children (SCF, 2003b). The Committee reconfirmed its view that carrageenan should not be used in infant formulae. However, the Committee had no objection to the use of carrageenan in follow-on formulae up to a maximum level of 0.3 g/L. 'The Committee further recommended maintaining the concept that if more than one of the three substances locust bean gum, guar gum or carrageenan are added to a follow-on formula, the maximum level established for each of those substances is lowered with that relative part as is present of the other substances together (SCF, 2003b)'.

Carrageenan (E 407) was also evaluated by JECFA in 1973, 1984, 2000, 2002, 2007, 2008 and 2015 (JECFA, 1974, 1984, 2000, 2002, 2007b, 2008, 2015). JECFA allocated an ADI of 0–75 mg/kg bw (JECFA, 1974), which was changed to an ADI 'not specified' in 1984 (JECFA, 1984) on the basis of a number of toxicological studies on carrageenan from different sources.

Processed Eucheuma seaweed (E 407a) was evaluated by JECFA in 1987, 1992, 1993, 1995, 2000 and 2002 (JECFA, 1987, 1992, 1993, 1995, 2000, 2002). In 2000, JECFA concluded that 'the toxicities of processed Eucheuma seaweed and carrageenan were sufficiently similar for the ADI "not specified" for carrageenan to be extended to a temporary group ADI including processed Eucheuma seaweed, pending clarification of the significance of the tumour promotion of known experimental colon carcinogens by carrageenan observed in experiments in rats'.

In 2002, JECFA re-evaluated the cancer-promoting activity using recent data on carrageenan (JECFA, 2002). In relevant studies, it was shown that the administration of carrageenan at concentrations of up to 5% in the diet did not promote colon carcinogenesis. Therefore, the Committee considered that the intake of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives was of no concern. In this JECFA evaluation, a group ADI 'not specified' was allocated to the sum of carrageenan (E 407) and processed Eucheuma seaweed (E 407a).

In 2007, JECFA reviewed all the available toxicological data, including data related to the safety assessment for infants from exposure via infant formula. JECFA estimated margins of exposure (MOEs) for infants and was of the view that 'it is inadvisable to use carrageenan or processed Eucheuma seaweed in infant formula intended for infants up to and including 12 months of age', because the database was not sufficient to draw conclusions on (1) effects of carrageenan on the immature gut, (2) absorption of carrageenan by the immature gut and (3) effects of carrageenan on the immune response of the gastrointestinal tract (JECFA, 2007b, 2008). Therefore, the group ADI 'not specified' for the sum of carrageenan and processed Eucheuma seaweed was maintained for food additive uses in foods other than infant formula (JECFA, 2007b).

In its latest evaluation in 2015, JECFA reviewed data published since 2007 taking into account new studies relevant to the evaluation of the use of carrageenan in infant formula and formula for special

medical purposes, including studies on absorption and toxicity in both the neonatal minipig and neonatal pig. JECFA also considered other data available in the literature related to carrageenan and to the signalling pathways involved in inflammation. The Committee noted that although the MOEs were small, these were derived from a neonatal pig study in which no adverse effects on the gut or on immune parameters were observed at the highest dose tested. JECFA also took into account the previous toxicological database on carrageenan, which did not indicate other toxicological concerns. Overall, JECFA concluded that 'the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1,000 mg/L is not of concern' (JECFA, 2015).

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are two of the food additives that composed jelly mini-cups which were suspended in 2004 by the European Commission to be placed on the market and import (Commission Decision 2004/37/EC, European Commission, 2004), following the measures taken and information provided by different Member States. Jelly mini-cups are defined as 'jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the minicups or mini-capsule to project the confectionery into the mouth'.

In 2004, the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) prepared a scientific opinion on a request from the European Commission related to the use of certain food additives derived from seaweed or non-seaweed origin, including carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in jelly mini-cups (EFSA AFC Panel, 2004). The AFC Panel concluded that any of these gel-forming additives or of 'any other type that gave rise to a confectionery product of a similar size, with similar physical and/or physicochemical properties and that could be ingested in the same way as the jelly mini-cups, would give rise to a risk for choking' (EFSA AFC Panel, 2004). The use of these additives in jelly mini-cups is not authorised in the EU.[9]

In 2005, the EFSA AFC Panel prepared a scientific opinion on the risks posed by semicarbazide in all types of food. According to EFSA (2005), processed Eucheuma seaweed (E 407a) is sometimes bleached with calcium hypochlorite resulting in higher concentrations of the reaction by-product semicarbazide; industry initiated trials to replace calcium hypochlorite with an alternative bleaching agent for the production of processed Eucheuma seaweed (E 407a). For processed Eucheuma seaweed prepared using a bleaching step, for the 25 batches reported by Marinalg to the AFC Panel the range for semicarbazide was 9–380 μg/kg with a mean of 65 μg/kg. According to the AFC Panel, 'for the food additive carrageenan, that may become contaminated with semicarbazide at a mean concentration of 65 μg/kg from use of hypochlorite in the production process, if consumption was up to the ADI for carrageenan of 75 mg/kg bw per day then the intake of semicarbazide from this source could be up to 0.005 μg/kg bw per day'. Based on the overall weight of evidence, the AFC Panel concluded that 'the weak genotoxicity exerted by semicarbazide *in vitro* is not expressed *in vivo*…. A large margin of at least 5 orders of magnitude exists between the dose causing tumours in experimental animals and human exposure, including that of infants'. Therefore, the AFC Panel concluded that 'the issue of carcinogenicity is not of concern for human health at the concentrations of semicarbazide encountered in food' (EFSA, 2005).

In 2006, the EFSA AFC Panel prepared a scientific opinion on use of formaldehyde as a preservative during the manufacture and preparation of food additives. The Panel estimated that exposure to gelling additives such as carrageenans containing residual formaldehyde at the levels of 50 mg/kg of additive would be of no safety concern (EFSA, 2007). Maximum limits (not more than 50 mg/kg) are established in the current EC Regulation for formaldehyde in several thickening food additives from algae origin including carrageenan (EU No 231/2012).

Regarding the food additive carrageenan (E 407), the International Agency for Research on Cancer (IARC), in 1983 classified carrageenan in group 3 considering that the available data did not provide evidence that carrageenan is carcinogenic to experimental animals, in the absence of human data. IARC also evaluated 'degraded carrageenan' and concluded that 'experiments in rats with doses of degraded carrageenan comparable to those used to test carrageenan provide sufficient evidence for the carcinogenicity of degraded carrageenan in rats. No data on humans were available' (category 2B).

A review of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) was published from the Nordic Council of Ministers. In the report, it was concluded for carrageenan (E 407) that: 'Carrageenan has been extensively studied and in most studies seems to pose no toxicological problem. However the question about the potential promotion of colon carcinogenesis by carrageenan

[9] Annex II to Regulation (EC) No 1333/2008.

should be clarified. SCF has expressed the wish to review carrageenan in the light of this aspect. JECFA, however, at its 57th meeting June 2001, withdrew the temporary status and allocated a full ADI "not specified". It may be necessary to clarify whether all types of carrageenan are sufficiently covered by the toxicological evaluation'. It was further concluded for processed Eucheuma seaweed (PES, E 407a) that: 'Because of the similarities in the nature of processed Eucheuma seaweed and the conventionally processed carrageenans and in the effects they caused in the recent 90-day study it can be concluded that PES can be included in the evaluation of carrageenans. processed Eucheuma seaweed as defined by the specifications is covered by the toxicological evaluation' (TemaNord, 2002).

2. Data and methodologies

2.1. Data

The Panel was not provided with a newly submitted dossier. EFSA launched public calls for data,[10,11,12] to collect relevant information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to the last Working Group meeting before the adoption of the opinion.[13] Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based, however, not always these were available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[14]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online database which was used for checking the labelling of products containing carrageenan (E 407) and processed Eucheuma seaweed (E 407a) within the EU's food products as GNPD shows the compulsory ingredient information presented in the labelling of products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The ANS Panel assessed the safety of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance document: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012a,b).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is expressed as equivalent. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels (MPLs) and/or reported use levels and analytical data submitted to EFSA following a call for data. Different

[10] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 22 November 2009. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[11] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption - Extended deadline: 30 September 2014 Available online: http://www.efsa.europa.eu/sites/default/files/consultation/140310.pdf

[12] Call for technical data on certain thickening agents permitted as food additives in the EU - Extended Deadline: 31 December 2015. Available online: http://www.efsa.europa.eu/it/data/call/141219

[13] 6/3/2018.

[14] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

exposure scenarios were calculated (see Section 3.4.1). Uncertainties on the exposure assessment were identified and discussed.

In the context of this re-evaluation, the Panel considered the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010 (EFSA, 2014).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substances

3.1.1.1. Carrageenan (E 407)

According to the Commission Regulation (EU) No 231/2012[1], 'carrageenan (E 407) is obtained by extraction with water or dilute aqueous alkali of strains of seaweeds of *Gigartinaceae*, *Solieriaceae*, *Hypneaceae* and *Furcellariaceae*, families of the class *Rhodophyceae* (red seaweeds). It consists chiefly of the potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The prevalent polysaccharides in carrageenan are designated as κ, ι, λ depending on the number of sulphate by repeating unit (i.e. 1,2,3 sulphate). Between κ and ι there is a continuum of intermediate compositions differing in number of sulphates per repeat units between 1 and 2. During the process, no organic precipitant shall be used other than methanol, ethanol and propan-2-ol. The wording "carrageenan" is reserved for the non-hydrolysed or otherwise chemically degraded polymer. Formaldehyde may be present as an adventitious impurity up to a maximum of 5 mg/kg'.

Three major types of carrageenan can be distinguished by the number and position of the sulfate groups on the disaccharide repeating unit (Marburger, 2003; Draeger et al., 2011):

1) κ-carrageenan with one sulfate group at C4 of the β-D-galactose,
2) ι-carrageenan with one sulfate group at C4 of the β-D-galactose and one sulfate group at C2 of the α-3,6-anhydro-D-galactose, and
3) λ-carrageenan with one sulfate group at C2 of the β-D-galactose and two sulfate groups at C2 and C6 of the α-D-galactose.

The names κ-, ι- and λ-carrageenan do not reflect definitive chemical structures but only general differences in the composition and degree of sulfation at specific locations in the polymer (JECFA, 2001).

The structural formulae of the major structure units are presented in Figure 1.

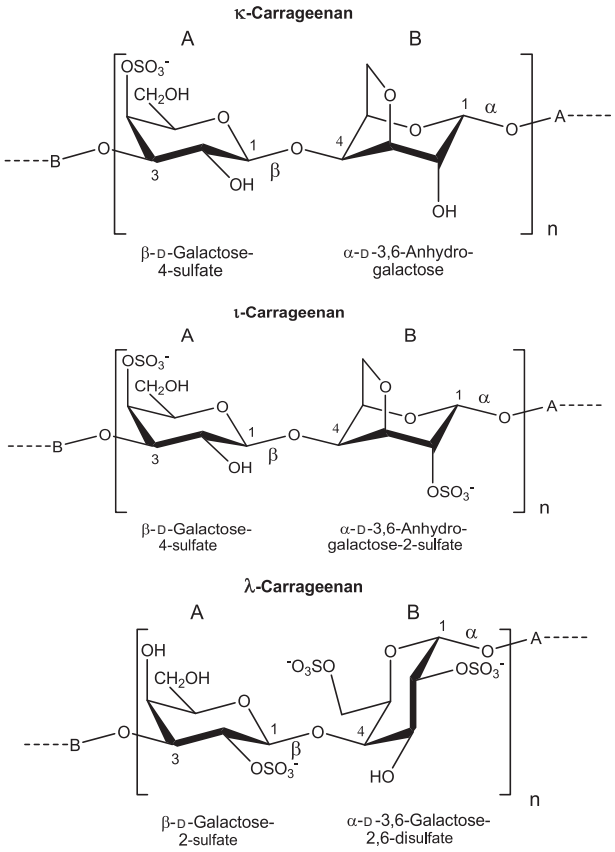


Figure 1: Structural formula of κ-, ι- and λ-carrageenan

In addition to sulfate, substitution by methyl ethers and pyruvate or sugars, such as xylose, glucose and uronic acid, may be present in minor amounts (Voragen et al., 2007). According to JECFA (2014), 'articles of commerce may include sugars for standardisation purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes'.

The chemical name of carrageenan is sulfate esters of polygalactose (Commission Regulation (EU) No 231/2012).

It appears as a yellowish to colourless, coarse to fine powder which is practically odourless (Commission Regulation (EU) No 231/2012; JECFA, 2014). The EINECS number for carrageenan is 232-524-2, for κ-carrageenan is 234-350-2, for ι-carrageenan is 232-949-3 and for λ-carrageenan is 232-953-5. CAS number for carrageenan is 9000-07-1, 11114-20-8 for κ-carrageenan, 9062-07-1 for ι-carrageenan and 9064-57-7 for λ-carrageenan.

Carrageenan is usually considered as water soluble, the solubility depends on the type of carrageenan, temperature, pH and the counter ion. While λ-carrageenan is readily water-soluble, κ-carrageenan is the most difficult to dissolve. The sodium salt of κ-carrageenan is soluble in cold water, whereas the potassium salt is soluble only by heating. ι-carrageenan has an intermediate solubility (van de Velde and De Ruiter, 2002). All types of carrageenan are insoluble in organic solvents including alcohols and ketones (Marburger, 2003). For additional information on solubility, see the EU and JECFA specifications (Section 3.1.2).

According to Commission Regulation (EU) No 231/2012, carrageenan is known under several synonyms such as Irish moss gelose (only synonym for *Chondrus*-derived carrageenan), Eucheuman (from *Eucheuma* spp.), Iridophycan (from *Iridaea* spp.), Hypnean (from *Hypnea* spp.), Furcellaran or Danish agar (from *Furcellaria fastigiata).* Other references gave the synonym carrageenin for carrageenan (Merck Index, 2006; Martindale, 2014).

Over time, the sources for the production of carrageenan have changed as have also for certain species their names (see Appendix I). Thus, the original source was *Chondrus crispus*, which still is used to a limited extend, while today most carrageenan is produced from the two sources *Eucheuma cottonii (synonym: Kappaphycus alvarezii)* and *Eucheuma spinosum (synonym: Eucheuma denticulatum*) (FAO, 2003).

Different seaweeds produce different types of carrageenan, the composition being strongly dependent on the algal species (Marburger, 2003; Draeger et al., 2011; FAO, 2003, Table 1).

Table 1: The carrageenan composition in selected red seaweeds (FAO, 2003[(a)])

Chondrus crispus	Mixture of κ and λ
Eucheuma cottonii	Mainly κ
Eucheuma spinosum	Mainly ι
Gigartina skottsbergii	Mainly κ, some λ
Sarcothalia crispata	Mixture of κ and λ

(a): FAO, 2003 (also available on: http://www.fao.org/docrep/006/y4765e/y4765e0a.htm)

One of the interested parties (Documentation provided to EFSA n. 17) provided a list of representative seaweed species with their carrageenan components used in the production of carrageenan and processed Eucheuma seaweed (Table 2).

Table 2: List of representative seaweed species with their carrageenan components

Seaweed species	Extract type	Carrageenan components
Chondrus crispus	E 407	κ/λ[(a)]
Chondrus ocellatus	E 407	κ/λ*
Gigartina stellata	E 407	κ/λ*
Gigartina pistillata	E 407	κ/λ*
Gigartina acicularis	E 407	κ/λ*
Gigartina radula	E 407	κ/λ*
Eucheuma cottonii	E 407/E 407a	κ
Furcellaria fastigiata	E 407	κ
Hypnea musiformis	E 407	κ
Eucheuma spinosum	E 407/E 407a	ι

(a): Ratio of κ:λ varies by season and location from about 90:10 to about 60:40 with a typical average of about 75:25.

According to one of the interested parties (Documentation provided to EFSA n. 19), carrageenan is:

- 'comprised of a mixture of polysaccharides ranging in size from 30 kDa to as high as 5,000 kDa';
- 'a polydisperse molecule and as such is always discussed in terms of its weight-average-molecular weight';
- 'defined as having a weight-average-molecular weight of 200–800 kDa'.

The Panel noted that in the literature the information on the molecular weight of an individual carrageenan preparation is often unprecise mixing the terms 'molecular weight', 'weight-average molecular weight' and 'number-average molecular weight' which from a chemical point of view are not synonymous.[15] In this opinion, the term describing the molecular weight of the carrageenan preparation tested is used as reported by the authors of the studies.

According to Uno et al. (2001a), a survey of 29 samples of food-grade carrageenan representing κ-, ι- and λ-carrageenan determined a number-average molecular weight of 193–324 kDa and a weight-average molecular weight of 453–652 kDa. The molecular weight distributions of 29 samples of food-grade purified carrageenan were studied by the combined gel permeation/inductively coupled plasma (GPC/ICP) method. According to the authors, 'no obvious peak of poligeenan[16] was detected'. According to Uno et al. (2001a), the 'detection limit' for poligeenan was about 5% in the sample of carrageenan.

The percentage below 50 kDa was referred to by various authors as low molecular-weight tail (LMT) and quantification of the LMT remains difficult and inaccurate by all currently available

[15] According to http://polymerdatabase.com/polymer%20physics/Molecular%20Weight.html, "A polymeric material, such as carrageenan, cannot be characterized by a single molecular weight like an ordinary substance. Instead, a statistical average calculated from the molecular weight distribution has to be used". "The weight-average molecular weight is a good measure for the expected statistical size of the polymer, whereas the number-average molecular weight is a measure for the chain length".

[16] Authors use the term poligeenan for degraded carrageenan with molecular weight: 20–30 kDa.

conventional analytical procedures (Blakemore et al., 2014; Documentation provided to EFSA n. 12; Documentation provided to EFSA n. 18). According to information from one of the interested parties (Documentation provided to EFSA n. 19), 'in native carrageenan, this LMT region represents less than 10% of the total carrageenan molecule'.

In this EFSA opinion, the term 'low molecular-weight tail' is used to describe the fraction of low weight-average molecular weight carrageenan also referring to other cut off values higher than 50 kDa.

Poligeenan, chemical name 3,6-anhydro-4-*O*-β-D-galactopyranosyl-α-D-galactopyranose-2,4′-bis (potassium/sodium sulfate)-(1-3′)-polysaccharide (CAS No. 53973-98-1) is a non-naturally occurring polymer produced by subjecting carrageenan to extensive acid hydrolysis at low pH (0.9–1.3) and high temperatures (> 80°C) for an extended period of time until the weight-average molecular weight is reduced to the range 10–20 kDa (Cohen and Ito, 2006; McKim, 2014; JECFA 2015; USDA 2016). According to Scifinder,[17] poligeenan is produced from ι-carrageenan. It was developed in the 1960s to treat pain associated with peptic ulcers, and its only application today is as a component of x-ray imaging diagnostic products (Watson, 2008; USDA, 2016). The name 'poligeenan' was confirmed in 1988 by the United States Adopted Names (USAN) Council (USAN, 1998). Prior to 1988, 'poligeenan' was referred to as 'degraded carrageenan' in the scientific literature. The terms 'poligeenan' and 'degraded carrageenan' were often used interchangeably in research articles and reports. It has been used in research studies as a surrogate for the low molecular weight fraction of carrageenan (JECFA, 2015; Weiner et al., 2015). Poligeenan is distinct from food-grade carrageenan, and according to the EC specifications the term carrageenan is reserved only for the non-hydrolysed or otherwise chemically degraded polymer. According to information gathered for this opinion, 'although the average molecular weights of carrageenan and poligeenan are significantly different, when the molecular weight distributions of the two substances are compared, there is a small overlap between the two at the lower molecular weight portion of the carrageenan distribution and the higher molecular weight portion of the poligeenan distribution, with the range of interest being between 20 and 50 kDa' (Figure 2) (JECFA, 2015). From Figure 2, the Panel noted that based on the data provided by the industry (Documentation provided to EFSA n. 18) the overlapping between the low weight-average molecular weight tail of carrageenan and the high weight-average molecular weight tail of poligeenan is between approximately 30 kDa to up to approximately 200 kDa, respectively.

The Panel also noted that poligeenan is a non-naturally occurring breakdown product of ι-carrageenan consisting of the same sulfated galactose and sulfated anydrogalactose units, linked by α-1,3- and β-1,4-glycosidic bonds as in ι-carrageenan (see Figure 1). Therefore, the Panel considered that the molecular backbone of poligeenan and of carrageenan do not differ. According to Figure 2 (Documentation provided to EFSA n. 18), poligeenan comprises polysaccharide molecules of molecular weight ranging from approximately 2 to 200 kDa, and the weight-average molecular weight of poligeenan ranges from 10 to 20 kDa. Based on the 'nearly identical molecular structure as carrageenan' and 'similar weight-average molecular weight' poligeenan has been used in experimental studies, as a surrogate for the LMT of carrageenan (Weiner et al., 2015).

[17] Available on: https://www.cas.org/products/scifinder

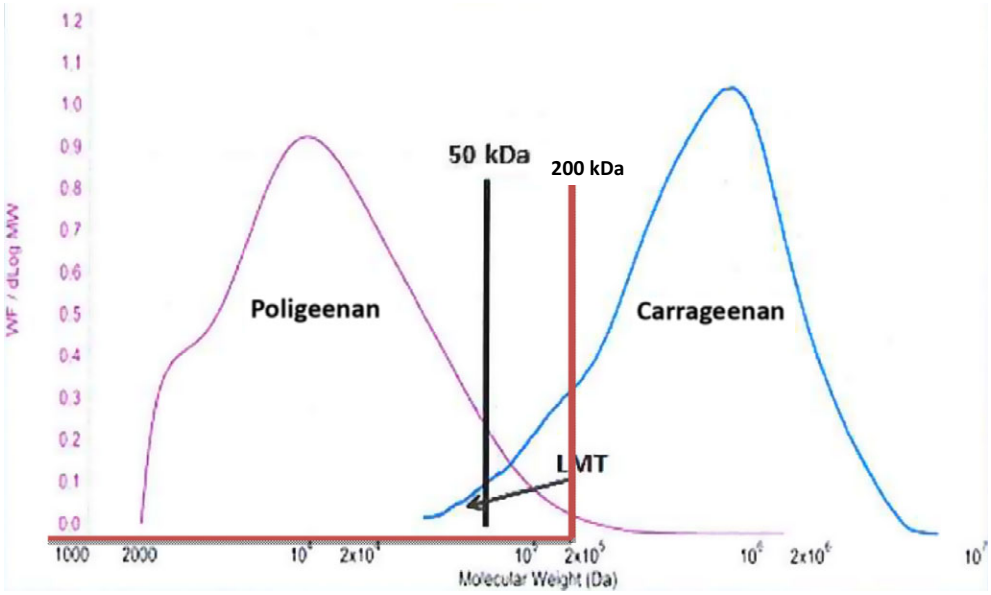


Figure 2: Examples of molecular weight profiles of poligeenan and carrageenan preparations analysed by size exclusion chromatography (SEC) and light scattering (LS). For clarity, the 200 kDa point of reference has been added. For the presented preparation of carrageenan, the weight-average molecular weight is approximately 770 kDa (Documentation provided to EFSA n. 18 and 19)

The Panel noted that no raw data supporting the molecular weight distributions as presented in Figure 2 were provided and that the provided profile is not representative for all kinds of carrageenan products (E 407) on the market.

C16 is a commercially available degraded ι-carrageenan produced by mild acid hydrolysis (prepared from *E. spinosum*, Glaxo Laboratories, France, whereas native ι-carrageenan was a commercial product from Societe Auby, France), reported to have a weight-average molecular weight of 20–30 kDa (Anderson and Soman, 1966) and a number-average molecular weight of 16–19 kDa, (Blakemore and Dewar, 1970).

Recent work has demonstrated that κ-carrageenan also may be acid hydrolysed, using milder conditions (0.1 M H_2SO_4 at 60°C for 1.5 h), to yield exclusively odd-numbered oligosaccharides (Yang et al., 2009). Products from such treatments and from other kinds of mild treatments for degradation of κ-carrageenan have been investigated for their ability to, e.g. protect from recrystallisation of ice cream (Kaminska-Dworznicha et al., 2015a; Kaminska-Dworznicka et al., 2015b, 2016). The authors proposed the designation 'new poligeenan'[18] for such products (Kaminska-Dworznicha et al., 2015a).

For powder commercial carrageenan samples, one of the interested parties (Documentation provided to EFSA n. 16) provided data on mean particle size and distribution for regular mesh (100 mesh) and fine mesh (270 μm mesh) using laser diffraction technology. The smallest particles detected in all samples were 300 nm. Furthermore data were also provided on particle size and distribution of commercial carrageenan product from the two production lines using laser diffraction technology (Documentation provided to EFSA n. 16).

According to the interested party, when used in food applications, all carrageenan powder particles are either dissolved completely into solution, making mean particle size powder data meaningless with respect to the nanoscale, or hydrated with water to swollen particles that are at least ten times greater in size than the powder particles, which takes their mean particle size even further away from the nano-scale (Documentation provided to EFSA n. 16).

Based on the information provided, the Panel noted that nanosized particles (with one or more dimensions below 100 nm) are not present in the powder commercial carrageenan used as a food additive.

Aqueous solutions of carrageenan are highly viscous. Viscosity increases with concentration and the molecular weight, and decreases with temperature. Commercial carrageenan is available in viscosities

[18] No toxicological studies have been identified with this product.

ranging from about 5 mPa·s to 800 mPa·s when measured at a concentration of 1.5% and 75°C (Stanley, 1987; van de Velde and De Ruiter, 2002). Gels are formed by cooling hot solutions of κ- or ι-carrageenan, in the presence of potassium or calcium cations, gelation is accompanied with formation of helices. κ-Carrageenan forms strong, rigid gels with potassium salts and brittle gels with calcium salts. ι-Carrageenan forms elastic gels with calcium salts, while λ-carrageenan has no gel formation ability with these ions (van de Velde and De Ruiter, 2002; Marburger, 2003; Voragen et al., 2007; Draeger et al., 2011; JECFA, 2015; FAO, 2003). In contrary, Running et al. (2012) reported gelation of λ-carrageenan in the presence of trivalent iron ions. The pH values of aqueous solutions are 6.52 (κ-carrageenan), 5.34 (ι-carrageenan) and 5.67 (λ-carrageenan) (Yaseen et al., 2004).

Data on infrared (IR) and raman spectra are presented for carrageenan by European Phamacopoeia (2011); Pereira et al. (2003); van de Velde and De Ruiter (2002). Data on ^{1}H- and ^{13}C-NMR spectra were reported for carrageenan by Bhattacharjee et al. (1978), Pereira et al. (2003) and van de Velde and De Ruiter (2002). Ackloo et al. (2001) reported data on matrix-assisted laser desorption/ionisation and electrospray ionisation mass spectra.

Carrageenan shows physicochemical synergistic interaction with other hydrocolloids, such as xanthan gum, locust bean gum and agar, resulting in an increase in viscosity or gel strength and leading to the ability to impart a desirable elastic character and to retard syneresis in these gels (Klose and Glicksman, 1990; Copetti et al., 1997; Barak and Mudgil, 2014). The synergistic effects occurring when combining carrageenan with locust bean gum, agar or xanthan gum are technologically utilised in food production (Wielinga, 2010).

When carrageenan is present in water at less than approximately 0.1% with no dietary solids or protein, the carrageenan molecules are in disorganised random conformation and available for maximum interaction with other molecules. When carrageenan is dissolved in water at higher concentrations, the carrageenan molecules may be in the disordered (random) or in the ordered (helical) conformation, depending on the carrageenan type and cations present. Only for κ-carrageenan, these helices will aggregate to form a more tightly closed structure. λ-Carrageenan is non-gelling at all concentrations and cation balances, being therefore always in the disorganised conformation in water. Normally λ-carrageenan is present as a minor component in combination with κ-carrageenan in commercial products and increases the gelling matrix through physical (void filling) and chemical (hydrogen-bonded helix cross-linkages) means. The gelation of κ- and ι-carrageenan requires heating to about > 60°C to dissolve the carrageenan at which temperature the carrageenan is in a random conformation (open structure). On cooling to about < 35°C, these carrageenan solutions will gel, first forming double helices (closed structure), and secondly, for κ-carrageenan, these helices will aggregate, as shown in Figure 3 (Blakemore and Harpel, 2010; FMC Corporation 2013; Blakemore et al., 2014; JECFA, 2015).

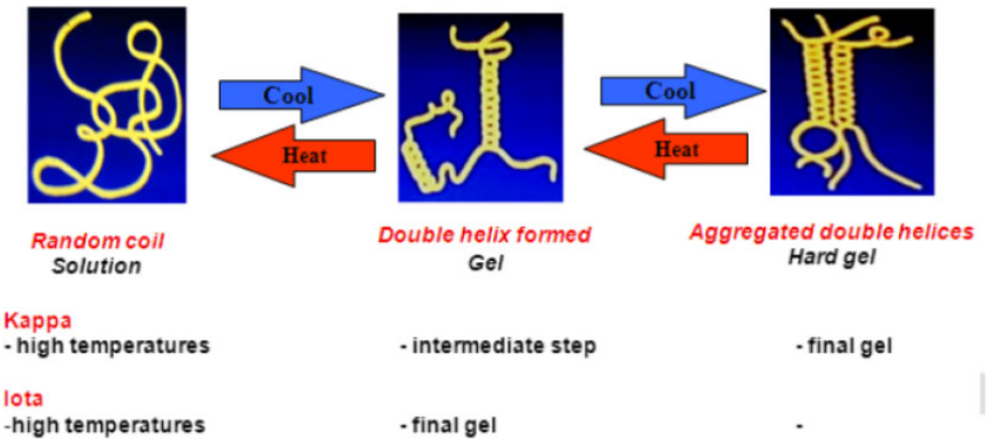


Figure 3: Gelation Mechanism of Carrageenan (re-produced from Blakemore and Harpel, 2010; Documentation provided to EFSA n.18)

Overall the Panel noted that based on the weight-average molecular weight degraded carrageenan, such as poligeenan or C16, is not an authorised food additive and it is different from the authorised food additive carrageenan (E 407). However, in experimental studies, poligeenan has been used as a surrogate for the LMT of carrageenan, based on the similarity of molecular structure and weight-

average molecular weight (Weiner et al., 2015[19]), and therefore, studies with degraded carrageenan are also described in this opinion. It has been confirmed by industry (Documentation provided to EFSA n. 19), that a commercial carrageenan (E 407) may have a weight-average molecular weight of 200 kDa. In view of the Panel, the molecular weight distribution of such a carrageenan product may have a considerable fraction of molecules with a weight-average molecular weight lower than 50 kDa (see section 3.6).

3.1.1.2. Processed Eucheuma seaweed (E 407a)

Processed Eucheuma seaweed (E 407a) is, according to the Commission Regulation (EU) No 231/2012, 'obtained by aqueous alkaline (KOH) treatment at high temperature of the strains of seaweeds *Eucheuma cottonii* and *Eucheuma spinosum*, of the class *Rhodophyceae* (red seaweeds) followed by fresh water washing to remove impurities and drying to obtain the product. Further purification may be achieved by washing with an alcohol. The alcohols authorised are restricted to methanol, ethanol or propane-2-ol. The product consists chiefly of the potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharide. Up to 15% algal cellulose is also present in the product. The wording "processed eucheuma seaweed" is reserved to the non- hydrolysed or otherwise chemically degraded polymer. Formaldehyde may be present up to a maximum of 5 mg/kg'. Neither CAS No nor EINECS No has been assigned to processed Eucheuma seaweed (407a).

Based on structural evaluation, processed Eucheuma seaweed (407a) and carrageenan (E 407) are closely related. Both substances are extracted from red seaweeds (Rhodophyceae), and the main component of processes Eucheuma seaweed (407a) is carrageenan (Marinalg, 2006; Blakemore and Harpel, 2010). Processed Eucheuma seaweed (407a) is distinguished from carrageenan (E 407) by its higher content of cellulosic matter (15%). In addition, the manufacturing does not include solubilisation and precipitation during processing (JECFA, 2007a).

Processed Eucheuma seaweed (407a) is produced from *E. cottonii* which is primarily containing κ-, while the carrageen content from *E. spinosum* is primarily ι- (Marinalg, 2006; JECFA, 2007a).

Processed Eucheuma seaweed (E 407a) is known under several synonyms such as Philippine natural grade (PNG) carrageenan, PES and semi-refined carrageenan (SRC), alternatively refined carrageenan (ARC) (Blakemore and Harpel, 2010).

Processed Eucheuma seaweed (E 407a) appears as tan to yellowish, coarse to fine powder, which is practically odourless (Commission Regulation 231/2012; JECFA, 2007a).

The physicochemical properties of processed Eucheuma seaweed (E 407a) are mainly dependent on its carrageenan content (Marinalg, 2006).

Processed Eucheuma seaweed (E 407a) forms cloudy viscous suspensions in water, it is insoluble in ethanol for a 1.5% solution (Commission Regulation 231/2012). According to JECFA (2007a), '1 g sample disperses and partially dissolves in 100 ml of water at 80°C giving a cloudy opalescent solution. The sample disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water'.

The Panel noted that no raw data showing the molecular weight distribution of processed Eucheuma seaweed (E 407a) were provided.

3.1.2. Specifications

Carrageenan (E 407)

The specifications for carrageenan (E 407) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2014) are listed in Table 3.

[19] As reported from Weiner et al. (2015): 'Since PGN, a low Mw polygalactan (Mw ~ 20 kDa), prepared from CGN by severe acid hydrolysis at elevated temperatures, and with a nearly identical molecular structure as CGN, and similar Mw as the LMT, does not bind as strongly to plasma proteins and can be quantitatively extracted from plasma, it is an appropriate and ideal surrogate for the detection of the LMT of CGN for plasma toxicokinetic analysis (Blakemore et al., 2014)' (CGN, carrageenan; Mw, weight-average molecular weight; PGN, poligeenan).

Table 3: Specifications for carrageenan (E 407) according to Commission Regulation (EU) No 231/2012 and JECFA (2014)

	Commission Regulation (EU) No 231/2012	**JECFA (2014)**
Synonyms	Products of commerce are sold under different names such as: Irish moss gelose; Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); Carrageenan (from *Chondrus* and *Gigartina* spp.)	Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); INS No. 407
Definition	Carrageenan is obtained by extraction with water or dilute aqueous alkali of strains of seaweeds of Gigartinaceae, Solieriaceae, Hypneaceae and Furcellariaceae, families of the class Rhodophyceae (red seaweeds). Carrageenan consists chiefly of the potassium, sodium, magnesium and calcium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharide. These hexoses are alternately linked α-1,3- and β-1,4 in the copolymer. The prevalent polysaccharides in carrageenan are designated as κ, ι, λ depending on the number of sulfate by repeating unit (i.e. 1,2,3 sulfate). Between κ and ι there is a continuum of intermediate compositions differing in number of sulfates per repeat units between 1 and 2. During the process, no organic precipitant shall be used other than methanol, ethanol and propan-2-ol. The wording carrageenan is reserved for the non hydrolysed or otherwise chemically degraded polymer. Formaldehyde may be present as an adventitious impurity up to a maximum of 5 mg/kg	A substance with hydrocolloid properties obtained from certain members of the class Rhodophyceae (red seaweeds). The principal commercial sources of carrageenans are the following families and genera of the class of Rhodophyceae: Furcellariaceae such as *Furcellaria* Gigartinaceae such as *Chondrus*, *Gigartina*, *Iridaea* Hypneaceae such as *Hypnea* Phyllophoraceae such as *Phyllophora*, *Gynmogongrus*, *Ahnfeltia* Solieriaceae such as *Eucheuma*, *Anatheca*, *Meristotheca* Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant. The prevalent polysaccharides in carrageenan are designated as κ-, ι- and λ-carrageenan. κ-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; ι-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between κ-carrageenan and ι-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In λ-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked). Carrageenan is obtained by extraction from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol. Articles of commerce may include sugars for standardisation purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes

	Commission Regulation (EU) No 231/2012	JECFA (2014)
Description	Yellowish to colourless, coarse to fine powder which is practically odourless	Yellowish or tan to white, coarse to fine powder that is practically odourless
EINECS	232-524-2	–
CAS number	–	9000-07-1
Chemical name	Sulfate esters of polygalactose	–
Functional uses	–	Thickener, gelling agent, stabiliser, glazing agent
Identification		
Test for galactose and anhydrogalactose	Passes test	Proceed as directed in Vol. 4 (under 'General Methods, Organic Components, Gum Constituents Identification') using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present
Test for sulfate	Passes test	Dissolve a 100 mg sample in 20 mL of water (with heating if necessary), and add 3 mL of barium chloride TS and 5 mL of hydrochloric acid, dilute TS; filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate appears
Solubility	Soluble in hot water; insoluble in alcohol for a 1.5% dilution	Insoluble in ethanol; soluble in water at a temperature of about 80°, forming a viscous clear or slightly opalescent solution that flows readily; disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water
Identification of hydrocolloid and predominant type of copolymer	–	Add 4 g of sample to 200 mL of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 mL of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ('brittle') gel indicates a carrageenan of a predominantly κ-type, and a compliant ('elastic') gel indicates a predominantly ι-type. If the solution does not gel, the carrageenan is of a predominantly λ-type
Infrared absorption	–	Passes test
Purity		
Solvent residues	Not more than 0.1% of methanol, ethanol, propan-2-ol, singly or in combination	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination
Viscosity	Not less than 5 mPa s (1.5% solution at 75°C)	Not less than 5 cp at 75° (1.5% solution)
Loss on drying	Not more than 12% (105°C, 4 h)	Not more than 12% (105° to constant weight)
pH	–	Between 8 and 11 (1 in 100 suspension)
Sulfates	Not less than 15% and not more than 40% on the dried basis (as SO_4)	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis
Ash	Not less than 15% and not more than 40% determined on the dried basis at 550°C	Not less than 15% and not more than 40% on the dried basis

	Commission Regulation (EU) No 231/2012	JECFA (2014)
Acid-insoluble ash	Not more than 1% on the dried basis (insoluble in 10% hydro-chloric acid)	Not more than 1% Use the ash from the Total ash test
Acid-insoluble matter	Not more than 2% on the dried basis (insoluble in 1% v/v sulfuric acid)	Not more than 2% Use 2 g of sample obtained from part (a) of the procedure for sulfate determination
Low molecular weight carrageenan (Molecular weight fraction below 50 kDa)	Not more than 5%	–
Arsenic	Not more than 3 mg/kg	Not more than 3 mg/kg Determine using an AAS (hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under 'General Methods, Metallic Impurities')
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg Determine using an AAS (electrothermal atomisation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under 'General Methods, Metallic Impurities').
Mercury	Not more than 1 mg/kg	Not more than 1 mg/kg Determine using AAS (cold vapour generation technique). The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under 'General Methods, Metallic Impurities').
Cadmium	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an AAS/(electrothermal atomisation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under 'General Methods, Metallic Impurities').
Microbiological criteria		Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 mL of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender
Total plate count.	Not more than 5,000 colonies per gram	Not more than 5,000 CFU/g
Yeast and moulds	Not more than 300 colonies per gram	–
Escherichia coli	Absent in 5 g	Negative in 1 g
Salmonella spp.	Absent in 10 g	Negative per test

Processed Eucheuma seaweed (E 407a)

Specifications of processed Eucheuma seaweed (E 407a) have been defined in Commission Regulation (EU) No 231/1012 and by JECFA (2007a). The available specifications are listed in Table 4.

Table 4: Specifications of processed Eucheuma seaweed (E 407a) according to Commission Regulation (EU) No 231/2012 and JECFA (2007a)

	Commission Regulation (EU) No 231/2012 (E 407a)	**JECFA (2007a) (INS 407a)**
Synonyms	PES (acronym for processed Eucheuma seaweed). The processed Eucheuma seaweed obtained from *Eucheuma cottonii* is generally called κ-processed Eucheuma seaweed and the processed Eucheuma seaweed from *Eucheuma spinosum* ι-PES	PES, PNG-carrageenan, semi-refined carrageenan; INS No. 407a
Definition	Processed Eucheuma seaweed is obtained by aqueous alkaline (KOH) treatment at high temperature of the strains of seaweeds *E. cottonii* and *E. spinosum*, of the class Rhodophyceae (red seaweeds) followed by fresh water washing to remove impurities and drying to obtain the product. Further purification may be achieved by washing with an alcohol. The alcohols authorised are restricted to methanol, ethanol or propan-2-ol. The product consists chiefly of the potassium, sodium, magnesium and calcium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharide. Up to 15% algal cellulose is also present in the product. The wording processed Eucheuma seaweed is reserved to the non hydrolysed or otherwise chemically degraded polymer. Formaldehyde may be present up to a maximum of 5 mg/kg	A substance with hydrocolloid properties obtained from either *E. cottonii* or *E. spinosum* (from the *Rhodophyceae* class of red seaweeds). In addition to carrageenan polysaccharides, processed Eucheuma seaweed may contain up to 15% of insoluble algal cellulose and minor amounts of other insoluble matter. Articles of commerce may include sugars for standardisation purposes or salts to obtain specific gelling or thickening characteristics. It is distinguished from carrageenan (INS No. 407) by its higher content of cellulosic matter and by the fact that it is not solubilised and precipitated during processing. The functional component of the product obtained from *E. cottonii* is κ-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose). From *E. spinosum* it is ι-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose-2-sulfate). Processing consists of soaking the cleaned seaweed in alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying, and milling to a powder. Alcohols that may be used during purification are restricted to methanol, ethanol, and isopropanol
Description	Tan to yellowish, coarse to fine powder which is practically odourless	Light tan to white coarse to fine powder
Identification		
Tests for galactose and anhydrogalactose	Passes test	Proceed as directed in Volume 4 (under 'General Methods, Organic Components, Gum Constituents Identification') using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present
Test for sulfate	Passes test	Dissolve a 100 mg sample in 20 mL of water. Heat to boiling, cool to room temperature, and add 3 mL of barium chloride TS and 5 mL of hydrochloric acid, dilute TS. Filter the mixture. Boil the filtrate for 5 min. A white, crystalline precipitate appears

	Commission Regulation (EU) No 231/2012 (E 407a)	JECFA (2007a) (INS 407a)
Solubility	Forms cloudy viscous suspensions in water. Insoluble in ethanol for a 1.5% solution	Forms cloudy viscous suspensions in water; insoluble in ethanol A 1 g sample disperses and partially dissolves in 100 mL of water at 80° giving a cloudy opalescent solution. (The sample disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water)
Identification of hydrocolloid and predominant type of copolymer	–	Add 4 g of sample to 200 mL of water, and heat the mixture in a water bath at 80°, with constant stirring until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. The solution becomes viscous and may form a gel. To 50 mL of the solution or gel, add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ('brittle') gel indicates a carrageenan of a predominantly κ-type. A compliant ('elastic') gel indicates a predominantly ι-type.
Infrared absorption	–	Passes test
Purity		
Solvent residues	Not more than 0.1% of methanol, ethanol, propane-2-ol, singly or in combination	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination
Viscosity	Not less than 5 mPa s (1.5% solution at 75°C)	Not less than 5 cp at 75° (1.5% solution)
Loss on drying	Not more than 12% (105°C, 4 h)	Not more than 12% (105° to constant weight)
pH	–	Between 8 and 11 (1 in 100 suspension)
Sulfate	Not less than 15% and not more than 40% on the dried basis (as SO_4)	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis
Ash	Not less than 15% and not more than 40% determined on the dried basis at 550°C	Not less than 15% and not more than 30% on the dried basis
Acid-insoluble ash	Not more than 1% on the dried basis (insoluble in 10% hydrochloric acid)	Not more than 1% Use the ash from the Total ash test
Acid-insoluble matter	Not less than 8% and not more than 15% on the dried basis (insoluble in 1% v/v sulfuric acid)	Not less than 8% and not more than 15% on the dried basis. Use 2 g of sample obtained from part (a) of the procedure for sulfate determination
Low molecular weight carrageenan (Molecular weight fraction below 50 kDa)	Not more than 5%	–
Arsenic	Not more than 3 mg/kg	Not more than 3 mg/kg Determine by the atomic absorption hydride technique. Use Method II for sample preparation
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under 'General Methods, Metallic Impurities')

	Commission Regulation (EU) No 231/2012 (E 407a)	JECFA (2007a) (INS 407a)
Mercury	Not more than 1 mg/kg	Not more than 1 mg/kg Determine by the cold vapour atomic absorption technique
Cadmium	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under 'General Methods, Metallic Impurities')
Microbiological criteria		Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 mL of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high speed blender
Total plate count	Not more than 5,000 colonies per gram	Not more than 5,000 CFU/g
Yeast and moulds	Not more than 300 colonies per gram	–
Escherichia coli	Absent in 5 g	Negative in 1 g
Salmonella spp.	Absent in 10 g	Negative per test

Concerning the concentration range of main components of the food additive E 407, the interested party provided information that E 407 produced by alcohol process comprises approximately 90% anhydrous carrageenan, 8% moisture, and 2% inorganic salts (mainly chlorides), while E 407 manufactured by gel press process comprises about 77% anhydrous carrageenan, 8% moisture and up to 15% inorganic salts (mainly chlorides) (Documentation provided to EFSA n. 15). (For details on manufacturing processes see the chapter 3.1.3.1).

However, no such information was provided on the concentration range of main components of the food additive E 407a.

According to the JECFA specifications, 'Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes'. This is also supported with the information from the literature that finished products are made by blending one or more extracts, with or without standardising agents, in order to maintain consistent quality from lot to lot' (FAO, 2003; Blakemore and Harpel, 2010). The Panel noted that this information should be included in the EU specification.

One of the interested parties has provided information in the form of aggregated ranges of concentration and other chemistry data based upon analyses of dozens of samples of the material from member companies for: viscosity (7.0–1,712.0 mPa s), loss on drying (4–12%), sulfates (17.0–36.0%), pH (7.1–11), total ash (19–39%), acid-insoluble ash (0.18–0.89%), acid-insoluble matter (0.34–1.15%), residual alcohol (0.001–0.100%), arsenic (0.005–2.000 mg/kg), lead (0.0001–2.12 mg/kg), mercury (0.0001–1.0000 mg/kg), cadmium (0.005–1.090 mg/kg), total plate count (< 10–5,000 CFU/g), moulds and yeasts (1–300 CFU/g), Coliform bacteria (negative in 5 g) and *Salmonella* (negative in 10 g), (Documentation provided to EFSA n. 15). In addition, the same interested party (Documentation provided to EFSA n. 17) provided raw data on the concentration of toxic elements in 10 non-consecutive batches of carrageenan (E 407) from 5 different manufactures (arsenic: 0.03–1.50 mg/kg, lead: 0.02–< 2.00* mg/kg, cadmium: 0.13–< 2.00* mg/kg and mercury < 0.005*–< 0.05*, and 10 non-consecutive batches of processed Eucheuma seaweed (E 407a) from 6 different manufacturers (arsenic: 0.47–1.70 mg/kg, lead: < 0.04*–< 2.00* mg/kg, cadmium: 0.68–< 2.00* mg/kg and mercury < 0.005*–0.05*. The data provided demonstrated that identity and purity of analysed products complied with the EC specifications (Documentation provided to EFSA n. 15).[20]

In addition, information on range of protein content (N × 6.25%) of 0.06–1.38% was also provided (Documentation provided to EFSA n. 15). According to industry, the proteins in E 407 are denatured during processing from seaweed (high heat + alkali + alcohol). The Panel noted that limits for proteins should be included in the EC specifications (Documentation provided to EFSA n. 15).

[20] * is LOD for specific manufacturer and laboratory used.

According to this interested party (Documentation provided to EFSA n. 15, any enzymatic activity within the live seaweed is either destroyed in the process of the seaweed being dried and/or denatured during manufacturing process, where the seaweed is subjected to high heat and alkaline conditions as well as alcohol precipitation.

Pesticides are not used, nor needed in the production of seaweeds and according to industry (Documentation provided to EFSA n. 15), available analytical data showed that the presence of pesticides was not detectable.

According to Commission Regulation (EU) No 231/2012, formaldehyde may be present as an adventitious impurity up to a maximum of 5 mg/kg. The safety of use of formaldehyde as processing aid during the storage and manufacturing of thickening agents from algae origin was evaluated by EFSA (2007) and it was concluded that exposure to gelling additives containing residual formaldehyde at the levels of 50 mg/kg of additive would be of no safety concern. The Panel noted that maximum limits for formaldehyde (up to a maximum of 5 mg/kg) are established in the current EU Regulation (Reg. EU No 231/2012) for carrageenan (E 407) and processed Eucheuma seaweed (E 407a).

The Panel also noted that according to the information provided to EFSA by industry (Documentation provided to EFSA n. 15 and 17), carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are only produced from dry seaweed and formaldehyde is not used neither for treatment of seaweed nor during the manufacturing process.

Formaldehyde has also been shown to be a natural component of most marine algae including red seaweeds. The levels detected in fresh red seaweeds used for carrageenan extraction were 44 mg/kg for *C. crispus* and 289 mg/kg for *Gigartina stellata* (*Mastocarpus stellatus*) (Yang et al., 1998).

A single laboratory validated colorimetric method that does not use acid hydrolysis (Documentation provided to EFSA n. 48) has been developed for measuring residual formaldehyde in alginate products. According to industry (Documentation provided to EFSA n. 17), attempts to apply this method for measuring formaldehyde in carrageenan (E 407) or processed Eucheuma seaweed (E 407a) were unsuccessful, including validation.

A new high-performance liquid chromatography (HPLC) method for analysis of formaldehyde in carrageenan or processed Eucheuma seaweed has been developed by Hornshøj et al. (2015). Samples are extracted with 2-propanol and reacted with 2,4-dinitrophenylhydrazine (DNPH) to form chromophore formaldehyde-DNPH which is quantified by reversed-phase HPLC with UV detection. The method is single laboratory validated and it has been found to have limit of detection (LOD) of 0.05 mg/kg and limit of quantification (LOQ) of 0.2 mg/kg. Recoveries from spiked samples were 95–107%. Only one sample of E 407 of 20 of E 407 and E 407a tested showed a detectable content of formaldehyde equal to 0.4 $\pm$ 0.06 mg/kg. Based on the results obtained, authors concluded that the formaldehyde content of commercial carrageenan and processed Eucheuma seaweed products are well below the EU maximum limit of 5 mg/kg.

The Panel noted that, according to the data provided, low levels of formaldehyde may be present in the final product in the quantities below the limit of 5 mg/kg set in the EU specifications.

Because of its polysaccharidic nature, carrageenan and processed Eucheuma seaweed can be a substrate for microbiological contamination during storage. The latter has been recently demonstrated by the finding of mycotoxin contaminations of gums (Zhang et al., 2014). The Panel noted that there are only minor differences in the microbiological criteria for carrageenan and processed Eucheuma seaweed between the specifications given by the EU Regulation and those given by JECFA.

The levels of impurities as lead, cadmium, mercury and arsenic, in the four batches analysed, were all below the levels as defined in the Commission Regulation (EU) No 231/2012.

The Panel noted that, according to the EU specifications for carrageenan (E 407) and processed Eucheuma seaweed (E 407a), impurities of the toxic elements arsenic, cadmium, lead and mercury are accepted up to concentrations of 3, 2, 5 and 1, mg/kg, respectively. Contamination at such levels could have a significant impact on the exposure to these metals, for which the exposures already are close to the health-based guidance values or benchmark doses (lower confidence limits) established by the EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012a,b,c, 2014).

The Panel also noted that, according to the point (7) of the preamble of the Commission Regulation (EU) No 231/2012: 'Specific purity criteria currently applicable should be adapted by reducing maximum limits for individual heavy metals of interest where feasible and where the JECFA limits are lower than those currently in force. . . . That approach should be departed from for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) (cadmium content) as manufacturers have declared that compliance with stricter Union provisions, reflecting JECFA limits, would not be technically feasible'.

The Panel noted that carrageenan (E 407) and processed Eucheuma seaweed (E 407a) must not contain more than 5% 'low molecular weight carrageenan' with weight-average molecular weight less than 50 kDa. This restriction is not indicated in the JECFA specifications (JECFA, 2014). It is also not clearly stated if the 5% of 'low molecular weight carrageenan' is expressed on the dried basis, as according to the data provided by the industry, E 407 contains 8% moisture (Documentation provided to EFSA n. 15). According to the industry, the Technical Working Group has been unable to develop a validated analytical method in response to this specification during 12 years of research using several methods based on size exclusion chromatography (SEC) and light scattering (LS). None have given reliable and reproducible results. The targets for accuracy and reproducibility were set to achieve a consistent relative standard deviation (RSD) on triplicate sample analysis of not more than 15% and recovery validation within 95–105% of added low molecular weight tail (LMT) of carrageenan (Documentation provided to EFSA n. 15). Six laboratories participated in the preliminary study with the procedure details approved by the Technical Working Group. According to industry, 'Eleven different commercial carrageenan samples, representing different sulphated polygalactose types (nominally κ, λ and ι) made by five different producers, were tested by all laboratories under "Round Robin" conditions. It appears that even under optimum SEC conditions, light scattering detector signal to noise ratio (S/N) in the LMT region is extremely low, and it is this signal upon which molecular weight determination is based' (Documentation provided to EFSA n. 17). According to Spichtig and Austin (2008), 'methods employing light scattering have been unsuccessful in producing reproducible results, probably due to the poor detector response at low masses'.

The molecular weight distributions of 29 samples of food-grade refined carrageenan were studied by the combined GPC/ICP method. According to the authors, no obvious peak of 'poligeenan' was detected, and the LOD was about 5% for 'poligeenan' in the sample of carrageenan (Uno et al., 2001a,b). The Panel noted that the method is not enough sensitive (LOD of 5% degraded carrageenan) and therefore not applicable for quantitative determination of the levels of not more than 5% of low molecular weight carrageenan (molecular weight fraction below 50 kDa) required by the EU specifications.

A single laboratory partially validated method based on high-performance size exclusion chromatography coupled to a refractive index detector (HPSEC-RI) has been employed for the measurement of the low molecular weight fraction of carrageenan in food-grade carrageenan ingredients and in a carrageenan-containing finished product (a jelly). Their use of carrageenan standards makes the application of light scattering (LS) unnecessary, thus removing the signal to noise ratio problem. However, as indicated by the authors, degraded carrageenan standards are difficult to make and may spoil on storage. LOD of the method was not reported. Over the course of half a year, 19 measurements were made on a reference carrageenan that demonstrated the method had good reproducibility (coefficient of variation (CV) for the low molecular weight fraction was 2.9%) in a single laboratory. Accuracy was difficult to assess due to the poor sensitivity of the two reference methods (HPSEC-RI-MALS systems) below 50 kD. According to the authors, a robust solution would be to have available a supply of well characterised carrageenan standards for HPSEC-RI calibration. An interlaboratory study, such as the one carried out by one of the interested parties, would be required to ascertain that the system also gives reproducible results in other laboratories. Applied to a number of different food-grade carrageenan samples, it was found that, in general, the low molecular weight fraction represents less than 8% of the total carrageenan and that around half of the tested samples was within the specified 5% limit (Spichtig and Austin, 2008; Documentation provided to EFSA n. 18). The Panel noted that, according to the data presented, half of the samples of carrageenan tested by Spichtig and Austin (2008) did not comply with the EU specification for low molecular weight carrageenan.

Investigation of ultrafiltration membranes in an attempt to separate and quantify the LMT carrageenan concluded that current commercially available membranes are not porous enough for the size and shape of LMT molecules (Documentation provided to EFSA n. 17).

According to information from one of the interested parties (Documentation provided to EFSA n. 19), 'in native carrageenan, this LMT region represents less than 10% of the total carrageenan molecule. Although there is a Commission specification of not more than 5% at less than 50 kDa, in practice there is no validated analytical method that can accurately quantify the percent of LMT present'.

The Panel thus noticed that, according to the interested parties, an interlaboratory validated analytical method to accurately measure the low molecular weight distributions of carrageenan was not fully developed or available at present.

Based on the data provided following general and specific calls for data (Documentation provided to EFSA n. 17 and 19), the overlapping between the low weight-average molecular weight tail of carrageenan and high weight-average molecular weight tail of poligeenan is expected to increase with

the decrease of the weight-average molecular weight of the carrageenan preparation. For example, for the carrageenan with the weight-average molecular weight of around 770 kDa (Figure 2), the overlap would encompass from approximately 30 kDa up to approximately 200 KDa molecules. Although for a commercially available carrageenan with a weight-average molecular weight of 200 kDa, as referred to by an interested party, the preparation would encompass a larger fraction with a molecular weight below 50 kDa.

Based on the information provided on weight-average molecular weights of commercially available carrageenan preparations, the Panel concluded that it cannot be excluded that carrageenan preparations encompassing considerable amounts of low molecular weight molecules could be used as food additive.

Therefore, with respect to the definition of carrageenan E 407 and Eucheuma seaweed (E 407a) in the Commission Regulation (EU) No 231/2012, the weight-average molecular weight range should be specified in a narrow way avoiding a significant overlap with the molecular weight range of poligeenan.

With respect to the purity criteria for carrageenan E 407 and processed Eucheuma seaweed (E 407a) in the Commission Regulation (EU) No 231/2012, the Panel noted the urgent need to develop a validated method to quantify the low molecular weight carrageenan with the defined limit of 5%.

3.1.3. Manufacturing process

3.1.3.1. Carrageenan (E 407)

According to Commission Regulation (EU) No. 231/1012, carrageenan is obtained from algae of the families Gigartinaceae, Solieriaceae, Hypneaceae and Furcellariaceae. Generally, the industrial sources of carrageenan are various species of red *algae* of the class Rhodophyceae, mainly *E. cottonii, E. spinosum, C. crispus, Chondrus ocellatus, Hypnea musciformis* and several *Gigartina, Iridaea* and *Furcellaria* species (Imeson, 2000; van de Velde and De Ruiter, 2002; Marburger, 2003). The algae are harvested either in nature or produced by seaweed farming (van de Velde and De Ruiter, 2002).

According to the information provided by an interested party (Documentation provided to EFSA n. 15 and 17), carrageenan (E 407) is only produced from dry seaweed and formaldehyde is not used in the production of carrageenan (E 407), neither for treatment of seaweed nor during the manufacturing process.

After the seaweeds are identified and selected to make particular extract they are washed to remove sand, salts and other foreign materials. It is then heated with water containing an alkali, for several hours in order to increase gel strength in the final product. Non-dissolved seaweed is removed by centrifugation or by a coarse filtration. The solution is then filtered again, in a pressure filter and at this stage, the solution contains 1–2% carrageenan and it is usually concentrated to 2–3% by vacuum distillation and ultrafiltration. There are two methods ('alcohol-precipitation method' and 'gel method') for recovering a solid. 'An alcohol-precipitation method' can be used for any of the carrageenan, while 'a gel method' can be used exclusively for κ-carrageenan. In the alcohol-precipitation method, isopropanol is added until all the carrageenan is precipitated as a fibrous coagulum that is then separated by centrifugation or by sieving through a fine sieve. The coagulum is pressed to remove solvent, washed with alcohol and then dried and milled to an appropriate particle size (80 mesh or finer). The 'gel method' relies on the ability of κ-carrageenan to form a gel with potassium salts. The gel may be formed in various ways. For the freeze-thaw process, it is convenient to form it as spaghetti-like pieces by passing the carrageenan solution through fine holes into a potassium chloride solution. The fine 'spaghetti' is collected and washed with potassium chloride, pressed to remove excess liquid and then frozen. When allowed to thaw, separation of water occurs by syneresis, the pieces are washed with potassium chloride, chopped up and dried in a hot air dryer. Inevitably the product contains some potassium chloride. The alternative to freeze-thaw is to squeeze out water from the gel by applying pressure to it. After squeezing for several hours the sheets of gel are chopped, dried in a hot air dryer and milled to an appropriate particle size. Finished products are made by blending one or more extracts, with or without standardising agents, in order to maintain consistent quality from lot to lot (FAO, 2003; Blakemore and Harpel, 2010).

According to the information provided by one of the interested parties (Documentation provided to EFSA n. 17), calcium hypochlorite is not used as a bleaching agent in the manufacturing process of the food additive carrageenan (E 407). Provided results of analysis of the content of semicarbazide in 10 commercial batches of carrageenan from 5 manufacturers were in the range of < 1–2 μg/kg. At maximum use in food of 2%, maximum semicarbazide contribution by carrageenan with 2 μg/kg semicarbazide to any food product would be 0.08 μg/kg (Documentation provided to EFSA n. 17).

The following flow chart (Blakemore and Harpel, 2010) (figure 4) details the manufacturing process for E 407.

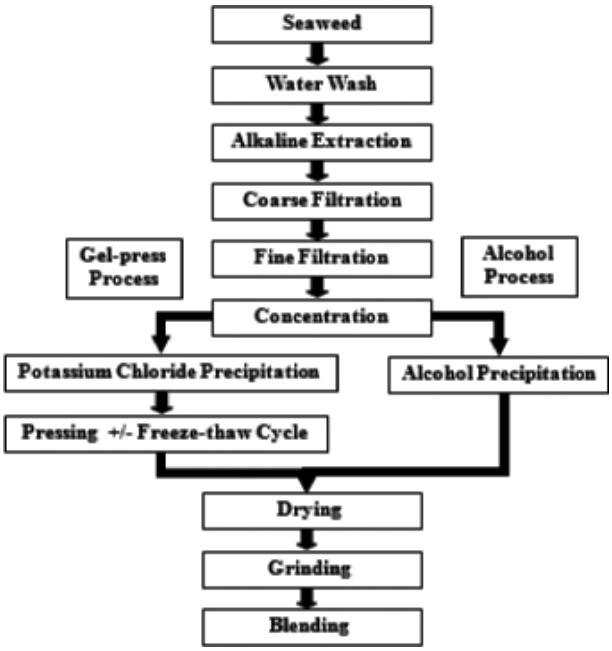


Figure 4: Carrageenan manufacturing process flow chart (re-produced from Blakemore and Harpel, 2010). (Documentation provided to EFSA n. 15)

3.1.3.2. Processed Eucheuma seaweed (E 407a)

According to Commission Regulation (EU) No 231/2012, the industrial sources of processed Eucheuma seaweed are strains of the seaweeds *E. cottonii* and *E. spinosum*, of the class Rhodophyceae.

According to the information provided by one of the interested parties (Documentation provided to EFSA n. 15 and 17), processed Eucheuma seaweed (E 407a) are only produced from dry seaweed and formaldehyde is not used in the production of processed Eucheuma seaweed (E 407a), neither for treatment of seaweed nor during the manufacturing process.

For the manufacturing process of processed Eucheuma seaweed (E 407a), the seaweed is treated with a potassium hydroxide solution for a short time at 70–80°C. This temperature is not high enough to allow the carrageenan to be extracted, but most of the low-molecular-mass material is removed. According to some information found in the literature, 'the treated material is then thoroughly washed with water to remove residual salts and further washed with alcohol, dried and milled to a powder. The alcohols that may be used during purification are restricted to methanol, ethanol and isopropanol' (JECFA, 2001; van de Velde and De Ruiter, 2002).

The manufacture processes of processed Eucheuma seaweed (E 407a) and carrageenan (E 407) are similar. While during carrageenan production the carrageenan is solubilised to remove solids, the processed Eucheuma seaweed process leaves carrageenan within the seaweeds cellulosic structural matrix. Therefore, processed Eucheuma seaweed contains algal cellulose, in contrast to carrageenan. All further purification steps are comparable (Blakemore and Harpel, 2010).

According to EFSA AFC Panel (EFSA, 2005), it is stated that processed Eucheuma seaweed (E 407a) is sometimes bleached with calcium hypochlorite resulting in higher concentrations of the reaction by-product semicarbazide; industry initiated trials to replace calcium hypochlorite with an alternative bleaching agent for the production of processed Eucheuma seaweed (E 407a).

According to information received by industry (Documentation provided to EFSA n. 17), three manufacturers of processed Eucheuma seaweed (E 407a) were identified as currently using calcium hypochlorite for bleaching and one manufacturer uses hydrogen peroxide for bleaching. Commercial samples from these manufacturers were analysed for semicarbazide for comparison, along with unbleached batches. The results are shown in Table 5. At maximum use in food of 2%, maximum semicarbazide contribution by processed Eucheuma seaweed (E 407a) with 39 μg/kg semicarbazide to any food product would be 0.78 μg/kg (Documentation provided to EFSA n. 17).

Table 5: Semicarbazide levels in processed Eucheuma seaweed (E 407a) after different bleaching treatments from different manufactures

Manufacture	Treatment	Semicarbazide (μg/kg)
–	(all lots washed after treatment)	
F	No calcium hypochlorite/peroxide	8
G	No calcium hypochlorite/peroxide	4
H	No calcium hypochlorite/peroxide	12
I	No calcium hypochlorite/peroxide	11
J	No calcium hypochlorite/peroxide	5
G	800 mg/L calcium hypochlorite	37
G	1,000 mg/L calcium hypochlorite	20
J	1,000 mg/L calcium hypochlorite	39
H	5,000 mg/L peroxide	17
H	2,500 mg/L peroxide	22

For processed Eucheuma seaweed prepared using a bleaching step, for the 25 batches reported by industry to EFSA AFC Panel (2005), the range for semicarbazide was 9–380 μg/kg with a mean of 65 μg/kg.

According to the EFSA AFC Panel (2005), 'for the food additive carrageenan, that may become contaminated with semicarbazide at a mean concentration of 65 μg/kg from use of hypochlorite in the production process, if consumption was up to the Acceptable Daily Intake (ADI) for carrageenan of 75 mg/kg bw per day then the intake of semicarbazide from this source could be up to 0.005 μg/kg bw per day'. Based on the overall weight of evidence, the AFC Panel concluded that 'the weak genotoxicity exerted by semicarbazide *in vitro* is not expressed *in vivo*. A large margin of at least 5 orders of magnitude exists between the dose causing tumours in experimental animals and human exposure, including that of infants'. Therefore, the AFC Panel concluded that 'the issue of carcinogenicity is not of concern for human health at the concentrations of semicarbazide encountered in food'.

The Panel noted that the carcinogenic effects of semicarbazide referred to by the AFC Panel were observed in a sensitive mouse strain but not in rat (Davies et al., 2000), and that semicarbazide was classified by IARC in Group 3 based on 'limited evidence' of carcinogenicity in experimental animals and 'no data' in humans (IARC, 1987). The Panel further noted that the currently reported levels on semicarbazide are below levels reported to the AFC Panel and thus would not be of toxicological concern referring to the existing ADI. The Panel also noted results from other studies (Maranghi et al., 2009, 2010) investigating effects in juvenile animals and on endocrine disruption, which did not allow the derivation of a NOAEL without further investigations.

The following flow chart (Blakemore and Harpel, 2010) (Figure 5) details the manufacturing process for processed Eucheuma seaweed (E 407a).

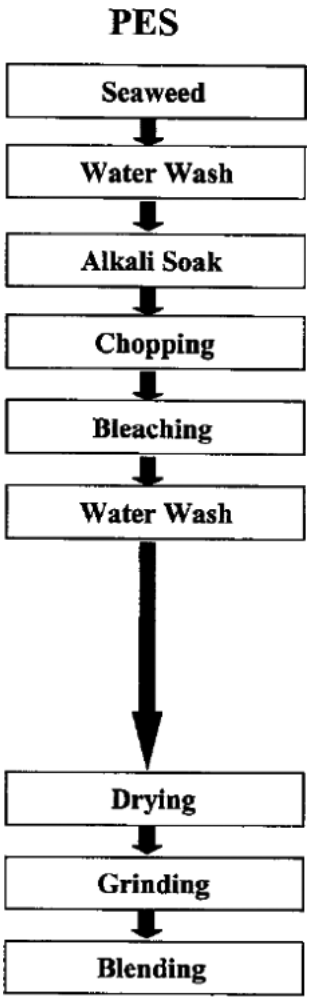


Figure 5: Processed Eucheuma seaweed manufacturing process flow chart (re-produced from Blakemore and Harpel, 2010; Documentation provided to EFSA n. 15)

3.1.4. Methods of analysis in food

3.1.4.1. Carrageenan (E 407)

A single-laboratory validated method to analyse carrageenan mixed into the diet for animals of an experimental study was verified (Documentation provided to EFSA n. 37). Carrageenan concentration in powdered rodent diet was determined, after extraction with water at approximately 75°C and shaking in water bath for 20 min. After centrifugation, the viscosity of supernatant was measured (no information on the method used). A linear calibration line was obtained, and the limit of detection (LOD) for the carrageenan was determined to be 0.42 g/100 g diet.

There is a number of analytical methods available for determination of carrageenan in foodstuff, including: gas chromatography (GC), HPLC, colorimetry, electrophoresis, immunoassays, and potentiometry. An overview of these methods was presented in a review by Roberts and Quemener (1999).

Gas chromatography

An analytical method for carrageenan and further polysaccharides (locust bean gum, guar gum, gum arabic, tragacanth, arabinogalactan, agar, furcellaran, xanthan) in dairy products was described by Glueck and Thier (1980). Thereby polysaccharides are extracted from foodstuff, and then fat, starch, milk proteins and carbohydrates are removed by extraction or degradation. The resulting polysaccharide fraction is analysed by GC after hydrolysis with trifluoroacetic acid, derivatisation of the resulting monosaccharides with hydroxylamine hydrochloride and acetic acid anhydride to form the aldonitrilacetate derivatives. The polysaccharides can be qualitatively identified by their characteristic monosaccharide pattern, and quantified via the single monosaccharide peaks. In the case of

carrageenan, the only hydrolysis product is galactose. For the polysaccharides, recoveries of 80–90% were obtained when adding 0.05% of the thickeners to skim milk or 1–2% to mixtures of ice cream or pudding constituents (Glueck and Thier, 1980). The analytical method without extraction from foodstuff was previously described by Mergenthaler and Scherz (1976).

In a later investigation the analytical procedure described by Glueck and Thier (1980) was improved by Preuss and Thier (1982, 1983). Changes in the separation of interfering substances (fats, proteins and starch) allowed the quantitative determination of carrageenan in a variety of foods like blancmanger powder, glaze, fruit ice, and cream cheese. Recoveries for most of the thickeners and gums are about 60–85% with a CV of 2–8 %. The Panel noted that both methods described do not allow to distinguish between agar and carrageenan as both polysaccharides consist only of galactose units.

Mergenthaler and Scherz (1980) developed an analytical method which allows to distinguish carrageenan from agar (and from other polysaccharides). After extraction of fat and proteins, the polysaccharides are eluted through a chromatographic column filled with diethylaminoethyl (DEAE)-cellulose, where carrageenan is separated from other polysaccharides inclusive agar. The resulting fractions can be further analysed either by thin layer chromatography or, after derivatisation, by GC.

The Panel noted that the above mentioned methods have been published without indication of the LOD.

Size exclusion chromatography methods

Hunziker and Miserez (1980) published a method for quantitative determination of different polysaccharides using gel permeation chromatography. After extraction of fat, carrageenan is purified by precipitation with methanol, and the precipitate is further washed and dried. The polysaccharide is chromatographed as 0.04 % solution.

A method based on HPSEC-RI has been employed for the measurement of the low molecular weight fraction of carrageenan in food-grade carrageenan ingredients and in a carrageenan-containing finished product (a jelly) (Spichtig and Austin, 2008). According to the authors, 'over the course of half a year, 19 measurements were made on a reference carrageenan and the results demonstrated that the method had good reproducibility (CV for the low molecular weight fraction was 2.9%) in a single laboratory. Applied to a number of different carrageenan ingredients, it was found that, in general, the low molecular weight fraction represents less than 8% of the total carrageenan in ingredients, and under the correct conditions increases little during food processing'.

Electrophoretic methods

An electrophoretic method for qualitative and quantitative analysis of gelling agents in food (pudding, milk-based baby food, sugar fruits, ice cream, ketchup, cream stabiliser) was published by Pechanek et al. (1982). After removal of fat, starch and proteins, carrageenan is precipitated and separated via electrophoresis using cellulose acetate membrane and stained with toluidine blue. The polysaccharides are quantified using a scanner. Lowest reported LOD for carrageenan was 40 ng.

Colorimetric methods

Soedjak (1994) analysed carrageenan in milk as methylene blue complex which can be determined photometrically. The interaction between methylene blue and polyanions is reversible, electrostatic, and stoichiometric (1:1 ratio between the anionic sites and the bound dye molecules). The method was found to be sensitive, capable of determination between 0.2 and $2.0 \cdot 10^{-3}$% polyanion of the sample. Due to the high sensitivity of the method, potentially interfering compounds (e.g. acids, sugars, salts, milk, proteins, dyes, emulsifiers, and neutral hydrocolloids) may be diluted out.

A similar method using alcian blue as complexation agent was published by Yabe et al. (1991). Carrageenan-alcian blue complex is precipitated, the precipitate dissolved in monoethanolamine and determined colorimetrically at 615 nm. Recoveries from jellies and salad dressings were > 90%, the detection limit was 0.05%.

Potentiometric methods

Carrageenan was determined at the concentration levels of 0.2–1.5% (m/m) in a variety of food products (e.g. cream, chocolate, caramel, ice cream, and salad dressing) using potentiometric titration of carrageenan with protamine titrant. For monitoring potentiometric titration polymeric matrix tubular membrane sensors were used. Reported limit of detection (LOD) was 0.7 μg/mL (Hassan et al., 2002).

Immunoassay methods

Haines and Patel (2005) developed an enzyme-linked immunosorbent assay (ELISA) for quantification of carrageenan in different foods (water jelly, milk jelly, salad dressing, chocolate milk, and meat pate). After extraction of fats and digesting proteins with protease, carrageenan is bound on a solid phase column containing sheep immunoglobulin G (IgG) antibody to carrageenan, and then eluted for measurement. No cross reaction with competing polysaccharides was observed. The method was inter-laboratory validated in accordance with an International AOAC PVM-approved procedure (AOAC, 1998). The LOD was 0.5 μg/mL and the mean recovery was in the range 69–102%. The results were highly repeatable (< 10% CV) and reproducible (< 15% CV). The Panel noted that it was not reported whether the anti-carrageenan antibody also recognised poligeenan or other 'degraded' carrageenan.

The Panel noted that acceptable methods were available for the determination of carrageenan in foodstuff.

3.1.4.2. Processed Eucheuma seaweed (E 407a)

There were no validated methods available which are specific for the analysis of processed Eucheuma seaweed (E 407a) in foodstuff and discriminate between processed Eucheuma seaweed and carrageenan.

However, an analysis method of processed Eucheuma seaweed (E 407a) mixed into the diet for animals of an experimental study was verified by (Documentation provided to EFSA n. 37). For details, see Section 3.1.4.1.

In addition, a number of other analytical methods were developed for the determination of carrageenan, the main component of processed Eucheuma seaweed (E 407a). These methods include GC, HPLC, colorimetry, electrophoresis, immunoassays and potentiometry. An overview is presented in the review of Roberts and Quemener (1999). See Section 3.1.4.1.

3.1.5. Stability of the substance, and reaction and fate in food

According to JECFA (2015), 'the stability of carrageenan in foods is influenced by several factors, such as pH, direct structural bridging between the negatively charged carrageenan and positively charged protein sites, and indirect structural bridging with negatively charged protein sites via divalent cations such as calcium, through hydrogen bonding and through carrageenan–carrageenan helical interactions' (JECFA, 2015).

According to Weiner (2014), a direct polyanion carrageenan and polycation protein interaction can occur below the isoelectric point of the protein, resulting in gelation or precipitation. Also, this direct carrageenan–protein bonding occurs above the isoelectric point, but to a lesser degree as the pH is increased. Above the isoelectric point, positively charged cations, such as calcium or other polyvalent cations, form a bridge across the negatively charged sulfate groups on carrageenan to the negatively charged carboxyl groups on the protein, forming stable gels. In addition, carrageenan–carrageenan molecules interact to form helical bridges, adding strength to the gels. In the absence of protein, carrageenan forms a loose random conformation in water solution rather than the structured helical coil conformation formed in the presence of protein (Blakemore and Harpel, 2010).

According to Spichtig and Austin (2008), pH seems to be a critical factor during food processing with respect to possible formation of low molecular weight carrageenan and pH levels less than 4.0 should be avoided.

According to industry, 'the presence of other food components can have significant positive impacts on carrageenan stability, particularly those that interact (cross-link) with the carrageenan (e.g. proteins, locust bean gum) or tighten the carrageenan molecular structure (e.g. salts, high solids). These food conditions therefore provide carrageenan added stability and protection from degradation' (Documentation provided to EFSA n. 17;18).

Carrageenan is sensitive to acid and oxidative breakdown. Acid-catalysed hydrolysis occurs at the glycosidic linkage, leading to depolymerisation (van de Velde and De Ruiter, 2002). According to industry, at pH values above 6, carrageenan is very stable even at higher temperatures. However, no detailed information was provided. Isolated carrageenan powders are slightly alkaline (pH 8–10), they are highly stable during storage. The industry reported that, 'when carrageenan is stored under cool dry conditions it is stable in excess of two years' (Marinalg, 2006).

κ-Carrageenan is the most sensitive type of carrageenan to exposure at low pH. The rate of hydrolysis (as determined by specific viscosity measurements) of κ-carrageenan is dependent on

carrageenan state. The use of buffers (e.g. citrate) is recommended for lower pH applications, and hydrolysis rates will show minor variations depending on the buffer system and concentration used (Documentation provided to EFSA n. 17).

Rasmussen (1974) confirmed that heat treatment at pH 7.0 of carrageenan at 100 and 121°C did not affect gel functionality. Processing at 85°C for 20 min at pH 3.5 gave a 50% decrease in gel strength for κ-carrageenan but no decrease in gel strength for ι-carrageenan.

A hydrolytic effect on carrageenan was demonstrated in a synthetic milk salt system with an initial pH of 6.70. The average molecular weight decreased by 42% after 20 min of heat treatment at 122°C (Badui, 1978). When heating carrageenan in sodium cacodylate buffer (pH 7) for 60 min at 122°C, the average molecular weight decreased by 17.3% (Desai and Hansen, 1986).

There were no studies on the stability of processed Eucheuma seaweed (E 407a) available. However, the main components of processed Eucheuma seaweed (E 407a) are κ- and ι- carrageenan that are subject to the same stability considerations. The cellulose in processed Eucheuma seaweed (E 407a) is also very stable in the pH range of processed foods (Marinalg, 2006).

The Panel noted that degradation of κ-carrageenan under mild acid conditions (0.1 M sulfuric acid at 60°C for 1.5 h) has been described by Yang et al. (2009).

The Panel further noted that only limited information on the stability of carrageenan in food was available. No adequate data on stability of carrageenan and/or processed Eucheuma seaweed addressing the usual variation of parameters (temperature, pH) relevant for the authorised food uses were available. Information on possible degradation products under acidic conditions in relevant food products was missing.

3.2. Authorised uses and use levels

Maximum levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) have been defined in Annex II to Regulation (EC) No 1333/2008[2] on food additives, as amended. These levels are defined by the Panel as 'maximum permitted levels (MPLs)' in this document.

Currently, carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised food additives in the EU at *quantum satis* (QS) in almost all foods. Specifically, carrageenan (E 407) is authorised in jam, jellies and marmalades and sweetened chestnut puree (04.2.5.2), other similar fruit or vegetable spreads (04.2.5.3) at MPL 10,000 mg/Kg and follow-on formulae (13.1.2), other foods for young children (13.1.4), dietary foods for infants for special medical purposes and special formulae for infants (13.1.5.1) and dietary foods for babies and young children for special medical purposes (13.1.5.2) at MPL 300 mg/Kg. Carrageenan E 407 and processed Eucheuma seaweed (E 407a) are included in the Group I of food additives authorised at (QS).

Table 6 summarises foods that are permitted to contain carrageenan (E 407) and processed Eucheuma seaweed (E 407a) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 6: MPLs of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in foods according to the Annex II to Regulation (EC) No 1333/2008

FCS category number	Food category names	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		Quantum satis
01.4	Flavoured fermented milk products including heat-treated products	Group I		*Quantum satis*
01.5	Dehydrated milk as defined by Directive 2001/114/EC	E 407		*Quantum satis*
01.6.1	Unflavoured pasteurised cream (excluding reduced fat creams)	E 407		*Quantum satis*
01.6.2	Unflavoured live fermented cream products and substitute products with a fat content of less than 20%	E 407		*Quantum satis*

FCS category number	Food category names	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
01.6.3	Other creams	Group I		*Quantum satis*
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	*Quantum satis*
01.7.5	Processed cheese	Group I		*Quantum satis*
01.7.6	Cheese products (excluding products falling in category 16)	Group I		*Quantum satis*
01.8	Dairy analogues, including beverage whiteners	Group I		*Quantum satis*
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		*Quantum satis*
02.3	Vegetable oil pan spray	Group I		*Quantum satis*
03	Edible ices	Group I		*Quantum satis*
04.2.1	Dried fruits and vegetables	Group I		*Quantum satis*
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		*Quantum satis*
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		*Quantum satis*
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	E 407	Maximum individually or in combination with E 400–404, E 406, E 407, E 410, E 412, E 415 and E 418	*10,000 mg/kg*
04.2.5.3	Other similar fruit or vegetable spreads	E 407	Maximum individually or in combination with E 400–404, E 406, E 407, E 410, E 412, E 415 and E 418	*10,000 mg/kg*
04.2.5.4	Nut butters and nut spreads	Group I		*Quantum satis*
04.2.6	Processed potato products	Group I		*Quantum satis*
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Group I	Only energy-reduced or with no added sugars	*Quantum satis*
05.2	Other confectionery including breath refreshening microsweets	Group I	E 407 and E 407a may not be used in jelly mini-cups, defined, for the purpose of this Regulation, as jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth	*Quantum satis*
05.3	Chewing gum	Group I		*Quantum satis*
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		*Quantum satis*
06.2.2	Starches	Group I		*Quantum satis*

FCS category number	Food category names	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
06.3	Breakfast cereals	Group I		*Quantum satis*
06.4.2	Dry pasta	Group I	Only gluten free and/ or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	*Quantum satis*
06.4.4	Potato gnocchi	Group I	Except fresh refrigerated potato gnocchi	*Quantum satis*
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		*Quantum satis*
06.5	Noodles	Group I		*Quantum satis*
06.6	Batters	Group I		*Quantum satis*
06.7	Pre-cooked or processed cereals	Group I		*Quantum satis*
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	*Quantum satis*
07.2	Fine bakery wares	Group I		*Quantum satis*
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	E 407/ E 407a	Only preparations in which ingredients have been injected; meat preparations composed of meat parts that have been handled differently: minced, sliced or processed and that are combined together. Except bifteki, soutzoukaki, kebap gyros and souvlaki	*Quantum satis*
08.3.1	Non-heat-treated processed meat	Group I		*Quantum satis*
08.3.2	Heat-treated processed meat	Group I	Except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	*Quantum satis*
08.3.3	Casings and coatings and decorations for meat	Group I		*Quantum satis*
09.2	Processed fish and fisheries products including molluscs and crustaceans	Group I		*Quantum satis*
09.3	Fish roe	Group I	Only processed fish roe	*Quantum satis*
10.2	Processed eggs and egg products	Group I		*Quantum satis*
11.2	Other sugars and syrups	Group I		*Quantum satis*
11.4.1	Table-top Sweeteners in liquid form	E 407		*Quantum satis*
11.4.2	Table-top Sweeteners in powder form	E 407		*Quantum satis*
12.1.2	Salt substitutes	Group I		*Quantum satis*
12.2.2	Seasonings and condiments	Group I		*Quantum satis*
12.3	Vinegars and diluted acetic acid (diluted with water to 4–30% by volume)	Group I		*Quantum satis*
12.4	Mustard	Group I		*Quantum satis*
12.5	Soups and broths	Group I		*Quantum satis*
12.6	Sauces	Group I		*Quantum satis*

FCS category number	Food category names	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
12.7	Salads and savoury-based sandwich spreads	Group I		*Quantum satis*
12.8	Yeast and yeast products	Group I		*Quantum satis*
12.9	Protein products, excluding products covered in category 1.8	Group I		*Quantum satis*
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC	E 407	If more than one of the substances E 407, E 410 and E 412 is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff	*300 mg/kg*
13.1.4	Other foods for young children	E 407	If more than one of the substances E 407, E 410 and E 412 is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff	*300 mg/kg*
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 407	If more than one of the substances E 407, E 410 and E 412 is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff	*300 mg/kg*
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 407	If more than one of the substances E 407, E 410 and E 412 is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff	*300 mg/kg*

FCS category number	Food category names	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/ EC (excluding products from food category 13.1.5)	Group I		*Quantum satis*
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group I		*Quantum satis*
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	*Quantum satis*
14.1.2	Fruit juices as defined by Directive 2001/ 112/EC and vegetable juices	Group I	Only vegetable juices	*Quantum satis*
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	*Quantum satis*
14.1.4	Flavoured drinks	Group I		*Quantum satis*
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	*Quantum satis*
14.2.3	Cider and perry	Group I		*Quantum satis*
14.2.4	Fruit wine and made wine	Group I		*Quantum satis*
14.2.5	Mead	Group I		*Quantum satis*
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	*Quantum satis*
14.2.7.1.	Aromatised wines	Group I		*Quantum satis*
14.2.7.2.	Aromatised wine-based drinks	Group I		*Quantum satis*
14.2.7.3.	Aromatised wine-product cocktails	Group I		*Quantum satis*
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group I		*Quantum satis*
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		*Quantum satis*
15.2	Processed nuts	Group I		*Quantum satis*
16	Desserts excluding products covered in category 1, 3 and 4	Group I		*Quantum satis*
17.1[(a)]	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		*Quantum satis*
17.2[(a)]	Food supplements supplied in a liquid form	Group I		*Quantum satis*
17.3[(a)]	Food supplements supplied in a syrup-type or chewable form	Group I		*Quantum satis*
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	Group I		*Quantum satis*

MPL: maximum permitted level.

(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex II of Regulation (EC) No 1333/2008, carrageenan (E 407) and processed Eucheuma seaweed (E 407a) may be standardised with sugars, on condition that this is stated in addition to the number and designation.

According to Annex III, Part 1 of Regulation (EC) No 1333/2008, carrageenan (E 407) is authorised as carrier in all food additives at the level of QS.

According to Annex III, Part 3, carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised in enzyme preparations at the level of QS.

In addition, according to Annex III Part 5 of Regulation (EC) No 1333/2008, carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised in nutrients except nutrients intended to be used in foodstuffs for infants and young children at the level of QS.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a)

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. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[21] for food additive usage level and/or concentration data in food and beverages intended for human consumption, including old call for data[22] on carrageenan (E 407) and Eucheuma seaweed (E 407a). In response to these calls, updated information on the actual use levels of carrageenan (E 407) and Eucheuma seaweed (E 407a) in foods was made available to EFSA by industry. Only few analytical data on the concentration of carrageenan (E 407) in foods were made available by Member States.

Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 457) of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) (n = 105) in foods for 50 out of the 79 food categories in which E 407 and 28 out of the 71 food categories in which E 407a are, respectively, authorised.

Updated information on the actual use levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in foods was made available to EFSA by the Association of the European Self-Medication Industry (AESGP) (Documentation provided to EFSA n. 20), Associazione Industriali delle Carni e dei Salumi (Documentation provided to EFSA n. 21), BABBI Confectionary Industry (Documentation provided to EFSA n. 22), Delixia s.r.l. (Documentation provided to EFSA n. 23, Interested party 1 (Documentation provided to EFSA n. 28), EUROGUM A/S (Documentation provided to EFSA n. 24), Fabricante Embutidos del centro SA (España) (Documentation provided to EFSA n. 25), FoodDrinkEurope (FDE) (Documentation provided to EFSA n. 26), the International Chewing Gum Association (ICGA) (Documentation provided to EFSA n. 27), Intertek Scientific & Regulatory Consultancy (Documentation provided to EFSA n. 29), Rudolf Wild GmbH & Co (Documentation provided to EFSA n. 30), and Specialised Nutrition Europe (SNE) (Documentation provided to EFSA n. 31).

The Panel noted that some data providers (Documentation provided to EFSA n. 24) are not a food industry using carrageenan and processed Eucheuma seaweed in their food products but food additive producers. Usage levels reported by food additive producers are not considered at the same level as those provided by food industry. Food additive producers might recommend usage levels to the food industry but the final levels might, ultimately, be different. Therefore, unless food additive producers confirm that these levels are used by food industry, they are not considered in the refined exposure scenario. Data from food additive producers will only be used in the *maximum level exposure assessment scenario* in case of QS authorisation when no data are available from food industry. In this way, the most complete exposure estimates are calculated.

For instance, for one of the data provider (Documentation provided to EFSA n. 24), all the submitted data are theoretical amounts suggested or recommended; they are 'based on their own

[21] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Available online: https://www.efsa.europa.eu/en/dataclosed/call/datex140310

[22] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents Published: 23 May 2010. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

technical know-how regarding adequate/recommended levels of use in different food applications'. This data provider (Documentation provided to EFSA n. 24) provided three identical levels on meat products. These levels were not considered in the current estimates.

Usage levels from the call for data from 2010[10] were also not used in this opinion, since the food categories on which they were reported for are well covered by the most recent data received.

Appendices A and B present data on the use levels of carrageenan (E 407) and processes Eucheuma seaweed (E 407a) in foods as reported by industry.

Summarised data on concentration levels in food submitted by Member States

In total, only two analytical results were reported to EFSA on carrageenan (E 407) by one country (Germany). These data were on liquid cow milk (FCS 01.1). Foods were sampled in year 2003.

Both analytical results on carrageenan (E 407) were left-censored: not quantified (< LOQ). Complete information on the methods of analysis (e.g. validation) was not made available to EFSA, but both samples were derived from accredited laboratories. The Panel noted that both analytical results were reported in a food category in which carrageenan (E 407) is not authorised for direct addition according to Annex II of Regulation (EC) No 1333/2008. Other authorised uses of carrageenan (E 407) according to Annex III to Regulation (EC) No 1333/2008 (Part 1 and 3) may have resulted in carry-over and its detection in this food category. It should be noted that carrageenan (E 407) was not quantified in these food categories and, therefore, it was not considered for the exposure calculation.

Appendix C shows the analytical results of carrageenan (E 407) in foods as reported by Member States.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel GNPD is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over two million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.[23]

For the purpose of this Scientific Opinion, the Mintel GNPD[24] was used for checking the labelling of products containing carrageenan (E 407) and processed Eucheuma seaweed (E 407a) within the EU's food products, as the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel, carrageenan (E 407) is labelled on 16,890 and processed Eucheuma seaweed (E 407a) on 653 foods, drinks and food supplement products published between 2012 and 2017.

Appendix D presents the percentage of the food products labelled with E 407 and E 407a between 2012 and 2017, out of the total number of food products per food sub-categories according to the Mintel food classification.

3.3.3. Food consumption data used for exposure assessment

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 (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys recently[25] added in the Comprehensive database were also taken into account in this assessment.[14]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless,

[23] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[24] http://www.gnpd.com/sinatra/home/ accessed on 5/10/2016 (up to the date of the work done on the opinion).
[25] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 7).

Table 7: Population groups considered for the exposure estimates of carrageenan (E 407) and processed Eucheuma seaweed (E 407a)

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

(a): The term 'toddlers' in the EFSA Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.

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

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

Food categories selected for the exposure assessment of carrageenan (E 407) and processed Eucheuma seaweed (E 407a)

The food categories in which the use of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

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

- 2.3 Vegetable oil pan spray;
- 6.4.4 Potato Gnocchi;
- 6.6 Batters;
- 6.7 Pre-cooked or processed cereals, only pre-cooked cereals;
- 8.3.3 Casings and coatings and decorations for meat;
- 12.1.2 Salt substitutes;
- 14.2.5 Mead;
- 14.2.7.2. Aromatised wine-based drinks;
- 14.2.7.3 Aromatised wine-based cocktails.

For the following food categories, the restrictions/exceptions which apply to the use of Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) could not be taken into account, and therefore, the whole food category was considered in the exposure assessment. This may result in an overestimation of the exposure:

- 5.1 Cocoa and cocoa products, only energy-reduced or with no added sugar;
- 5.2 Other confectionery including breath refreshening microsweets, except in jelly mini-cups, defined, for the purpose of this Regulation, as jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth;
- 7.1 Bread and rolls, except products in 7.1.1 and 7.1.2;
- 8.2 Meat preparations as defined by Regulation (EC) No 853/2004, only preparations in which ingredients have been injected; meat preparations composed of meat parts that have been handled differently: minced, sliced or processed and that are combined together. Except bifteki, soutzoukaki, kebap gyros and souvlaki;
- 8.3.2 Heat-treated meat products, except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben;
- 9.3 Fish roe, only processed fish roe.

The FCs 17.1/17.2/17.3 Food supplements, in solid, liquid, syrup-type or chewable form, the form cannot be differentiated and the same use level was applied to the whole FC 17.

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are also allowed in FC 13.2, 13.3 and 13.4. Food items under food categories 13.2, 13.3 and 13.4 consumed by population groups- children, adolescents, adults and the elderly- may be very diverse and, in addition, there is very limited information on their consumption. Therefore, eating occasions belonging to the food categories 13.2, 13.3 and 13.4 were reclassified under food categories in accordance to their main component.

The use levels available for food categories 13.2, and 13.3 (there were no reported data for FC 13.4) were not considered for the exposure assessment.

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are also authorised in FC 18 (Processed foods not covered by categories 1 to 17, excluding foods for infants and young children). Considering that FC 18 is extremely unspecific (e.g. composite foods, processed foods, prepared or composite dishes belonging to this food category were reclassified under food categories in accordance to their main component and included as such in the exposure assessment). The use levels available for food categories 18 were not considered for the exposure assessment.

No information on the usage levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) has been reported to EFSA for 19 food categories and therefore these could not be taken into account (Appendix E). This may have resulted in an underestimation of the exposure if E 407 and E 407a are used in these food categories. Comparing these food categories with the data extracted from the Mintel database (information on the use of E 407/E 407a was found for the categories 04.2.5.3, 06.3, 06.4.2, 06.5, 12.3, 14.1.3, 15.1), the Panel noted that the use of carrageenan and processed Eucheuma seaweed in these food categories was limited.

Overall, for both the maximum level exposure and for the refined scenarios 41 food categories were included, in the present exposure assessment to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) (Appendix E).

3.4. Exposure estimate

3.4.1. Exposure to carrageenan E 407 and to processed Eucheuma seaweed E 407a from their use as food additives

The Panel estimated chronic exposure for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Based on information from the Mintel's GNPD, the Panel considered that E 407 and E 407a are not likely to be used in combination in the same food product and, therefore, the exposure assessment of E 407 and E 407a was performed considering the reported use level for either E 407 and E 407a independently per food category (Appendix D). Dietary exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) was calculated by multiplying carrageenan (E 407) and processed Eucheuma seaweed (E 407a) concentrations for each food category (Appendix E) with their respective consumption amount per kilogram of body weight for

each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only one day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual average exposure per survey and population group (Table 7). Based on these distributions, the mean and 95th percentile of exposure were calculated per survey for the total population and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) was carried out by the ANS Panel based on two different sets of concentration data: (1) MPLs as set down in the EU legislation or maximum levels of data provided to EFSA when E 407 and E 407a are authorised at QS (defined as the *regulatory maximum level exposure assessment scenario*); and (2) reported use levels (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below.

These scenarios do not consider the consumption of food supplements (FC 17.1, 17.2 and FC 17.3) or FSMP which are covered in added scenarios detailed below.

A possible additional exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives authorised in accordance with Annex III to Regulation (EC) No 1333/2008 was not considered in any of the above exposure assessment scenarios.

In addition, formaldehyde exposure was calculated for the non-brand-loyal scenario.

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 6. As carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised according to QS in almost all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels provided by industry, excluding exposure via food supplements and FSMP, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

The Panel considers the exposure estimates derived following this scenario as the most conservative since it is assumed that the consumer will be continuously (over a lifetime) exposed to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in food at the maximum reported use levels.

Refined exposure assessment scenario

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

Appendix E summarises the concentration levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels or the maximum of the analytical results, whichever was highest or available, for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results for all food categories.

Specific exposure assessment scenarios

- **Food supplement consumers only scenario**

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised in the food category 17 Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children. As exposure via food supplements may deviate largely from the one via food, and that the number of food supplement consumers may be low depending on populations and surveys, an additional scenario was calculated in order to reflect additional exposure to food additives from food supplements compared to exposure to food additives excluding these sources. This scenario will be estimated as follow:

- Consumers only of food supplements were assumed to be exposed to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) present at the maximum reported use level on a daily basis via consumption of food supplements.
- For the remaining food categories, the mean of the typical reported use levels was used.

As food category 17 does not consider food supplements for infants and toddlers, as defined in the legislation, exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from food supplements was not estimated for these two population groups.

- **FSMP consumers only scenario**

As carrageenan (E 407) is also authorised in the food categories 13.1.5.1 and 13.1.5.2 with MPL, an additional exposure assessment scenario taking into account these two food categories was performed to estimate the exposure of infants and toddlers who may eat and drink these foods for special medical purposes (FSMP).

The consumption of these foods is not reported in the EFSA Comprehensive database. To consider potential exposure to carrageenan (E 407) via these foods, the Panel assumes that the amount consumed of FSMP in infants and toddlers resembles that of comparable foods in infants and toddlers from the general population. Thus, the consumption of FSMP categorised as food category 13.1.5 is assumed to equal that of formulae and food products categorised as food categories 13.1.1, 13.1.2, 13.1.3 and 13.1.4.

FSMP consumed in other population groups (children, adolescents, adults and the elderly) may be very diverse; they were not considered because of very limited information on consumption. Eating occasions belonging to the food categories 13.2, 13.3, 13.4 were therefore reclassified under food categories in accordance to their main component and no exposure estimates for these population groups were calculated.

This scenario was estimated as follows:

- Consumers only of FSMP were assumed to be exposed to carrageenan (E 407) present at the maximum reported use level (data provided by food industry, reported values equal to the MPL) on a daily basis via consumption of the food categories 13.1.5.1 and 13.1.5.2 (infant formulae, follow-on formulas and processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC).
- For the remaining food categories, the mean of the typical reported use levels was used.

Dietary exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a)

Table 8 summarises the estimated exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives in six population groups (Table 7) according to the different exposure scenario's. Detailed results per population group and survey are presented in Appendix G.

Table 8: Summary of anticipated exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per 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)
Maximum level exposure assessment scenario						
Mean	21.8–86.7	135.3–581.4	109.6–487.0	63.5–264.6	34.7–138.6	24.7–89.4
95th percentile	64.9–324.8	354.3–913.2	245.0–999.1	152.7–534.7	92.5–326.8	58.1–175.0
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	15.1–64.3	99.4–374.9	70.1–296.9	38.9–175.9	22.0–88.9	14.2–50.8
95th percentile	42.4–256.9	262.5–758.6	143.8–690.8	103.8–397.2	63.3–247.2	29.8–116.8
Non-brand-loyal scenario						
Mean	5.2–18.7	19.2–70.7	18.3–57.8	9.5–27.1	5.0–16.3	4.4–15.6
95th percentile	19.4–49.0	55.0–121.6	36.0–109.8	17.8–50.3	10.2–34.7	8.4–27.5

From the maximum level exposure assessment scenario, the mean exposure to Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives ranged from 21.8 mg/kg bw per day in the infants to 581.4 mg/kg bw per day in toddlers. The 95th percentile of exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) ranged from 58.1 mg/kg bw per day in the elderly to 999.1 mg/kg bw per day in children.

From the refined estimated exposure scenario, in the brand-loyal scenario, mean exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives ranged from 14.2 mg/kg bw per day in the elderly to 374.9 mg/kg bw per day in toddlers. The high exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) ranged from 29.8 mg/kg bw per day in the elderly to 758.6 mg/kg bw per day in toddlers. In the non-brand-loyal scenario, mean exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives ranged from 4.4 mg/kg bw per day in the elderly to 70.7 mg/kg bw per day in toddlers. The 95th percentile of exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) ranged from 8.4 mg/kg bw per day in the elderly to 121.6 mg/kg bw per day in toddlers.

From the exposure scenario taking into account the foods for special medical purposes, for consumers only, mean exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives ranged from 2.5 mg/kg bw per day to 30.9 mg/kg bw per day for infants and from 0.3 mg/kg bw per day to 19.4 mg/kg bw per day for toddlers. The 95th percentile of exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) for infants ranged from 21.9 mg/kg bw per day to 49.4 mg/kg bw per day and for toddlers from 9.8 mg/kg bw per day to 33.5 mg/kg bw per day.

From the refined estimated exposure scenario taking into account the consumption of food supplements, for consumers only, among children, adolescents, adults and the elderly, mean exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives ranged from 9.9 mg/kg bw per day in adults to 77.9 mg/kg bw per day in children. The 95th percentile of exposure ranged from 23.9 mg/kg bw per day in the elderly to 88.0 mg/kg bw per day in children (see Appendix H).

Main food categories contributing to exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) using the maximum level exposure assessment scenario

From the maximum level exposure assessment scenario, the main contributing food categories to the total mean exposure estimates for infants were flavoured fermented milk products and fine bakery wares. For toddlers, children and adolescents, the main contributing food categories were flavoured fermented milk products, fine bakery wares and flavoured drinks; while, for adults and the elderly, the main contributing food categories were fine bakery wares and flavoured drinks.

The main food categories contributing to the combined exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) using the maximum level exposure assessment scenario are presented in Appendix G.

Main food categories contributing to exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) using the refined exposure assessment scenario

In the brand-loyal scenario, the main contributing food categories were flavoured fermented milk products and fine bakery wares for infants; for toddlers, children and adolescents the main contributing food categories were flavoured fermented milk products, fine bakery wares and flavoured drinks. For adults and elderly, the main contributing food categories were fine bakery wares and flavoured drinks.

In the non-brand-loyal scenario, the main contributing food categories were: flavoured fermented milk products and processed fruit and vegetables for infants; flavoured fermented milk products and fine bakery wares for toddlers and children; fine bakery wares for adolescents, adults and the elderly.

The main food categories contributing to the combined exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) using the refined exposure scenarios are presented in Appendix G.

Exposure to formaldehyde from the occurrence in carrageenan (E 407) and processed Eucheuma seaweed (E 407a)

According to the EU specifications, the content of formaldehyde is limited to a maximum of 5 mg/kg in carrageenan products. Information from literature suggested that residual levels of formaldehyde in five carrageenan products tested using the method provided by one of the interested parties (Documentation provide to EFSA n. 48) are in the range 0.5–1.5 mg/kg.

Based on these values, the Panel calculated the exposure to formaldehyde from the occurrence of carrageenan products as food additives in different foodstuffs for the highest exposure estimates in toddlers based on the refined brand loyal exposure scenario. The highest P95th exposure to residuals levels of formaldehyde was 3.8 μg/kg bw per day using the EU specification of 5 mg/kg down to 1.14 μg/kg bw per day using the highest residual levels of 1.5 mg/kg reported in Documentation provide to EFSA n. 48.

The Panel evaluated the risks associated with methanol and its subsequent metabolism to formaldehyde in the opinion on aspartame (EFSA ANS Panel, 2013). Because the increase of formaldehyde above the baseline level due to 4 mg formaldehyde/kg bw per day was a small fraction of the natural occurring variation, the Panel concluded that this level of formaldehyde did not constitute a significant additional risk. The Panel noted that the exposure at the limit of specifications for formaldehyde in carrageenan products was 3.8 μg/kg bw per day which is around 1,100 times lower than the amount arising from aspartame.

Therefore, the Panel concurred with the previous EFSA AFC opinion (EFSA, 2007) that exposure to carrageenan as additives containing residual formaldehyde, at the EU purity criteria level of 5 mg/kg, would be of no safety concern.

Uncertainty analysis

Uncertainties in the exposure assessment of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 9.

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

Sources of uncertainties	Direction[a]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey covering only a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–

Sources of uncertainties	Direction[a]
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 9 out of 79 food categories)	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 7 out of 79 food categories)	+
Food categories selected for the exposure assessment: data not available for certain food categories which were excluded from the exposure estimates (n = 19 out of 79 food categories)	–
Foods which may contain the food additive according to Annex III to Regulation (EC) No 1333/2008 not taken into account	–
Maximum level exposure assessment scenario: • exposure calculations based on the maximum reported use levels (reported use from industries)	+
Refined exposure assessment scenarios: • scenario including only the food categories according to Annex II to Regulation (EC) No 1333/2008 • exposure calculations based on the maximum or mean levels (reported use from industries)	– +/–
Food supplements consumers only scenario: • exposure calculations based on consumers only • exposure calculations based on the mean levels (reported use from industries for all foods except food supplements	+ –/+
FSMP, consumers only scenario: • exposure calculations based on consumers only • exposure calculations based on the MPL levels for the FSMP and mean reported use levels for all other foods • foods which may contain the food additive according to Annex III to Regulation (EC) No 1333/2008 not taken into account	+ + –
Uncertainty in possible national differences in use levels of food categories	+/–

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

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised as a Group I food additive in 79 food categories and have specific authorised uses in six other categories (Table 6). Since the majority of food categories correspond to the general Group I food additives authorisation, carrageenan (E 407) and processed Eucheuma seaweed (E 407a) may not necessarily be used in some of these food categories. This may explain why use levels for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) were not reported by the food industry for 19 food categories. However, the Panel noted that information from the Mintel GNPD (Appendix D) indicated that seven of these 19 food categories were labelled with carrageenan and processed Eucheuma seaweed (fruit and vegetable spreads, breakfast cereals, noodles etc.).

Overall, the Panel considered that the uncertainties identified in both the maximum level exposure scenario and the refined scenario would, in general, result in an overestimation of the real exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in European countries considered in the EFSA European database. This consideration is based on the assumption that the food additive is not used in those food categories in which it is permitted but for which no usage data were provided by the stakeholders (see Annexes A.1 and A.2).

For FSMP, the Panel noted that one industry reported a use of carrageenan as food additive at the level of the regulation in one niche product which is not aligned with information reported from the Mintel GNPD (Appendix E) where no FSMP products seems labelled on the market. If this information is confirmed, it means that uncertainties arising from the FSMP exposure scenario would lead to an overestimate of the current exposure to carrageenan products as food additives calculated by the Panel.

3.4.2. Exposure via other sources

Pharmaceutical and other uses

Information on pharmaceutical uses was obtained by searches of the literature, the websites of national competent authorities for medicinal products and publicly available SmPC (summary of product characteristics) on the nationally available authorised products indicated to EFSA by the European Medicines Agency (EMA) communication (Documentation provided to EFSA n. 3).

Carrageenan is used in pharmaceutical products as well-known excipient, e.g. as pseudo-emulsifier or as thickening and gelling agent (Teuscher, 1997). It is used in solid, semi-solid and liquid dosage forms (Blaschek et al., 2016).

In former times, carrageenan was used as mucilage in cases of diarrhoea and as bulk-foaming laxative to treat obstipation. The dosage was given with 1.5 g herbal substance/cup of water as decoction. Gastrointestinal discomfort including gas in the intestine is mentioned as possible undesired effect when carrageenan is taken in very high amounts, as for all bulk forming substances (Blaschek et al., 2014; Martindale, 2014).

Carrageenan is used in some countries in combination products (Martindale, 2014).

For carrageenan as an active ingredient, only few authorised medicinal combination products exist within the EU but not for oral use.

There is no additional information available regarding any use of processed Eucheuma seaweed.

3.5. Biological and Toxicological data

In the present opinion, the term 'carrageenan' is used when the test compound in the biological and toxicological studies has been described by the authors as 'native carrageenan' or 'undegraded carrageenan' or 'conventionally processed carrageenan'. For the degraded carrageenan, the terms used by the authors of the studies, e.g. poligeenan, C16, were reported.

Taking into account that the studies performed with carrageenan date from back to the 1940s up to present as well as the fact that the source description has changed during this time and differs between authors (e.g. Weiner et al., 2017), the Panel decided, that, an interpretation of the identity (expressed in type(s) of carrageenan) was necessary for each study in order to make it possible to compare the results of different studies. An explanation of how this has been performed is in Appendix J.

The Panel noted that in most studies no adequate information on the molecular weight distribution of carrageenan was available, and therefore it was in most cases not possible to assess if the carrageenan tested complied with the EU specifications (the limit of low molecular weight carrageenan (below 50 kDa) of 5%).

As mentioned above, the information on the molecular weight distribution in the tested individual carrageenan preparations is often missing or unprecise mixing the terms 'molecular weight', 'weight-average molecular weight' and 'number-average molecular weight'. In this opinion, the term describing the molecular weight of the carrageenan preparation tested, is used as reported by the authors of the studies.

The Panel further noted that according to Weiner (2014, 2016), the vehicle employed to administer carrageenan was critical for understanding the toxicological effects and their interpretation because the conformation of the carrageenan molecule changes depending on the vehicle. Most animal studies administered carrageenan in diet or in drinking water and some studies were conducted by oral gavage in water. When carrageenan was dissolved in water at dilute concentration (50.1%) with no added potassium or calcium ions, dietary solids or protein, the polymer independent of type exists in a disorganised random conformation (Blakemore and Harpel, 2010). Under these conditions, which are not representative of the use of the food additive E 407, all the carrageenan molecules are available for maximum interaction with the gastrointestinal (GI) tract cells.

3.5.1. Absorption, distribution, metabolism and excretion of Carrageenan

No data were available on absorption, distribution, metabolism and excretion (ADME) of processed Eucheuma seaweed. The Panel noted that in a review, it was concluded that there was no reason to expect processed Eucheuma seaweed to behave differently than carrageenan, based on their chemical structure (Cohen and Ito, 2002).

In vitro studies

The rate of degradation of eight batches of κ-carrageenan (Copenhagen Pectin Factory Ltd., Denmark, interpreted by the Panel with a ratio ι:κ:λ type 0:100:0) in simulated gastric juice (at pH 1.2 or 1.9) was determined by Ekstrom (1985). The various batches of κ-carrageenan used were similar in terms of weight-average molecular weight (167 ± 7 kDa) and shape of the distribution curve of the molecular weight. An average of 25% of the carrageenan in the batches had a mass around 100 kDa and 9% less than 50 kDa. After 2 h in simulated gastric juice at pH 1.2, almost 90% of the carrageenan had a mass less than 100 kDa and 25% had a mass less than 20 kDa. At pH 1.9, the rate of degradation was lower; after 2 h, 65% of the carrageenan had a mass less than 100 kDa and 10% than 20 kDa. The presence of pepsin did not affect the rate of the degradation although carrageenan has a strong protein-binding tendency. According to the author, the acidity and the rate of passage through the stomach will determine the degree of degradation of carrageenan.

Capron et al. (1996) investigated the stability of κ- and ι-carrageenan (Sanofi, interpreted by the Panel with a ratio ι:κ:λ type 0:100:0 and ι:κ:λ type 100:0:0) to depolymerisation in batch experiments (for 3 or 6 h at pH 1.2, 2, 3 or 8) or using an artificial stomach. Degradation was measured by using SEC and multiangle laser LS data. No hydrolysis of κ-carrageenan was observed at pH 8. Under the most drastic conditions (6 h, pH 1.2), the weight-average molecular weight remains higher than 200 kDa and only 20% has a molecular weight less than 100 kDa. Carrageenan in helical form (with potassium) was less susceptible to acid hydrolysis whereas ι-carrageenan was more resistant than the κ-type. Moreover, in conditions mimicking gastric digestion using simulated gastric juice, the formation of fragments of low molecular weight (less than 100 KDa) from well-defined food-grade carrageenan (E 407) is quite limited (less than 10%). The Panel noted that these results are in contrast to the previous findings of Ekstrom (1985) for which a quite complete degradation of κ-carrageenan was observed in solutions of low pH.

A total of 154 strains from 22 species of *Bifidobacterium, Peptostreptococcus, Lactobacillus, Ruminococcus, Coprococcus, Eubacterium a*nd *Fusobacterium*, which occur in high concentrations in the human colon, were tested for their ability to ferment 21 different complex carbohydrates. Among them, carrageenan (unspecified type; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) was not fermented by any strains of the investigated species (Salyers et al., 1977).

Fermentations of 10 polysaccharides including carrageenan (unspecified type; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30), by 24 species of the family Enterobacteriaceae (Klebsielleae and other gram-negative facultative bacilli) were examined by Ochuba and von Riesen (1980). Carrageenan was fermented by 15 species including *Klebsiella* (*K. oxytoca* and *K. pneumoniae*), *Aeromonas*, *Enterobacter*, *Pectobacterium*, *Serratia* and Vibrio strains. According to the authors, this study demonstrated the possible fermentation of carrageenan by enteric bacteria.

In vivo studies

Rats

In groups of three to seven male and female rats (no further information about the strain) fed with 2, 5, 10, 15, or 20% (about 2,000–20,000 mg/kg bw per day) of κ/λ-carrageenan from *C. crispus* (commercial product – Seakem, type 6, from Marine Colloids, Rockland, Maine; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio ι:κ:λ type 0:70:30, 'molecular weight distribution not indicated') over 23–148 days via the diet, more than 90% of the ingested carrageenan was excreted via faeces (Hawkins and Yaphe, 1965).

A comparable result was obtained in groups of five young male Charles River CD rats fed with 0% and 5% (equivalent to 6,000 mg/kg bw per day) of ι-carrageenan (commercial product from Auby, Neuilly-sur-Seine, France; interpreted by the Panel as isolated from *E. spinosum* with an approximate ratio ι:κ:λ type 100:0:0, weight-average molecular weight: 360–540 kDa) (Dewar and Maddy, 1970) or 5% of degraded carrageenan (C16, degraded ι-carrageenan, prepared from *E. spinosum* seaweed, commercial product from Glaxo, Paris, with weight-average molecular weight between 20 and 30 kDa) over 10 days. The faecal levels of carrageenan or degraded carrageenan were calculated from the excreted quantities of 3,6-anhydrogalactose. The treatment had no adverse effects on feed intake. Faecal excretion of carrageenan amounted to 80% of the administered dose of native carrageenan and faecal excretion of degraded carrageenan amounted to 86% of the administered dose of degraded carrageenan (C16). According to the authors, the low value for the percentage of carrageenan

excreted might have been due to inaccuracy or errors in measuring the daily feed intake and in assessing the background level of the 3,6-anhydrogalactose content of the faeces from rats on control diet.

Studies conducted with Sprague–Dawley rats fed diets containing 4% κ/λ-carrageenan (commercial product calcium carrageenan, Sea Kem, type II, Marine Colloids, Co.; interpreted by the Panel as *C. crispus* with an approximate ratio ι:κ:λ type 0:70:30, 'molecular weight distribution not indicated'), separate absorption studies were done with adult males (Tomarelli et al., 1974). The animals (n = 8) were placed in metabolism cages, fed a basal diet for 7 days, followed by 5 days of feeding the carrageenan diet, and then 2 more days on the basal diet. By using an improved colorimetric method, 99% of the ingested carrageenan was found to be excreted via faeces.

A study including different trials was performed in rats by Pittman et al. (1976). In a first trial, Sprague–Dawley rats of both sexes (n = 3) were given carrageenan from *E. spinosum* (number-average molecular weight: 8.7–145 kDa, ratio ι:κ:λ type 100:0:0, Marine Colloids, Co) in distilled water at a dose of 500 mg/kg bw per day via gavage daily for 9 months. At the end of this time, rats were killed and the livers were retained for analysis. In another experiment weanling Sprague–Dawley rats of both sexes (n = 5) were fed control diet or a diet containing 5% (about 5,000 mg/kg bw per day) of carrageenan from *C. crispus* (number-average molecular weight: 186–214 kDa ratio ι:κ:λ types 0:71:29 to 0:88:12, Stauffer Chemicals, Inc). After 13 weeks, five animals of each group and sex were killed and samples of liver were analysed for carrageenan. Faecal samples were also collected for analysis. Faecal and liver samples were examined qualitatively by gel electrophoresis, and quantitative measurements of carrageenan were carried out on samples of liver and urine. For the high molecular carrageenan (> 100 kDa) no or only little absorption was noted based on the absence of carrageenan from the livers of rats. By contrast, evidence for the presence of carrageenan in the livers of rats was obtained only in those animals given ι-carrageenan with number-average molecular weight of 88 kDa or less, for 9 months. The Panel noted that low molecular carrageenan was partly absorbed in rats given dietary ι-carrageenan.

Chen et al. (1981) used a gel electrophoresis method developed for the detection of carrageenan (unspecified type; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, 'molecular weight distribution not indicated') in rat liver homogenates (measurable limit of 15 μg carrageenan/g liver tissue). No carrageenan was found in the livers of rats fed 25% (about 25,000 mg/kg bw per day) over one month. Further details were not reported.

In the subchronic study of Abraham et al. (1985), three groups of 30 Sprague–Dawley rats were exposed to 1% or 5% carrageenan (commercial product *Iridaea* carrageenan from Hercules Inc., Copenhagen Pectin Fabrik Ltd., Denmark; interpreted by the Panel as isolated from *Iridaea ciliate* = *Sarcothalia crispata,* mixture of κ and λ, 'molecular weight distribution not indicated') via diet (equivalent to 900 and 4,500 mg/kg bw per day or with cellulose at a 5% dietary level (13 males and 17 females) for 13 weeks. Storage of carrageenan or carrageenan-like material was investigated by the presence of metachromatic material following toluidine blue staining, increase in lysosomal enzyme activity in sites similar to ultrastructural localisation of fibrillar material and chemical analysis. According to the authors, this study showed no retention or storage of native carrageenan in the gut or liver of chronically administered rats. The absence of tissue retention of native carrageenan was also observed in an additional chronic experiment in which two strains of rats received this carrageenan for 39 weeks.

Arakawa et al. (1986, 1988) found that dietary κ-carrageenan with a molecular weight > 100 kDa (commercial product of κ-carrageenan CS-47, from San-ei Chemical Ind., Co., Osaka; interpreted by the Panel from *E. cottonii* or other sources with an approximate ratio ι:κ:λ type 0:100:0) was not metabolised to lower molecular weight material in the faeces of rats and that 97–98% of the ingested carrageenan dose (4 g/kg) was excreted in the faeces as unchanged carrageenan.

Nicklin et al. (1988) investigated the fate of radiolabelled ^{3}H-ι-carrageenan in rats (n = 3) administered by gavage in water (50 μg/mL per rat). ι-Carrageenan (commercial ι-carrageenan from Hercules Ltd., UK; interpreted by the Panel with an approximate ratio ι:κ:λ type 100:0:0, 'molecular weight distribution not indicated') was incubated with galactose oxidase and tritiated borohydride, leading to a tritiated molecule labelled on CH_2OH group. Some radioactivity was observed in the intestinal wall, Peyer's Patches, mesenteric lymph nodes, caecal lymph nodes, and serum. Autoradiographs of tissues indicated that the radioactivity would be associated with the macrophages. The authors suggested that carrageenan could enter the body via the macrophages of the Peyer's Patches and caecal lymph nodes. The majority of the radioactivity was excreted in the faeces.

However, according to the authors, exchange of the tritium label could occur with the hydrogen of body water. The Panel considered this study of low relevance.

Conventional rats with normal gut microbiota and rats (n = 10) with their gut microbiota replaced by human fecal microbiota were given 2.5% κ-carrageenan (commercial product of κ-carrageenan CS-47, from Sigma, Type 1, C-1013, batch 86F0698; interpreted by the Panel from *E. cottonii* or other sources with an approximate ratio ι:κ:λ type 0:100:0) in water as a gel (Taché et al., 2000). Faecal samples were collected and then heated at 80°C for 30 min in water (pH 8) and centrifuged. The molecular weight of carrageenan in faeces was measured in supernatants by SEC consisting of an HPLC system equipped with a multiangle laser LS detector providing the absolute molecular weight of the eluted polymers. No carrageenan was detected in faecal samples from untreated control rats. The molecular weight of steam-heated dietary carrageenan ranged from 310 to 320 KDa. In treated rats, the average molecular weights of the carrageenan found in faeces were 346 KDa and 307 KDa in conventional rats and in rats with human faecal microbiota, respectively. This slight difference was not statistically significant. According to the authors, the average molecular weight of carrageenan was not changed to a great extent during its passage through the GI tract.

Food-grade λ-carrageenan (information given in Japanese interpreted by the Panel as from *Chondrus* or *Gigartina* sp. with a ratio ι:κ:λ unknown) was included in the diet of rats at a level of 5% (Uno et al., 2001b). After an overnight deprivation of food, animals (one male and one female) were given access to the treated diet for one day and then to the untreated basal diet for 2 days. Faeces were collected for each rat at three time points each day and analysed by using GPC to separate high and low molecular weight λ-carrageenan components in faeces. ICP atomic emission spectroscopy (ICP-AES) was used for direct detection of the sulfur of the carrageenan present in faeces. The molecular weights of the carrageenan found in the faeces after day 1 and day 2 were similar to that of the carrageenan in the blended diet: 782 kDa in faeces compared to 832 kDa in diet. No carrageenan was detected in faeces excreted on day 3. According to the authors, dietary λ-carrageenan was excreted in the faeces without any decomposition to lower molecular weight fractions.

Guinea pigs

In the study of Pittman et al. (1976), female guinea pigs were divided into groups (n = 6) and fed control diet or a diet containing 2% (500–1,000 mg/kg bw per day) of carrageenan from *E. spinosum* (number-average molecular weight of 5–145 kDa, ratio ι:κ:λ type 100:0:0, Marine Colloids Inc. or Glaxo Lab.) for a period of 7–10 weeks after which they were killed and samples of livers were obtained for analysis. In two later experiments, other groups of female guinea pigs (n = 3) were given by gavage, carrageenan from *E. spinosum* with number-average molecular weight ranging from 5–145 kDa (ratio ι:κ:λ type 100:0:0, Marine Colloids Inc.) or from *C. crispus* (number-average molecular weight of 8.5–314 kDa, ratio ι:κ:λ types 0:100;0; 0:0:100 or 0:70:30, Marine Colloids Inc.) as 1% solutions (about 1,000–2,000 mg/kg bw per day) via drinking water for 2 weeks. At the end of the first of these latter experiments, samples of liver, faeces and bladder urine were analysed, while at the end of the second experiment samples of liver and faeces were examined. In all trials, faecal and liver samples were examined qualitatively by gel electrophoresis, and quantitative measurements of carrageenan were carried out on samples of liver and urine. For the high molecular weight carrageenan (> 100 kDa), no or only little absorption was noted based on the absence of carrageenan in the livers or the urine of guinea pigs. By contrast, significant amounts of carrageenan were found in livers of animals given carrageenan with number-average molecular weight lower than 88 kDa, whatever the type of carrageenan was. According to the authors, urinary excretion of carrageenan was limited to low-molecular-weight material (number-average molecular weight: < 20 kDa). Qualitative and quantitative evidence indicated that there was an upper limit to the size of carrageenan molecules absorbed, but estimates of this upper limit ranged from 10 to 85 kDa depending upon the analytical approach. Absorption of low molecular carrageenan from the drinking-water was generally higher than following dietary administration. The Panel noted that low molecular carrageenan would be partly absorbed, particularly when administered by gavage and given via drinking water.

Minipigs and pigs

The studies in neonatal minipigs and in neonatal pig (Documentation provided to EFSA n. 32; Documentation provided to EFSA n. 33; also described in Weiner et al., 2015) employed a liquid chromatography with tandem mass spectrometry (LC–MS/MS) analytical method developed using 'poligeenan' as a surrogate for detection of the low molecular weight carrageenan in blood.

In the neonatal minipig study (Gottingen minipigs) (Documentation provided to EFSA n. 32), following daily oral administration of carrageenan (the test compound is assumed to be identical with that described below in the study by Weiner et al., 2015) at doses of 0, 300 or 3,000 mg/kg formula (equal to 60–66 or 600–666 mg/kg bw per day) for 10 days, starting on postnatal day (PND) 2, there were no quantifiable serum poligeenan concentrations in animals receiving the 600–666 mg/kg bw per day on both evaluation days. According to the authors, the unique reported values for two animals (one control and one receiving 60–66 mg/kg bw per day) may represent interfering compounds rather than actual exposure to low molecular weight carrageenan.

In the study in neonatal pigs (Domestic Yorkshire Crossbred Swine) (Documentation provided to EFSA n. 33; Weiner et al., 2015), the test material sample of κ/γ-carrageenan (FMC Lot. 90303011) (ratio κ/λ unknown) was fully characterised for molecular weight, percentage of molecular weight below 50 kDa, known as LMT as per European requirements. The weight-average molecular weight of the test material sample was 664–732 kDa with a LMT of 0.3–3.9%. The test substance was administered at doses of 0, 300, 1,000 or 2,250 mg/kg formula for 28 days starting on PND 3 (highest dose equal to 430–448 mg/kg bw per day). The authors reported an analytical signal in male piglets, corresponding to that expected for the low molecular weight carrageenan. According to the author, this pattern was not consistent with the expected exposure based on the calculated compound consumption of carrageenan. This is supported by the fact that (1) a positive signal was elicited in all males in the control group; (2) similar values were observed in all treated males at 300, 1,000, and 2,250 mg/kg; (3) the signal did not produce a dose response effect in the treated groups (300, 1,000 and 2,250 mg/kg) and (4) a positive signal was almost exclusively limited to male animals. These data would indicate that the signal in the plasma samples is not a low molecular carrageenan.

Considering the study in neonatal minipigs and neonatal pigs, the Panel noted the use of appropriate animal models for representing the immature gastrointestinal tract in human infants. However, due to problems in the outcomes of analytical assays, the Panel was unable to conclude on a possible *in vivo* degradation of carrageenan.

Primates

In the short-term study of Abraham et al. (1972), male and female rhesus monkeys (*Macaca mulatta,* n = 10) were given a type of κ/λ-carrageenan derived from *C. crispus* (carrageenan mixture of κ and λ with number-average molecular weight of approx. 800 kDa obtained from Marine Colloids, Inc. Rockland, ME, Lot. Beta282400; interpreted by the Panel as *C. crispus* with an approximate ratio ι:κ:λ type 0:70:30) via drinking water as a 1% solution (about 1,300 mg/kg bw per day) for 7–11 weeks. There was no evidence of carrageenan storage by contrast to the administration of degraded ι-carrageenan (C16) (prepared by hydrolysis of an extract of *E. spinosum* (C16), provided by Laboratoires Glaxo, Paris, with the weight-average molecular weight in the ranges 20–30 kDa) which produced extensive reticuloendothelial retention of degraded material that was still present in Kupffer cells 6 months after administration of C16 was stopped.

In the long-term toxicity study (Documentation provided to EFSA n. 34), rhesus monkeys (19 males and 21 females) were divided into four groups with 10 animals each and dosed with 0, 50, 200, 500 mg/kg bw per day κ/λ-carrageenan (commercial product Gelcarin HMR from Marine Colloids, Rockland, Maine; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, molecular weight distribution not indicated) by gavage daily on 6 days a week for the first 5 years, whereas for the remaining 2.5 years, carrageenan was incorporated into diet and given to monkeys. In this study, there was also no evidence for the uptake of fibrillar, metachromic, carrageenan-like material in the cells of the reticular-endothelial system in the liver, lymph nodes, caecum, colon or anorectal junction.

In the study of Pittman et al. (1976), rhesus monkeys of both sexes (n = 2) were given carrageenan from *C. crispus* (number-average molecular weight: 185 kDa, ratio ι:κ:λ type 0:70:30, Marine Colloids Inc.), daily in doses of 50, 200 or 500 mg/kg bw per day by gavage. Overnight collections of faeces were obtained after 11 months. Overnight collections of urine were obtained from two of the monkeys on the high dose after 15 months. Two female rhesus monkeys received a single dose of 1 g (about 200 mg/kg bw) of low molecular weight carrageenan from *E. spinosum* (number-average molecular weight: 9.9 kDa, ratio ι; κ; λ type 100:0:0, Glaxo Lab.) by gavage and complete first-day and second-day urine collections were made. In animals receiving carrageenan of number-average molecular weight: 185 kDa no or only traces of carrageenan were excreted in urine. By contrast, when the monkeys were given low molecular weight carrageenan (number-average molecular weight: 9.9 kDa), this type of carrageenan was excreted in urine. From these studies, the Panel noted

that by contrast to carrageenan with a number-average molecular weight of 185 kDa, the lower molecular weight carrageenan would be more absorbed and further excreted in urine. The Panel further noted that the degraded carrageenan used in this study was derived from the ι-carrageenan only and not from the native κ/λ-carrageenan to which it was compared, making inconsistencies in this comparative study and in another study using C16 (Abraham et al., 1972).

Overall, the Panel noted the absence of metabolic studies of carrageenan in humans. In several *in vivo* studies in rats, guinea pigs, neonatal minipigs, neonatal pigs and monkeys, faecal excretion of carrageenan was shown to be 98–100% of the ingested amount. These studies included rats with normal gut microflora and rats with their gut microflora replaced by human faecal microflora. Several short-term and long-term studies performed in rats dosed via diet containing different types of carrageenan confirmed the absence of tissue storage of this carrageenan. However, degraded carrageenan like C16 or low molecular weight carrageenan (number-weight molecular weight of 88 kDa or less) have been described to be absorbed and to be present in various tissues, namely the liver, and the urine of animals given degraded carrageenan mainly when given in drinking water but also when administered via the diet. However, the Panel noted that in these studies, degraded C16 type derived from ι-carrageenan was compared to native κ/λ-carrageenan, making the comparison inconsistent. Due to the high percentage of administered carrageenan recovered in faeces, its degradation to lower molecular weight carrageenan should be very limited.

The Panel noted that the conditions (duration, pH, temperature) for potential hydrolysis of carrageenan in the GI tract are less extreme than the 'mild' acidic conditions which resulted in the formation of chemically degraded carrageenan (C16). The theoretical possibility that more limited degradation could occur under conditions representative of the *in vivo* situation remains an uncertainty although this has not been observed to any significant extent in the *in vivo* ADME studies reported.

The Panel noted that the tested carrageenan differed in the molecular weight distribution, and the Panel considered that existing results did not allow to evaluate kinetic differences between the different types of native carrageenan (κ, λ and ι) and their corresponding low molecular weight fractions.

3.5.2. Acute toxicity of carrageenan

For rats, the oral LD_{50} for sodium or calcium carrageenan (interpreted by the Panel as salts of carrageenan isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) was 5,650–6,250 and 5,140–5,660 mg/kg bw, respectively (JECFA, 1974). For mice, the oral LD_{50} for sodium or calcium carrageenan was reported to be 8,730–9,670 and 8,710–9,590 mg/kg bw, respectively (JECFA, 1974). For ι-carrageenan (food-grade), a LD_{50} of > 5,000 mg/kg bw was reported in rats (Weiner, 1991). For calcium carrageenan, an oral LD_{50} value of > 10,000 mg/kg bw was determined in Sprague–Dawley rats (Documentation provided to EFSA n. 35). No data were available on acute oral toxicity of degraded carrageenan and processed Eucheuma seaweed.

3.5.3. Short-term and subchronic toxicity

Short-term toxicity studies with carrageenan and degraded carrageenan

Rats

Groups of six male albino rats (no further information about the strain; initial body weights of 48–58 g) were dosed with 0%, 5%, 10% or 20% κ/λ-carrageenan (interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) (equivalent to 0, 5,00, 11,800 or 23,600 mg/kg bw per day) via the diet for 10 weeks (Nilson and Schaller, 1941). No indication about weight-average molecular weight was given. Although treated rats showed more laxative condition, in general the animals grew well. Rats dosed with 20% required significantly more feed and water per gram of weight gain than the control group, and in this group, the weight gain also was about 30% less compared with controls. Three of six rats in this dose group died within 14 days after initiation (most probably due to a change in the bacterial flora), but the necropsies were essentially negative. Other parameters were not examined.

Rats (3–4 weeks old, strain not specified) were divided into groups of 10 males and 10 females, dosed for 50 days with carrageenan (commercial product Carasty-S, from the South Portland Maine Plant (USA), food ingredient product lot no. 10036; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) via diet at dose levels of 0%, 15% or 25% (equivalent to 0, 18,000 or 30,000 mg/kg bw per day) (Documentation provided to EFSA n. 36). No

indication about weight-average molecular weight was given. Body weights were recorded twice weekly and at final sacrifice on day 50. An interim sacrifice was done on day 31 (5 males and 5 females from each group) and at gross examination special attention was given to the liver. The rate of feed consumption for the test animals was generally less than the controls, resulting in a decreased weight gain especially in the early part of the study. In all dosed animals diarrhoea was noted after 4 days in study, which continued throughout the remaining period of the study, and the symptoms were more pronounced at a dietary level of 25%. Only at this dose level, male and female rats also excreted bloody stools. In addition, animals receiving carrageenan started losing hair from the dorsal mid-line surfaces on the back during days 8–50, and this finding was more severe in females of the 25% group. At interim sacrifice, it was noted that dosed animals appeared smaller compared with controls and also internal organs were correspondingly smaller. This effect was most prominent in males dosed with 25%. The livers of all animals were normal in texture and surface appearance, and no histopathological differences caused by dosing with carrageenan were seen. At terminal sacrifice on day 50, no gross pathological abnormalities were noted. Many of the livers examined had a mottled appearance, but this finding also occurred in controls. A histopathological examination of the livers gave no indication of adverse effects in all dose groups. Detailed statistics were not reported in this study.

Pigs

In a study with pigs aged 61–75 days, groups of 3 male and 3 female Danish Landrace pigs were given 0, 50, 200, or 500 mg/kg bw per day of κ-carrageenan (commercial product Carrageenan Genulacta type K 100 degrees, batch 039240, from Copenhagen Pectin Factory Ltd., Denmark; number-average molecular weight of 200 kD; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) over 83 days (Poulsen, 1973). No compound-related deaths were observed; the behaviour, appearance, feed intake and growth rate remained normal. No significant changes were reported in haematological, clinical chemical or urinary parameters. At necropsy, there were no changes in relative organ weights. Histopathological examination was performed on principal organs and tissues. No ulceration or erosions were seen in the gastrointestinal tract. Three pigs (one in the 200 mg/kg bw per day group and two in the 500 mg/kg bw per day group) showed slight changes in the mucous membrane of the large intestine, including a few focal areas with irregular surface and some shift in cellular infiltration pattern. Bacteriological examination of the microbiota of the jejunum, caecum, colon and ampulla of the rectum from all animals, showed that treatment resulted in changes in intestinal microbiota. The total counts of aerobic bacteria were decreased in the colon and rectum and the number of lactobacilli was reduced in the rectum.

Monkeys

Rhesus monkeys (*M. mulatta*; 3 males and 3 females) were given solutions of 1% carrageenan composed of κ- and λ-carrageenan (commercial product from Marine Colloids, Inc., Rockland; lot n. 282400, number-average molecular weight of 800 kD; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) or of degraded carrageenan (2%, 1% or 0.5%) (commercial product from Glaxo, Paris, Batch L4006; interpreted by the Panel as prepared from ι-carrageenan originally isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0, with a number-average and weight-average molecular weights of 16–19 kDa and 20–30 kDa, respectively) in drinking water over a period of 7–11 weeks, followed by recovery for up to 24 weeks (Abraham, 1972). There was no evidence of storage of carrageenan, in contrast to degraded carrageenan which produced extensive reticuloendothelial retention of material that was still present in Kupffer cells 6 months after administration of degraded carrageenan was stopped. The stored material was PAS positive and metachromatic; it occupied sites corresponding to the presence of acid phosphatase and P-glucuronidase. Ultrastructurally, the hepatocytes appeared normal in all groups. The results demonstrated that degraded carrageenan is taken up by, and selectively stored in lysosomes of the reticuloendothelial system, notably in the liver, providing evidence of absorption of the sulfated polysaccharide. Parenchymal cells of liver, kidney, or other organs did not reveal the presence of stored material. In contrast to degraded carrageenan, carrageenan administration did not give rise to stored material in reticuloendothelial cells nor in hepatocytes.

In a study in male and female rhesus monkeys (*Macaca mulatta*), the effects of κ/λ-carrageenan, (commercial product from Marine Colloids, Inc., Rockland; as Gelcarin HMR, lot n. 282400; approximate number-average molecular weight of 800 kDa; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of κ:λ type 70:30), have been compared with those of a

degraded carrageenan (C16: commercial product from Glaxo, Paris, Batch L4006; interpreted by the Panel as prepared from ι-carrageenan originally isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0), with a number-average molecular weight of about 20 kDa (Benitz et al., 1973). Both types of carrageenan were administered in drinking-water for 7–14 week. Monkeys given 1% carrageenan (providing an intake of about 1,300 mg/kg bw per day) all gained weight and stayed in good condition. After 7 and 11 weeks, two animals were sacrificed and the remaining ones were put back on plain tap-water and allowed to recover for 11 weeks. To these animals, increasing daily doses of carrageenan ranging from 50 to 1,250 mg/kg bw per day were given for up to 12 weeks. Five males and five females received various concentrations of C16 in the drinking-water up to week 14. Six animals received a 2% solution, two animals a 1% solution and the remaining two received a 0.5% solution of C16. The corresponding average daily intakes of C16 were calculated by the authors to be 2,900, 1,400 and 700 mg/kg bw per day. For the native carrageenan (κ-/λ- carrageenan), soft stools were reported with sporadic positive results for faecal occult blood with a more or less similar pattern in the control monkeys. Gross and microscopic examination of the intestinal tract revealed no changes attributable to the administration of κ-carrageenan. Of the two animals sacrificed at 7 and 14 weeks, only distal colon focal capillary hyperaemia and sometimes a slight degree of mucosal oedema was reported. All other parts of the gastro-intestinal tract were microscopically normal.

Monkeys given 0.5% or 1.0% degraded carrageenan (C16) solution gained weight, but in those on 2.0% (an intake of approximately 2.9 g/day) weight-losses were considerable. All monkeys on C16 lost blood frequently from the intestinal tract in a dose-related degree and developed some degree of anaemia. The authors reported that the animals receiving the 1% C16 solution appeared to pass blood in faeces continuously, while the two monkeys on 0.5% C16 had positive occult blood in faeces at lower frequency. Pathological changes seen in the colon ranged from shallow mucosal erosions to ulceration associated with cellular infiltration, granulation tissue in the lamina propria and formation of multiple crypt abscesses. The severity of these effects was dose-dependent, and some reversal was indicated in monkeys given 2% C16 for 14 week and then allowed to recover on tap-water for 20–24 week. At necropsy, the intestinal tract in these animals was reported as grossly and microscopically normal although some of these animals showed in the colonic wall ulcers on which surrounding mucosa was hyperaemic and contained markedly dilated capillaries. There were reports of remnants of small, usually almost obliterated crypts, some of them containing a considerable number of red blood cells. Results from the recovery period after C16 treatment demonstrated that C16 was the responsible agent for the reported effects on the colon. These studies in rhesus monkeys demonstrated a definite difference between the effects of degraded carrageenan (C16) and carrageenan. The authors concluded that 'administration of the degraded ι-carrageenan (C16) with a number-average molecular weight of about 20,000 (Blakemore and Dewar, 1970) led to intestinal blood loss and anaemia. The morphological abnormalities were seen in the colon, including the colonic portion of the ileocolic valve, and resembled various stages of human ulcerative colitis, an aspect referred to below. Minor degrees of pathological change such as oedema, hyperaemia and an increased number of macrophages were also found in the caecum. All the effects seen in this group were dose-dependent'. The high molecular carrageenan appeared to be well tolerated, whereas low-molecular degraded carrageenan (C16) induced adverse effects in the intestines characterised by various degrees of ulceration and granuloma formation. The LOAEL of the low molecular weight, degraded carrageenan was approximately 750 mg/kg bw per day, whereas the NOAEL of carrageenan was 1,300 mg/kg bw per day, the only dose tested.

Subchronic toxicity studies with carrageenan

Rats

In groups of 3–5 male and 5–7 female rats (no further information about the strain) fed 2%, 5%, 10%, 15% or 20% (equivalent to 1,800, 4,500, 9,000, 13,500 and 18,000 mg/kg bw per day) of κ/λ-carrageenan (commercial product Seakem, type 6, Marine Colloids Inc., Rockland, Main; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) over 23–148 days via diet, apart from a decrease in weight gain in rats dosed with ≥ 10% no adverse effects were reported (Hawkins and Yaphe, 1965). No indication about weight-average molecular weight was given. As ≥ 90% of the ingested carrageenan was excreted via faeces, the authors concluded that it had no direct nutritional value.

Three groups (15 males and 15 females each) of 30 Sprague–Dawley rats of 4 weeks were exposed to 1 or 5% carrageenan (commercial product *Iridaea* carrageenan from Hercules Inc.,

Copenhagen Pectin Fabrik Ltd., Denmark; interpreted by the Panel as isolated from *Iridaea ciliate* = *Sarcothalia crispate,* mixture of κ and λ) via diet (equivalent to 900 and 4,500 mg/kg bw per day) or with cellulose at a 5% dietary level (13 males and 17 females) for 13 weeks (Abraham et al., 1985). No adverse effects on mortality, food consumption, body weight, haematology, clinical chemistry, urinalysis, faeces, or organ weights were noted in animals exposed to 1% carrageenan. No mortality was observed in animals exposed to 5% carrageenan. No indication about weight-average molecular weight was given. At this dose, one male rat showed diarrhoea in week 8 and 5/30 rats, both males and females (exact numbers not given) showed soft stools during weeks 10–13 indicating gastrointestinal disturbances. An increase in food consumption was observed in males at the highest dose (5%) compared to controls. At the highest dose (5%), in both sexes haemoglobin values were decreased compared with controls, while haematocrit levels and erythrocyte and leukocyte counts were comparable with controls. Decreased levels of Na^+ and K^+ and increased SGPT (serum glutamic pyruvic transaminase) levels were observed in females. The faecal consistency at termination was comparable for rats of all groups and no occult blood was detected. There were also no changes in organ weights between treated and control animals. Histopathological examination revealed no changes attributable to the ingestion of carrageenan for 13 weeks. The Panel considered 4,500 mg/kg bw per day, the highest dose tested as the NOAEL in this study.

Three types of carrageenan (differing in viscosity, cation content, and ratios of κ to λ) were tested on Sprague–Dawley rats: (1) TYPE S-70:30 κ:λ ratio, high viscosity (270 cP) and high sodium (6%), (2) TYPE C-80:20 κ:λ, viscosity of 190 cps and high potassium (6%) and (3) TYPE AX-90:10 κ:λ, low viscosity (26 cP) and high calcium (6%) (Abraham et al., 1985) (for the three types, commercial products *Carastay* carrageenan, from Stauffer Chemical Company; sources unknown; interpreted by the Panel as an approximate ratio of ι:κ:λ type 0:70:30; 0:80:20 and 0:90:10). No indication about weight-average molecular weight was given. Eight groups of 50 animals (25 males and 25 females) received the following treatment via their diets for 13 weeks: group 1 was given ground rodent diet and served as untreated control, group 2 was given 5% cellulose and served as a control, group 3 was given 1% (equivalent to 900 mg/kg bw per day) Type S carrageenan, group 4 was given 5% (equivalent to 4,500 mg/kg bw per day) Type S carrageenan, group 5 was given 1% (equivalent to 900 mg/kg bw per day) Type C carrageenan, group 6 was given 5% (equivalent to 4,500 mg/kg bw per day) Type C carrageenan, group 7 was given 1% (about 900 mg/kg bw per day) Type AX carrageenan, and group 8 was given 5% (about 4,500 mg/kg bw per day) Type AX carrageenan. The remaining 10 males and 10 females of group 9 were killed before the start of the study for the determination of baseline haematology and blood chemistry. All parameters classically recommended in OECD 408 were measured in this study. In total, 8 out of 400 rats from groups 2, 3, 4, 7 and 8 died during the study and the cause of death (pneumonia, cage trauma, technician error) was incidental in each case and not related to treatment with carrageenan. There were no adverse effects on body weights, and food consumption was comparable within all groups. Haematological and clinical chemistry parameters gave no significant differences among the nine groups of rats and there was no increase in faecal occult blood. At necropsy, there were no gross changes caused by the treatment and both qualitative and quantitative analyses of liver tissues gave no indication for tissue residues of carrageenan (Abraham et al., 1985). The Panel considered the NOAEL of this study to be 4,500 mg/kg bw per day for all types of carrageenan tested, the highest doses tested.

A 90-day study was performed in compliance to GLP in Fischer 344 rats (groups of 20 rats per sex) exposed via diet at 2 doses of κ-carrageenan (commercial product lot no. 6371-03; interpreted by the Panel as isolated from *E. cottonii* with an approximate ratio of ι:κ:λ type 0:100:0) with an average molecular weight range of 196–257 kDa (not specified if number- or weight-average molecular weight) and low molecular weight tail of 1.9% and 12% < 50 kDa (mean 7%), respectively (Weiner et al., 2007; Weiner, 2014). Animals were exposed to 25,000 and 50,000 mg/kg diet (equal to 1,656 and 3,394 mg/kg bw per day for males and 1,872 and 3,867 mg/kg bw per day for females). Apart from very limited clinical signs (soft faeces, more pronounced in high dose rats), no treatment-related effects were reported on body weights, urinalysis, haematology or clinical chemistry parameters, or on organ weights or ophthalmic, macroscopic or microscopic findings. In addition, the GI tract seemed normal as reported in the detailed histopathological evaluation. According to the authors, the NOAEL was equal to 3,394–3,867 mg/kg bw per day, the highest dose tested in males and females, respectively. The Panel agreed with this NOAEL. The Panel noted that in this study the test material was well characterised and almost complied with the EU specification for E 407.

Subchronic toxicity studies with processed Eucheuma seaweed

The subchronic toxicity of processed Eucheuma seaweed was studied in rats and was compared with effects of carrageenan (Documentation provided to EFSA n. 38). Processed Eucheuma seaweed (interpreted by the Panel as isolated from *E. cottonii* (Ec) with an approximate ratio of ι:κ:λ type 0:100:0 or from *E. spinosum* (Es) with an approximate ratio of ι:κ:λ type 100:0:0) was fed to groups of 20 male and 20 female Sprague–Dawley rats per group (5 weeks old at initiation) at dose levels of 0, 0.5% (equal to 415 and 493 mg processed Eucheuma seaweed -Ec/kg bw per day or 424 mg and 437 processed Eucheuma seaweed-Es/kg bw per day for males and females, respectively), 1.5% (equal to 1,268 and 1,465 mg processed Eucheuma seaweed-Ec/kg bw per day or 1,304 mg and 1,471 mg processed Eucheuma seaweed-Es/kg bw per day for males and females, respectively), or 5% (equal to 4,342 and 4,992 mg processed Eucheuma seaweed-Ec/kg bw per day or 4,484 mg and 5,068 processed Eucheuma seaweed-Es/kg bw per day for males and females, respectively) in the diet for 91–94 days. No indication about the weight-average molecular weight was given. Control groups of 30 male and 30 female Sprague–Dawley rats were given the basal diet for the same period. In additional reference groups of 10 male and 10 female Sprague–Dawley rats, the animals were fed diets for the same treatment period containing 5% carrageenan derived also from *E. cottonii* (carrageenan-Ec; equal to 4,363 and 5,039 mg/kg bw per day for males and females, respectively) or *E. spinosum* (carrageenan-Es; equal to 4,455 and 5,048 mg/kg bw per day for males and females, respectively). In further five groups, the reversibility of effects was studied after a post-exposure observation period; these additional groups of 10 male and 10 female rats were fed the control diet or 5% processed Eucheuma seaweed (processed Eucheuma seaweed-Ec or processed Eucheuma seaweed-Es) or 5% carrageenan (carrageenan-Ec or carrageenan-Es) diets for 90 days and then basal diet for 28 days.

No treatment-related clinical signs were detected in any group as well as no ophthalmological abnormalities. No effects on the body weight of male rats were measured in the low- and mid-dose groups. A slight but statistically significant reduction in the body weight was reported in male rats receiving 5% processed Eucheuma seaweed-Ec (approximately 4% lower than the control; which was also present after the recovery period) or 5% processed Eucheuma seaweed-Es (approximately 5% lower than the control; no effects in the recovery period). The reference group exposed to 5% carrageenan-Ec showed no reduced body weight in males but not the group exposed to 5% carrageenan-Es did (maximum 10% lower than the control, also after the recovery period). No effects were detected in any group of female rats fed processed Eucheuma seaweed-Ec or processed Eucheuma seaweed-Es as well as females receiving 5% carrageenan-Ec. A slight but significant reduction of body weight was found in females exposed to 5% carrageenan-Es (4–6% lower than the controls, also in the reversal phase). The food consumption of treated male rats showed no consistent changes in groups fed with processed Eucheuma seaweed-Ec or carrageenan-Ec. In the functional testing battery at week 12, no dose-related changes were recorded in any treatment group. The urine analysis revealed a significantly higher refractive index and a lower pH value in male rats fed 5% carrageenan-Es. In female rats, the urine volume was significantly increased after exposure to 5% processed Eucheuma seaweed -Es. The same effect plus a lowered refractive index were seen after 5% carrageenan-Ec. Significantly lower levels of ketones were excreted from females fed 5% carrageenan-Ec and 5% carrageenan-ES. After the recovery period, significantly lower urine pH was seen in the male 5% processed Eucheuma seaweed–Ec-treated group and lower proportions of urates in the urine of females from the 5% processed Eucheuma seaweed-Ec and 5% CP-Ec treatment groups. Other effects in urinalysis were not dose-dependent. In the JECFA evaluation (1999), it was concluded that the urinalysis provided no evidence of any toxicologically relevant effects. The Panel agreed with this conclusion. Statistically significant alterations in haematological parameters were reported (e.g. reduced erythrocyte or monocyte counts in males exposed to 5% processed Eucheuma seaweed-Ec; reduced eosinophil counts in males after 5% processed Eucheuma seaweed -ES; reduced haemoglobin, haematocrit, erythrocyte and white blood cell counts in females fed 5% processed Eucheuma seaweed-Ec). Historical control data of this laboratory were not presented. However, the Panel considered the toxicological relevance of these findings questionable since the differences to the concurrent control were marginal, as was also the case with carrageenan-Ec and carrageenan-Es. Clinical chemistry data revealed no effects in any treatment group. In this subchronic study with processed Eucheuma seaweed and carrageenan, no treatment-related clinical signs were detected and only minor effects on body weight or on parameters measured in the functional testing battery, urinalysis, haematology and clinical chemistry were seen. These effects as well as the increase in the

relative organ weights of the full and empty caecum, which was regarded by the Panel as the consequence of accumulation of poorly absorbed material in the intestinal tract, were considered to be without toxicological relevance. No treatment-related histopathological changes were found. Comparing the effects of processed Eucheuma seaweed with the carrageenan of the same seaweed species there was no evidence for any difference in toxicological properties after repeated oral administration. Both preparations of processed Eucheuma seaweed and carrageenan altered the nutrition level available to rats at a concentration of 5% in the diet resulting in increased food intake due to the low nutrient value.

Conclusions for short-term and subchronic studies with carrageenan, degraded carrageenan and processed Eucheuma seaweed

Overall, short-term and subchronic administration of carrageenan was well tolerated and induced no adverse effects in the GI tract in rats, guinea pigs, pigs and monkeys.

Monkeys given degraded carrageenan showed histopathological lesions in the colon which varied from slight mucosal erosions at the low dose (750 mg/kg bw per day) to ulceration associated with inflammatory infiltration of the lamina propria at the high dose (2,900 mg/kg bw per day). The Panel noted a LOAEL of 750 mg degraded ι-carrageenan (C16)/kg bw per day.

The NOAEL for processed Eucheuma seaweed was approximately 4,500 mg/kg bw per day, the highest dose tested.

3.5.4. Genotoxicity

Carrageenan

In vitro

Calcium carrageenan (commercial product FDA 71-5. 977052-14-4; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, (number- or weight-average molecular weight not specified) was assessed for its mutagenicity in a bacterial mutation assay with *Salmonella* Typhimurium tester strains TA1535, TA1537 and TA1538 and for mitotic recombination in *Saccharomyces cerevisiae* (strain D-4) both with and without S9 metabolic activation (Documentation provided to EFSA n. 39). At the tested concentrations of 0.55%, 1.1% or 2.2% for *S.* Typhimurium and 0.24%, 0.49% or 0.98% for *S. cerevisiae* D4, negative results were obtained. However, the Panel noted that the study bears significant shortcomings in the experimental design which include the use of a limited number of *S.* Typhimurium strains, and that mitotic recombination in *S. cerevisiae* (strain D-4) is considered obsolete and currently not employed for the evaluation of genotoxicity of dose levels employed. On this basis, the Panel considered this study of limited reliability.

Semi-refined carrageenan 'SRC-EC' or 'SRC-ES' (commercial product from Seaweed Industry Association of the Philippines; interpreted by the Panel as isolated from *E. cottonii* with an approximate ratio of ι:κ:λ type 0:100:0, and from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0, number- or weight-average molecular weight not specified, respectively) were assayed for their mutagenicity in a bacterial reverse mutation assay according to the relevant OECD TG Guideline 471 and in compliance with GLP regulations. At the tested concentrations of 0.001–10 mg/plate, both with and without metabolic activation, there was no indication for mutagenicity in the tester strains TA1535, TA1537, TA98, TA100 and TA102 (Documentation provided to EFSA n. 40).

Food-grade carrageenan (commercial product purchased on market place as food grade and analysed by the South Korean advanced Food Research Institute according to Korea Food Additive Code; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, number- or weight-average molecular weight not specified) was tested in a bacterial reverse mutation assay. At the tested concentrations of 156.3–5,000 μg/plate, both with and without metabolic activation, there was no indication for mutagenicity in the tester strains of *S.* Typhimurium TA1535, TA1537, TA98, TA100 and in *Escherichia coli* WP2uvrA (Chung et al., 2009). The Panel noted that the study meets the requirements of the relevant OECD TG Guideline no. 471.

Sodium carrageenan (interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, number- or weight-average molecular weight not specified) was assessed for its capability to induce chromosomal aberrations in anaphase in human embryonic lung cells (WI38) at dose levels of 8, 80 and 800 μg/mL in the absence of S9 metabolic activation only, and no cytogenetic effects were reported (Documentation provided to EFSA n. 41). In contrast, calcium carrageenan (not specified further) assayed in a different laboratory (Documentation provided to EFSA n. 42) produced

severe adverse effects at chromosomal level (mainly multipolar spindle mitosis) in the anaphase chromosomes of human embryonic lung cells (WI38) at 10, 100 and 1,000 μg/mL, at the 24 h sampling time. However, at the two higher dose levels, the authors noted a marked discrepancy in the values of individual scoring, indicating that a technical error could have occurred in which two replicate cultures from the intermediate and highest dose levels were reversed. According to the authors, if this was the case, calcium carrageenan would have then yield significant levels of aberrant cells only at one dose level (high dose level), while having no effect at the other two levels. This prompted the authors to repeat the study using the same dose level but inexplicably using an extended sampling time of 42 h. Under these experimental conditions, an extensive chromosome damage irrespective of the tested dose was observed. However, the Panel noted that, in addition to the possible technical error occurred, the positive outcome for chromosomal aberrations at both treatment times could be related to indirect cytotoxic effects not clearly evaluated in this study and therefore the results obtained are questionable.

The Panel also noted that the test on induction of chromosomal aberrations in anaphase did not receive further validation and is currently not employed in genetic toxicology testing. The Panel also noted that the unique positive results in this assay have been reported by this laboratory with another high molecular weight polysaccharide (EFSA ANS Panel, 2017), and overall considered the results from this study not relevant for the risk assessment.

Food-grade carrageenan (commercial product purchased on market place as food grade and analysed by the South Korean Food Research Institute according to Korea Food Additive Code; the origin cannot be interpreted by the Panel, number- or weight-average molecular weight not specified) was assessed for the induction of chromosomal aberrations in Chinese hamster lung fibroblast (CHL), and for induction of micronuclei and DNA breakage by means of the alkaline comet assay in the L5178Y mouse lymphoma cells (Chung et al., 2009). For the induction of chromosomal aberrations, the assay consisted of short-term (6 h) both in the absence and presence of S9 metabolic activation and continuous (24 h) treatments in the absence of S9 metabolic activation at dose levels of 150, 300 and 600 μg/mL. Approximately 22 h after the beginning of the treatment, colcemid was added to each culture at a final concentration of 0.25 μg/mL. 200 metaphases (100 metaphases from each duplicate culture) were selected and analysed for each treatment group and the results were expressed as mean aberrant metaphases excluding gaps per 100 metaphases. At the tested concentrations of 150–600 μg/mL, there was no indication of a clastogenic effect. The Panel noted that the study is compliant with the OECD TG Guideline no 473.

For the induction of micronuclei, L5178Y mouse lymphoma cells were treated with carrageenan at concentrations of 0, 50, 100 or 200 μg/mL for 3 h, both, with and without S9 mix and harvested after a 21-h recovery period, or treated at concentrations of 0, 50, 100 or 200 μg/mL for 24 h without S9 mix and harvested immediately. No duplicate cultures were used and micronuclei were scored in 2,000 cells per culture. Slight but not significant increases in micronucleated cells compared to the concurrent vehicle control were observed at all dose levels and treatment performed. The observed increases were considered by the authors to be of no biological relevance (Chung et al., 2009). The Panel agrees with this conclusion and noted that the study is compliant with the OECD TG no. 487, with the exception that the highest dose level selected was not sufficiently high due to the absence of cytotoxicity and did not show any precipitation of test compound in culture medium as observed in the chromosomal aberration assay. In addition, no historical control range values were reported.

For the comet assay, L5178Y mouse lymphoma cells were similarly treated with carrageenan at concentrations of 0, 50, 100 or 200 μg/mL for 3 h, both, with and without S9 metabolic activation and harvested after a 21-h recovery period, or treated at concentrations of 0, 50, 100 or 200 μg/mL for 24 h without S9 metabolic activation and harvested immediately. Duplicate cultures were used and comets were examined from 100 randomly selected nuclei (50 nuclei per culture) using a fluorescence microscope. Images of selected nuclei were analysed using image-analysis software and tail intensity (% of tail DNA) and Olive tail moment were used to measure the damage of DNA damage. Slight but statistically significant increases in % tail DNA and Olive tail moment compared to the concurrent vehicle control were sporadically found. The increases were not dose-related and the observed values were similar to those commonly observed in the untreated controls for this cell line (Chung et al., 2009). However, the Panel noted some shortcomings which include the absence of historical control range values, a limited number of cells scored, a limitation in the selection of the highest dose level which did not cause adequate cytotoxicity, and no precipitation was observed in culture medium as it was in the chromosome aberration assay.

The Panel considered that carrageenan proved to be negative in all end-points tested in this study (Chung et al., 2009).

In vivo

Sodium carrageenan (interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, number- or weight-average molecular weight not specified) was assessed for its genotoxic properties in the host-mediated assay *in vivo* in mice, in the rat bone marrow chromosomal aberration assay, and in the rat dominant lethal assay. In all assays performed, the treatment regime used consisted of three dose levels 30, 2,500 and 5,000 mg/kg bw administered acutely by oral gavage, as single dose or subacutely using the same dosages as those in the acute study, each day for 5 consecutive days, 24 h apart (Documentation provided to EFSA n. 41).

In the host-mediated assay, 6–10 mice were allocated to each of the five groups for acute treatment and the four groups for subacute treatments. Three dose levels as described above and negative controls for both acute and subacute treatments were employed. Positive control groups were also included in the acute treatment. The indicator organisms used in this study were two histidine auxotroph *S.* Typhimurium strains (his G-46, TA1530) for induction of reverse mutation and a diploid strain (D3) of *S. cerevisiae* for mitotic recombination. In the acute treatment, all animals, immediately after treatment received 2 mL of indicator organism by intraperitoneal injection containing 3×10^8 cells for *S.* Typhimurium strains and 5×10^8 cells for *S. cerevisiae*. Three hours later animals were sacrificed, indicator organisms removed from peritoneal cavity and appropriately plated for scoring of mutant colonies. Results obtained indicated that tester strains *S.* Typhimurium TA1530, G-46 and *S. cerevisiae* D3 did not show any increase in revertants and mitotic recombination, respectively, both in the acute and subacute treatments. The Panel noted that this assay did not receive further validation and is not currently employed for genotoxicity testing.

In the rat bone marrow chromosomal aberration assay, 59 animals in the acute treatment and 18 in the subacute treatment were employed. In the acute studies, animals were sacrificed 6, 24 and 48 h after dosing and in the subacute treatment 6 h after the last dose. Bone marrow erythrocytes were used to prepare cytogenetic slides for chromosome analyses. Fifty metaphase spread for animal were scored for chromosomal aberration analyses. Results obtained show that the frequency of chromosomal damage observed in the sodium carrageenan animal treated groups was within the range of values observed for the concurrent vehicle control groups (0–6%) both in the subacute and acute treatments.

In the rat dominant lethal assay, a total of 10 male random-bread rats were allocated to each of the five groups, (e.g. three dose levels as described above and positive and negative control) for both acute and subacute treatments. Following treatment, the males were sequentially mated to 2 untreated virgin females per 5 days a week for 8 weeks. At the end of 5 days, females were removed from the males and housed separately until sacrifice. Females were sacrificed at 14 days after separation from males and at necropsy the uterus were analysed for early deaths, late fetal deaths and total implantations. Results obtained showed clear negative results.

Similarly, for calcium carrageenan (interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, number- or weight-average molecular weight not specified) the genotoxic potential was evaluated in the host-mediated assay *in vivo* in mice, in the rat bone marrow chromosomal aberration assay and in the rat dominant lethal assay. In all assays performed, the treatment regime used consisted of three dose levels 30, 2,500 and 5,000 mg/kg bw administered acutely by oral gavage, as single dose or sub acutely using the same dosages as those in the acute study, each day for 5 consecutive days, 24 h apart (Documentation provided to EFSA n. 35).

In the host-mediated assay, a number of 6–10 mice were allocated to each of the five groups for acute treatment and the four groups for subacute treatments. Three dose levels as described above and negative controls for both acute and subacute treatments were employed. Positive control groups were also included in the acute treatment. The indicator organisms used in this study were two histidine auxotroph *S.* Typhimurium strains (his G-46, TA1530) for induction of reverse mutation and a diploid strain (D3) of *S. cerevisiae* for mitotic recombination. In the acute treatment, all animals, immediately after treatment received 2 mL of indicator organism by intraperitoneal injection containing 3×10^8 cells for *S.* Typhimurium strains and 5×10^8 cells for *S. cerevisiae*. Three hours later animals were sacrificed, indicator organisms removed from peritoneal cavity and appropriately plated for scoring of mutant colonies. Results obtained indicated that tester strains *S.* Typhimurium TA1530, G-46 and *S. cerevisiae* D3 did not show any increase in revertants and mitotic recombination, respectively,

both in the acute and subacute treatments. The Panel noted that this assay did not receive further validation and is not currently employed for genotoxicity testing.

In the rat bone marrow chromosomal aberration assay, a total of 59 animals in the acute treatment and 18 in the subacute treatment were employed. In the acute studies, animals were sacrificed 6, 24 and 48 h after dosing and in the subacute treatment 6 h after the last dose. Bone marrow erythrocytes were used to prepare cytogenetic slides for chromosome analyses. Fifty metaphase spread for animal were scored for chromosomal aberration analyses Results obtained indicate that calcium carrageenan did not induce statistically significant increases of chromosomal aberrations at any dose level and sampling time employed both in the subacute and acute treatments.

In the rat dominant lethal assay, a total of 10 male random-bread rats were allocated to each of the five groups, (e.g. three dose levels as described above and positive and negative control) for both acute and subacute treatments. Following treatment, the males were sequentially mated to two untreated virgin females per 5 days a week for 8 weeks. After 5 days, females were separated from males and housed separately until sacrifice. Females were sacrificed 14 days after separation from males, and at necropsy the uterus were analysed for early deaths, late fetal deaths and total implantations. Results obtained showed clear negative results. The results obtained in these *in vivo* assays indicated that both sodium and calcium carrageenan were not systemically genotoxic. However, the Panel noted that these results could be anticipated due to the negligible absorption of both sodium and calcium carrageenan.

In summary, carrageenan (native, food-grade, sodium and calcium salts) has been tested in several *in vitro* and *in vivo* genotoxicity tests. Negative results from *in vitro* studies of high relevance and OECD guideline compliance were observed in bacterial gene mutation assays with semi-refined carrageenan from *E. cottonii* or *E. spinosum* sources (Documentation provided to EFSA n. 40) and with food-grade carrageenan (Chung et al., 2009).

Similarly, in mammalian cells, food-grade carrageenan proved to be negative for induction of chromosomal aberrations in CHL and micronuclei in L5178Y mouse lymphoma (Chung et al., 2009). The Panel noted that this combination of tests fulfilled the basic requirements to cover the three genetic endpoints (e.g. point mutation, structural and numerical chromosomal aberrations) required for the assessment of the genotoxicity of a test compound (EFSA Scientific Committee Guidance, 2011).

Negative results from limited studies were also observed *in vitro* in a bacterial gene mutation assay for calcium carrageenan (Documentation provided to EFSA n. 39), in a chromosomal aberration assay in anaphase in human embryonic lung (WI38) cells (Documentation provided to EFSA n. 41) for sodium carrageenan and *in vivo* in a host mediated assay in mice and in a chromosomal aberration and a dominant lethal assays in rats (Documentation provided to EFSA n. 41; Documentation provided to EFSA n. 35) for both sodium and calcium carrageenan.

Overall, carrageenan did not show genotoxic activities from a robust data set.

Processed Eucheuma seaweed

In vitro

Semi-refined carrageenan (commercial product from Seaweed Industry Association of the Philippines; interpreted by the Panel as isolated from *E. cottonii* with an approximate ratio of ι:κ:λ type 0:100:0, and from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0, number- or weight-average molecular weight not specified) from two sources (e.g. *E. cottonii* or *E. spinosum*), respectively, were assayed for their mutagenicity in a bacterial reverse mutation assay according to the relevant OECD TG Guideline 471 and in compliance with GLP. At the tested concentrations of 0.001–10 mg/plate, both with and without metabolic activation, there was no indication for mutagenicity in the tester strains TA1535, TA1537, TA98, TA100 and TA102 (Documentation provided to EFSA n. 40).

In the study by Sylianco et al. (1993), processed Eucheuma seaweed (identified as PNG carrageenan, without no further details) was assayed for its mutagenicity in a bacterial reverse mutation assay at concentrations of 25, 50 or 100 mg/mL in the absence of S9 metabolic activation only. The assay was performed using *S.* Typhimurium TA100 tester strain. The Panel noted that the study has severe limitations which included the absence of treatment in the presence of S9 metabolism and the use of the single *S.* Typhimurium TA100 tester strain. Concentration were only reported in limited tables as 25, 50 or 100 mg/mL and therefore it was not known which concentration was administered to the plates, the number of plates was not indicated, the number of colonies in the positive control was not reported. No data were given on cytotoxicity. Overall, the Panel concluded that the study was not suitable to be considered for risk assessment.

In the rec-assay using *Bacillus subtilis* rec$^-$ and *B. subtilis* rec$^+$, no DNA damaging activity was found at concentrations of 25, 50 or 100 mg/mL of processed Eucheuma seaweed (identified as PNG carrageenan, without no further details) (Sylianco et al., 1993). The Panel noted that the study has severe limitations which include the absence of treatment in the presence of S9 metabolism, concentration were only reported in limited tables as 25, 50 or 100 mg/mL and therefore it is not known which concentration was administered to the plates and the *B. subtilis* rec$^-$ and rec$^+$ employed were not reported. Overall, the Panel concluded that the study was not suitable to be considered for risk assessment.

In vivo

In the mouse bone marrow micronucleus assay (Sylianco et al., 1993), Swiss Webster mice (sex and number of animals not stated) were administered by oral gavage twice with processed Eucheuma seaweed (identified as PNG carrageenan, without further details) at dose levels of 625, 1,250 or 2,500 mg/kg bw, 24 h apart. Vehicle (water) and positive control (dimethylnitrosamine) treatment groups were also included. Six hours after the last treatment, the mice were sacrificed and bone marrow was processed for analysis of micronucleated polychromatic erythrocytes (no data about cytotoxicity clinical signs or number of cells analysed). In all treatment groups, the number of micronucleated cells was not increased apart from the positive (no data about the dose). The Panel noted that the results of this study are of limited relevance due to the absence of information on the number of polychromatic erythrocytes scored for the induction of micronuclei and cytotoxicity not evaluated. In addition, the Panel noted that PNG carrageenan is poorly absorbed.

No mutagenic properties were reported in the host-mediated assay (Sylianco et al., 1993). Swiss Webster mice (sex and number of animals not stated) received via gavage 0 (vehicle control, water), 625, 1,250 or 2,500 mg/kg bw processed Eucheuma seaweed (number- or weight-average molecular weight not specified) and the indicator organisms (*S.* Typhimurium strain G46) were injected into the peritoneum (no further details). The positive control benzo(a)pyrene resulted in a fivefold increase of the mutation frequency. The Panel noted that the study was poorly reported and that it has not received further validation and it is presently considered obsolete.

In summary, processed Eucheuma seaweed derived from either *E. cottonii* or *E. spinosum* was not mutagenic in a OECD guideline compliant bacterial reverse mutation assay (Documentation provided to EFSA n. 40). Additional *in vitro* studies (bacterial reverse mutation test and rec assay) resulted also in no genotoxic activity (Sylianco et al., 1993). However, these studies suffered from methodological weaknesses and limited information. Similarly *in vivo*, in studies with limited reliability, no clastogenic or aneugenic activity and no mutagenic activities were found in a mouse bone marrow micronucleus assay and in an host mediated assay, respectively (Sylianco et al., 1993).

Overall, despite the limitation of the available data the Panel considered processed Euchema seaweed to be of no genotoxic concern.

Degraded carrageenan

In vitro

Degraded carrageenan (not further specified) was tested for its mutagenicity in a bacterial reverse mutation assay. At the tested concentrations of 100–5,000 μg/plate, both with and without metabolic activation, there was no indication for mutagenicity in the tester strains of *S.* Typhimurium TA98 and TA100 (Wakabayashi, 1981; article in Japanese and summary in English). The Panel considered the negative results observed as reliable with limitations since an incomplete set of *S.* Typhimurium tester strains (only two strains) was employed.

In the study by Mori et al. (1985), degraded carrageenan, with a sulfate content of about 30% and an average molecular weight of 20–40 kDa (number- or weight-average molecular weight not specified), obtained from Glaxo Laboratories (Paris, France, not further characterised), was assayed for induction of gene mutation in *S.* Typhimurium strains TA98 and TA100, both in the absence and presence of S9 metabolic activation at dose levels of 8.900 and 26.700 μg/plate, much higher than the upper limit indicated in the relevant OECD Guideline 471. The results obtained did not show any increase in revertant numbers compared to the concurrent vehicle control. The Panel considered the negative results observed as reliable with limitations since an incomplete set of *S*. Typhimurium tester strains (only two strains) was employed.

In the study by (Wakabayashi, 1981; article in Japanese and summary in English), degraded carrageenan (not further specified) was tested for induction of point mutation in V79 Chinese hamster

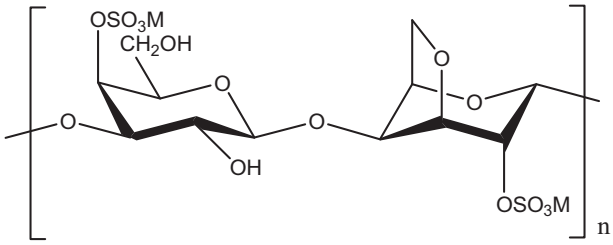
cells at the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus, only in the absence of S9 metabolism, at dose levels of 10, 100 and 1,000 μg/mL and negative results were obtained. The Panel noted that the study bears some shortcomings which include the use of a top dose level lower than that commonly recommended despite the absence of any cytotoxicity. In addition, treatments were performed only in the absence of S9 metabolism. On this basis, the Panel considered the results of this study of limited relevance.

In the study by Mori et al. (1985) degraded carrageenan, with a sulfate content of about 30% and an average molecular weight of 20–40 kDa (not specified if number- or weight-average molecular weight), obtained from Glaxo Laboratories (Paris, France, not further characterised), was assayed for induction of DNA repair using the *in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes and intestinal mucosal cells from ileum and colon/rectum at dose levels of 10 and 100 μg/mL as limited by the solubility of the test compound. The results obtained indicated that degraded carrageenan did not produce any evidence of UDS in either the hepatocyte or intestinal mucosal cells. The Panel noted that this assay detects only compounds which specifically trigger DNA excision repair following DNA adduction.

In silico

The evaluation of structural alerts for genotoxicity in the basic chemical structure of the monomer component of degraded carrageenan (as displayed in the figure below adapted from Wakabayashi) was performed with the OECD QSAR Tool box (2.6.13) using the following profilers: 'Alerts for *in vitro* mutagenicity (Ames test) by ISS', 'DNA alerts for Ames, MN, and CA by OASIS v.1.3', 'Alerts for DNA binding (by OASIS v.1.3 and OECD)' and 'Alerts for *in vivo* mutagenicity (Micronucleus) by ISS'.

No relevant structural alerts for genotoxicity were identified with the exception of the alert namely 'H-acceptor-path3-H-acceptor' detected by the profiler 'Structure Alerts for the *in vivo* micronucleus assay' The 'H-acceptor-path3-H-acceptor' refers to the possibility of non-covalent binding to DNA or proteins as a result of the presence of two bonded atoms connecting two hydrogen bond acceptors. However, the Panel noted that the positive predictivity of such alerts for *in vivo* genotoxicity was quite low, ranging from 'none' (34%) to 63% depending on the database, with a high incidence of false positives (Benigni et al., 2010, 2012).



M=Na or K

Summary of findings for degraded carrageenan

Degraded carrageenan (not further specified) was not mutagenic in *S.* Typhimurium TA 98 and TA 100 or in Chinese hamster V79 cells (Wakabayashi, 1981). Mori et al. (1985) confirmed the absence of mutagenicity in *S.* Typhimurium TA 98 and TA 100 tester strains and showed that degraded carrageenan did not exhibit genotoxic activity in DNA repair tests employing rat hepatocytes and intestinal mucosal cells. However, despite the limitations of the above mentioned studies, the absence of genotoxic effects was also indicated by the results of *in silico* evaluation.

Conclusion for genotoxicity

Overall, the Panel concluded that carrageenan and processed Eucheuma seaweed did not raise a concern with respect to genotoxicity. The Panel also noted that degraded carrageenan was not of genotoxic concern; this further supported that the food additives E 407 and E 407a, even if they contain some low molecular weight carrageenan, were not of genotoxic concern.

3.5.5. Chronic toxicity and carcinogenicity on carrageenan and degraded carrageenan

Mice

Male and female mice (10 animals/group, strains not specified) were exposed for the lifetime to diets containing carrageenan (Nilson and Wagner, 1959). Carrageenan (commercial product Krim-Ko-gel, high viscosity, now designated Sea Kem, type 5; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) used in the study (performed from 1951 to 1953) was purchased on the open market except for several lots which were supplied by the manufacturer. The first two lots analysed 76% and 75% hydrocolloid, 12% and 15% moisture, 25% and 22% mineral matter by weight, respectively. The hydrocolloid content was based on precipitating a 2% solution of the sample with 2 volumes of 99% isopropanol. No indication about the weight- average molecular weight was given. The arsenic content varied from 0.04 to 10.4 ppm (10.4 mg/kg). Carrageenan was mixed into the diet at 1%, 5%, or 15% levels (equivalent to 1,500, 7,500, 22,500 mg/kg bw per day) at the expense of equal weights of ground yellow corn and at the 25% (equivalent to 37,500 mg/kg bw per day) level for a fixed ration of ground yellow corn, wheat and oats. The animals were weighed once weekly and also food and water intake was determined once a week. No haematological and clinical parameters were measured. The mice were kept until death and at necropsy a gross examination was performed. If possible, organ sections or entire organs of each animal were preserved, sectioned and examined microscopically. No adverse effects on growth, survival, gross and histopathology were reported in any of the dosed animals.

The Panel noted that different lots of carrageenan have been used during the study, that the presence of arsenic at higher level than EU specifications in the substance has been detected. Moreover, the study has been conducted until the death of animals and there are differences in the feed composition for animals exposed to 25%. Consequently, this study cannot be used for the hazard characterisation.

Rats

A similar study has been performed by Nilson and Wagner (1959) with groups of 10 male and female rats. The strain was not specified. Carrageenan (commercial product Krim-Ko-gel, high viscosity, now designated Sea Kem, type 5; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) was mixed into the diet at 1%, 5% or 15% (equivalent to 500, 2,500, or 7,500 mg/kg bw per day) at the expense of equal weights of ground yellow corn, and at 25% (equivalent to 37,500 mg/kg bw per day) for a fixed ration of ground yellow corn, wheat and oats. No indication about the weight-average molecular weight was given. No adverse effects were noted except at the highest dose (25%) which exhibited lesions suggesting hepatic cirrhosis. According to the authors, these observations could indicate some dietary deficiency or insufficiency since the bulk handled was large and faeces often very loose indicating that mechanical loss of nutrient could not be discounted for this effect.

The Panel noted that different lots of carrageenan have been used during the study, and that the presence of arsenic in the test material was at higher level than EU specifications for the substance. Moreover, the study was conducted until the death of animals and there were differences in the feed composition for animals exposed to 25% carrageenan. Consequently, the Panel noted that this study was not in agreement with the current guidelines for a long-term study used for the hazard characterisation. The Panel noted that JECFA used this study in 1974 to allocate an ADI of 75 mg/kg bw per day, based on a NOAEL of 15% equal according to JECFA to 7,500 mg/kg bw per day. In 1978, the SCF endorsed this value and confirmed it until the latest evaluation in 2003.

Nutritional studies were conducted with Sprague–Dawley rats fed diets containing 4% κ/λ-carrageenan (interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) (equivalent to 2,000 mg/kg bw per day) that had been mixed into skimmed milk at a concentration equal to that of the protein and heat sterilised under conditions routinely used in the manufacture of canned liquid milk (Tomarelli et al., 1974). No indication about the weight-average molecular weight of the tested material was given. The authors only refer to a Food and Drug Administration review (FDA, 1972) in which, to ensure against the introduction of degraded carrageenan into the food supply, have amended the existing Regulation by stipulating that the carrageenan have an average molecular weight exceeding 100 kDa, as indicated by a specified test.[26]

[26] FDA, Fed. Regist., 15434 (2 August 1972).

Two experiments of 6 months duration each were conducted to determine the effect of feeding relatively large amounts of processed carrageenan for prolonged periods. In the first experiment, two dietary groups were maintained: a control group fed the basal diet and a carrageenan group fed the basal diet in which equivalent amounts of the skimmed milk powder and cellulose were replaced by the processed milk-carrageenan product. In the second experiment, a third dietary group was included, a group fed the basal diet in which dextrose was substituted for the cellulose. This group served as a control to assess the influence of the relatively large amounts of indigestible carbohydrate presented by the carrageenan or cellulose. The dietary groups of the first experiment consisted of 25 males and 25 females, while in the second there were 12 males and 12 females per group. In the second experiment half of the rats of each dietary and sex group were killed at the fourth month. The rats were weighed twice weekly. At intervals, 6–8 rats from each of the groups were located in metabolism cages during 5–7 days for studying the absorption of nutrients. At necropsy, the organs were examined for gross lesions with particular attention directed to the lower gastrointestinal tract. The authors reported that, 'when fed in a simulated milk powder diet, the processed carrageenan at a dietary level of 4% had no influence (compared to glucose or cellulose) on general health, growth rate, diet energy efficiency, absorption of protein, fat, calcium, blood coagulation, utilization of protein for growth, or utilization of iron. Gross and microscopic examination of the caecum and colon revealed no abnormalities'.

The Panel considered 4% of carrageenan in the diet (equivalent to 2,000 mg/kg bw per day), which did not induce nutritional imbalance, as a NOAEL.

Several carrageenan products (commercial products from Carrageenans Hercules Inc) extracted from *C. crispus* (77.7% κ, 22.3% λ), *G. acicularis* (23.3% κ, 76.7% λ), and *E. spinosum* (100% ι) were tested in rats by oral administration (Documentation provided to EFSA n. 44). No indication about the weight-average molecular weight was given. Alphacel was used as a bulk replacement control diet. Seven groups of 30 Sprague–Dawley rats (15 males, 15 females) with an age of about 4 weeks were dosed via diet with 1 or 5% (equivalent to 500 and 2,500 mg/kg bw per day) of the carrageenan or with 5% Alphacel over 40 weeks. Mortality and morbidity were recorded daily and animal weights were recorded weekly. Haematological tests included haemoglobin, haematocrit, erythrocyte count, and total and differential leukocyte count. Clinical tests consisted of blood glucose, blood urea nitrogen, serum glutamate pyruvate transaminase, Na^+, K^+, Cl^-, and examination of stools from six males and six females of each group was performed after 40 weeks. At termination, a full complement of tissue specimens was obtained from six male and six female rats dosed with 5% of the different carrageenan and these specimens were prepared and evaluated histologically and also liver portions were processed for electron microscopy. None of the dosed rats died during the study and there was no evidence of toxicity. There were also no differences in mean body weights between dosed rats and controls receiving 5% Alphacel, and the treatment had no adverse effects on haematological and clinical chemistry parameters. The faecal consistency at study termination was comparable for rats of all groups, and occult blood was detected less frequently among rats dosed with carrageenan compared with those rats treated with Alphacel. The dosing with the different carrageenan caused no significant changes of absolute liver weights in dosed rats and the histopathological examination gave no indications for alterations in morphology or for storage of metachromatic material in either Kupffer cells or hepatocytes. The Panel considered that the NOAEL of this study was equal to 5% equivalent to 2,500 mg/kg bw per day the highest dose tested, whatever the carrageenan tested.

Carrageenan (commercial product Gelcarin HMR from Marine Colloids, Inc. Rockland, Ma, Lot No 102012; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) was fed to two strains of weanling rats (Sprague–Dawley albino or Osborne-Mendel) at a 5% equivalent to (2,500 mg/kg bw per day) dietary level in two different basal diets (ground Wane Lab Blox [AMC] or FDA diet) for 39 weeks (Documentation provided to EFSA n. 42). Alphacel was used as a bulk replacement control diet. No indication about the weight-average molecular weight was given. Cellulose fibre was prepared at a 5% dietary level in two basal diets and fed to two strains of rats which served as controls. Rats were assigned to 8 groups of 18 males and 18 females and the rats were fed their respective diets for 39 weeks in a multiple-cross design: group l – Osborne-Mendel rats fed 5% cellulose in FDA diet, group 2 – Osborne-Mendel rats fed 5% carrageenan in FDA diet, group 3 – Osborne-Mendel rats fed 5% cellulose in AMC diet; group 4 – Osborne-Mendel rats fed 5% carrageenan in AMC diet; group 5 – Sprague–Dawley rats fed 5% cellulose in FDA diet; group 6 – Sprague–Dawley rats fed 5% carrageenan in FDA diet; group 7 –Sprague–Dawley rats fed 5% cellulose in AMC diet and group 8 – Sprague–Dawley rats fed 5% carrageenan in AMC diet. Mortality

and morbidity were recorded twice daily, and body weight, physical appearance, food and water consumption, and stool consistency (soft, firm or semifluid) were recorded at intervals. The presence of faecal occult blood was also monitored. The animals were killed and grossly examined at 30 days (3 males and 3 females per group) and 90 days (5 males and five females per group), while the surviving 160 rats were killed after 38 weeks and examined grossly. In addition, final body weights and absolute and relative liver weights were noted. Portions of the liver from all rats killed after 39 weeks were prepared for histochemical and ultrastructural determination of carrageenan storage and also a microscopic examination was undertaken. The treatment caused no mortality and all rats were in good health. Initially the body weights of Osborne-Mendel rats were lower compared with Sprague–Dawley rats, however after 39 weeks the Osborne-Mendel rats weighed more than the Sprague–Dawley strain. The dosing of carrageenan in AMC diet caused lower body weights in Sprague–Dawley males (group 8) at 4 weeks and in Osborne-Mendel females (group 4) throughout the experimental period. Soft faeces were noted in rats of all carrageenan groups. Occult blood in the faeces was sporadic in all groups (in control and treated groups) in time of appearance and quantity; occult blood was detected less frequently among rats dosed with carrageenan compared with those rats in the Alphacel group. Only Osborne-Mendel rats fed 5% carrageenan in FDA diet showed significantly increased (not further specified) relative liver weights after 39 weeks. At interim sacrifice on day 90, all rats given cellulose diet were grossly normal. Only two carrageenan fed rats (one male from group 8 and one female from group 2) had gross hepatic changes characterised as lobular flattening with a rough capsular surface. At the histopathological examination, both the 5% cellulose and the 5% carrageenan diets caused hepatocellular pericentral atrophy, hepatocellular necrosis and apoptosis. In some instances, the mitotic activity either was moderate or increased. There was a moderate degree of sinusoidal hyperaemia and varying degrees of hepatocyte vacuolisation. Bile duct proliferation was observed only in the livers of rats given carrageenan. For all of these findings, no statistics were given (Documentation provided to EFSA n. 42). The Panel considered that the 5% in the diet equivalent to 2,500 mg/kg bw per day, the highest dose tested, was the NOAEL.

Groups of 15 male and female Sprague–Dawley rats were dosed with two extracts of carrageenan (one with molecular weight estimate of 244 kD, interpreted by the Panel as isolated from *H. musciformis* similar to κ; or the second one with molecular weight estimate of 252 kD, interpreted by the Panel as isolated from *S. crispata* (synonym: *Iridaea crispata*) lying between λ and κ properties) at a concentration of 0%, 1% or 5% (equivalent to 0, 500 and 2,500 mg/kg bw per day) in the diet for one year (Documentation provided to EFSA n. 43). No indication about weight-average molecular weight was given. Controls received 5% Alphacel in the diet. In rats dosed with 1% there were no changes in the stools, while female rats dosed with 5% carrageenan from *S. crispata* (*I. crispate)* and males dosed either carrageenan at the 5% concentration had loose stools. Blood was observed sporadically in the stools, but the frequency was lower than in the controls. At the end of the study, body weights of the carrageenan-treated groups were decreased by 20% or 25% in the 1% and 5% carrageenan groups, respectively. In the group dosed with 1% carrageenan from *H. musciformis* the absolute and relative liver weights of males were comparable to the controls, while the absolute and relative liver weights of females were decreased when compared to the controls (absolute: 9.0 vs 15.6 g; relative 3.0 vs 4.0 g). In the group dosed with 5% carrageenan from *H. musciformis*, the absolute liver weights of males and females were decreased compared to the controls (males: 14.9 vs 18.7 g; females 8.4 vs 15.6 g) and the relative liver weight of females was decreased compared to the controls (2.9 vs. 4.0 g). In the group dosed with 1% carrageenan from *S. crispata* (*I. crispate)* the absolute liver weight of males was comparable to the controls but the relative weigh was increased (3.1 vs. 2.7 g). For females, both the absolute and relative liver weights were decreased compared to the controls (absolute: 9.8 vs 15.6 g; relative 3.2 vs 4.0 for the controls). In the group dosed with 5% carrageenan from *S. crispata* (*I. crispata*) the absolute liver weights of the females were lower than in the controls (8.2 vs 15.6 g). For this group the relative liver weights of the males were higher than the controls (3.0 vs 2.7 g) and the relative liver weight of the females was lower compared to the controls (2.9 vs 4.0 g). Some incidental effects were observed at macroscopical and histopathological examination of the liver. The dosing gave no indication for storage of carrageenan-like material in the liver cells of any of the treated rats, and no fibrillar material was seen by electron microscopy. Except for gross changes in the livers, no other adverse effects were observed. The Panel considered that the LOAEL in this study was 1% in the diet (equivalent to 500 mg/kg bw per day) for both carrageenan preparations.

Groups of 30 male and 30 female MRC outbred rats were dosed lifetime with 0%, 0.5%, 2.5% or 5% of carrageenan (commercial product Gelcarin HMR from Marine Colloids, Inc., Rockland, ME, Lot.

No 102012; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30), in the diet (Rustia et al., 1980). Carrageenan (Gelcarin) was reported as having a molecular weight of approximately 800 kDa. No indication about weight-average molecular weight was given. The average daily carrageenan intake was equal to 0, 360, 1,998 and 4,022 mg/kg bw. As untreated controls, 100 females and 100 males were used. The animals were inspected routinely two times a day and food consumption was recorded twice a week for a 2-day period. Animals were allowed to die spontaneously or killed when moribund. Complete autopsies were performed on all experimental and control animals, except for a few that were cannibalised or showed advanced post-mortem deterioration. Histological specimens were taken from all visceral organs and from all tumours and pathologically altered tissues. All segments of the gastrointestinal tract including the oesophagus, glandular steroids and forestomach, small intestine/duodenum, jejunum and ileum, cecum, colon and rectum were examined for lesions. The other tissues were fixed, processed and stained routinely with haematoxylin and eosin. The data including tumour and death rates were statistically analysed. The animals tolerated the carrageenan well at all dose levels and exhibited no clinical signs of excessive toxicity (e.g. significant body weight reduction). Animals occasionally developed a soft stool consistency, particularly in the initial experimental stages, and also diarrhoea was noted in some animals. No ulcerative alterations or neoplastic lesions of the intestinal tract were observed and the number of tumour-bearing animals, tumours per tumour-bearing animal and the overall incidence (i.e. number of tumours in test and control animals) and average latency periods did not differ significantly among test and control groups). According to the Panel, the results of this study, demonstrated no carcinogenic effects of carrageenan in rat. The Panel considered that the NOAEL of this study was 4,022 mg/kg bw per day, the highest dose tested.

Syrian golden hamsters

In a study with Syrian golden hamsters, groups of 30 male and 30 female hamsters were dosed livelong with 0%, 0.5%, 2.5% or 5% of carrageenan (commercial product Gelcarin HMR from Marine Colloids, Inc., Rockland, ME, Lot. No 102012; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) (Rustia et al., 1980). Carrageenan (Gelcarin) was reported as having a molecular weight of approximately 800 kDa. No indication about weight-average molecular weight was given. The average daily carrageenan intake was equal to 0, 370, 2,162 or 3,719 mg/kg bw per day. As untreated controls, 100 females and 100 males were used. The animals were inspected routinely two times a day and food consumption was recorded twice a week for a 2-day period. Animals were allowed to die spontaneously or killed when moribund. Complete autopsies were performed on all experimental and control animals, except for a few that were cannibalised or showed advanced post-mortem deterioration. Histological specimens were taken from all visceral organs and from all tumours and pathologically altered tissues. All segments of the gastrointestinal tract including the oesophagus, glandular steroids and forestomach, small intestine/duodenum, jejunum and ileum, cecum, colon and rectum were examined for lesions. The other tissues were fixed, processed and stained routinely with haematoxylin and eosin. The data including tumour and death rates were statistically analysed. The animals tolerated the carrageenan well at all dose levels and exhibited no clinical signs of excessive toxicity (e.g. significant body weight reduction). Animals given the highest dose level of carrageenan had soft stools during the initial weeks of the experiment. No ulcerative alterations or neoplastic lesions of the intestinal tract were observed and the number of tumour-bearing animals, tumours per tumour-bearing animal and the overall incidence (i.e. number of tumours in test and control animals) and average latency periods did not differ significantly among test and control groups). From the results of this study, carrageenan demonstrated no carcinogenic effects in Syrian golden hamsters. The Panel considered that the NOAEL of this study was equal to 3,719 mg/kg bw per day the highest dose tested.

Monkeys

A long–term toxicity study (7.5 years) has been performed on 40 rhesus monkeys (19 males and 21 females with initial weights of 2–8.22 kg) which were divided into 4 groups with 10 animals each and dosed with 0, 50, 200, 500 mg/kg bw per day κ/λ-carrageenan (commercial product Gelcarin HMR from Marine Colloids, Rockland, Maine; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30) by gavage daily on six days a week for the first 5 years (after about one year 10/19 males became aggressive and were dosed via diet) (Documentation provided to EFSA n. 34). No indication about weight-average molecular weight was given. Thereafter, for the remaining 2.5 years, carrageenan was incorporated into diet and given to monkeys. Observations on

appearance and behaviour were done on 6–7 days/week, while body weights were recorded weekly for the first month, and monthly thereafter and before termination. Clinical chemistry was done twice prior to start and at 2–12 months, in years 4–7 and prior to termination. A hepatic biopsy was performed on selected monkeys after 9 months and in years 2, 3.75, 5 and 6 of the study. Necropsy and gross examination was done in 6 of 10 monkeys which died or were killed (2–3 animals within all groups) for humane reasons during the study and at termination. The organ weights of liver, kidneys, heart, spleen, ovaries, testes, brain, adrenals and pituitary were determined and a microscopic examination of all major relevant organs was done. Histopathological examination (staining with toluidine blue) and electron microscopy was done in portions of the liver, large and small intestine, caecum and lymph nodes from each monkey. No statistical differences in mean survival times were detected in any group as compared to controls and there were also no gross or microscopic changes in the examined tissues. During years 5–7.5, in females dosed with 50 mg/kg bw per day a significant decrease in body weights was seen. Loose stools, chronic intestinal disorders, poor appetite and emaciation were observed in an apparently random distribution. Stool consistency was decreased in a dose-related manner over 7.5 years (the incidence of soft stools as a frequency of total observations was 9.1%, 18.7%, 20.3% and 24.4% in controls, low, mid- and high-dose groups) while positive faecal occult blood findings were increased in a similar fashion; the values obtained ranged from 16.8% in controls to 25.4% in the high-dose group. Faeces of monkeys given carrageenan were found to contain degraded carrageenan. Therefore, the Panel noted that it cannot be excluded that the presence of occult blood in animals is treatment-related. There were no changes in haematology, clinical chemistry values, organ weights, gross pathology, microscopic and ultrastructural morphology. There was also no evidence for the uptake of fibrillar, metachromic, carrageenan-like material in the cells of the reticular-endothelial system in the liver, lymph nodes, caecum, colon or anorectal junction. Three neoplastic changes were noted at termination: one perianal tumour in one control female, a cervical polyp in one female at 50 mg/kg bw per day and a fibroma of the thigh in one male at 200 mg/kg bw per day).

The Panel noted that the chronic toxicity studies were almost exclusively conducted with κ/λ-carrageenan and that no or no adequate description of the molecular weight distribution of the tested carrageenan preparations was given. There was no indication of carcinogenicity related to the treatment.

Initiation–Promotion studies

Several initiation–promotion studies with equivocal data were identified in the scientific literature by the Panel. Overall, the Panel considered that the results of the initiation–promotion studies did not demonstrate consistent modulating effects of carrageenan on the initiation and/or promotion phase of colon carcinogenesis induced in rats by well-known carcinogenic compounds such as azoxymethane (AOM) (Watanabe et al., 1978; Corpet et al., 1997; Taché et al., 2000) and dimethyhydrazine (DMH) (Arakawa et al., 1986; Hagiwara et al., 2001). Watanabe et al. (1978) and Arakawa et al. (1986) used doses up to 15% of the diet and were in excess of those accepted as maximum (5%) for a chronic bioassay (OECD, 1998). Aberrant crypt foci were used as putative preneoplastic (surrogate) markers for the development of colon cancer in the studies of Corpet et al. (1997) and Taché et al. (2000). They found a significant decrease in the number of aberrant crypt foci. Furthermore, no plausible mechanism of action has been proposed that could explain the results observed.

Overall, the Panel considered that there was no concern with respect to the carcinogenicity of carrageenan in several chronic toxicity studies in rats performed with carrageenan (mostly κ/λ-type, molecular weight distribution not adequately described). From the available rats studies, NOAELs up to 7,500 mg/kg bw per day, the highest dose tested, were identified (Nilson and Wagner, 1959). However, in another study in rats given carrageenan preparations with a molecular weight of 244 kDa and 252 kDa, a LOAEL of 1% in the diet (equivalent to 500 mg/kg bw per day) was identified by the Panel (Documentation provided to EFSA n. 43). The Panel noted that the characterisation of the test material in all the chronic studies was limited.

No data were available on chronic oral toxicity and carcinogenicity of processed Eucheuma seaweed. Studies with degraded carrageenan showing carcinogenicity have been described under Section 3.5.8. (Other studies).

3.5.6. Reproductive and developmental toxicity

Reproductive toxicity studies

High molecular calcium carrageenan (κ-λ) (commercial product Gelcarin-HMR from Marine Colloids, Rockland, Maine; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30, no indication of molecular weight distribution) was tested in a combined three-generation reproduction toxicity and developmental study (the latter dealt with in section Developmental Toxicity) with groups of 40 male and 40 female Osborne-Mendel rats (Collins et al., 1977a). The just weaned animals were dosed with diets containing calcium carrageenan at levels of 0, 5,000, 10,000, 25,000 or 50,000 mg/kg diet. The authors calculated daily carrageenan consumption for adult males was 0, 290, 592, 1,555 or 3,212 mg/kg bw per day and for adult females 0, 350, 711, 1,832 or 3,805 mg/kg bw per day. After weaning, all animals were fed carrageenan in their diets for 12 weeks before mating. Animals of both F_{1A} and F_{1B} litters were sexed and weighed at birth and weighed also on days 4, 7, 14 and 21. Checking for abnormalities in all dams and pups was done until weaning at PND 21, when F_{1A} rats were autopsied and discarded. Randomly selected F_{1B} and F_{2B} rats were mated to produce the F_2 and F_3 generations, respectively. F_{2A}, F_{2B}, F_{3A} and F_{3B} animals were weaned and autopsied comparable to F_{1A} rats, except that randomly selected rats from F_{3A} were used in a chronic feeding study. On gestation day (GD) 20, F_{2C} and F_{3C} litters were exposed by caesarean section. Randomly selected F_0, F_{1B} and F_{2B} parents were kept on the same dietary level for 9 months followed by a histopathological examination. Representative weanlings from F_{2A}, F_{2B}, F_{3A} and F_{3B} also were examined for pathology. All adult rats were weighed weekly and food consumption was measured at the same time. All litters of more than 10 offspring were reduced to 10 by a randomised selection on PND 4. When killed, all adult and weanling rats were autopsied. The treatment had no effect on mortality, but there was a noticeable deterioration in the physical state of rats dosed with 5% via diet, i.e. these rats were smaller and thinner than the controls, and had rough hair coats. Diarrhoea was marked in animals fed the two highest dose levels. The consumption of the diet containing carrageenan was increased at ≥ 2.5% in both sexes. The cumulative weight gain of males at week 12 of the premating period was significantly decreased at the 5% level in F_0, at the three highest dose levels in F_{1B} and at all four dose levels in F_{2B} generation. In females, apart from a significant decrease in the F_{2B} generation at the 5% level, the cumulative weight gain during the premating period was unaffected. For the fertility index, there was no dose-related difference at any dose level. There were no adverse effects concerning average litter size or viability of the pups. The viability index gave no indication for adverse effects of carrageenan and also the average number of liveborn pup surviving to PND 4 indicated no significant postnatal effect at any dose level. The treatment appeared to have no effect on the viability of the pups from birth to weaning at PND 21. Birth weight of the pups were incidentally decreased (2.5 and 5% level F_{1B} female pups and at 5% level F_{3A} male and female pups). Dose-related and statistically significant decreased body weights of weanlings were noted at all dose levels. However, decreased pup weight on PND 21 in the 0.5% dose level group was only significant in the female pups of the F_{1B} litter. Overall, up to the highest dose tested, no effect was observed on reproductive or developmental parameters apart from the effect on pup weight on PND 21. Based on the decreased body weight gain of the male animals and the decreased pup weight at dose levels of ≥ 1%, the authors proposed a NOAEL of 0.5% (equal to about 290 or 350 mg calcium carrageenan/kg bw per day for male or female animals). The Panel agreed with this NOAEL. However, decrease in pup weight was observed in the presence of maternal effects (diarrhoea) which may have affected lactation.

In an one-generation reproductive and behavioural toxicity study with Sprague–Dawley rats, male and female rats were dosed orally with 0–18,000 mg/kg diet of calcium carrageenan (not specified; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30; no indication of molecular weight distribution) (equal to 0, 500, 1,000 or 2,100 mg/kg bw per day for males and for females 0, 400, 700 or 1,500 mg/kg bw per day during the gestation period and 0, 600, 1,300 or 2,600 mg/kg bw per day during the lactation period) (Vorhees et al., 1979). The substance was continuously administered after the acclimation period and included: 14 days prior to mating, 1–4 days during breeding (males were discarded following conception), throughout gestation (22 days), lactation (21 days) and post-weaning testing (69 days), i.e. from weaning at 21 days of age until the termination of the experiment at 90 days of age. Developmental measures in the offspring included physical development (pinna detachment, incisor eruption, eye opening, and growth), behaviour and histopathology of the brain. Calcium carrageenan produced a few isolated and inconsistent effects

(e.g. delay in the appearance of pivoting, advance in the appearance of full locomotion or increase in cell density in the olfactory bulbs); however, there was no clear pattern of behavioural dysfunction and the observed effects were not dose-related. No other fertility or developmental effects were observed although the description of these parameters was limited.

Developmental studies

In all studies described below (Documentation provided to EFSA n. 45), body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. To test the methodology and the sensitivity of the laboratory animals' positive controls were tested. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live fetuses were recorded. All fetuses were examined grossly for sex distribution and for external abnormalities (one-third detailed visceral examination and two-third stained and examined for skeletal defects). In the following developmental toxicity studies, the source and type of carrageenan used were not specified. The Panel interpreted the results as being obtained from carrageenan isolated from *C. crispus* with an approximate ratio ι:κ:λ type 0:70:30. The molecular weight distribution of the tested carrageenan preparations was not indicated.

Mice

Pregnant CD-1 mice (22–27 animals/group) were treated via gavage with 0, 10, 45, 470 or 900 mg/kg bw per day of sodium carrageenan (unspecified) in corn oil (the use of this vehicle was described in Documentation provided to EFSA n. 46) on GD 6–15 (Documentation provided to EFSA n. 45). The dams were sacrificed on GD 17. Mortality was 1, 1, 0, 2 and 9 in the 0, 10, 45, 470 or 900 mg carrageenan/kg bw per day group. The dosing caused an apparent increase in the number of resorptions with or without corresponding decrease in the number of live fetuses in the two highest doses. At the two highest doses, there was a decrease in the fetal weight (approximately 7% and 13%). In these groups also, retardation of skeletal maturation (increased incidence of incomplete or missing sternebrae and incomplete skull closure) was observed. The other soft and skeletal tissue abnormalities observed were comparable to the control group. Due to the high mortality in the high dose group, the Panel considered this study not relevant for risk assessment.

Rats

Pregnant Wistar rats (21–27 animals/group) were treated via gavage with 0, 40, 100, 240 or 600 mg/kg bw per day of sodium carrageenan (unspecified) in corn oil (the vehicle as described in Documentation provided to EFSA n. 46) on GD 6–15 (Documentation provided to EFSA n. 45). The dams were sacrificed on GD 20. The dosing caused an apparent increase in the number of resorption sites with or without corresponding decrease in the number of live fetuses in the two highest dose levels. At the highest dose group, there was a decrease in the fetal weight (15%). In the two highest dose groups also, retardation of skeletal maturation (incomplete or missing sternebrae missing hyoid) was observed. The other soft and skeletal tissue abnormalities were comparable to the control group.

In a follow-up study of the above one (Documentation provided to EFSA n. 46), groups of 21–24 Wistar-derived albino rats were exposed to sodium or calcium carrageenan (unspecified) via diet at concentrations of 0, 1 or 5% (equal to 0, 600 or 3,000 mg/kg bw per day) on GD 6–15. The motivation was that treating by the intubation route entailed a physiological stress and therefore embryo/fetal disorders. The dams were sacrificed on GD 20. No mortalities were observed. For both sodium and calcium carrageenan, the dosing up to 5% caused no adverse effects on maternal or fetal survival, on the number of implantations, or on the degree of maturation of fetuses. The incidence seen of either soft or skeletal tissue defects did not differ between the test groups and the controls. The NOAEL for developmental and maternal toxicity was 5% (3,000 mg/kg bw per day, sodium and calcium carrageenan) the highest dose tested.

The aforementioned three-generation reproductive toxicity study included a developmental part (Collins et al., 1977b). See for details the section reproductive toxicology. The parental animals were F_{1B} and F_{2B} of the three-generation reproductive study, and the offspring were F_{2C} and F_{3C}, respectively. All dams were subjected to caesarean section on GD 20, and the number of corpora lutea, early and late resorptions sites, implantation sites, the live and dead fetuses and the body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, half of the fetuses underwent detailed visceral examinations and the other half were stained and examined for skeletal defects. The average daily high molecular calcium carrageenan (κ-λ) consumption (0%,

0.5%, 1.0%, 2.5% and 5.0%) of the dams of the F_{2C} generation was calculated by the authors to be 0, 309, 609, 1,563 or 3,184 mg/kg bw per day and of the F_{3C} 0, 290, 592, 1,524 or 3,060 mg/kg bw per day, respectively. There were no adverse effects on the average numbers of corpora lutea, implantations, and early or late deaths and the average resorptions (in %) per litter. The Panel noted that there were some incidental inconsistent findings but considered these findings not of biological significance. The Panel considered that the dosing up to 5% (3,060 mg/kg bw per day, the highest dose tested) in the diet gave no indications for maternal abnormalities or fetal external, skeletal and soft-tissue anomalies.

Hamsters

Pregnant Golden hamsters (24–30 animals/group) were treated via gavage with 0, 10, 45, 470 or 900 mg/kg bw per day of sodium carrageenan (unspecified) in corn oil (vehicle as described in Documentation provided to EFSA n. 46) on GD 6–10 (Documentation provided to EFSA n. 45). The dams were sacrificed on GD 14. Mortality was 1, 0, 0, 0, 2 in the 0, 10, 45, 470 or 900 mg sodium carrageenan/kg bw per day group. The number of resorption sites was increased in the 45 mg/kg bw per day group with a corresponding decrease in the number of live fetuses. This effect was not observed at the higher dose levels and therefore not considered to be treatment-related. The treatment had no effect on implantation in the treated groups. At the two highest dose levels, a delayed skeletal maturation (missing sternebrae and incomplete ossification of extremities) and an increased number of malformations (scoliosis, exencephaly and cleft palate) was observed.

In a follow-up study (Documentation provided to EFSA n. 46), groups of 21–26 young adult pregnant Golden hamsters were dosed with sodium or calcium carrageenan (unspecified) via diet at concentrations of 0%, 1% or 5% (calculated by the Panel to be equivalent to 0, 1,000 or 5,000 mg/kg bw per day, Swirsky Gold et al., 1984) on GD 6–10. No adverse effects on maternal or fetal survival, on the number of implantations. The incidence seen of either soft or skeletal tissue defects or skeletal retardations did not differ between the test groups and the controls. In hamsters dosed with 5% calcium carrageenan, there was a reduction in the pregnancy rate, while the dosing of sodium and calcium carrageenan gave no indications for developmental effects. The effect on pregnancy rate may be incidental as it was not observed in the group dosed with sodium carrageenan. Furthermore, dosing was performed after implantation. The Panel considered 5% in the diet (5,000 mg/kg bw per day, sodium or calcium carrageenan, the highest dose tested) as the NOAEL.

Pregnant Golden hamsters (21–26 animals/group) were treated by oral intubation with sodium or calcium carrageenan in distilled water at dose levels of 0, 10, 40, 100 or 200 mg/kg bw per day on GD 6–10 (Collins et al., 1979). On GD 14, one day before expected parturition, the animals were subjected to caesarean section and the numbers of corpora lutea, implantations, resorptions sites, and live and dead fetuses and bodyweights of live pups were recorded. All fetuses were examined grossly for sex distribution and for external abnormalities; approximately half of the fetuses from each litter for detailed visceral examination and half stained and examined for skeletal defects. The dosing with sodium or calcium carrageenan caused no adverse effects in dams and offspring.

Rabbits

Pregnant Dutch-belted rabbits (12–13 animal/group) were treated by intubation one daily from GD 6 to 18 with doses of 0, 40, 100, 240 or 600 mg/kg bw per day sodium carrageenan in corn oil (vehicle as described in Documentation provided to EFSA n. 46) (Documentation provided to EFSA n. 45). Caesarean section was performed on GD 29. Mortality was 4, 3, 0, 2, 3 in the 0, 40, 100, 240 or 600 mg sodium carrageenan/kg bw per day. High mortality was also observed in the control group. The Panel considered this effect was not related to carrageenan but probably to the use of the vehicle (corn oil) or to the general condition of the animals at the start of the study. Live fetuses of each litter were placed in an incubator for 24 h for an evaluation of neonatal survival. Even the highest dose level of 600 mg/kg bw per day caused no effect on implantation on fetal survival. There was also no indication for developmental disorders in either soft or skeletal tissues in treated animals compared with the controls. However, the number pregnant does at caesarean section was low (9, 8, 12, 9 and 7 for the of 0, 40, 100, 240 or 600 mg sodium carrageenan/kg bw per day) and therefore this study cannot be used for risk assessment.

Overall, the Panel noted that the NOAEL for reproductive effects of calcium κ/λ-carrageenan (high molecular) observed in a dietary three-generation reproduction toxicity study in rats was 3,212 mg/kg bw per day, the highest dose tested and 290 mg/kg bw per day for parental and developmental effects based on decreased parental body weight gain and pup weight (Collins et al., 1977a).

Reproductive and neurodevelopmental effects were not observed in a one-generation study in rats up to doses of 2,600 mg sodium κ/λ-carrageenan/kg bw per day (Vorhees et al., 1979). The prenatal developmental studies performed with sodium κ/λ-carrageenan by gavage suspended in corn oil were considered by the Panel not suitable for the safety evaluation (Documentation provided to EFSA n. 45). The Panel considered that the high maternal mortality observed in mice and rabbits was not ascribed to κ/λ-carrageenan but to the vehicle corn oil used and to the method of dosing. The NOAEL for developmental effects found in dietary studies in prenatal developmental toxicity studies was 3,000 and 5,000 mg/kg bw per day sodium or calcium κ/λ-carrageenan for rats and hamsters, respectively (Documentation provided to EFSA n. 46), and 3,060 mg calcium κ/λ-carrageenan/kg bw per day for rats (Collins et al., 1977a) the highest dose levels tested.

The Panel noted that the reproductive and developmental toxicity studies were exclusively conducted with κ/λ- carrageenan and that the molecular weight distribution of the tested carrageenan preparations were not indicated.

No data are available on reproductive and developmental toxicity of ι-carrageenan and processed Eucheuma seaweed.

3.5.7. Hypersensitivity, allergenicity and food intolerance

Animals

Weiner et al. (2015) evaluated the potential effects of carrageenan after 28 days of administration of piglet-adapted formula to Domestic Yorkshire Crossbred Swine (6 males, 6 females per group) from lactation day 3 for 28 consecutive days on several endpoints. The endpoints evaluated included emphasis on the GI tract and the immune system including immunophenotyping, circulating cytokines and immunohistochemistry of the GI tract. Animals were exposed to 0, 300, 1,000 and 2,250 mg/kg diet equal to 0, 52, 193, and 430 mg/kg bw per day and 0, 56, 203, and 448 mg/kg bw per day, for male and female, respectively. The test material sample of κ/λ-carrageenan (FMC Lot. 90303011) (ratio κ/λ unknown) was fully characterised for, molecular weight, percentage of the molecular weight below 50 kDa, known as the low molecular weight tail (LMT) as per European requirements, and met internationally recognised carrageenan food additive specifications. The weight-average molecular weight of the test material sample was 664–732 kDa with a LMT of 0.3–3.9% and a viscosity of 80 cP. Some animals show minor findings, such as faecal changes but clinical pathology parameters, including haematology, clinical chemistry and coagulation were unaffected by the treatment. The results of the immunological evaluations did not reveal any treatment-related changes at the concentrations tested as compared to the vehicle control (swine adapted infant formula).

Humans

The α-1,3-galactosidic linkage, which is present in the three types of carrageenan is specifically recognised by human IgG and IgM anti-galactose natural antibodies. Binding of these antibodies to this saccharide epitope may lead to an immune reaction and, for instance, they were involved in acute rejection by humans of pig xenografts (Galili et al., 1985; Thibaudeau et al., 1996; Klein et al., 1998). These antibodies developed with age in human; they are not present in the neonates but develop within a few months, as soon as the newborn's gastrointestinal tract becomes colonised by microorganisms.

The Panel noted that an ELISA was available for the detection/quantification of carrageenan in various foods (Haines and Patel, 1997, 2005), which indicated that carrageenan could elicit the production of and react with, specific antibodies thus revealing the presence of immunogenic sites in carrageenan. It was not known whether these antibodies also recognised poligeenan and other degraded carrageenans.

Vojdani and Vojdani (2015) reported that 27%, and 17% of the sera from 288 healthy, asymptomatic volunteers contained specific IgG and IgE antibodies to carrageenan, respectively. Fixation of IgE but not of IgG antibodies was largely inhibited by several common food antigens, indicating that these IgE may be formed in response to cross-reacting antigens present in common foods.

The Panel noted that despite the presence in human serum of antibodies capable to recognise saccharidic epitopes on the carrageenan molecules, no case reports of significant allergic and/or anaphylactic reactions after ingestion of foods containing carrageenan were identified in the available literature.

3.5.8. Special studies with carrageenan and degraded carrageenan on the mechanisms of inflammation

A number of studies with samples of carrageenan and 'degraded' carrageenan, which were not always adequately characterised analytically, has been performed in order to evaluate if these compounds were able to induce or increase the production of proinflammatory mediators by various cells. These cell models (mostly intestinal or macrophage-like) were selected by the authors on the ground of their relevance to the oral administration of the additive E 407. Only the most recent ones are reported below as described by their authors.

***In vitro* studies**

Borthakur et al. (2007) reported that when human (intestinal epithelial cells from colonic surgeries, cell line NCM 460) cells, and normal rat ileal epithelial cells were treated for 1–96 h with λ-carrageenan (not analytically characterised) at a concentration of 1 μg/mL, increased BCL10, nuclear and cytoplasmic nuclear factor κB (NF-κB), and IL8 secretion were observed.

In a series of studies with various cell models (RAW 264.7 and 23ScCr mouse macrophage cell lines, NCM 460 human colonic epithelial cell line, primary cultures of human colonic cells), Bhattacharyya et al. (2008b, 2010) studied the mechanisms of carrageenan-induced intestinal inflammation. Toll-like receptor (TLR) and the protein B-cell lymphoma/leukaemia 10 (BCL10) were identified as important components of the cell membrane for binding of carrageenan to the cell membrane. The authors reported that in their studies, binding of carrageenan (purity and composition not reported) to the receptor resulted in stimulation of NF-κB, and activation of interleukin-8 (IL-8) production and of a reactive oxygen species (ROS)-mediated pathway.

Choi et al. (2012) evaluated the effects of carrageenan on proinflammatory transcription factor NF-κB (subfamily RelA/p65) and early growth response gene 1 product (EGR-1) in relation to human intestinal epithelial barrier integrity. Human colonic cancer cell lines HCT-8, HT-29 and Caco-2 were exposed to 0 or 1 μg/mL of carrageenan (type not specified) for up to 24 h. NF-κB activation (but not EGR-1 activation) was involved in the induction of proinflammatory cytokine IL-8. According to the authors, the results showed that both NF-κB and EGR-1 play a role in maintaining the epithelial barrier integrity in response to carrageenan.

Another study assessed 'whether binding of carrageenan to TLR4 was specific or due to the mechanical coating of the membrane as a result of the large molecular weight of carrageenan and of the conditions used *in vitro* (Documentation provided to EFSA n. 47). Human embryonic kidney cells transfected with a TLR4 reporter system were employed to determine the binding, using alkaline phosphatase (SEAP) as the reporter molecule. Cells were exposed for 24 h to various types of carrageenan, all compliant with the JECFA specifications: food-blend carrageenan from a manufacturer, commercial λ, κ and ι-carrageenan, and a commercial mixture of κ- and ι-carrageenan, at concentrations of 0, 0.1, 1, 10, 50, 100, 500, 1,000 or 5,000 ng/mL'. Positive (LPS) and negative (clarified locust bean gum (CLBG) and sodium alginate (SA)) controls were included. Cell viability, as measured by the levels of cellular adenosine triphosphate and released lactate dehydrogenase released, was unaffected by any of the carrageenan or by CLBG, SA or LPS at any exposure concentration tested. Overall, no measurable changes in cell viability were observed under these test conditions, and the three types of carrageenan tested were not TLR4 agonists or antagonists.

McKim et al. (2015) evaluated the ability of the different types of carrageenan (κ-, ι- and ʎ-, food-grade purity 51–85%) to bind and activate TLR4 signalling by using a TLR4/MD-2/CD14/NF-κB/SEAP reporter construct in a HEK293 cell line. The carrageenan used was characterised with respect to identity and purity, and it was observed that commercial carrageenan samples contained sugars (dextrose or sucrose). Cells were exposed for 24 h to concentration of 0.1, 1, 10, 50, 100, 500, 1,000, and 5,000 ng/mL of the various compounds. The results showed that carrageenan did not bind to TLR4 and was not cytotoxic to the HEK293 cells at the concentrations and experimental conditions tested. In addition, it was shown that carrageenan binds tightly to serum proteins, in particular proteins from the fetal serum used in the cell cultures. The Panel noted that the authors claimed that carrageenan was described as having a weight average molecular weight (mw) of 200–800 kDa but that no data presenting the MW profile of the different forms of carrageenan used in the study were reported in this publication.

In another study, McKim et al. (2016) evaluated the intestinal permeability, cytotoxicity and carrageenan-mediated induction of proinflammatory cytokines using a standard Caco-2 absorption model and both ι-, κ- and λ-carrageenan. They found no carrageenan permeability or cytotoxicity at

concentrations of 100, 500 and 1,000 μg/mL. In two human intestinal cell lines (HT-29 and HCT-8) carrageenan (0.1, 1.0 and 10.0 μg/mL) did not induce IL-8, IL-6 or MCP-1 nor produced cellular toxicity at 24 h. The authors concluded that carrageenan did not cross the intestinal epithelial cells and were not cytotoxic to these cells. Carrageenan did not increase cellular oxidative stress nor did they induce expression of proinflammatory genes. Positive control substances produced the expected results indicating that the test systems were responsive. According to the authors, this study was unable to reproduce any of the previously reported *in vitro* findings that carrageenan may cause inflammation or disrupt insulin signalling pathways. They also reported that when a commercial batch of ʎ-carrageenan was carefully characterised for identity and purity, only 64% of the material sold was carrageenan, with 34% being comprised of sugars. The Panel noted that the authors claimed that carrageenan was described as having a weight average molecular weight (mw) of 200–800 kDa but that no data presenting the MW profile of the material used in this study were reported in the publication.

Chan et al. (2017) studied the influence of the three major types of carrageenan polysaccharides on monocyte (human THP1) behaviour *in vitro*, only the λ-type induced monocyte adhesion and only in the presence of serum. Further analyses indicated λ-carrageenan bound IL-8 in the serum and activated the cultured monocytes through an IL-8-dependent pathway.

Fahoum et al. (2017) investigated if 'carrageenan' may modify gastric proteolysis and if 'physiologically (partially) digested carrageenan' (i.e. carrageenan incubated in a simulated gastric fluid (pd carrageenan = pdCGN) may affect gut epithelial structure and function. Food-grade κ-, ι- and λ-types of carrageenan (no indication of molecular weight distribution) were used.

The authors reported that:

- native carrageenan bound milk, soya or egg proteins,
- native carrageenan impaired digestibility by pepsin of these proteins,
- in Caco 2 cells, pdCGNs induced increased expression of inflammatory markers and affected the epithelial structure,
- Some of these effects were associated with the degree of sulfation of the different carrageenan forms, with kappa being less potent.

The Panel noted that:

- the simulated gastric fluid used was close to the 'physiologic' conditions and distinctly different from the treatment used to yield 'polygeenan' or C 16 from carrageenan,
- there was no information on the changes in MW of carrageenan after partial digestion,
- when looking at the biological effects, it was unclear what the partially digested material used in the experiments with 'pdCGNs' was: either 'protein-carrageenan complexes', or 'pure carrageenan' (not in complex with a protein),
- as regards the biological effects (e.g. secretion of mediators of inflammation), there was a difference between the types of pdCGN i.e. iota and lambda having an effect in contrast to kappa. The results with native iota carrageenan were negative.

Therefore, the Panel considered that although the reported effects may have been relevant for this evaluation, it was not possible to use the data from this study.

Applying Caco 2, THP-1 and HT-29 cells as models, the same group (Jiang et al., 2013; Wu et al., 2017), reported the effects of κ-carrageenan on the inflammatory reaction *in vitro*. The results showed that κ-carrageenan could induce significant increase in secretion of various inflammatory cytokines including tumour necrosis factor-α (TNF-α), IL-1β, IL-6, and to participate in the Bcl10-NF-κ B-mediated pathway to enhance LPS stimulated secretion of IL-8 in HT-29 cells. The Panel noted that the MW distribution of the κ-carrageenan used was not given by the authors who only indicated that: 'the average molecular weight of 1×10^6 of kappa carrageenan was used in this experiment' (Wu et al., 2017).

Overall, the interaction of carrageenan with inflammatory pathways at the molecular level was investigated in several *in vitro* cell models. These studies were based on signalling pathways involving the transcription protein nuclear factor κB (NF-kB), which, among others, regulates the expression of genes associated with inflammation. In many studies, the three types of carrageenan (commercial material, data on purity not available) were reported to activate inflammatory cascades that are related to innate immunity and to the generation of ROS, which may lead to inflammation. A recent study conducted with κ-, ι– and λ-carrageenan suggested that the difference in their capacity to react with food and to induce the expression of inflammatory markers may be due to the difference in sulfation of the different forms (Fahoum et al., 2017). The Panel noted that the available *in vitro*

studies with 'carrageenan' were discussed several times (McKim, 2014; Weiner, 2016, 2017). Main pitfalls were that the studies on signalling pathways did not establish neither concentration-related responses, nor direct and specific binding to receptors or entry of carrageenan inside the cells. The author considered that high molecular weight carrageenan might coat the receptors. The Panel acknowledged that the *in vitro* studies are useful to investigate the mechanisms of toxicity. However, the Panel also noted that they were often conducted using established cell lines, which are not fully representative of the normal human intestinal epithelium *in vivo,* and that several confounding factors, including poor characterisation of the material used and limited consideration of the interactions with food were identified in the protocols used.

In vivo studies

Bhattacharyya et al. (2013) investigated the pathways for induction of inflammation in Bcl10 wild-type, heterozygous and null mice. Groups of 3–8 male mice were given drinking-water containing 0 or 10 μg/mL of carrageenan (commercial λ- and κ-types) for 14 weeks, amounting to averaged 50 μg carrageenan/mouse (equivalent to 1.7 mg/kg bw per day). Markers of intestinal inflammation (faecal calprotectin), blood cytokines (including KC, the mouse analogue of human IL-8) and markers of the inflammatory cascade were measured. Body weights were unchanged. Faecal calprotectin and circulating KC were significantly increased in both wild-type and heterozygous mice exposed to carrageenan compared with controls. In all three types of mouse, serum IL-6 and monocyte chemotactic protein-1 (MCP-1) were similarly increased by carrageenan treatment. No treatment-related effects were observed on serum levels of cytokines TNF-α, interferon gamma (IFN-γ), IL-1β, IL-10, IL-12 and IL-23. No gross changes or macroscopic lesions were apparent in the intestine of carrageenan-treated mice, except in one wild-type mouse. Microscopically, in carrageenan-exposed mice, the extent of inflammatory infiltrate throughout the intestine was greater in the wild-type than in the Bcl10 null mice, being significantly greater in the small intestine than in the colon and rectum for each of the groups. IL-10-deficient mice exposed to carrageenan showed an increase in activation of NF-kB (RelA) activation, but no increase in RelB or phospho-Bcl10. According to the authors, these findings demonstrated 'a requirement for Bcl10 to obtain maximum development of the inflammatory pathway by carrageenan and lack of complete suppression by IL-10 of activation of the inflammatory pathway by carrageenan'.

In the study by Shang et al. (2017) C57/BL/6J mice (6/groups) were given normal autoclaved water or λ, κ or ι-carrageenan (purchased from Sigma Shangai; no further precision) in drinking water (20 mg/L) for 6 weeks. Blood (for the estimation of serum TNF-α, IL-1β, IL-6 and IL-10), cecum and colon were collected at the end of the treatment period. A sample of the colon was fixed in 10% formaldehyde then the degree of inflammation was scored after staining with haematoxylin and eosin. The colonic content was collected aseptically for analysis of the microbiota by DNA amplification and high-throughput sequencing. The authors reported that the treatment with carrageenan induced disruption of the epithelium of the colon, colitis, and increased (about three times) the serum TNF-α levels, whatever the type of carrageenan used; serum levels of IL-1β, IL-6 and IL-10 were unchanged. The treatment with λ, κ and ι-carrageenan had different effects on the various components of the gut microbiota but all of them were able to decrease the amount of a bacterium known to have anti-inflammatory properties (*Akkermensia muciniphila*).

Wu et al. (2017) used the *Citrobacter freundii* DBS100-induced intestinal inflammation model with NHS male and female mice to study if κ-carrageenan administered by gavage in water for 1 week at daily doses of 1.7 mg/kg bw, 8.3 mg/kg bw or 41.7 mg/kg bw, could aggravate the inflammatory reaction of the colon to pathogen exposure. They reported that κ-carrageenan modulated cytokine production, down regulated the proportion of T regs and up regulated NF-κB. According to the authors, these results suggested that κ-carrageenan acted as a potential inflammatory agent that could magnify existing intestinal inflammation. The Panel noted that the source of kappa carrageenan used in this study was not indicated. The authors stated that: 'the average molecular weight of 1×10^6 was used in this experiment', but no indication was given about the molecular weight distribution. The Panel also noted that in the absence of LPS stimulation, kappa carrageenan alone did not significantly modify the responses of the cells.

Overall, the Panel noted that the material used for most of the *in vivo* experiments presented was commercial carrageenan, which was however generally not further characterised in particular as regards the amount of low weight-average molecular weight carrageenan, in the region of 50 kDa. In addition, the Panel noted that carrageenan was given in drinking water, which was not representative of the dietary exposure where carrageenan is bound to protein. Therefore, the extent to which these

studies might be representative of the situation when carrageenan is used as a food additive, and consequently the possible use of these studies for the safety assessment of the food additive carrageenan (E 407) where, according to the EU specifications, the amounts of compounds with molecular weight below 50 kDa must be low (< 5%), was deemed limited.

The Panel also noted that intake of carrageenan has been reported to influence the composition of the gastro-intestinal microflora Nilson and Schaller, 1941; Poulsen, 1973; Shang et al., 2017). Interplays between the bacterial gut microflora and sulfated glycans (Mallett et al., 1985; Shang et al., 2016a,b) seem to play a significant role in the development of inflammation in the gut (Pomim, 2017). Because sulfate and undigested sulfur compounds have been implicated in the aetiology of ulcerative colitis (Pitcher and Cummings, 1996; Roediger et al., 1997; Magee et al., 2000; Kamada et al., 2013), the sulfate moiety of carrageenan might be involved in the proinflammatory effects (Pomin, 2015, Fahoum 2017) through the production in the colon of hydrogen sulfide from sulfate, by sulfate-reducing bacteria (Gibson, 1990). Finally, from the reported studies, the purity of the test material used appeared to be of primary importance, and the amount of low molecular weight carrageenan it contained likely influenced the capacity of the test material to induce some effects. However, there is still uncertainty about the 'safe' level of this low molecular weight material. These considerations raised the issue of how the samples used in the studies could be considered as representative of the food additive E 407 as sold in the market. The Panel could not conclude on the potential of carrageenan used as a food additive (E 407) to induce immunotoxic and/or inflammatory effects in the absence of relevant studies using well characterised carrageenan.

3.5.9. Other *in vivo* studies on the effects of carrageenan and degraded carrageenan on the gastro-intestinal tract

Rats

In two consecutive studies (Grasso et al., 1973; Grasso 1975), groups of Wistar rats (males and females) were exposed to carrageenan (5% in diet equivalent to 4,050 mg/kg bw per day) or degraded carrageenan (0.25%, 0.5%, 1% and 5% in drinking water equivalent to 225, 450, 890 and 4,450 mg/kg bw per day) for 21 or 56 days (commercial products for both types of carrageenan were from Glaxo Laboratories, Paris; interpreted by the Panel as isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0). Carrageenan was a fine cream-coloured powder prepared from *E. spinosum* (moisture content, 6%; viscosity of a 0.5% aqueous solution at 25°C, 22 cP; total heavy metals, 20 ppm) and degraded carrageenan was a fine cream-coloured powder prepared by controlled hydrolysis of carrageenan extracted from *E. spinosum* (moisture content, 5%; viscosity of a 0.5% aqueous solution at 25°C 7 cP; sulfate content, 33.2%; total heavy metals, 20 ppm). Rats developed diarrhoea with watery stools when given 5% degraded carrageenan. Only a slight diarrhoea, marked chiefly by faeces which were semi-solid in consistency, was observed in rats given 1% degraded carrageenan or 5% carrageenan. The whole of the GI tract was examined macroscopically for ulceration, but no adverse effects were noted. The Panel considered that the NOAEL for ι-carrageenan was 5% in the diet equivalent to 4,050 mg/kg bw per day, the highest dose tested.

Groups of 10 male and 10 female Sprague–Dawley rats were given either a 5% solution of degraded ι-carrageenan (C16; interpreted by the Panel as low weight-average molecular weight commercial product produced via mild acid hydrolysis of an extract of *E. spinosum* obtained from Laboratories Glaxo, France) as drinking water (daily intake of degraded carrageenan was 6–10 g/kg bw) or a dose of 5 g degraded carrageenan per kg bw in aqueous solution by gavage (once daily; 6 days/wk; 0.5–5 g/kg/bw per day) for 15 months (Fabian et al., 1973). Number-average and weight-average molecular weights for this carrageenan fraction were reported to be in the range of 16–19 kDa and 20–30 kDa, respectively. Groups of 5 rats/sex given distilled water as drinking fluid by gavage served as controls. Rats were necropsied at intervals ranging from 1 to 15 months. At 15 months, treatment of one female and one male which were given degraded carrageenan via drinking water or gavage stopped. These rats were necropsied at 17 and 16.5 months, respectively. After 6 months, squamous metaplasia of the rectal mucosa was reported, together with accumulation of metachromic material (presumed by the authors to be carrageenan) in macrophages. No lesions in the caecum were found, whereas ulcerative lesions in the distal colon could explain the occurrence of occult blood in the stools.

Germfree (n = 9) and conventional (n = 12) female Wistar rats were exposed to degraded carrageenan with a sulfate content of about 30% and an average molecular weight of 20–40 kDa (obtained from Glaxo Labs and interpreted by the Panel produced by mild acid hydrolysis of carrageenan; Hirono et al., 1981). No information on the type of carrageenan is reported. The

degraded carrageenan used in this study was reported as having an average molecular weight of 20–40 kDa. Degraded carrageenan was added at a level of 10% to the basal diet. The germfree rats were necropsied on day 7 (n = 3); day 20, 35 and 63 (n = 2). Three conventional rats (n = 3) were necropsied according the same schedule. Histopathological examination of Swiss-roll preparations of the intestines. Mucosal lesions associated with erosion of the large intestine induced by degraded carrageenan were much more extensive in germfree than in conventional rats.

Six pregnant Wistar rats received drinking water with 5% of degraded carrageenan as (Marcus and Watt, 1971). The carrageenan was derived from the red seaweed *E. spinosum* and was degraded by mild acid hydrolysis to retain a 29% sulfate content, interpreted by the Panel to be mainly ι-carrageenan. No indication about the weight-average molecular weight was given. Six pregnant rats received drinking water without degraded carrageenan. Twelve female offspring from each group were separated and the exposure to 5% degraded carrageenan via drinking water was continued for 6 months. Ulceration of the caecum was observed in 4 of the twelve treated rats. In two of the four rats, the ulceration was accompanied by inflammatory cell infiltration (polymorphonuclear cells and macrophages) and occasionally by glandular hyperplasia at the ulcer margins. In the remaining 2 rats, the ulcers were healed, the mucosa showing atrophy, distortion of glands and fibrosis of the lamina propria.

Degraded carrageenan derived from the red seaweed *E. spinosum* (obtained by mild acid hydrolysis to retain 30% sulfate content and an average molecular weight of 20–40 kDa; interpreted by the Panel to be mainly ι-carrageenan) was given to Sprague–Dawley rats (Wakabayashi et al., 1978). In experiment I, 4 groups of 30 males and 30 females was given a diet with 0%, 1%, 5% or 10% degraded carrageenan *ad libitum* for 24 months (equivalent to 0, 500, 2,500 or 5,000 mg/kg bw per day). In experiment II, 2 groups of 20 males and 20 females was given 0% or 5% aqueous solution of degraded carrageenan *ad libitum* as drinking water for 15 months (equivalent to 0 or 2,500 mg/kg bw per day). In experiment III, 3 groups of 15 males and 15 females were given 0, 1,000 or 5,000 mg degraded carrageenan/kg bw in aqueous solution by gavage for 15 months. Degraded carrageenan induced consecutively: colitis, secondary metaplasia, then tumours (squamous cell carcinomas, adenocarcinomas, adenomas). When degraded carrageenan was given by gavage, the incidence of malignant tumours was lower than when given by diet or drinking water. The Panel noted that this study has been taken into consideration in IARC's evaluation of carcinogenicity of low molecular weight degraded carrageenan (category 2B) (IARC, 1983).

Carrageenan derived from red seaweed *E. spinosum*, and degraded by acid hydrolysis to retain 30% sulfate content, and obtained from Glaxo, France (interpreted by the Panel to be ι-carrageenan; no indication about the weight-average molecular weight was given) was given to male Fischer 344 rats via the diet (10%) for varying periods of time: group I (n = 39) for 2 months (equivalent to 8,100 mg/kg bw per day); group II (n = 42) for 6 months and group III (n = 42) for 9 months (equivalent to 5,000 mg/kg bw per day) (Oohashi et al., 1981). The degraded carrageenan was reported as having an average molecular weight of 20–40 kDa. After termination of the administration on degraded carrageenan, all the rats were given the normal basal diet until 18 months. At that time all rats were autopsied. The controls (n = 46) received normal diet for 18 months. Colorectal squamous metaplasia (100%) and squamous cell carcinomas, anaplastic carcinomas and adenomas (incidence 5/39 animals) were seen after exposure to degraded carrageenan for 2 months. The incidence of colorectal tumours increased with exposure time and was 17/42 (40.5%) after exposure to degraded carrageenan for 9 months. The Panel noted that this study was taken into consideration in IARC's evaluation of carcinogenicity of low molecular weight degraded carrageenan (category 2B) (IARC, 1983).

Guinea pig

In two consecutive studies, Watt and Marcus (1969, 1971) exposed groups of 10 male adult guinea pigs (strain not given) exposed to a 1% drinking solution (not more than 1,500 mg/kg bw per day according to the authors) of ι-carrageenan (commercial product from Cumming & Son Ltd., Salford) via drinking water, or to 5% aqueous drinking solution of degraded carrageenan (commercial product from Laboratoires Glaxo-Evans, Paris; both types interpreted by the Panel as isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0) (not more than 2,000 mg/kg bw per day according to the authors) during 20 or 30 days. No indication about weight-average molecular weight was given. In animals exposed to ι-carrageenan, 2/4 showed ulcerative lesions in the caecum after 20 days and the other 6 animals had lesions after 30 days. No such effects were seen in controls (drinking water). In animals exposed to degraded ι-carrageenan, most of the animals showed looseness of the stools by the end of 10 days; from 20th to the 30th days, all had occult blood in the faeces. Incidence of

ulcerative colitis was 100%. In five animals killed between the 20th and 25th days the lesions were mainly in caecum, while in the remaining animal, killed between the 26th and 30th days, ulceration had extended into the lower colon and rectum. The ulcerations involved mainly the mucosa and showed features of both acute and subacute inflammatory infiltration as well as crypt abscesses.

Multiple caecal ulcerations were noted in guinea pigs (strain not given) dosed with 5% (about 2,000 mg/kg bw per day) carrageenan (mainly ι carrageenan; interpreted by the Panel as isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0) over 2–4 weeks via diet (Sharratt et al., 1970). No indication about the weight-average molecular weight was given. Sequential studies showed that the lesion first develops as an accumulation of macrophages in the lamina propria and subsequently in the submucosa leading to the formation of pale raised areas which can easily be seen macroscopically. Ulceration of the mucosa then occurs, particularly in these areas. The ulcers were small and superficial and affected only the mucosa. A mixed cellular infiltrate, consisting predominantly of macrophages accompanied by polymorphonuclear, lymphocytes, and plasma cells, surrounded the ulcerated area.

In two consecutive studies (Grasso et al., 1973, 1975), white guinea pigs (males and females) were exposed to carrageenan (5% in diet equivalent to 5,900 mg/kg bw per day for rats) or degraded (0.25%, 0.5%, and 2% in drinking water equivalent to approximately 300, 600 and 2,500 mg/kg bw per day) carrageenan for 21–45 days. For both types, commercial products from Laboratoires Glaxo, Paris; interpreted by the Panel as isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0). No indication about the weight-average molecular weight of the tested compound was given. Details of the compounds used are reported in the rat studies described above (Grasso et al., 1973, 1975). Groups of four male animals were exposed to 1% degraded carrageenan in drinking water during 3 weeks and sacrificed week 4, 7, 11, 15. Multiple pin-point caecal and colonic ulcerations were developed after 3–5 week of treatment in guinea pigs given 5% carrageenan in the diet or 2% degraded carrageenan in the drinking water. Ulceration was not observed in the animals receiving degraded carrageenan 0.25% in drinking water. Ulcers appeared as extensive macrophage infiltration at the base, cover a thin layer of fibrin; polymorphonuclear cells and lymphocytes occurred in considerable numbers. Macrophages, polymorphonuclear and lymphocytes deeply infiltrated the epithelium around the ulcer. In some animals, microabscesses were formed close to the ulcerated areas due to substantial polymorphonuclear cell infiltration. No caecal or colonic ulceration was observed in animals sacrificed 1–4 weeks after the end of the treatment, but distinct granulomas with evident carrageenan within the macrophages were observed. In animals scarified at a later stage, the histology of the caecum and colon was unchanged compared to the controls, and no carrageenan could be shown in the macrophages within the lamina propria.

Guinea pigs (8 females of the Hartley strain, average weight 360 g) given 2% partially degraded i-carrageenan (C16) *(*commercial products from Glaxo); interpreted by the Panel as isolated from *E. spinosum* with an approximate ratio of ι:κ:λ type 100:0:0) in sterile distilled drinking water for 2 weeks developed caecal ulceration (Abraham et al., 1974). No indication about the weight-average molecular weight was given. This effect was not observed when the same level of C16 was given in milk, or when lower levels of C16 (0.02% or 0.2%) were given in drinking water for 12 or 10 months, respectively. The authors reported that the ability of macrophage lysosomes in the lamina propria of the guinea pig to endocytose and store C16, which was not seen in the other species, was apparently closely related to the caecal ulceration observed in guinea pigs species.

Engster and Abraham (1976) compared the caecal response of various degraded carrageenan (Marine colloids, 7 ι-fractions of number-average molecular weight between 5 and 145 kDa from *E. spinosum*, 3 κ-fractions of number-average molecular weight between 8.5 and 314 kDa and 3 λ-fractions of number-average molecular weight between 20.8 and 275 kDa from *C. crispus*) administered to female guinea pigs as a 1% solution in the drinking water for 2 weeks. Six ι-fractions were also given to female guinea pigs in the diet at a 2% level for 10 weeks. When administered in the drinking water, all ι-fractions, with the exception of those of lowest (5 kDa) and highest (145 kDa) number-average molecular weights, were absorbed and inflammation, erosion or ulceration were observed in the caecum. By contrast, κ- and λ- carrageenan fractions given in drinking water or ι-fractions given in the diet for 10 weeks produced no caecal damages. According to the authors, these results indicated that caecal ulceration in the guinea pig was caused only by certain molecular weight ι-fractions and when administered in drinking water.

Rabbits

Twenty male Californian rabbits (average body weight 2,950 g) received drinking water with 0, 0.1, 1 or 5% degraded carrageenan derived by mild acid hydrolysis of i-carrageenan from *E. spinosum*

(Glaxo, France, retaining about 29% sulfate) for 6–12 weeks (Watt and Marcus, 1970). No indication about the weight-average molecular weight was given. Animals fed degraded carrageenan at 5% concentration in their drinking water received an average dose of 1,400 mg/kg bw). Diarrhoea associated with occult blood in the faeces developed by the end of 7 days and persisted. All animals of this group showed severe ulceration of the colon. One rabbit fed degraded carrageenan at 1% (average daily dose of 800 mg/kg bw) over a 7-week period developed diarrhoea, occult blood in the faeces was present in all animals after 2 weeks. All animals showed moderate ulceration in the colon. Animals fed 0.1% degraded carrageenan (daily dose of 70 mg/kg bw) for 12 weeks, developed no diarrhoea but occult blood was seen in three rabbits by the end of week 10. Multiple ulcers were found in the colon in three of five rabbits.

Fifteen mature rabbits (not further specified) weighing about 2,200 g and sensitised intramuscularly with 1 mg λ-degraded carrageenan in 1 mL, were administered λ-degraded carrageenan (approximately 30 kDa; not further specified) dissolved in drinking water at a concentration of 1%. No indication about weight-average molecular weight of the tested compound was given. Ten rabbits were given the substance continuously for 12 months and the remaining five were treated for 28 months. They were sacrificed at the end of the treatment period. Histopathological examination showed mild inflammatory changes of the colonic mucosa in all animals and a focal high-grade dysplasia involving the mucosal epithelium in 3/5 animals treated for 28 months. Tumours of the colorectum were not observed (Kitano et al., 1986).

Overall, degraded carrageenan administered in water is known to be a model for the development of colitis in various animal species (Elson et al., 1995). Degraded ι-carrageenan given in drinking water or diet induced ulceration in the caecum and colon of rats, guinea pigs and rabbits. A mixed cellular infiltrate consisting predominantly of macrophages, polymorphonuclear cells, lymphocytes, and plasma cells, surrounded the ulcerated area. Preulcerative changes consisted mainly in accumulation of macrophages in the lamina propria and they were associated with the presence of material that was assumed to be degraded carrageenan in the subepithelial tissue. Rats exposed to degraded ι-carrageenan (weight-average molecular weight reported as 20–30 kDa, or average molecular weight as 20–40 kDa) via drinking water, diet or by gavage developed in first instance colitis, secondary metaplasia and finally tumours (squamous cell carcinomas, adenocarcinomas, adenomas).

3.5.10. Studies reporting other biological effects of carrageenan and degraded carrageenan

In vitro studies

κ- ι-carrageenan (E 407) or a semi-refined κ- (processed Eucheuma seaweed derived from *E. cottonii*) were added to cultured rats hepatocytes and haematopoietic progenitors at concentrations of 0, 1, 10 or 100 μg/L (Parent-Massin et al. 1993). After 20 h of treatment, the morphology and the levels of protein content of hepatocytes, and the lactate dehydrogenase activity revealed no difference compared with the control values for each compound tested. Proliferation of granulo-monocytic progenitors (CFU-GM) was not changed in the presence of κ-processed Eucheuma seaweed and κ- refined carrageenan, while ι- carrageenan induced a light delay in proliferation of haematopoietic progenitors at the highest concentration tested. The authors concluded that these compounds, as well as refined (E 407) and semi-refined (processed Eucheuma seaweed) had no effect in rat hepatocytes *in vitro* and in the rat haematopoietic progenitors clonogenic assay.

Bhattacharyya et al. (2008a) reinvestigated the ability of carrageenan to induce cell death and to decrease cell proliferation, using cells of the NCM460 line derived from normal colonic mucosa, and primary human colonic epithelial cells. λ-carrageenan (from Sigma-Aldrich of unknown source) was used for most of the experiments; cell proliferation was also assessed using commercial κ-carrageenan and ι-carrageenan, as well as degraded carrageenan of molecular weight less than 5 kDa. No indication about the weight-average molecular weight was given. Cells were exposed for 1–8 days to a concentration of 0, 1 or 10 μg/mL of each type of carrageenan. The treatment of cells with λ-carrageenan increased cell death, reduced cell proliferation and caused cell cycle arrest, compared with control cells. There was no indication of apoptosis, and cell death was attributed to necrosis. Different types of carrageenan lead to similar results on cell proliferation. Apart from a dose-related increase in cell death, the results showed comparisons only between controls and 1 μg/mL of λ-carrageenan, therefore it was not possible to determine whether other parameters were also affected in a dose-related manner.

Zainal Ariffin et al. (2014) investigated the cytotoxicity of degraded carrageenan (no further information) and κ- and ι-carrageenan in human intestine and liver cell lines. No indication about weight-average molecular weight of the tested compound was given. Degraded carrageenan was found to inhibit cell proliferation in Caco-2, FHs74 Int, HepG2 and Fa2N-4 cell lines, and the antiproliferative effect was related to apoptosis together with inactivation of cell proliferation genes as determined by morphological observation and molecular analysis. No cytotoxic effect was found with carrageenan towards normal and cancer intestine and liver cell lines.

In the study by Shahrul Hisham et al. (2014), food-grade κ-carrageenan, dried sheet κ-carrageenan, commercial grade κ-carrageenan, food-grade ι-carrageenan and commercial grade i-carrageenan were dissolved in hydrochloric acid and water in order to prepare degraded carrageenan and carrageenan, respectively. The effects of these compounds were assessed using various normal and cancer human intestinal cell lines. Degraded κ-carrageenan inhibited cell proliferation in Caco-2, FHs 74 Int, HepG2 and Fa2N-4 cell lines; on the contrary, carrageenan was not cytotoxic to normal and cancer intestine and liver cell lines.

In vivo studies, including studies in juvenile animals

Mice

Bhattacharyya et al. (2012) investigated a possible relationship with markers related to the development of diabetes following observations on the interaction of carrageenan with TLR4, as systemic inflammation in Type 2 diabetes might be mediated by the TLR4 pathway. Groups of six male mice were administered carrageenan (commercial κ-λ-type, no indication on weight-average molecular weight was given.) in drinking- water at 0 or 10 μg/mL (equal to 1.7 mg/kg bw per day). A glucose tolerance test (GTT) and an insulin resistance test (ITT) were performed in the same mice at 12 and 14 weeks of age, respectively. No treatment-related effects on body weight or water consumption were observed. In the GTT, blood glucose was statistically significantly increased by carrageenan at all time points. In the ITT, blood glucose levels were statistically significant decreased by more than 80% in controls, but by only 43% in the carrageenan-treated mice, at all time points.

The Panel agreed with JECFA who noted a number of limitations in this study: 'the animals underwent an 18-h overnight fast, which is a considerable length of time for mice that are nocturnal feeders and is known to enhance reactions to insulin in mice (Ayala et al., 2010); measurements of blood glucose extended to only 90 min after dextrose injection rather than 120 min, which is recommended as one of the critical time points for comparisons between treated and control groups' (Ayala et al., 2010 as cited in JECFA, 2015).

According to the results of a 1-year study in mice (Bhattacharyya et al., 2015), exposure to carrageenan may lead to fasting hyperglycaemia and in combination with high fat diet exacerbation of glucose intolerance and hyperlipidemia without effect on weight. No indication about the weight-average molecular weight was given.

Rats

Harmuth-Hoene and Schelenz (1980) investigated the effect of polysaccharides, including carrageenan (unspecified type; interpreted by the Panel as isolated from *C. crispus* with an approximate ratio of ι:κ:λ type 0:70:30; Copenhagen Pectin Factory Ltd), given at the 10% level in a semi-synthetic diet (equivalent to 1,200 mg/kg bw per day), on absorption of Ca, Fe, Zn, Cu, Cr and Co, on weight gain and on faecal dry matter excretion, in five groups of 12 weanling male rats each and compared to a control group, over a period of 8 days. No indication on weight-average molecular weight was given. Carrageenan reduced significantly the absorption of all minerals tested and increased both their faecal excretion and that of faecal dry matter.

Minipigs and pigs

A toxicity and toxicokinetic feeding study has been performed on carrageenan in neonatal minipigs and neonatal pigs in accordance with GLP and based on current guidelines and guidance documents for preclinical juvenile studies for drugs (Documentation provided to EFSA n. 32; Documentation provided to EFSA n. 33; also described in Weiner et al., 2015) (for details of the protocol see section 3.5.7. Some animals did show minor findings, such as faecal changes that are not uncommon at this age. The results of the urinalysis did show glucosuria, which was limited to a few animals at 2,250 mg/kg group (1/6 males, 3/6 females). This observation was not considered to have had an adverse impact on the animals affected, as supported by a lack of adverse changes, which included

clinical findings comparable to controls, and good growth and development during the dosing period. In addition, the other functional clinical parameters, including blood glucose, were unaffected by treatment at this dose level and the microscopic evaluation of the kidneys and other tissues did not reveal any changes indicative of the treatment related effect. Therefore, the finding of glucosuria was not considered to be toxicologically meaningful by the authors. The Panel considered the highest dose tested 430 mg/kg bw per day as the NOAEL. The Panel noted that the test compound complied with the EU specifications and noted that glucosuria was also observed in other studies (Bhattacharyya, 2015; USDA, 2016) and needs clarification.

In a piglet model, induction of colitis using 1% carrageenan solution caused intestinal bacterial dysbiosis according to the authors (Munyaka et al., 2016). The solution used in this study was prepared by acid hydrolysis was supposed to contain carrageenan of average molecular weight of Mn 2×10^4 to 3×10^4 as described by the authors. The authors reported that 'certain bacterial shifts observed in the distal GI tract as a result of CG treatment are consistent with previous findings in patients with intestinal bowl disease'. Bacterial diversity was significantly influenced by carrageenan treatment. Especially in the caecum, ascending, and descending colon notable microbial changes in the percentages of the major phyla Firmicutes, Bacteroidetes, and Proteobacteria were induced. Weiner et al. (2017) criticised the description of the carrageenan used in this study and stipulated that the carrageenan used should be mentioned 'degraded carrageenan with a weight average molecular weight (MW) of 20,000–40,000 Da'.

Monkeys

Three groups of three males and five females infant baboons (*Papio cynocephalus*) received infant formula containing nominal concentrations of 0, 300 and 1,500 mg carrageenan/l from birth to 112 days of age (McGill et al., 1977), being the estimated intake about 86 and 432 mg/kg bw per day. The test material was described as native carrageenan (no indication about weight-average molecular weight), it was heated in the formula and bound to protein to stabilise the suspension of fat, protein and other nutrients. Baboons were fed formula five times per day for the first 14 days, four times per day for the next 14 days, three times per day for the next 56 days and twice per day for 28 days until 112 days of age. No other food or fluid were given to the animals. The baboons were observed daily and the body weight was measured daily. Stools were checked weekly for occult blood. Blood samples were taken monthly, and organ weights were measured at necropsy. On the night before necropsy, each baboon was given water only *ad libitum*. At necropsy under anaesthesia, the GI tract was removed and fixed in 10% buffered formalin. The gut was observed minute-by-minute as fresh and after fixation. Samples were taken for both histological section and for microscopic analysis (from all major viscera, muscle, lymph nodes and the central nervous system). Microscopic examination of the colon showed reddening areas beneath the epithelium and to a lesser degree deeper in the *lamina propia* on which in few instances, deposits of granular brown pigment representing hemosiderin were reported. Occult blood in the faeces was also reported. Although the authors could not explain the cause or origin of the occult blood in faeces they concluded that it was not associated with carrageenan content of the formula. There were no effects on: organ or body weights, urine and faeces, haematological or clinical chemical variables. Hyperplasia of lymphatic tissue and the inflammatory lesions of the colon were present in all animals, regardless of the presence or concentration of carrageenan. The authors attributed these findings to possible antigenic stimulation by exogenous milk proteins.

3.5.11. Human data

Infants and children

Sherry et al. (1993; letter to the journal editor) assessed whether there was a correlation between the frequency of symptomatic upper respiratory tract infections and the type of formula during the first 6 months of life in full term-infants. No indication about weight-average molecular weight was given. In this study, 1,269 infants given liquid formula containing carrageenan at 300 mg/L (0.03%) were compared with 149 infants given powder-based formula without carrageenan. A slightly higher proportion of infants (53.4%) given liquid infant formula containing carrageenan were illness-free during the first 6 months of life compared to those receiving powder-based formula without carrageenan (47.6%). A slight difference in the number of months in which at least one upper respiratory tract infection occurred was also found in favour of the infants receiving liquid formula (0.9 month) compared to powdered formula (1 month). However, the differences between the two groups

were no statistically significant. Based on the results, the authors suggested that the carrageenan-containing liquid infant formula was not immunosuppressive. The Panel considered that the details of the study reported in the letter to the journal were too limited to draw a reliable conclusion.

Another masked, randomised, parallel growth and tolerance study was conducted on healthy infants (aged 0–9 days at the time of enrolment) during the first four months of life (Borschel et al., 2002). A total of 195 infants were given, from enrolment until 112 days of age, extensively hydrolysed casein–based formulas in two different forms: powdered (PWD) that did not contain carrageenan (95 infants), and liquid ready-to-feed (RTF) containing carrageenan (100 infants). No indication about the weight-average molecular weight was given. One hundred and thirty-seven infants completed the study, whereas 58 exited the study in early stages. Among these, 21 subjects from the RTF group and 16 from the PWD group left the study due to intolerance symptoms. Intake, stool patterns and anthropometric measurements (e.g. weight, length and head circumference) were monitored at entry and on days 14 (only anthropometric parameters), 28, 56, 84 and 112 of the study. Overall, no statistically significant differences between the two groups were reported in weight gain, length, head circumference or tolerance to the formulas.

Adults

Bonfils (1970) did not report any evidence of additional gastrointestinal disease in any of the 200 patients with peptic ulcers treated with Ebimar (degraded carrageenan). In a further experiment designed to determine whether degraded carrageenan is absorbed from the colon in patients with ulcerative colitis, 10 such patients were given 10 g Ebimar for 10 days, equivalent to twice the therapeutic dose. No absorption could be demonstrated at this dosage and regular sigmoidoscopic examinations revealed no adverse changes with respect to colonic ulceration or mucosal bleeding. The author concluded that, based on his 12 years experience of the clinical use of degraded carrageenan, as well as the toxicological and pharmacological investigations he carried out, in human beings the therapeutic use of degraded carrageenan is free from side-effects and from risk of toxicity to the colon.

No adverse effects have been described after pharmaceutical uses of degraded carrageenan.

A randomised, double-blind, placebo-controlled, multicenter, clinical trial was conducted to assess if patients with ulcerative colitis in remission would have a longer interval to relapse if they followed a diet without carrageenan (Bhattacharyya et al., 2017). No indication about the weight-average molecular weight of the tested material was given. It is reported that most of the food-grade carrageenan is comprised of κ- and λ-carrageenans of molecular weight 200–800 kDa. Twelve patients completed the study questionnaires. There was relapse in three patients who received carrageenan-containing capsules. None of the patients who received placebo-containing capsules relapsed ($p = 0.046$, log-rank test). Laboratory tests showed increases in faecal calprotectin ($p = 0.06$; paired t-test, two-tailed) and interleukin-6 ($p = 0.02$, paired t-test, two-tailed) between the beginning and the end of study participation in the carrageenan-exposed group, but not in the placebo group. The authors concluded that carrageenan intake contributed to an earlier relapse in patients with ulcerative colitis in remission. The Panel noted that the study is of limited relevance due to the low number of patients involved.

3.6. Discussion

The present opinion deals with the re-evaluation of the safety of food-grade carrageenan (E 407) and processes Eucheuma seaweed (E 407a) used as food additives.

According to the Commission Regulation (EU) No 231/2012[1], 'carrageenan (E 407) is obtained by extraction with water or dilute aqueous alkali of strains of seaweeds of *Gigartinaceae*, *Solieriaceae*, *Hypneaceae* and *Furcellariaceae*, families of the class Rhodophyceae (red seaweeds). It consists chiefly of the potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The prevalent polysaccharides in carrageenan are designated as κ-, ι-, λ- depending on the number of sulphate by repeating unit (i.e. 1,2,3 sulphate)'.

Processed Eucheuma seaweed (E 407a) is, according to the Commission Regulation (EU) 231/2012, 'obtained by aqueous alkaline (KOH) treatment at high temperature of the strains of seaweeds *E. cottonii* and *E. spinosum*, of the class *Rhodophyceae* (red seaweeds) followed by fresh water washing to remove impurities and drying to obtain the product' complying with the EC specifications. Up to 15% algal cellulose is also present in the product. The main polysaccharide is κ-carrageenan.

Because of the structural similarities of processed Eucheuma seaweed and the conventionally processed food-grade carrageenan and the similarities of effects they caused in the comparative study (Documentation provided to EFSA n. 38), the Panel concluded that the re-evaluation of processed Eucheuma seaweed (E 407a) can be included in that of food-grade carrageenan (E 407).

According to one of the interested parties (Documentation provided to EFSA n. 19), carrageenan has a molecular weight distribution from 30 kDa to as high as 5,000 kDa and is defined as having a weight-average molecular weight between 200 and 800 kDa. Furthermore, it has been confirmed by industry (Documentation provided to EFSA n. 19), that commercial carrageenan (E 407) may have a weight-average molecular weight as low as 200 kDa. In view of the Panel, the molecular weight distribution of such a carrageenan product may have a considerable fraction of molecules encompassing weight-average molecular weight of degraded carrageenan.

The Panel noted that in the literature the information on the molecular weight in the individual carrageenan preparation is often unprecise mixing the terms 'molecular weight', 'weight-average molecular weight' and 'number-average molecular weight'.

Uno et al. (2001a), in a survey of 29 samples of food-grade carrageenan representing κ-, ι- and λ-carrageenan determined a number-average molecular weight of 193–324 kDa and a weight-average molecular weight of 453–652 kDa. The molecular weight distributions of 29 samples of food-grade purified carrageenan were studied by combined gel permeation/inductively coupled plasma (GPC/ICP) method and 'no obvious peak of poligeenan was detected'. According to Uno et al. (2001a), the 'detection limit' for poligeenan was about 5% in the sample of carrageenan.

Validated analytical methods to accurately measure the low molecular weight distribution of carrageenan appear to be not fully developed or available to all industries (Cohen and Ito, 2006; Documentation provided to EFSA n. 15). According to information from one of the interested parties (Documentation provided to EFSA n. 19), 'in native carrageenan, this LMT region represents less than 10% of the total carrageenan molecule…Although there is a Commission specification of not more than 5% at less than 50 kDa, in practice there is no validated analytical method that can accurately quantify the percent of LMT present'.

The Panel noted that, according to the interested parties, an inter-laboratory validated analytical method to accurately measure the low molecular weight distributions of carrageenan is not fully developed or available at present.

The debate in literature with respect to the safety of native (undegraded) carrageenan has been related to the presence of degraded carrageenan. Degraded carrageenan is also known in forms of artificially produced product from carrageenan (e.g. poligeenan, C16) associated with adverse effects. Poligeenan is a much lower weight-average molecular weight polymer (10–20 kDa) generated by subjecting ι-carrageenan to the extreme conditions of acid hydrolysis at low pH (0.9–1.3) and high temperatures (> 80°C) for several hours (Cohen and Ito, 2006; McKim, 2014; JECFA, 2015; USDA, 2016). C16 is artificially formed from ι-carrageenan under conditions of acidic hydrolysis with 0.1 M sulfuric acid, at 60°C for 1.5 h (Glaxo Lab). Contradictory statements in the literature on carrageenan/degraded carrageenan stem largely from inadequate descriptions of the experiments performed and the results obtained in these experiments, and lead to confusion (Benitz et al., 1973).

The Panel emphasised that degraded carrageenan (e.g. poligeenan or C16) has not been authorised as a food additive in the EU. Information available from the US indicated that poligeenan is not used in any food applications (USDA, 2016). The Panel further noted that the term 'poligeenan' has been used in literature including test protocols in a broader sense. However, more specifically poligeenan is a breakdown product of ι-carrageenan consisting of the same sulfated galactose and sulfated anydrogalactose units, linked by α-1,3- and β-1,4-glycosidic bonds as in all types of carrageenan (see Figure 1). According to Figure 2 (Documentation provided to EFSA n. 18), poligeenan comprises polysaccharide molecules of molecular weight ranging from approximately 2 to 200 kDa, and the weight-average molecular weight of poligeenan ranges from 10 to 20 kDa. The Panel noted that based on the data provided (Documentation provided to EFSA n. 18) the overlapping between the low molecular weight tail of carrageenan and high molecular weight tail of poligeenan is increasing with the decrease of the weight-average molecular weight of the carrageenan preparation.

The Panel noted that in most toxicity studies no adequate information on the molecular weight distribution of carrageenan was available. Therefore, it was not possible to assess if the carrageenan tested complied with the EU specifications (specially the limit of low molecular weight carrageenan < 5% below 50 kDa). Furthermore, the Panel noted the methodological limitations of *in vivo* studies, in which carrageenan was given in drinking water, which is not representative of the dietary exposure where food-grade carrageenan will be bound to protein. Therefore, the extent to which drinking water

studies might be representative for food-grade carrageenan used as a food additive and consequently of the relevance of these studies for its safety assessment was deemed limited.

In several *in vivo* studies in rats, guinea pigs, minipigs, pigs and monkeys faecal excretion of high molecular weight carrageenans were shown to be 98–100% of the ingested amount. These studies included rats with normal gut microflora and rats with their gut microflora replaced by human faecal microflora. Several short-term and long-term studies performed in rats dosed via diet containing different high molecular weight types of carrageenan confirmed the absence of tissue storage of these types of carrageenan. However, degraded carrageenan like C16 or low molecular weight carrageenan (number-weight molecular weight of 88 kDa or less) have been described to be absorbed and to be present in various tissues, namely the liver, and the urine of animals given degraded carrageenan, mainly when given in drinking water but also when administered via the diet. Due to the high percentage of administered high weight-average molecular weight carrageenan recovered in faeces, its degradation to lower weight-average molecular weight carrageenan should be very limited. The Panel noted that the conditions (duration, pH, temperature) for potential hydrolysis of carrageenan in the GI tract are less extreme than the 'mild' acidic conditions which resulted in the formation of chemically degraded carrageenan (C16). The theoretical possibility that more limited degradation could occur under conditions representative of the *in vivo* situation remains an uncertainty although this has not been observed to any significant extent in the *in vivo* ADME studies reported.

The Panel further noted that the tested carrageenan differed in their molecular weight distribution, and the Panel considered that existing results did not allow to evaluate kinetic differences between the different types of native carrageenan (κ-, λ- and ι-) and their corresponding low weight-average molecular weight fractions.

The acute toxicity of carrageenan was low.

Short-term and subchronic administration of carrageenan was well tolerated and induced no adverse effects in the gastrointestinal tract of mice, rats, guinea pigs, pigs and monkeys in the majority of studies. However, one study in rats (Morgan and Joiner, 1975) receiving commercial carrageenan reported diarrhoea in all dosed animals accompanied by the excretion of bloody stools in male and female rats at the highest dose tested. The Panel considered 4,500 mg/kg bw per day as the NOAEL for short-term and subchronic administration of carrageenan and processed Eucheuma seaweed. This was the highest dose tested in a subchronic oral toxicity study in rats. Also in a 90-day study in rats, performed according to the OECD guidelines with κ-carrageenan with an average molecular weight range of 196–257 kDa (not specified if number- or weight-average molecular weight) almost complying with the EU specifications (LMT of 1.9–12% (mean 7%) < 50 kDa) the NOAEL was the highest dose tested (3,394 mg/kg bw per day in males, and 3,867 mg/kg bw per day in females) (Weiner et al., 2007).

Monkeys given degraded ι-carrageenan (C16) for 7–14 weeks in drinking water showed histopathological lesions in the colon, which varied from slight mucosal erosions at the low dose (750 mg/kg bw per day) to ulceration associated with inflammatory infiltration of the lamina propria at the high dose (2,900 mg/kg bw per day). In this study, all monkeys on C16 lost blood frequently from the intestinal tract in a dose-related degree and developed some degree of anaemia (Benitz et al., 1973). The Panel considered 750 mg degraded ι-carrageenan (C16)/kg bw per day the LOAEL. Guinea pigs given degraded ι-carrageenan via drinking water developed caecal ulceration. This was not seen when degraded ι-carrageenan was given via the diet. Fractions of degraded κ- and λ-carrageenan given to guinea pigs either via drinking water or diet did not cause gastrointestinal tract damages. The Panel noted, that based on the 'nearly identical molecular structure as carrageenan' and 'similar weight-average molecular weight' poligeenan has been used in experimental studies, as a surrogate for the LMT of carrageenan (Weiner et al., 2015).

Carrageenan (different types) has been tested for genotoxicity in several *in vitro* and *in vivo* test systems. For calcium carrageenan severe adverse effects (aberrations in the anaphase chromosomes of human embryonic lung WI38 cells) were reported in an *in vitro* study. However, when tested *in vivo* (host-mediated assay with male mice and dominant lethal test in rats), calcium carrageenan was considered to be non-mutagenic. The Panel considered that there was no evidence for a genotoxic potential of carrageenan.

Processed Eucheuma seaweed derived from either *E. cottonii* or *E. spinosum* was not mutagenic in a bacterial reverse mutation assay. The negative studies (bacterial reverse mutation test, bone marrow micronucleus test, host mediated assay) performed by Sylianco et al. (1993) were considered by JECFA to be inadequate. However, JECFA concluded that no further studies of genotoxicity were required. The Panel agreed with this conclusion.

Degraded carrageenan showed no evidence of unscheduled DNA synthesis in studies for genotoxicity using DNA repair tests with cultured rat hepatocytes and intestinal mucosal cells. Degraded carrageenan was not mutagenic in the Salmonella mutagenicity test. These observations suggested that the carcinogenicity of degraded carrageenan observed in two rat studies (Wakabayashi et al., 1978; Oohashi et al., 1981) and leading to an IARC classification 2B (IARC, 1983), was most probably due to a non-genotoxic (thresholded) mechanism (Mori et al., 1985).

Overall, the Panel concluded that carrageenan and processed Eucheuma seaweed did not raise a concern with respect to genotoxicity. The Panel further noted that degraded carrageenan was not of genotoxic concern; this further supported that the food additives E 407 and E 407a, even if they contain some low molecular weight carrageenan, were not of genotoxic concern.

Several chronic toxicity studies in rats have been performed with carrageenan (mostly κ/λ-type, molecular weight distribution not adequately described). From the available rat studies, NOAELs up to 7,500 mg/kg bw per day, the highest dose tested, were identified (Nilson and Wagner, 1959). However, in another study in rats given carrageenan preparations with a molecular weight of 244 kDa and 252 kDa, a LOAEL of 1% in the diet (equivalent to 500 mg/kg bw per day) was identified by the Panel (Documentation provided to EFSA n. 43). The Panel noted that the characterisation of the test material in all the chronic studies was limited.

In Rhesus monkeys dosed with a commercial carrageenan composed of κ/λ-carrageenan types at a ratio of 70:30 (from *C. crispus*) amounting up to 500 mg/kg bw per day for 7.5 years, occult blood was observed in treated animals (Abraham et al., 1983). Furthermore, faeces of monkeys given carrageenan were found to contain degraded carrageenan. Since the faeces of monkeys given carrageenan were found to contain degraded carrageenan, the Panel noted that it cannot be excluded that the presence of occult blood in animals is treatment-related.

Overall, the Panel considered that the information available suggested that the colon haemorrhagic properties reported in some studies having tested carrageenan, could be associated with the presence of low molecular weight carrageenan in the test preparation. Therefore, the particular low molecular weight range of degraded carrageenan should be avoided in the specifications for the food additives carrageenan (E 407) and processed Euchema seaweed (E 407a).

From the results of the chronic toxicity studies, which were mostly conducted with κ/λ-carrageenan, the Panel considered that food-grade carrageenan (E 407) was not carcinogenic.

In dietary prenatal developmental studies, no developmental effects were observed at 3,000 and 5,000 mg/kg bw per day for sodium or calcium carrageenan (κ/λ-type) for rats and hamsters (Documentation provided to EFSA n. 46), and at 3,060 mg calcium carrageenan/kg bw per day for rats (Collins et al., 1977a), the highest doses tested. The Panel noted that the NOAEL for reproductive effects of calcium κ/λ-carrageenan, (no indication of molecular weight distribution) observed in a dietary three-generation reproductive toxicity study in rats was 3,212 mg/kg bw per day, the highest dose tested, and 290 mg/kg bw per day for parental and developmental effects, based on decreased parental body weight gain and pup weight (Collins et al., 1977a). Reproductive and neurodevelopmental effects were not observed in one-generation study in rats up to doses of 2,600 mg sodium carrageenan/kg bw per day, the highest dose tested (Vorhees et al., 1979).

The Panel noted that testing for chronic toxicity and reproductive and developmental toxicity was performed almost exclusively with κ/λ-carrageenan of which the molecular weight distribution was not or not adequately described. That ι-carrageenan is rarely characterised for these endpoints is noted against the well known inflammatory effects of degraded ι-carrageenan, which was shown to induce colitis and finally tumours in the rat.

In a well-conducted feeding study with κ/λ- carrageenan (test substance (ratio κ/λ unknown), fully characterised for molecular weight, percentage of molecular weight below 50 kDa, known as LMT as per European requirements) in neonatal pigs (Domestic Yorkshire Crossbred Swine, 6 males, 6 females per group), administration in piglet-adapted formula from lactation day 3 for 28 consecutive days), the Panel identified a NOAEL of 430 mg/kg bw per day, the highest dose tested. The weight-average molecular weight of the test material sample was 664–732 kDa with a LMT of 0.3–3.9%. No effects were reported on various parameters exploring the immune system (Documentation provided to EFSA n. 33; Weiner, 2015).

In two brief reports on studies in human infants, given either a formula containing carrageenan at 300 mg/L or a formula for special medical purposes containing carrageenan at 1,000 mg/L, no differences in comparison to the control groups were described.

The available database on processed Eucheuma seaweed was limited to the endpoints of subchronic toxicity and genotoxicity but was sufficient for the evaluation of the similarities between processed Euchema seaweed and food-grade carrageenan.

In 1974, JECFA used the results of a chronic (life-time) dietary study in rats (Nilson and Wagner, 1959) to allocate an ADI of 75 mg/kg bw per day based on a NOAEL of 15% carrageenan in the diet (equal to 7,500 mg/kg bw per day). In 1978, the SCF endorsed this value and confirmed it in the latest evaluation in 2003. The Panel, however, noted several flaws in that study (use of different lots of carrageenan, presence of high levels of arsenic in the test material), and lack of agreement with the current guidelines for long term studies used for the hazard characterisation.

The Panel noted that the α-1,3-galactosidic linkage, which is present in all the carrageenan molecules is specifically recognised by human anti-Gal IgG and IgM antibodies. Binding of these natural antibodies to this saccharide epitope may lead to an immune reaction and, for instance, are involved in acute rejection by humans of pig xenografts (Galili et al., 1985; Thibaudeau et al., 1996; Klein et al., 1998). The Panel noted that these antibodies developed with age in humans; they are absent in the neonates but develop within a few months, as soon as the newborn's gastrointestinal tract becomes colonised by microorganisms. The Panel noted that despite the presence in human serum of antibodies capable to recognise saccharidic epitopes on the carrageenan molecules, no case reports of significant allergic and/or anaphylactic reactions after ingestion of foods containing carrageenan were identified in the available literature.

The Panel noted that acid degraded ι-carrageenan (C16 and poligeenan), which are clearly different from the food-grade additive E 407, have been often used to induce inflammations and ulcerations in the intestinal tract of rats, guinea pigs and rabbits, in order to develop an experimental model of human inflammatory bowel disease (Watt and Marcus, 1971; Elson et al., 1995; Martino et al., 2017).

Tobacman (2001) published a review on the association between exposure to carrageenan and the development of colonic ulcerations and gastrointestinal neoplasms in animal experiments. This author suggested that (native) undegraded carrageenan can be degraded to 'poligeenan' via acid hydrolysis in the stomach or by enzyme digestion in the intestines or by the microbiota of the gut. Critiques of the paper noted that Tobacman ascribed results of animal feeding studies with poligeenan to carrageenan and disregarded how the method of administration could affect the results (Watson, 2008). Weiner (2014) published a critical review of the available *in vivo* safety studies in which the author considered that existing studies on immune system parameters were conducted mainly with κ-carrageenan in drinking water, which provided data that were not directly applicable to dietary exposure, where carrageenan is tightly bound to protein. According to this author, based on the available studies conducted with carrageenan in feed, carrageenan has not been shown to produce immunotoxicity. The Panel agreed with this conclusion.

In many of the studies reporting the development of an inflammatory reaction after ingestion of 'carrageenan', the material used was not clearly characterised analytically and in particular, the distinction was not undoubtedly made between 'native carrageenan', degraded carrageenan C16 (material prepared to contain a significant amount of low molecular weight material), and poligeenan (ι-carrageenan treated under specific conditions of temperature and pH to fully hydrolyse high molecular weight carrageenan). These weaknesses in the identification of the test sample resulted in confusion in the interpretation of the data.

The Panel noted that intake of carrageenan has been reported to influence the composition of the gastrointestinal microflora (Nilson and Schuller, 1941; Poulsen, 1973; Shang et al., 2017). Interplays between the bacterial gut microflora and sulfated glycans (Mallett et al., 1985; Shang et al., 2016a,b) seem to play a significant role in the development of inflammation in the gut (Pomim, 2017). Because sulfate and undigested sulfur compounds have been implicated in the aetiology of ulcerative colitis (Pitcher and Cummings, 1996, 1996; Roediger et al., 1997; Magee et al., 2000, 2000; Kamada et al., 2013), the Panel considered that the sulfate moiety of carrageenan might be involved in the proinflammatory effects (Pomin, 2015; Fahoum, 2017) through the production in the colon of hydrogen sulfide from sulfate, by sulfate-reducing bacteria (Gibson, 1990).

Before reaching a definitive conclusion on the possible inflammatory and immunotoxic effects of carrageenan used as a food additive, the Panel considered that these effects and the presence in human plasma of natural antibodies, which recognised galactose with a α-1,3-galactosidic linkage such as present in carrageenan, deserve further investigation.

In the view of the Panel, results from a limited study, suggesting that carrageenan intake may contribute to an earlier relapse in patients with ulcerative colitis in remission compared to untreated controls, need further investigation.

The Panel also noted, that experimental results of *in vitro* and animal studies showing a possible influence of carrageenan exposure on glucose tolerance need clarification.

Taking into consideration the lack of adequate data and the existing uncertainties, the Panel was unable to establish a new ADI and decided to change the existing ADI to a temporary ADI, which should only be maintained until the data gaps are addressed.

For the food additives E 407 and E 407a, the fraction of low molecular weight carrageenan (< 50 kDa) is limited in the EC specifications by the purity criteria. This fraction has been associated with potential health hazards similar to those reported for preparations of degraded carrageenan such as poligeenan, to which this fraction shows similarity in molecular structure and in weight-average molecular weight. No data are available to demonstrate that poligeenan shows also similarities in conformations. Against this background, the Panel emphasised the need to establish a fully interlaboratory validated analytical method to detect low molecular weight carrageenan in the food additives E 407 and E 407a at the given limit. To overcome the limitation of the available analytical methods, the possibility of extending the molecular weight of the existing 5% limit for low molecular weight carrageenan from 50 kDa to up to 200 kDa may be considered. Furthermore, in the definitions for carrageenan (E 407) and processed Eucheuma seaweed (E 407) in the Commission Regulation (EU) No 231/2012 the weight-average molecular weight range should be specified in a narrow way avoiding a significant overlap with the molecular weight range of poligeenan.

To assess the dietary exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives, the exposure was calculated based on (1) maximum use levels provided to EFSA (defined as the maximum level exposure assessment scenario) and (2) reported use levels (defined as the refined exposure assessment scenario, brand-loyal and non-brand-loyal).

Carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised in a wide range of foods. The Panel did identify brand loyalty for specific food categories, e.g. flavoured fermented milk products for infants and toddlers, thus the Panel considered this scenario as the most appropriate and realistic scenario for risk characterisation for all population groups.

A maximum estimated exposure assessment scenario taking into account the food for special medical purposes for infants and young children (FC 13.1.5.1. Dietary foods for infants for special medical purposes and special formulae for infants, and FC 13.1.5.2. Dietary foods for babies and young children for special medical purposes as defined by Commission Directive 1999/22/EC) was also performed using food industry data to estimate exposure for infants and toddlers who may be on a specific diet. This exposure scenario considered products belonging to food categories 13.1.2 and 13.1.4 excluding food category 13.1.1 (infant formulae) and 13.1.3 (processed cereal-based foods) where carrageenan (E 407) and processed Eucheuma seaweed (E 407a), according to EU regulation, are not authorised. Considering that this diet is required due to specific needs, it is assumed that consumers are loyal to the food brand, therefore, the maximum reported use level for the FSMP was used and the mean of the typical reported for the remaining food categories.

A refined estimated exposure assessment scenario taking into account the consumption of food supplements was also performed to estimate exposure for children, adolescents, adults and the elderly, for consumers only, as exposure via food supplements may deviate largely from that via food, and the number of food supplement consumers may be low depending on the population and survey.

The refined estimates were based on 41 out of 79 food categories in which carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised. Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the real exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in European countries for both the maximum level exposure scenario and for the refined exposure assessment scenarios when considering only food additives uses for which data have been provided.

No information on the usage levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) has been reported to EFSA for 19 food categories. However, the Panel noted that, given the information from the Mintel GNPD, it may be assumed that carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are used in several food categories (n = 7) for which no data have been provided by food industry. The Panel noted that out of these 19 food categories, pasta and breakfast cereals are products highly consumed. If this would be confirmed, it would therefore result in an underestimation of the exposure.

A possible additional exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use directly by the consumer in adding special powder to thicken foods (soup, sauce, cream, dessert, pap for children) and from their use as food additives authorised in accordance with

Annex III to Regulation (EC) No 1333/2008 were not considered in any of the above exposure assessment scenarios.

The Panel also noted that the refined exposure estimates were based on information reported on the use levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) by food industry. If actual practices change, these refined estimates may no longer be representative and should be updated.

For FSMP, the Panel noted that one industry reported a use of carrageenan as food additive at the level of the MPL (300 mg/kg), according to the Annex II to Regulation (EC) No 1333/2008, in one niche product which is not align with information reported from the Mintel GNPD (Appendix D), where no FSMP products seem to be labelled on the market. If this information is confirmed, the uncertainties arising from the FSMP exposure scenario would lead to an overestimation of the current exposure to carrageenan products as food additives calculated by the Panel.

The present re-evaluation included the use of carrageenan (E 407) in foods for infants from 12 weeks of age and for young children.

In 2007, JECFA reviewed all the available toxicological data and concluded that the group ADI 'not specified' for the sum of carrageenan and processed Eucheuma seaweed was maintained for food additive uses in foods other than infant formula (JECFA, 2007b). In its latest evaluation in 2015, JECFA reviewed data published since 2007 and concluded that 'the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1,000 mg/L is not of concern' (JECFA, 2015).

The SCF maintained the original group ADI for food-grade carrageenan and processed Eucheuma seaweed of 0–75 mg/kg bw because of some uncertainty 'about the general immuno-reactive potential of the various carrageenan now in use as food additives' (SCF, 1996).

Concerning uses of carrageenan in food for infants and young children the Panel concurred with the SCF (SCF (1998a, 2003a,b) 'In the absence of any further information on possible absorption of carrageenan by the immature gut in the very young infant, the Committee reaffirms its earlier view (SCF, 1998a) that it remains inadvisable to use carrageenan in infant formulae that are fed from birth, including those in the category of foods for special medical purposes. The Committee has no objection to the use of carrageenan in foods for older infants, such as follow-on milks (SCF, 1983) and weaning foods (SCF, 2003a)'. 'The Committee further recommends maintaining the concept that if more than one of the three substances locust bean gum, guar gum or carrageenan are added to a follow-on formula, the maximum level established for each of those substances is lowered with that relative part as is present of the other substances together'.

Concerns have been raised regarding possible a greater sensitivity of infants to gastrointestinal effects of carrageenan when compared to adolescents or adults (Koletzko et al., 2005). The Panel noted that colitis was reported at high doses or after prolonged administration that were not comparable to the exposure expected from the use of carrageenan as a food additive. Furthermore, it was not clear from the reports whether the carrageenan used in these studies complied with the EU specifications. However, the Panel considered that the available data were not sufficient to rule out a possible increased sensitivity of infants to colitis induction by carrageenan.

4. Overall considerations and conclusions

In its evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a), the Panel noted that:

- according to industry carrageenan (E 407) is defined as having a weight-average molecular weight of 200–800 kDa;
- Uno et al. (2001a) determined in a survey on samples of food-grade carrageenan, representing κ-, ι- and λ-carrageenan, a weight-average molecular weight of 453–652 kDa and detected no obvious peak of poligeenan with a detection limit for poligeenan of about 5%;
- the ADME database was sufficient to conclude that high molecular weight carrageenan (e.g. κ/λ-carrageenan with a number-average molecular weight of approx. 800 kDa, study in rhesus monkeys) was not absorbed intact, while low molecular weight carrageenan (number-average molecular weight: 88 kDa or less) was found in tissues of rats or guinea pigs after administration of this material by gavage or diet;
- in one subchronic toxicity GLP study in rats performed with κ-carrageenan with an average molecular weight range of 196–257 kDa [not specified if number- or weight-average molecular weight; LMT of 1.9–12% < 50 kDa (mean 7%)], almost complying with the EU specification,

the NOAEL was equal to 3,394–3,867 mg/kg bw per day for males and females, respectively, the highest dose tested;

- no adverse effects have been detected in several chronic toxicity studies in rats with carrageenan (mostly κ/λ-type, no adequate indication of molecular weight distribution); from the available rat studies, NOAELs up to 7,500 mg/kg bw per day, the highest dose tested, were identified (Nilson and Wagner, 1959). However, in another study in rats given carrageenan preparations with a molecular weight of 244 and 252 kDa, a LOAEL of 1% in the diet (equivalent to 500 mg/kg bw per day) was identified by the Panel (Documentation provided to EFSA n. 43). The Panel noted that the characterisation of the test material in all the chronic studies was limited;
- carrageenan (different types) and processed Eucheuma seaweed did not raise a concern with respect to genotoxicity;
- there was no concern with respect to carcinogenicity for carrageenan (mostly κ/λ-type);
- the NOAEL of sodium and calcium κ/λ-carrageenan for developmental effects found in dietary prenatal developmental toxicity studies was 3,000 and 5,000 mg/kg bw per day for rats and hamsters (Documentation provided to EFSA n. 46), and 3,060 mg calcium carrageenan/kg bw per day for rats (Collins et al., 1977a), the highest doses tested;
- the Panel considered that for the safety evaluation of processed Eucheuma seaweed (E 407a) read across can be made from study results used in the toxicological evaluation of carrageenan (E 407).

In addition, the Panel observed that:

- from all the data received, data were adequate for a refined exposure assessment for 41 out of 79 food categories;
- in the general population, based on the reported use levels, a refined exposure, in the brand-loyal scenario of up to 758.6 mg/kg bw per day for toddlers (from 12 months up to and including 35 months of age) was estimated;
- for populations consuming foods for special medical purposes and special formulae, the 95th percentile of maximum exposure assessments calculated based on the maximum reported data from food industry (equal to the MPL) were up to 49.4 mg/kg bw per day for infants (from 12 weeks up to and including 11 months of age).

For the food additives E 407 and E 407a, the fraction of low molecular weight carrageenan is limited in the EC specifications by the purity criteria. The Panel was informed by one interested party that the material on the market does not necessarily comply with the EU specifications regarding the limit of the low molecular weight fraction. This fraction has been associated with potential health hazards similar to those reported for preparations of degraded carrageenan, such as poligeenan or C16, to which this fraction shows similarity in molecular structure and in weight-average molecular weight. Although, full identity of degraded carrageenan such as poligeenan or C16 with the low molecular weight fraction of carrageenan has not been specifically demonstrated.

Concerning degraded carrageenan (e.g. poligeenan, C16) the Panel therefore noted the following:

- degraded carrageenan has been described to be absorbed and to be present in various tissues, namely the liver, and the urine of animals when administered in drinking water or via the diet;
- degraded carrageenan did not raise concern with respect to genotoxicity;
- rats exposed to degraded ι-carrageenan (weight-average-molecular weight of 20–40 kDa) via drinking water, diet or by gavage for up to 24 months developed in first instance colitis, secondary metaplasia and finally tumours (squamous cell carcinomas, adenocarcinomas, adenomas);
- monkeys given degraded ι-carrageenan (C16) via drinking water showed histopathological lesions in the colon which varied from slight mucosal erosions at the low dose (750 mg/kg bw per day) to ulceration associated with inflammatory infiltration of the lamina propria at the high dose (2,900 mg/kg bw per day). In this study, all monkeys on C16 lost blood frequently from the intestinal tract in a dose-related degree and developed some degree of anaemia. A LOAEL of 750 mg degraded ι-carrageenan (C16)/kg bw per day was noted;
- toxicity studies have been mainly conducted with degraded ι-carrageenan, thus, degraded κ- and λ-carrageenan being sparsely toxicologically characterised.

The following uncertainties were noted by the Panel as regards the chemistry and fate of carrageenan (E 407) and processed Eucheuma seaweed (E 407a):

- no data are available showing the molecular weight distribution for different food-grade carrageenan preparations within the defined weight-average molecular weight range of 200–800 kDa;
- no data are available showing the molecular weight distribution for individual food-grade processed Eucheuma seaweed preparations;
- the weight-average molecular weight range of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) is not defined in the EU specifications allowing for the presence of a low weight-average molecular weight fraction of carrageenan. Based on the information provided on weight-average molecular weights of commercially available carrageenans, the low molecular weight material needs to be accurately quantified;
- in most of the toxicological studies the carrageenan used is not well specified and its weight-average molecular weight and its content of low molecular weight fragments are not given;
- although it has been claimed that there is no adequate analytical method available to measure the low molecular weight fraction, the Panel noted that there is indication of the percentage of the low molecular weight fraction in the food additive carrageenan (E 407) tested in a few toxicological studies;
- only limited information on the stability of carrageenan in food was available. No data on stability of carrageenan and/or processed Eucheuma seaweed addressing the usual variation of parameters (temperature, pH) relevant for the authorised food uses were available. Information on possible degradation products under acidic conditions in relevant food products is missing;
- studies investigating the hydrolysis of the κ-, ι- and λ- carrageenan showed contradictory results.

The Panel further noted the following uncertainty as regards the exposure assessment scenario:

- the refined estimates were based on 41 out of 79 food categories in which carrageenan (E 407) and processed Eucheuma seaweed (E 407a) are authorised and result in an overestimation of the real exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in European countries considering only food additives uses for which data have been provided were considered.

Among the uncertainties from the biological and toxicological data, the Panel considered the following:

- the lack of reliable comparative toxicokinetic and toxicological studies between the different types of carrageenan and their corresponding low molecular weight fractions;
- the theoretical possibility that limited degradation could occur under conditions representative of the *in vivo* situation;
- no firm conclusion on the other types of carrageenan could be drawn on the observation of occult blood in faeces of rhesus monkeys dosed with a commercial carrageenan;
- the characterisation of the test material in most of the toxicological studies was limited;
- there were no adequate toxicological studies performed with low weight-average molecular weight carrageenan (around 200 kDa), apart from one 90-day study (with an average molecular weight carrageenan in the range of 196–257 kDa, not specified if it was a number-average or a weight-average);
- testing for chronic toxicity and reproductive and developmental toxicity was performed almost exclusively with κ/λ-carrageenan; almost no data on ι-carrageenan was available;
- inadequate data on the possible relevance of carrageenan exposure for existing inflammatory bowel diseases in humans;
- the relevance for humans of the observations in animal studies pointing to the induction of glucose intolerance and glucosuria by carrageenan is unclear;
- the possible role of sulfate and the interactions of the various forms of carrageenans with the gut microflora in some of the reported inflammatory effects of carrageenans.

Altogether in most toxicological studies, the molecular weight distribution of the carrageenan tested is not or only inadequately described. The low molecular weight fraction of carrageenan is associated with potential adverse effects. This is due to its similarity in molecular structure and weight-average molecular weight with those of degraded carrageenan, such as poligeenan or C16, known to cause inflammatory intestinal effects. Moreover, results suggesting that carrageenan might enhance inflammatory bowel disease in humans need clarification. The test compounds used in a large number of the toxicity tests are thus not considered to reflect adequately the diversity of preparations of the

food additive on the market, particularly with respect to the broad range of weight-average molecular weights reported. The Panel noted that most of these issues were also raised and discussed in a recent review (David et al., 2018).

The Panel also considered other pending uncertainties regarding the relevance of the studies available to assess the safety of the authorised food additive carrageenan (E 407). The physicochemical properties of carrageenan depend on the chemical conformation (helical or random coil) in which it exists in the preparations and in foods (solid or liquids foods) and are influenced by the presence of cations, proteins and the pH and temperature. These conditions could affect the toxicity of carrageenan and are thus relevant for the safety assessment of the food additive in the authorised uses. Furthermore, findings in some studies suggest that carrageenan exposure could be related to the induction of glucose intolerance and glucosuria. In the view of the Panel, all this information still needs clarification.

Overall, taking into account the lack of adequate data to address the above mentioned uncertainties, the Panel concluded that the existing group ADI for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) of 75 mg/kg bw per day should be considered temporary, whilst the database should be improved within 5 years after publication of this opinion. Within the given time frame, high importance should be ascribed to the establishment of an adequate interlaboratory validated analytical method to quantify, at the existing 5% limit, the low weight-average molecular weight fraction of carrageenan, and to establish whether or not this fraction is associated with health risks.

The Panel further concluded that in the refined brand-loyal exposure assessment scenario the exposure estimates exceeded, in some cases by up to approximately 10-fold, the temporary existing ADI at the high level (95th percentile) for all population groups and at the mean for all population groups except for infants and the elderly. Although the exposure may be overestimated the extent of the exceedance of the ADI (10-fold) may be a safety concern.

5. Recommendations

The Panel recommended that the EC considers:

- in the definition for the food additives E 407 and E 407a in the Commission Regulation (EU) No 231/2012, specifying the weight-average molecular weight range in a narrow way avoiding a significant overlap with the molecular weight range of preparations of degraded carrageenan;
- with respect to the purity criteria in the Commission Regulation (EU) No 231/2012, the need of an interlaboratory validated analytical method to detect low molecular weight carrageenan in the food additives E 407 and E 407a at the limit specified in the Regulation;
- possibility of extending the molecular weight of the existing 5% limit for low molecular weight carrageenan from 50 kDa to up to 200 kDa for the food additives E 407 and E 407a in the Commission Regulation (EU) No 231/2012. This would take into account the limitation of the available analytical methods to quantify the percentage of low weight-average molecular weight carrageenan and will further make certain that the presence of low weight-average molecular weight carrageenan is at a low level; it should be indicated if the 5% limit refers to the food additive as such or on dried basis;
- the need of information on the stability of the food additives E 407 and E 407a in food. No data on stability of carrageenan (E 407) and/or processed Eucheuma seaweed (E 407a) addressing the usual variation of parameters (temperature, pH) relevant for the authorised food uses were available. Information on possible degradation products under acidic conditions in relevant food products was missing;
- revising the maximum limits for the impurities of toxic elements (lead, mercury, cadmium and arsenic) in the EC specification for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in order to ensure that the food additives will not be a significant source of exposure to these toxic elements in food;
- collecting monitoring data for the food categories contributing the most to the exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in order to allow for more refined estimates of exposure;
- establishing, as a general principle, numerical MPLs for all food authorised uses with special consideration of the main food categories contributing to the exposure (e.g. flavoured milk products, flavoured drinks, food supplements and fine bakery wares) to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives in order to reduce consumers exposure.

Documentation provided to EFSA

1) Pre-evaluation documents on carrageenan (E 407). Frauenhofer ITEM. December 2012.
2) Pre-evaluation documents on processed Eucheuma seaweed (E 407a). Frauenhofer ITEM. December 2012.
3) EMA (European Medicines Agency): communication to EFSA request for information on a certain group of substances used as food additives, May 2015.
4) Istituto superiore di sanita', 2010. Reply to EFSA: call for scientific data on food additives permitted in the EU and belonging to the functional class of miscellaneous food additives. Information on semicarbazide. Submitted on 14 June 2010.
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10) FDA (Food and Drug administration), 2014. Preliminary information on the new studies carried out on carrageenan in infant formulae. Submitted on January 2014.
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12) Marinalg International, 2015. Reply to EFSA: call for technical data on certain thickening agents permitted as food additives in the EU. Information on carrageenan (E 407) on: overview document "New and significant published studies and information critical to the EFSA re-evaluation of the safety of carrageenan for use as a food additive"; Appendix A and B, "Carrageenan studies published since 8 November, 2010 submission to EFSA re-evaluation" (spreadsheet and copies of the papers); Appendix C, "Status report on the work of Marinalg International to measure the molecular weight distribution of carrageenan and processed Eucheuma seaweed in order to meet the EU specification: less than 5% below 50 kDa; Submitted on 26 November 2015.
13) Marinalg International, 2016. Reply to EFSA. Additional studies published McKim et al. (2016), "*Effects of carrageenan on cell permeability, cytotoxicity, and cytokine gene expression in human intestinal and hepatic cell lines*". Submitted on 22 September 2016.
14) Marinalg International, 2015. Reply to EFSA: call for technical data on certain thickening agents permitted as food additives in the EU. Information on processed Eucheuma seaweed (E 407a) on: "A critical review and expert opinion on the safety of processed Eucheuma seaweed (PES)"; "90-day feeding study in the rat with semi-refined carrageenan from two sources, including a recovery phase" (unpublished BIBRA Report 1997); Appendix A and B, "List of bridging carrageenan studies and reviews, published or made available papers, since November 2010 submission of published papers to EFSA for re-evaluation (spreadsheet and copies of the papers); Appendix C, "Status report on the work of Marinalg International to measure the molecular weight distribution of carrageenan and processed Eucheuma seaweed in order to meet the EU specification: less than 5% below 50 kDa. Submitted on 26 November 2015.
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carrageenan (E 407a) on and processed Eucheuma seaweed (E 407a) on identity, specifications and manufacturing process, and a report on the measurement of the molecular weight distribution of carrageenan and processed Eucheuma seaweed. Submitted on 22 December 2015.
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20) AESGP (Association of the European Self-Medication Industry), 2014. Data on usage levels on carrageenan (E 407) and processed Eucheuma seaweed (E 407a) in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 3). Submitted to EFSA by 30 September 2013.
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Glossary and Abbreviations

AAS	atomic absorption spectroscopy
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AESGP	Association of the European Self-Medication Industry
AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ALT	alanine transaminase
ANS	Panel on Food Additives and Nutrient Sources added to Food

AOAC	Association of Analytical Communities
AOM	azoxymethane
ARC	alternatively refined carrageenan
ASEMESA	Asociacion Espanola de Exportadores e Industriales de Aceitunas de Mesa
AST	aspartate transaminase
BCL 10	B-cell lymphoma/leukaemia 10
CAS	Chemical Abstracts Service
CFU	colony forming unit
CHL	Chinese hamster lung fibroblast
CLBG	clarified locust bean gum
DEAE	diethylaminoethyl
DMH	dimethyhydrazine
DNPH	2,4-dinitrophenylhydrazine
EC	Evaluated under Commission
EDA	European Dairy Association
EINECS	European Inventory of Existing Commercial Chemical Substances
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FAO/WHO	Food and Agriculture Organization/World Health Organisation
FC	Food for weight control
FCS	Food Classification System
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FSMP	Foods for special medical purposes
GC	gas chromatography
GD	gestational day
GERD	gastroesophageal reflux disease
GI	gastrointestinal
GLP	good laboratory practice
GNPD	Global New Products Database
GPC/ICP	gel permeation/inductively coupled plasma
GTT	glucose tolerance test
HMPC	Committee on Herbal Medicinal Products
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HPSEC-RI	high-performance size exclusion chromatography coupled to a refractive index detector
IARC	International Agency for Research on Cancer
ICGA	International Chewing Gum Association
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
INS	International Numbering System for Food Additives
IOM	Institute of Medicine
IR	infrared
ITT	insulin resistance test
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, 50% i.e. dose that causes death among 50% of treated animals
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
LMT	low molecular weight tail
LS	light scattering
MALS	Multiple Angle
MoE	margins of exposure

MPL	maximum permissible level
NDA	Panel on Dietetic Products, Nutrition and Allergies
NF-κB	nuclear factor κB
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PES	processed Eucheuma seaweed
PND	postnatal day
PNG	Philippine natural grade
QS	*quantum satis*
ROS	reactive oxygen species
RSD	relative standard deviation
SA	sodium alginate
SCE	sister chromatid exchange
SCF	Scientific Committee for Food
SCFA	short-chain fatty acids
SEC	size exclusion chromatography
SGPT	serum glutamic pyruvic transaminase
SIDS	Screening Information Dataset
SmPC	Summary of product characteristics
SNE	Specialised Nutrition Europe
SRC	semi-refined carrageenan
TLR	Toll-like receptor
TNF-α	tumour necrosis factor-α
TNO	Netherlands Organisation for Applied Scientific Research
UDS	unscheduled DNA synthesis
USAN	United States Adopted Names
UV	ultraviolet
WHO	World Health Organisation

Appendix A – Summary of reported use levels (mg/kg or mg/L as appropriate) of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) provided by industry

Appendix B – Summary of reported use levels (mg/kg or mg/L as appropriate) of processed Eucheuma seaweed (E 407a) provided by industry

Appendix C – Summary of analytical results (mg/kg or mg/L as appropriate) of carrageenan (E 407) provided by Member States

Appendix D – Number and percentage of food products labelled with carrageenan (E 407) and processed Eucheuma seaweed (E 407a) out of the total number of food products present in the Mintel GNPD per food subcategory between 2012 and 2017

Appendix E – Concentration levels of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)

Appendix F – Summary of total estimated exposure of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix G – Main food categories contributing to exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure)

Appendix H – Summary of anticipated exposure to carrageenan (E 407) and processed Eucheuma seaweed (E 407a) taking into account the consumption of food supplements, for consumers only, among children, adolescents, adults and the elderly (minimum–maximum across the dietary surveys in mg/kg bw per day)

Appendix I – Sources for the production of carrageenan

Over the last few years, most of the seaweeds used and authorised according to the EU specification for the production of carrageenan have been reclassified by marine biologists as they gain more knowledge of their structure. If readers are to go beyond this publication for further information, they need to be familiar with the both the old and new names, as listed below:

- *Betaphycus gelatinum* was *Eucheuma gelatinae*.
- *Chondrus crispus* remains unchanged, and is commonly known as 'Irish moss'.
- *Eucheuma cottonii* is now *Kappaphycus alvarezii*, and commercially was and is called 'cottonii'.
- *Eucheuma denticulatum* was *Eucheuma spinosum* and commercially was and is called 'spinosum'.
- *Eucheuma gelatinae* is now *Betaphycus gelatinum*.
- *Eucheuma spinosum* is now *Eucheuma denticulatum* and commercially was and is called '*spinosum*'.
- *Gigartina canaliculata* remains unchanged.
- *Gigartina skottsbergii* remains unchanged.
- *Gigartina stellata*, mentioned in earlier articles but now not so important, is now *Mastocarpus stellatus.*
- *Hypnea musciformis* remains unchanged.
- *Iridaea ciliata* is now *Sarcothalia crispata*.
- *Iridaea laminaroides* is now *Mazzaella laminaroides*.
- *Kappaphycus alvarezii* was *Eucheuma cottonii* and commercially was and is called '*cottonii*'.
- *Mazzaella laminaroides* was *Iridaea laminaroides*.
- *Mastocarpus stellatus* was *Gigartina stellata*, mentioned in earlier articles but now not so important.
- *Sarcothalia crispata* was *Iridaea ciliata*.

Appendix J – Interpretation of the identity of samples used in ADME and Toxicity investigations

Introductory text from Section 3.5

The investigations used in the present opinion to disclose the ADME as well as the toxicity are spread over a time period from 1941 and to the present day. In this connection, the Panel noted, that:

- The sources of carrageenan have changed over time as already discussed in Section 3.1.1.1.
- The original source was *Chondrus crispus* which still is used.
- Today most carrageenan, however, is coming from the two algae *Eucheuma cottonii* and *Eucheuma spinosum*.
- Each biological source presents a unique composition of the three-known carrageenans.
- ι and κ (most often in natural mixtures) so far have been the most used carrageenans.
- Pure λ-carrageenan recently has become more used in the US (TCI America, Portland, OR, USA (at: http://www.tcichemicals.com/eshop/en/us/commodity/C3313/) and Modernist Pantry, Eliot, ME, USA (at: http://www.modernistpantry.com/carrageenan-λ.html).
- The way of describing samples used in investigations has changed during the long period of time mentioned.

Thus, taking the time as well as the source aspect into consideration, the Panel decided that in order to make it possible to compare the results of different studies, an interpretation of the identity (expressed in type(s) of carrageenan(s)) should be made for each sample.

General about the possibilities for, and methods to, identify a sample to an overall composition with regard to the three carrageenans, as maybe added information about the overall molecular size (mean and molecular weight distribution).

1. Each carrageenan used may only be characterised based on the information given in the publication (incl. appendices etc.).

   1.1. This information may include:

      1.1.1. Producer
      1.1.2. Commercial Brand name including information on subqualities (if any)
      1.1.3. Producers commodity (part) number
      1.1.4. Purity – regarding the occurrence of non-carrageenan compounds of organic and/or inorganic nature
      1.1.5. Biological origin (Algal species)
      1.1.6. Geographical origin
      1.1.7. Type of carrageenan according to the in general accepted subdivision into the types of ι-, κ- and λ-carrageenans. If given, this may be as one of the three types or as a mixture, with or without percentages of occurrence of each type.
      1.1.8. Molecular weight: as a number-average and/or as a weight-average molecular weight
      1.1.9. Molecular weight distribution (in weight percentage. In case the product is stated to be a mixture of two or three of the above-mentioned types of carrageenan, information about molecular weight (average and distribution) may be given separately for each type.
      1.1.10. Content if any of degraded carrageenan, their origin and characteristics

2. Working with older literature, most often many of the above pieces of information are lacking. However, in some cases, it is possible to deduce further information from those already given.

   2.1. An example would be the deduction of the type(s) of carrageenan(s) in a product for which the biological origin (algal species) is given.

      2.1.1. In this latter case, it should be noted, that the species may have changed name. Important name changes are summarised in Annex A.

3. Writing the present Opinion 'Re-evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives', the Panel as already decided to use all existing possibilities for deduction of information – further to that already directly provided - about the products used in the experiments incorporated into the Opinion. Thus, for each

experiment incorporated one will find a paragraph summarising The information provided and the interpretation of this, concerning the carrageenan used.

3.1. This above-mentioned paragraph will occur at the end of the text describing the experiment/test in question, and will present itself as follows: Carrageenan used (1) information provided: xxx (2) Interpretation of the information: xxx