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Re-evaluation of agar (E 406) as a food additive

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Abstract

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of agar (E 406) as a food additive. In the European Union (EU), agar (E 406) has been evaluated by the Scientific Committee for Food (SCF) in 1989, who allocated to agar a not specified acceptable daily intake (ADI), and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974, who considered very few data to conclude to a not limited ADI. According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010, the Panel considered that the safety assessment is limited to the use and use levels received from industry in 7 food categories for which data were considered in this opinion out of the 70 food categories in which agar (E 406) is authorised; an indicative high refined exposure assessment up to 26 mg/kg body weight (bw) per day has been calculated in toddlers at the 95th percentile (non-brand-loyal scenario); agar is unlikely to be absorbed unchanged and slightly fermented by intestinal microbiota; sufficient toxicity data were available; there was no concern with respect to the genotoxicity of agar; no carcinogenic effects were reported in carcinogenicity studies in mice and rats at the doses of 4,500 mg/kg bw per day and 2,500 mg/kg bw per day, respectively, the highest doses tested; oral intake of agar (4,500 mg/person corresponding to 64 mg/kg bw per day) was tolerated in humans for 12 weeks without noticeable side effects. Therefore, the Panel concluded that there is no need for a numerical ADI for agar and that there is no safety concern for the general population at the refined exposure assessment for the reported uses of agar as a food additive.

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Summary

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of agar (E 406) when used as a food additive.

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

Agar (E 406) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives. Specific purity criteria on agar (E 406) have been defined in Commission Regulation (EU) No 231/2012. In the EU, agar (E 406) has been evaluated by the Scientific Committee for Food (SCF) in 1989 (SCF, 1989) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 (JECFA, 1974). The SCF did not consider it necessary to specify an acceptable daily intake (ADI), and consequently, allocated to agar a not specified ADI. JECFA considered very few data (two animal studies, one human study) to conclude to a not limited ADI.

Agar is extracted from certain strains of marine algae of the families Gelidiaceae and Gracilariaceae and relevant red algae of the class Rhodophyceae (Commission Regulation (EU) No 231/2012). Agar (E 406) is defined as a hydrophilic colloidal polysaccharide consisting mainly of galactose units with a regular alternation of L- and D-isomeric forms. These hexoses are alternately linked with alpha-1,3 and beta-1,4 bonds in the copolymer. On about every tenth D-galactopyranose unit, one of the hydroxyl groups is esterified with sulfuric acid which is neutralised by calcium, magnesium, potassium or sodium.

Because of its polysaccharidic nature, agar can be a substrate of microbiological contamination during storage. The latter has been recently demonstrated by the mycotoxin contaminations of gums (Zhang et al., 2014). The Panel noted that the differences in the microbiological criteria for agar between the specifications given by the EU Regulation and those given by JECFA are not decisive.

Degradation of agar has been investigated in an *in vitro* human model. This study demonstrated that agar would be partially fermented during its passage through the large intestine by the action of the intestinal tract microflora. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that agar is unlikely to be absorbed unchanged, and that the limited extent of fermentation of agar would lead to products such as short-chain fatty acids (SCFA). Based on the available knowledge on the role of SCFA as end products of the fermentation of dietary fibres by the anaerobic intestinal microflora (Topping and Clifton, 2001; Den Besten et al., 2013), the Panel considered that their potential formation as fermentation products from agar does not raise any concern.

The acute toxicity of agar in mice, rats and rabbits is low.

No adverse effects have been identified up to 10,000 mg/kg body weight (bw) per day in mice and 4,500 mg/kg bw per day in rat, the highest dose tested, in two dose range-finding subchronic toxicity studies performed by the National Toxicology Program (NTP) in 1982 (Doc. provided to EFSA n.8).

No genotoxic activity was observed in the available *in vitro* and *in vivo* genotoxicity assays with agar. The Panel noted that the package of available studies was limited, but also that agar is routinely used as a substrate in *in vitro* gene mutation assays in bacteria and mammalian cells. Overall, considering its chemical structure and negligible absorption, the Panel concluded that there is no concern with respect to the genotoxicity of agar (E 406).

No chronic toxicity studies according to OECD guidelines have been identified by the Panel.

In carcinogenicity studies by NTP (Doc. provided to EFSA n.8, 1982; Melnick et al., 1983), no indication of a substance-related increased tumour incidence was reported. The authors stated that under the experimental conditions used, agar was not carcinogenic to mice and rat at the highest doses tested (up to 4,500 or 2,500 mg/kg bw per day, respectively). The Panel agreed with the authors.

No data on reproductive toxicity were available. In the prenatal developmental toxicity studies in rats and hamsters, no maternal and developmental effects were observed up to the highest dose tested (1,140 and 650 mg agar/kg bw per day, respectively) (Doc. provided to EFSA n.3).

Agar is used as an adjuvant in the treatment of diabetes and/or obesity (Maeda et al., 2005). This study did not show noticeable side effects in participants receiving a daily dose of 4.5 g agar for 12 weeks agar as an adjuvant in the treatment of diabetes and/or obesity (Maeda et al., 2005). No adverse effects were described for agar as a mild laxative in single doses from 2.75 to 5.5 g or in daily doses from 4 to 16.5 g administered with sufficient fluids, but intestinal stenosis was given as a contraindication (Heber, 2007).

Agar (E 406) is authorised in a wide range of foods but reported by industry to be used in a limited number of food categories (9/70). According to the Panel, it is not expected that brand loyalty will result in higher exposure in the general population, except in specific populations consuming foods belonging to the food categories 13.2, 13.3 and 13.4. The Panel therefore selected the non-brand-loyal refined scenario as the most relevant exposure scenario for this additive.

In the maximum level and refined scenarios, only seven food categories (out of 70 in which agar is authorised in the Regulation (EC) No 1333/2008) for which data have been reported by industry, were included. The Panel considered that the uncertainties identified would result in an overestimation of exposure for these seven food categories.

Considering information from the Mintel Global New Products Database (GNPD), only approximately 50% of the food products labelled with agar (E 406) belonged to food subcategories for which reported use levels were available, and consequently used in the exposure assessment. The Panel noted that given the information from the Mintel GNPD it may be assumed that agar is used in food categories for which no data have been provided by food industry. If this is confirmed, the present assessment would therefore result in an underestimation of the exposure.

The Panel further noted that the exposure to agar from its use according the Annex III (Part 1, 2, 3, 4 and 5 section A) to Regulation (EC) No 1333/2008 was not considered in the exposure assessment.

The Panel also noted that the refined exposure estimates are based on information provided on the reported level of use of agar (E 406). If actual practice changes, this refined estimates may no longer be representative and should be updated.

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- the safety assessment carried out by the Panel is limited to the use and use levels in seven food categories for which data were considered in this opinion out of the 70 food categories in which agar (E 406) is authorised;
- an indicative high refined exposure assessment up to 26 mg/kg bw per day has been calculated in toddlers at the 95th percentile (non-brand-loyal scenario);
- agar is unlikely to be absorbed unchanged and is slightly fermented by intestinal microbiota;
- sufficient toxicity data were available;
- there is no concern with respect to the genotoxicity of agar (E 406);
- no carcinogenic effects were reported in carcinogenicity studies in mice and rats at the doses of 4,500 mg/kg bw per day and 2,500 mg/kg bw per day, respectively, the highest doses tested;
- oral intake of agar (4,500 mg/person corresponding to 64 mg/kg bw per day) was tolerated in humans for 12 weeks without noticeable side effects,

the Panel concluded that there is no need for a numerical ADI for agar, and that there is no safety concern for the general population at the refined exposure assessment for the reported uses of agar as food additive.

The Panel recommended that the maximum limits for the impurities of toxic elements (lead, mercury, cadmium and arsenic) in the EC specification should be revised in order to ensure that agar (E 406) as a food additive will not be a significant source of exposure to those toxic elements in food, in particular for infants and children.

The Panel recommended that information on the possible use of formaldehyde should be provided since no such information was made available following the call for data.

Due to the discrepancies observed between the data reported from industry and the Mintel database, where agar is labelled in more products than in food categories for which data were reported from industry, the Panel recommended collection of data of usage and use levels of agar (E 406) in order to perform a more realistic exposure assessment.

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1. Introduction

The present opinion deals with the re-evaluation of the safety of agar (E 406) when used as food additives. Agar (E 406) is an authorised food additive in the European Union (EU) according to Annex II and Annex III of Regulation (EC) No 1333/2008[1].

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 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 EU. 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 EU before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the 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[3] of 2001. The report 'Food additives in Europe 2000' 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 EFSA 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 the Terms of Reference

This re-evaluation refers exclusively to the uses of agar (E 406) as a food additive in food, including food supplements, and does not include a safety assessment of other uses of agar as described in Section 3.4.2.

1.2. Information on existing evaluations and authorisations

Agar (E 406) is authorised as a food additive in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives. Specific purity criteria on agar (E 406) have been defined in Commission Regulation (EU) No 231/2012[4].

[1] 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.
[2] 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.
[3] COM(2001) 542 final.
[4] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1.

In the EU, agar (E 406) has been evaluated by the SCF in 1989 (SCF, 1989), who reviewed all the available studies on agar, including acute, subacute, developmental, carcinogenicity and mutagenicity studies. In view of the fact that agar is devoid of toxicity at the highest dose levels tested, the SCF did not consider it necessary to specify an acceptable daily intake (ADI), and consequently allocated to agar a not specified ADI.[5]

Agar (E 406) was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 (JECFA, 1974). JECFA considered very few data (two animal studies and one human study) to conclude to a not limited ADI. In 2006, JECFA updated the specifications of agar (JECFA, 2006a).

Agar (E 406) has also been reviewed by the Nordic Council of Ministers (TemaNord, 2002), who concluded that there is no need for a re-evaluation since the substance is widely used and there is a long history of safe intake of agar as a food.

Agar is one 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; EC, 2004), following the measures taken and information provided by the 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 mini-cups or mini-capsule to project the confectionery into the mouth'.

In 2004, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (EFSA AFC Panel, 2004) 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 agar (E 406) in jelly mini-cups. 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 (EC, 2004). The use of these additives in jelly mini-cups is not authorised in the EU.[6]

Agar is permitted as a binding and viscosity controlling agent in cosmetic products (European Commission database-CosIng[7]). Agar is included in the EU Register[8] of feed additives (Regulation (EC) No 1831/2003[9]).

2. Data and methodologies

2.1. Data

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier. EFSA launched public calls for data,[10,11,12] and, if relevant, contacted other scientific risk assessment bodies 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, these were not always available to the Panel.

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

[5] SCF precised in this opinion that when a numerical ADI value has not been specified for a substance, means that, on the basis of the available toxicological, biochemical and clinical data, the total daily intake of the substance arising from its use or uses at the level necessary to achieve the desired technological effect will not in any circumstances represent a hazard to health. For this reason, the establishment of a numerical limit for ADI is not considered necessary for these substances.

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

[7] Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple

[8] Available online: http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

[9] Regulation (EC) No 1831/2003 of the European Parliament and the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29–43.

[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: 23 May 2010. 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] 12–13/10/2016.

[14] Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of agar (E 406) in food products.

2.2. Methodologies

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

The ANS Panel assessed the safety of agar (E 406) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: 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, 2012).

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 body weight (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 (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 to'. 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 agar (E 406) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008[15] and reported use levels submitted to EFSA following calls for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

In the context of this re-evaluation, the Panel followed 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

According to Commission Regulation (EU) No 231/2012, agar is 'a hydrophilic colloidal polysaccharide consisting mainly of galactose units with a regular alternation of L- and D-isomeric forms. These hexoses are alternately linked with alpha-1,3 and beta-1,4 bonds in the copolymer. On about every tenth D-galactopyranose unit, one of the hydroxyl groups is esterified with sulfuric acid which is neutralised by calcium, magnesium, potassium or sodium. It is extracted from certain strains of marine algae of the families Gelidiaceae and Gracilariaceae and relevant red algae of the class Rhodophyceae.'

The Panel noted that not all families and species used in the production of agar are specified in the above definition. According to the reviews by Stanley (2006) and Armisen and Galatas (2000), agar can be extracted not only from species of the families Gracilariaceae, Gelidiaceae, but also from those of Phyllophoraceae and, Ceramiaceae and Ahnfeltiaceae families, all belonging to the red seaweeds (class Rhodophyta) algae. The majority of the raw material is harvested from wild growth (Stanley, 2006), although a commercial cultivation has been reported from Chile (OIiviera and Alveal, 1990).

The higher taxonomy of the red algae has long been disputed. Recent systematic systems were published by Saunders and Hammersand (2004), Adl et al. (2005) and Hwan Su Yoon Müller et al. (2006). Since these systems differ from each other and from previous systems in the higher taxa, in the present opinion we therefore only refer to genus and species of such algae.

Agar has the CAS Registry Number 9002-18-0, and the EINECS No 232-658-1.

[15] Commission 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.

Based on structural evaluations, agar is a linear polysaccharide built up of alternating 3-linked β-D-galactopyranose and 4-linked α-L-galactopyranose residues, a substantial part of the α-L-galactose residues may exist as a 3,6-anhydro derivative (Knutsen et al., 1994; Stanley, 2006). Thus, the basic structure of agar consists of a repeating disaccharide unit which is termed 'agarobiose' (Stanley, 2006); the structural formula is presented in Figure 1.

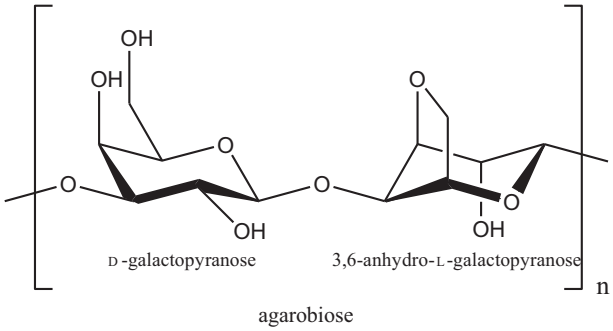


Figure 1: Structural formula of agar linear polysaccharide built up of the repeating disaccharide agarobiose units

Older investigations revealed that agar can be separated at least into two polysaccharides named 'agarose' and 'agaropectin' which are both agarobiose polymers (Araki, 1956). Agarose is a neutral gelling fraction and has a high molecular weight above 100,000 Da and a sulfate content usually below 0.15%, while agaropectin is a sulfated non-gelling fraction with a molecular weight of only around 14,000 Da and a sulfate content of 5–8% (Armisen and Galatas, 2000). However, more recent investigations suggest that agar consists not only of two well defined polysaccharides – agarose and agaropectin – but also it is in fact a complex mixture of polysaccharides, ranging from virtually uncharged molecules to various charged galactans, some rich in sulfate, others in pyruvate (Duckworth and Yaphe, 1971; Stanley, 2006; Doc. provided to EFSA n. 2 and 5). Thereby, the structure of agar is highly dependent on the seaweed sources and the manufacturing method (Stanley, 2006).

According to Commission Regulation (EU) No 231/2012, agar is odourless or has a slight characteristic odour. Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips, or in cut, flaked or granulated forms. It may be light yellowish orange, yellowish grey to pale yellow or colourless. It is tough when damp, brittle when dry. Powdered agar is white to yellowish white or pale yellow.

Agar is known under several synonyms such as agar-agar, gelose, Japan agar, Bengal, Ceylon, Chinese or Japanese isinglass, Layor Carang (JECFA 2006, Commission Regulation (EU) No 231/2012).

3.1.2. Specifications

Specifications of agar have been defined in Commission Regulation (EU) No 231/1012 and by JECFA (2006). The available specifications are listed in Table 1.

Table 1: Specifications of agar (E 406) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Definition	Agar is a hydrophilic colloidal polysaccharide consisting mainly of galactose units with a regular alternation of L- and D-isomeric forms. These hexoses are alternately linked with alpha-1,3 and beta-1,4 bonds in the copolymer. On about every tenth D-galactopyranose unit, one of the hydroxyl groups is esterified with sulfuric acid which is neutralised by calcium, magnesium, potassium or sodium. It is extracted from certain strains of marine algae of the families Gelidiaceae and Gracilariaceae and relevant red algae of the class Rhodophyceae	Agar is the dried hydrophilic, colloidal substance extracted from certain marine algae of the class Rhodophyceae. It is a polysaccharide, consisting primarily of D- and L-galactose units. About every tenth D-galactopyranose unit contains a sulfate ester group. Calcium, magnesium, potassium or sodium cations are also associated with the polysaccharide

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Assay	The threshold gel concentration should not be higher than 0.25%	Not higher than 0.25% for threshold gel concentration Prepare serial dilutions of the sample with known solids content (0.15%, 0.20%, 0.25%, etc.) and place in tubes, 150 mm long by 16 mm internal diameter, stoppered at both ends. Cool for 1 h at 20–25°C. Allow cylinders of gel to slide from the tubes to a level surface. The lowest concentration of gel that resists gravity without rupture for 5–30 s is the threshold concentration of the sample
Description	Agar is odourless or has a slight characteristic odour. Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips, or in cut, flaked or granulated forms. It may be light yellowish orange, yellowish grey to pale yellow or colourless. It is tough when damp, brittle when dry. Powdered agar is white to yellowish white or pale yellow. When examined in water under a microscope, agar powder appears more transparent. In chloral hydrate solution, the powdered agar appears more transparent than in water, more or less granular, striated, angular and occasionally contains frustules of diatoms. Gel strength may be standardised by the addition of dextrose and maltodextrins or sucrose	Odourless or has a slight characteristic odour. Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips, or in cut, flaked, granulated or powdered forms. It may be light yellowish orange, yellowish grey to pale yellow, or colourless. It is tough when damp, brittle when dry. Powdered agar is white to yellowish white or pale yellow
Identification		
Solubility	Insoluble in cold water; soluble in boiling water	Insoluble in cold water; soluble in boiling water
Gel formation with water	–	Prepare a 1.0% solution of the sample in boiling water in a flask and place the flask in water at 30°C for 15 min. A firm, resistant gel is formed. Place the flask in water at 70°C for 1 h, the gel is not molten. When heating the flask at higher temperature than 95°C, gel is liquefied to form a clear solution
Precipitate formation with ammonium sulfate solution	–	A warm (40°C) 0.5% solution of the sample gives a precipitate with half its volume of a warm (40°C) 40% ammonium sulfate solution. This test distinguishes agar from alginates, gum arabic, gum ghatti, karaya gum, pectin and tragacanth
Precipitate formation with lead acetate solution	–	A warm 0.5% solution of the sample gives a precipitate with one-fifth its volume of basic lead acetate TS. This test distinguishes agar from methyl cellulose
Microscopy	–	Place a few fragments of unground agar or some powder on a slide and add some drops of water or chloral hydrate TS. When examined under a microscope, agar in water appears granular and somewhat filamentous. In chloral hydrate TS, the powdered agar appears more transparent than in water
Purity		
Loss on drying	Not more than 22% (105°C, 5 h)	Not more than 22% after drying at 105°C until the difference between two weighings is less than 1 mg (about 5 h). Unground agar should be cut into pieces from 2 to 5 mm^2 before drying
Ash	Not more than 6.5% on the anhydrous basis determined at 550°C	Not more than 6.5% on the dried basis
Acid-insoluble ash (insoluble in approximately 3N hydrochloric acid)	Not more than 0.5% determined at 550°C on the anhydrous basis	Not more than 0.5% on the dried basis

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Insoluble matter (after stirring for 10 min in hot water)	Not more than 1.0%	–
Starch (and dextrins)	Not detectable by the following method: To a 1 in 10 solution of the sample, add a few drops of iodine solution. No blue colour is produced	Not detectable To a warm (40°C) 0.5% solution of the sample, add two drops of iodine TS. Where the drops fall, a red violet colour appears. After mixing, the solution should be golden brown and not blue or reddish
Gelatin and other proteins	Dissolve about 1 g of agar in 100 mL of boiling water and allow to cool of about 50°C. To 5 mL of the solution add 5 mL of trinitrophenol solution (1 g of anhydrous trinitrophenol/100 mL of hot water). No turbidity appears within 10 min	Not detectable To a warm (40°C) 0.5% solution of the sample, add 1 volume of warm (40°C) picric acid TS. No turbidity should appear within 10 min
Water absorption	Place 5 g to agar in a 100 mL graduated cylinder, fill to the mark with water, mix and allow to stand at about 25°C for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into a second 100 mL graduated cylinder. Not more than 75 mL of water is obtained	Place 5 g of the sample in a 100-mL graduated cylinder, fill to the mark with water, mix, and allow to stand at 25°C for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into a second 100-mL graduated cylinder. Not more than 75 mL of water should be obtained
Arsenic	Not more than 3 mg/kg	Not more than 3 mg/kg
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Foreign insoluble matter	–	Not more than 1% Boil 5 g of the sample with 500 mL of water and 12 mL of sulfuric acid under a reflux condenser for 2 h. Allow to cool and filter through a tared, fine, sintered glass crucible. Wash flask and filter with 50 mL of water, dry at 105°C to constant weight and weigh. Calculate as percentage
Microbiological criteria	Total plate count: Not more than 5,000 colonies per gram Yeasts and moulds: Not more than 300 colonies per gram *Escherichia coli*: Absent in 5 g *Salmonella* spp.: Absent in 5 g	Total plate count: Not more than 5,000 colonies per gram Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 mL of Butterfield's phosphate buffered dilution water and homogenising in a high speed blender Yeasts and moulds: Not more than 500 colonies per gram Coliforms: Negative by test *Salmonella*: Negative by test

The Panel noted that there is also a 'Foreign insoluble matter test' in JECFA specifications that is not present in the Commission specifications.

For pharmaceutical use, specifications are given in 8.4th European Pharmacopeia (2015).

The Panel noted that in the EU specifications the most recent nomenclature could be used in relation to the class of Rhodophyceae.

Because of its polysaccharidic nature, agar can be a substrate of microbiological contamination during storage. The latter has been recently demonstrated by the mycotoxin contaminations of gums (Zhang et al., 2014). The Panel noted that the differences in the microbiological criteria for agar between the specifications given by the EU Regulation and those given by JECFA are not decisive.

Information on the levels of toxic metals (e.g. lead, mercury, cadmium, and arsenic), as requested in the EFSA call for data[12] was not provided by interested parties. The Panel noted that, according to the EU specifications for agar (E 406), impurities of the toxic elements arsenic, cadmium, lead and mercury are accepted up concentrations of 3, 1, 5 and 1 mg/kg, respectively. Contamination at those levels could have a significant impact on the exposure to these metals for which the intake is already

close to the health-based guidance values established by the EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012).

No information on mean particle size and the particle size distribution was provided by interested parties.[12]

3.1.3. Manufacturing process

The industrial sources of agar used as a food additive are various species of red algae, the so-called agarophytes. In general, these algae belong to one of the two genera *Gelidium* or *Gracilaria*, respectively.

The number of species reported to be used worldwide in the commercial production of agar is 32 (Stanley, 2006), of which 12 are stated to be of primary commercial value (Stanley, 2006). The latter are (species as followed by producer countries): *Gelidium acerosa* (Japan, India), *Gelidium amansii* (Japan), *Gelidium cartilagineum* (USA, Mexico, South Africa), *Gelidium corneum* (South Africa, Portugal, Spain, Morocco), *Gelidium liatulum* (Japan), *Gelidium lingulatum* (Chile), *Gelidium pacificum* (Japan), *Gelidium pristoides* (South Africa), *Gelidium sesquidale* (Portugal, Morocco) and *Gracilaria confervoides* (South Africa) (Stanley, 2006). Even though many species of *Gelidium* are mentioned, according to McHugh (2003), *Gracilaria* algae still are the major agar source worldwide.

Industrially, agar is produced by a variety of extraction methods developed over the centuries (Stanley, 2006). In general, the production from harvest of the algae to the final packed agar in powder form consists of the following steps: harvesting and transport to coastal site for the preliminary drying as followed by bleaching, transport to processing plant, agar extraction process consisting of alkaline treatment (in the case of *Gracilaria* spp.), washing, soaking for extraction, filtration, gelling, freezing/thawing, drying, milling and packing (FAO, 2014-2015).

The preservation of the seaweeds, between the time of harvesting and their actual use by the agar manufacturer is important. The first step is preservation through dehydration, to avoid fermentation that first destroys the agar and then the seaweed. In general, the moisture content is reduced to about 20% by natural or artificial drying. This also reduces the risk of post-harvest contamination and growth of fungi, and subsequent production of mycotoxins. In recent (current) industrial practice of agar production, sodium hypochlorite (Li et al., 2008) and/or H_2O_2 (Stanley, 2006) are described as being used for bleaching. Positive experiments with photobleaching using sunlight have been described (Li et al., 2008).

It is necessary to avoid wetting during transportation and/or storage. The stability of agar contained in *Gelidium* species is higher than that of *Gracilaria agar. Gelidium* agar thus can be preserved in seaweeds indefinitely, provided that they have been treated properly. However, agar contained in *Gracilaria* can be hydrolysed both due to endogenous enzymes and to the growth of *Bacillus cereus*. According to Freile-Pelegrin (2000), some ways for preservation of *Gracilaria* algae during storage using formaldehyde have been reported. However, Freile-Pelegrin stated that such treatment has disadvantages since formaldehyde is highly toxic and causes pollution, and therefore, it should not be used (Freile-Pelegrin, 2000). No other references have been found on the use of formaldehyde in the production of agar. The Panel noted that no information on the use of formaldehyde following the call for data[12] has been provided by the interested parties.

Extraction may be accomplished under acidic or basic conditions, depending on the type of agarophyte and the product quality (Stanley, 2006). Extraction from *Gelidium* is performed under pressure (105–110°C for 2–4 h). *Gracilaria* is usually treated with water at 95–100°C for 2–4 h. The remainder of the process is the same for both types of raw material. Acidic extraction improves the yield of agar, whereas basic treatment modifies the structure of the carbohydrate chain to enhance the gelling properties. For the extraction of *Gracilaria* spp., alkali treatment is mandatory. Agar from these species is in general of low quality due to high natural sulfation. However, by treatment with alkaline solutions, the quality is improved, among others by conversion of L-galactose-6-sulfate to 3,6-anhydro-L-galactose, which enhances the gel-forming ability (Kumar and Fotedar, 2009). Also, a combination of basic treatment, which improves the gel strength, followed by acidic extraction may be used. For the alkali treatment, the seaweed is heated in 2–5% sodium hydroxide at 85–90°C for 1 h; the strength of the alkali varies with the species and is determined by testing on a small scale. After removal of the alkali, the seaweed is washed with water, and sometimes with very weak acid to neutralise any residual alkali. No matter the extraction process, the agar is recovered from the gelled extract by freezing and subsequent thawing which separates much of the water and dissolved salts (Stanley, 2006). The yield and the gel strength may depend not only on the extraction process but

also on the time of the year the harvest took place (Marinho-Soriano and Bourret, 2003, 2005). The latest developed method of extraction is the steam explosion technique where the agarophyte is heated in an autoclave to 140–190°C and then subjected to decompression. The exploded algae are then extracted with phosphate buffer (Stanley, 2006).

3.1.4. Methods of analysis in food

An analytical method for agar and further polysaccharides (locust bean gum, guar, gum arabic, tragacanth, arabinogalactan, carrageenan, 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 gas chromatography after hydrolysis with trifluoroacetic acid, derivatisation of the resulting monosaccharides with hydroxylaminhydrochloride 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 agar, 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). An alternative derivatisation method with trimethylchlorosilane is reported by Schmolck and Mergenthaler (1973).

In a later investigation, the analytical procedure described by Glueck and Thier (1980) was improved by Preuss and Thier (1983). Changes in the separation of interfering substances (fats, proteins and starch) allowed the quantitative determination of agar in a variety of foods like blancmange powder, glaze, fruit ice, and cream cheese. Recoveries for most of the thickeners and gums are about 60–85% with a coefficient of variation of 2–8%.

The Panel noted that both methods do not distinguish between agar and carrageenan as both consist only of galactose.

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

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, the polysaccharides are precipitated and separated via electrophoresis using polyacrylamide gel, agarose gel or a cellulose acetate membrane. Polysaccharides are then quantified using a TL-scanner.

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

Data on stability of food additive agar (E 406) during the shelf life were not available to the Panel. Agar quality and yield from the biomass of tropical agarophyte *Gracilaria cornea* after one and a half year of storage at a temperature of 22.1 ± 0.9°C and humidity of 59.8 ± 3.6% were within the range of food-grade agar (Freile-Pelegrin, 2000).

Agar in the dry state is not subject to contamination by microorganisms. However, agar solutions and gels are fertile media for bacteria and/or moulds and appropriate precautions should be taken to avoid the growth of microorganisms.[16]

Agar is a polysaccharide and therefore can suffer hydrolysis, which results in reduced molecular weight and lost of its gelling power. Acid hydrolysis appears as a result of lowered pH and becomes important if agar is extensively heated at pH below 5.5. Alkaline hydrolysis is important at pH above 8. The enzymatic hydrolysis is not relevant, as it is due to agarases (enzymes that break down agaroses) that are found in marine bacteria, in a few bacilli and schizosaccharomycetes that are not commonly found in food products where agar is used (Armisen and Galatas, 2000).

Due to the reactivity of agar with sugar, there is an increase in the gelling power of agar when used in products such as jams and jellies. The addition of humectants, such as glycerol or sorbitol, to agar gels reduces the drying of gels exposed to air. Tannic acid (pentadigalloyl glucose) present in some fruits like squash, apple and prune blocks the gelling process (Armisen and Galatas, 2000).

[16] Available on http://www.agargel.com.br/agar-tec-en.html

The high melting point of agar gels is also increased by the addition of salts (Saha and Bhattacharya, 2010). It has been conjectured that the enhancement of gel strength of agars by alkaline treatment may be in part due to removal of bound protein, thus producing a more regular polymer (Stanley, 2006).

In water or aqueous fluids, agar swells and may increase its volume by more than fivefold within 4 h (Teuscher et al., 2004; European Pharmacopeia, 2015), but, e.g. shows lower water binding capacity than guar gum or karaya gum. Agar is insoluble in cold water and in alcohol. However, agar is soluble in water at temperatures above the gel melting points (≥ 85°C). Then, it forms sols which can be cooled to much lower temperatures before setting to gels. Once the gel has set, reheating to higher temperature is required to melt it. This is known as 'gelation hysteresis' phenomenon. Viscosity of the sol is markedly dependent on the seaweed species from which the agar is extracted and on the extraction conditions employed (Stanley, 2006). Agar forms gels at very low concentrations, with a concentration threshold for gelation around 0.2% (Imeson, 2010).

According to Armisen and Galatas (2000), agar gelation occurs only in the neutral agarose fraction and does not need any other substance to gel. Agarose produces 'physical gels' which have all their structure formed only by the polymer molecules united solely by hydrogen bonds. Due to this unique gelling property, these gels hold in the interior network a great amount of water. The most remarkable property of 'physical gels' is their reversibility. They melt just by heating, but gel again upon cooling. The gelling temperature is around 38°C and the melting temperatures around 85°C.

In food some blended products of agar with other gelling agents are used to increase its gel strength, by modifying its texture or elasticity, or with antagonist to reduce the gel strength or block the gelling process. The use of a mixture of agar and locust bean gum reduces the loss of liquid from the gel (syneresis) during handling, transportation and storage (Armisen and Galatas, 2000). When mixed with konjac flour or glucomannan (which cannot form a gel alone), agar interacted synergistically to produce a more elastic gel (Aksowan, 2002).

3.2. Authorised uses and use levels

Maximum levels of agar (E 406) have been defined in Annex II to Regulation (EC) No 1333/2008[15] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, agar (E 406) is an authorised food additive in the EU at *quantum satis* (QS) in almost all foods apart from jam, jellies and marmalades and other fruit and vegetable spreads. Agar (E 406) is included in the Group I of food additives authorised at QS.

Table 2 summarises foods that are permitted to contain agar (E 406) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 2: MPLs of agar (E 406) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food categories name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		QS
01.4	Flavoured fermented milk products including heat treated products	Group I		QS
01.6.2	Unflavoured live fermented cream products and substitute products with a fat content of less than 20%	E 406		QS
01.6.3	Other creams	Group I		QS
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	QS
01.7.5	Processed cheese	Group I		QS
01.7.6	Cheese products (excluding products falling in category 16)	Group I		QS
01.8	Dairy analogues, including beverage whiteners	Group I		QS

Food category number	Food categories name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg)
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		QS
02.3	Vegetable oil pan spray	Group I		QS
03	Edible ices	Group I		QS
04.2.1	Dried fruit and vegetables	Group I		QS
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		QS
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		QS
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	E 406	(a)	10,000
04.2.5.3	Other similar fruit or vegetable spreads	E 406	(a)	10,000
04.2.5.4	Nut butters and nut spreads	Group I		QS
04.2.6	Processed potato products	Group I		QS
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	Group I	Only energy-reduced or with no added sugar	QS
05.2	Other confectionery including breath refreshening microsweets	Group I	E 406 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	QS
05.3	Chewing gum	Group I		QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		QS
06.2.2	Starches	Group I		QS
06.3	Breakfast cereals	Group I		QS
06.4.2	Dry pasta	Group I	Only gluten free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS
06.4.4	Potato Gnocchi	Group I	Except fresh refrigerated potato gnocchi	QS
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		QS
06.5	Noodles	Group I		QS
06.6	Batters	Group I		QS
06.7	Precooked or processed cereals	Group I		QS
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	QS
07.2	Fine bakery wares	Group I		QS
08.3.1	Non-heat-treated meat products	Group I		QS
08.3.2	Heat-treated meat products	Group I	Except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*	QS

Food category number	Food categories name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg)
08.3.3	Casings and coatings and decorations for meat	Group I		QS
09.2	Processed fish and fishery products including molluscs and crustaceans	Group I		QS
09.3	Fish roe	Group I	Only processed fish roe	QS
10.2	Processed eggs and egg products	Group I		QS
11.2	Other sugars and syrups	Group I		QS
12.1.2	Salt substitutes	Group I		QS
12.2.2	Seasonings and condiments	Group I		QS
12.3	Vinegars	Group I		QS
12.4	Mustard	Group I		QS
12.5	Soups and broths	Group I		QS
12.6	Sauces	Group I		QS
12.7	Salads and savoury-based sandwich spreads	Group I		QS
12.8	Yeast and yeast products	Group I		QS
12.9	Protein products, excluding products covered in category 1.8	Group I		QS
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	E 406	Only foods in tablet and coated tablet format	QS
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		QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	QS
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Group I	Only vegetable juices	QS
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	QS
14.1.4	Flavoured drinks	Group I		QS
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	QS
14.2.3	Cider and perry	Group I		QS
14.2.4	Fruit wine and made wine	Group I		QS
14.2.5	Mead	Group I		QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	QS
14.2.7.1	Aromatised wines	Group I		QS
14.2.7.2	Aromatised wine-based drinks	Group I		QS
14.2.7.3	Aromatised wine-product cocktails	Group I		QS
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		QS
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		QS
15.2	Processed nuts	Group I		QS

Food category number	Food categories name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg)
16	Desserts excluding products covered in category 1, 3 and 4	Group I		QS
17.1[(b)]	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		QS
17.2[(b)]	Food supplements supplied in a liquid form	Group I		QS
17.3[(b)]	Food supplements supplied in a syrup-type or chewable form	Group I		QS
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	Group I		QS

MPL: maximum permitted level.
(a): Maximum individually or in combination with E 400–404, E 406, E 407, E 410, E 412, E 415 and E 418.
(b): 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 III, Part 1 of Regulation (EC) No 1333/2008, agar (E 406) is authorised as a carrier in food additive at QS in all food additives.

According to Annex III, Part 2, to Regulation (EC) No 1333/2008, agar (E 406) is also authorised as food additive other than carriers in all foods additives at QS.

In addition, according to Annex III, Part 3 of Regulation (EC) No 1333/2008, agar (E 406) is also authorised as a food additive in food enzymes with a maximum level in the products (beverages or not) at QS.

According to Annex III, Part 4, to Regulation (EC) No 1333/2008, agar (E 406) is also authorised as food additive including carriers in all foods flavourings at QS.

Finally, according to Annex III, Part 5, Section A of Regulation (EC) No 1333/2008, agar (E 406) is also authorised at QS in all nutrients.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of agar (E 406)

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 public calls[17,18] for occurrence data (usage level and/or concentration data) on agar (E 406). In response to this public call, updated information on the actual use levels of agar (E 406) in foods was made available to EFSA by industry. No analytical data on the concentration of agar (E 406) in foods were made available by the Member States.

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

Industry provided EFSA with data on use levels (n = 21) of agar (E 406) in foods for 9 out of the 70 food categories in which agar (E 406) is authorised.

Updated information on the actual use levels of agar (E 406) in foods was made available to EFSA by FoodDrinkEurope (FDE) (Doc. provided to EFSA n. 12), the International Chewing Gum Association (ICGA) (Doc. provided to EFSA n. 13), Babbi confectionery industry (Doc. provided to EFSA n. 10), Biovegan GmbH (Doc. provided to EFSA n. 11) and Interested party 1 (Doc. Provided to EFSA n. 14).

[17] http://www.efsa.europa.eu//sites/default/files/consultation/140310.pdf
[18] http://www.efsa.europa.eu/sites/default/files/consultation/ans091123.pdf

The Panel noted that Biovegan GmbH provided data on levels of agar used in glazing, or in the gelling agent to be inserted then in jam, marmalades and confectionery. These levels were reported as food category 18. The Panel decided not to use the data from Biovegan GmbH (Doc. provided to EFSA n. 11).

Appendix A provides data on the use levels of agar (E 406) in foods as reported by industry.

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

The Mintel's Global New Products Database (GNPD) is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 800,000 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.[19]

For the purpose of this Scientific Opinion, the Mintel GNPD[20] was used for checking the labelling of products containing agar (E 406) within the EU's food products as the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel, agar (E 406) is labelled on more than 2,300 products of sauces, desserts, chewing gum and in decorations, coatings and fillings of foodstuff. Around 1,500 products were found to be published in this database between 2011 and 2016[15] out of which few drinks (n = 8): soy-based drinks, flavoured milk and ready-to-drink coffee were also published. The main foods labelled with agar are chilled desserts, chocolate coating confectionery and cakes.

The Panel noted that information from the Mintel GNPD (Appendix B) indicated that approximately 15 out of the 72 food subcategories, categorised according to the Mintel nomenclature, in which agar (E 406) was labelled, were included by the Panel in the current exposure estimates. These 15 food subcategories represented approximately 50% of the food products items labelled with agar (E 406) in the database. Agar (E 406) is authorised in 50 out of the remaining 57 food subcategories not included in the exposure assessment.

Appendix B presents the percentage of the food products labelled with agar (E 406) between 2011 and 2016, out of the total number of food products per food subcategories according to the Mintel food classification.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys recently[21] added in the Comprehensive database were also taken into account in this assessment.[22]

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, 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 3).

[19] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[20] http://www.gnpd.com/sinatra/home/ accessed on 05/10/2016.
[21] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm
[22] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Table 3: Population groups considered for the exposure estimates of agar (E 406)

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

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

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). The nomenclature from the FoodEx classification system has been linked to the food categorisation 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.

3.3.3.2. Food categories considered for the exposure assessment of agar (E 406)

The food categories in which the use of agar (E 406) is 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 was the case for nine food categories (Appendix C) and may have resulted in an underestimation of the exposure. The food categories which were not taken into account are described below (in the ascending order of the FCS codes):

- 01.6.3 Other creams
- 02.3 Vegetable oil pan spray
- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4 restricted to fillings, toppings and coatings for fine bakery wares and desserts
- 06.6 Batters
- 06.7 Precooked or processed cereals
- 08.3.3 Casings and coatings and decorations for meat
- 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products, only vegetable nectars
- 14.2.4 Fruit wine and made wine
- 14.2.5 Mead.

For the following food category, the restrictions/exceptions which apply to the use of agar (E 406) could not be taken into account, and therefore the whole food category was considered for the exposure estimations. This applies to two food categories (Appendix C) and may have resulted in an overestimation of the exposure:

- 05.1 Cocoa and Chocolate products as covered by Directive 2000/36/EC, only energy-reduced or with no added sugar
- 08.3.2 Heat-treated meat products, except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*.

Most of the food categories were not taken into account because no concentration data were provided to EFSA. For the remaining food categories, the refinements considering the restrictions/

exceptions as set in Annex II to Regulation No 1333/2008 were applied. Overall, for all scenarios, seven food categories were included in the present exposure assessment to agar (E 406) (Appendix C).

3.4. Exposure estimate

3.4.1. Exposure to agar (E 406) from its use as a food additive

The Panel estimated chronic exposure to agar (E 406) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to agar (E 406) was calculated by multiplying agar (E 406) concentrations for each food category (Appendix C) 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 1 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 exposure per survey and population group (Table 3). On the basis of these distributions, the mean and the 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy, and for toddlers from Belgium, Italy and Spain were not included.

The exposure assessment to agar (E 406) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario*) as provided by industry. These two scenarios are discussed in detail below.

A possible additional exposure from the use of agar (E 406) as a food additive in food additives, enzymes and nutrients in accordance with Annex III (Parts 1, 3 and 5) to Regulation (EC) No 1333/2008 was not considered in any of the exposure assessment scenarios, as no data were available.

No specific scenario for food supplements was considered by the Panel because it is authorised at QS in the Regulation (EC) No 1333/2008, and no usage data were provided by industries.

3.4.1.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As agar (E 406) is 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, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

The concentration data used in this scenario are listed in Appendix C.

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the population groups will be exposed to agar (E 406) present in food at the maximum reported use levels over a long period of time.

3.4.1.2. 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 C summarises the concentration levels of agar (E 406) 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 agar (E 406) present at the maximum reported use for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level;
  - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long term to agar (E 406) present at the mean reported use in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

3.4.1.3. Dietary exposure to agar (E 406)

Table 4 summarises the estimated exposure to agar (E 406) from its use as a food additive in six population groups (Table 3) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix D.

Table 4: Summary of dietary exposure to agar (E 406) from their use as a food additive 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 (12 weeks–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	0.01–3.8	1.0–17.0	1.9–13.9	0.9–5.1	0.3–2.6	0.2–2.6
95th percentile	< 0.01–20.9	5.6–48.7	7.3–37.6	3.2–15.8	1.3–10.0	0.7–9.8
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	0.01–3.6	0.9–14.8	1.7–11.6	0.8–4.2	0.3–2.3	0.3–2.3
95th percentile	< 0.01–19.6	5.1–44.5	5.5–34.2	2.7–14.1	1.0–8.8	1.0–8.8
Non-brand-loyal scenario						
Mean	0.01–2.0	0.2–8.4	0.2–6.6	0.1–2.1	0.05–1.1	0.02–1.2
95th percentile	< 0.01–10.5	1.5–26.2	0.6–19.6	0.5–7.7	0.2–4.8	0.1–4.9

From the *maximum level exposure assessment scenario*, the mean exposure to agar (E 406) from its use as a food additive ranged from 0.01 mg/kg bw per day in infants to 17.0 mg/kg bw per day in toddlers. The 95th percentile of exposure to agar (E 406) ranged from < 0.01 mg/kg bw per day in infants to 48.7 mg/kg bw per day in toddlers.

From the *refined estimated exposure brand-loyal scenario*, the mean exposure to agar (E 406) from its use as a food additive ranged from 0.01 mg/kg bw per day in infants to 14.8 mg/kg bw per day in toddlers. The high exposure to agar (E 406) ranged from < 0.01 mg/kg bw per day in infants to 44.5 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, the mean exposure to agar (E 406) from its use as a food additive ranged from 0.01 mg/kg bw per day in infants to 8.4 mg/kg bw per day in toddlers. The 95th percentile of exposure to agar (E 406) ranged from < 0.01 mg/kg bw per day in infants to 26.2 mg/kg bw per day in toddlers.

3.4.1.4. Main food categories contributing to exposure to agar (E 406) using the maximum level exposure assessment scenario (Table 5)

Table 5: Main food categories contributing to exposure to agar (E 406) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
01.4	Flavoured fermented milk products including heat-treated products	12.5–57.0 (6)	9–74.6 (10)	6.2–27.4 (14)	5.6–23 (11)	5.3–24.1 (15)	6.1–25.1 (13)
03	Edible ices	5.2–36.8 (3)	5.6–23.3 (7)	5.9–41.4 (18)	7–38.8 (17)	5.4–37.6 (16)	5.3–43.1 (12)
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	5.2–73.8 (5)	11.7–44.2 (9)	17.1–55.1 (18)	21.5–66.6 (17)	17–48.7 (17)	8.5–36.4 (14)
05.3	Chewing gum	–	–	5.8–13.9 (4)	5.2–28.6 (4)	11.9–27.4 (2)	6.2–17.9 (2)

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
08.3	Meat products	6.8–17 (3)	9.9–15.1 (2)	5.8–12.3 (8)	5.4–12.3 (9)	5.6–38 (12)	8.3–55 (8)
16	Desserts excluding products covered in categories 1, 3 and 4	58.6–79 (3)	7.9–74.1 (8)	10.3–64.2 (15)	6.3–56.5 (15)	5.8–62 (15)	5.2–76.7 (13)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

3.4.1.5. Main food categories contributing to exposure to agar (E 406) using the refined exposure assessment scenario (Tables 6 and 7)

Table 6: Main food categories contributing to exposure to agar (E 406) using the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
01.4	Flavoured fermented milk products including heat-treated products	12.8–58.3 (6)	9.8–82.3 (10)	5.3–34.6 (15)	5.9–28.7 (12)	6–27.3 (15)	6.6–27.4 (13)
03	Edible ices	12.7–37.7 (2)	7.8–23.6 (5)	5.3–42.7 (14)	6.5–41.8 (14)	5.5–39.7 (15)	5–45.4 (10)
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	5.6–73.8 (3)	6.8–44.7 (9)	8.5–57.2 (18)	14.5–68.7 (17)	11.8–50.7 (17)	5.4–37.4 (14)
05.3	Chewing gum	–	–	5.9–14.3 (4)	10.5–31.5 (3)	11.2–33.7 (2)	5.9–22.4 (2)
08.3	Meat products	14.1–14.1 (1)	13.2–13.2 (1)	8.6–8.6 (1)	5.4–6.9 (4)	5.3–35.1 (7)	5.1–55.7 (8)
16	Desserts excluding products covered in categories 1, 3 and 4	63.3–82.2 (3)	41.7–79.6 (7)	12.4–79.3 (15)	6.3–69.2 (15)	5.3–70.2 (16)	5.7–82.9 (13)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

Table 7: Main food categories contributing to exposure to agar (E 406) using the non-brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
01.4	Flavoured fermented milk products including heat-treated products	22.5–94.1 (6)	19.2–95.0 (10)	9.1–71 (17)	5.8–66 (17)	13.4–64.9 (16)	12.9–68.5 (13)
03	Edible ices	5.2 (1)	8.1 (1)	5.3–34 (9)	6.6–20 (6)	5.5–10.9 (5)	5.3–12.2 (3)
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	12.6 (1)	9.3 (1)	5–21.8 (9)	5.1–25.6 (12)	5.6–16.2 (10)	6.4–12.6 (3)
05.3	Chewing gum	–	5.1 (1)	7.2–21.7 (4)	5–47.1 (9)	7.0–51.3 (4)	5.9–31.4 (2)
08.3	Meat products	–	11.0 (1)	6.4–21.0 (2)	5.2–10.9 (6)	5.0–33.3 (7)	5.6–58.1 (4)

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
16	Desserts excluding products covered in categories 1, 3 and 4	53.3–76.6 (3)	8.4–78.3 (8)	7.5–84.6 (16)	6.3–80.5 (16)	9.8–75.5 (16)	9.8–84.1 (13)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

3.4.1.6. Uncertainty analysis

Uncertainties in the exposure assessment of agar (E 406) 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 8.

Table 8: 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 of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: • uncertainties to which types of food the levels refer to • use levels considered applicable for all foods within the food category	+/–
Food categories considered for the exposure assessment (all scenarios): • exclusion of food categories due to missing FoodEx linkage (n = 9/70 food categories) • inclusion of food categories without considering the restriction/exception (n = 2/70 food categories)	– +
Reported use levels: • data not available for many food categories which were excluded from the exposure estimates (n = 61/70 food categories i.e. representing max 16% by weight of the food which may contain agar) • uncertainty on the actual use of agar (E 406) in food categories authorised for the Group I food additives	– +/–
Maximum level exposure assessment scenario: • food categories which may contain agar (E 406) due to carry-over not considered • food categories listed under Annex II to Regulation (EC) No 1333/2008 considered at maximum reported use levels	– +
Refined exposure assessment scenarios: • food categories which may contain agar (E 406) due to carry-over not considered • exposure calculations based on the maximum or mean levels (reported use from industries)	– +/–
Uncertainty in possible national differences in use levels of food categories	+/–

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

Agar (E 406) is authorised as a Group I food additive in 66 food categories and has a specific authorised uses in four categories (Table 2). Since, the majority of food categories correspond to the general Group I food additives authorisation, agar (E 406) may not necessarily be used in some of these food categories. This may explain why reported use levels of agar (E 406) were only available for nine food categories. The Panel calculated that the seven food categories taken into account in the present estimates constitutes between 12% and 16%, depending of the population groups, by weight of the food which according to Annex II may contain agar as a food additive.

Furthermore, the Panel noted that information from the Mintel's GNPD (Appendix B) indicated that approximately 15 out of the 72 food subcategories, categorised according to the Mintel nomenclature, in which agar (E 406) was labelled, were included by the Panel in the current exposure estimates. These 15 food subcategories represented approximately 50% of the food products items labelled with agar (E 406) in the database. In the remaining 57 food subcategories, in which agar (E 406) was labelled but which were not included in the exposure assessment, agar (E 406) was authorised in 50 food subcategories.

Overall, the Panel considered that the uncertainties identified result in an overestimation of the exposure to agar (E 406) as a food additive in the European countries for the maximum level exposure scenario and for the refined scenario considering only food categories for which data have been reported (10% of the authorised uses).

However, considering information from the Mintel GNPD, only approximately 50% of the food products labelled with agar (E 406) belonged to food subcategories for which reported use levels were available, and consequently used in the exposure assessment. The Panel noted that given the information from the Mintel's GNPD, it may be assumed that agar is used in food categories for which no data have been provided by food industry. If this was confirmed, it would therefore result in an underestimation of the exposure.

The Panel noted that food categories which may contain agar (E 406) due to carry-over (Annex III, Parts 1, 2, 3, 4, 5 section A) were not considered in the current exposure assessment.

3.4.2. Exposure via other uses

Exposures to agar due to the following uses were not considered in this opinion.

Agar as an ingredient in slimming products and other foods

Uses of agar in slimming products and in products for supportive treatment of diabetes and/or obesity are described (Teuscher et al., 2004; Maeda et al., 2005; Haensel and Sticher, 2007; Imeson, 2010).

In these products, agar is regarded as a satiating material as it swells in the stomach to increase bulk and to give a feeling of fullness, leading to less intake of food. Agar, also known as kanten, is popular for dieting in the form of the 'kanten plan' or 'kanten diet' in Japan, which are also receiving publicity in the USA (Maeda et al., 2005; Imeson, 2010) (see Section 3.5.7).

The Panel noted that agar can also be used as an ingredient in the preparation of homemade foods.

Pharmaceutical uses

Agar is an excipient used in pharmaceutical products, e.g. as a tablet disintegrant or as an emulsifier. It is used in solid, semisolid and liquid dosage forms.

Several (older) textbooks and Pharmacopoeias mention the medicinal use of agar (British Pharmaceutical Codex (BPC), 1954; The United State Pharmacopeia (U.S.P) XVI, 1960; Teuscher, 1987). The daily dosage is given with 4–16 g, the average single dose is defined with 4 g (BPC, 1954; Sollmann, 1957; U.S.P. XVI, 1960; cited in JECFA, 1974). But also daily dosages up to 20 g are mentioned (List and Lörhammer, 1969). The action of agar is described as a mild laxative, due to its swelling properties. The intake of agar is thought to cause an increase in the bulk of the content of the intestine which stimulates the intestinal muscles, thereby aiding peristalsis. No adverse effects are described and the posology is given with three times daily 1–2 teaspoons (a teaspoon of 5 mL corresponds to 2.75 g agar) together with liquids (Heber, 2007). Agar is also used in combination products to treat constipation (e.g. with liquid paraffin and phenolphthalein); however, because of the often small amounts of agar in this combinations, it is only be judged as an emulsion stabiliser (Martindale, 2014).

From data provided by the European Medicines Agency (EMA), information was retrieved about the current medicinal use of agar and its use as an excipient in medicinal products (Doc. provided to EFSA n.1). For agar as an active ingredient, only few authorised medicinal products exist (or existed) within the EU (as combination with other active ingredients). In those combination products,[23] the daily dosage of agar is claimed to be up to 900 mg (up to three times daily 300 mg) as an adjuvant in treatment of obesity. The usage in children is not foreseen.

[23] http://www.medisite.fr/dictionnaire-des-medicaments-pseudophage-granule-pour-solution-buvable-en-sachet-dose.778863.8028.html

3.5. Biological and toxicological data

3.5.1. Absorption, distribution, metabolism and excretion

There is substantial evidence that high molecular weight dietary polysaccharides, could be broken down in the large intestine in man. In addition to intermediate metabolites such as lactate, acrylate or fumarate, the main end products of this colonic anaerobic digestive process are short-chain fatty acids (SCFA), such as acetic, propionic and butyric acids, which are absorbed from the colon (Cummings and Englyst, 1987). Only limited *in vitro* data on microbial fermentation of agar polysaccharides were available.

***In vitro* data**

In an *in vitro* study, low molecular weight polysaccharides derived from agar were fermented using human faeces from three adult donors as the source of inoculum (Ramnani et al., 2012). These polysaccharides were prepared by use of either acid hydrolysis or degradation using hydrogen peroxide. During fermentation assays, organic matter disappearance and SCFA production were measured after 5, 10 or 24 h of anaerobic incubation. After 24 h of incubation, acetate and propionate productions were 3.4 times lower in case of *Gelidium*-derived agar powder (14.4 mM) than for agar powder derived from *Gracilaria* (48.7 mM). The Panel noted that agar powders were less fermented than inulin (61.5 mM) and more fermented than cellulose (2.0 mM).

***In vivo* data**

Digestibility of agar was investigated in an *in vivo* study (five albino rats, no further information about the strain) dosed with basal diet supplemented with agar (750 mg agar/rat per day) over 7 days; a digestibility of 21% was calculated (Booth et al., 1963). These authors defined the digestibility as the ratio of the total intake of the test material minus increase in faecal weight to the total intake of test material. The Panel noted that these data on digestibility, as defined by the authors, are not relevant for assessing the absorption process of agar.

Overall, the *in vitro* degradation of agar has been investigated in an *in vitro* human model. This study indicated that agar could be partially fermented during its passage through the large intestine by the action of the intestinal tract microflora. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that agar is unlikely to be absorbed unchanged and that the limited extent of fermentation of agar would lead to products such as SCFA. Based on the available knowledge on the role of SCFA as end products of the fermentation of dietary fibres by the anaerobic intestinal microflora (Topping and Clifton, 2001; Den Besten et al., 2013), the Panel considered that their potential formation as fermentation products from agar does not raise any concern.

3.5.2. Acute toxicity

Oral acute lethal dose (LD_{50}) values for agar have been reported of 11,400 and 15,700 mg/kg bw in rats and mice, respectively (Bailey and Morgareidge, 1976, cited in NTP, 1982, Doc. provided to EFSA n.8).

An acute oral toxicity study was conducted with F344 rats and B6C3F1 mice. Groups of five males and five females of each species were administered a single dose of agar (630, 1,750, or 2,500 mg/kg bw) in distilled water by gavage. No chemical-related effects were observed at necropsy for either rats or mice (Doc. provided to EFSA n.8).

Groups of 30 female F344 rats were dosed over 1 week with diet containing 3% agar (equivalent to 3,600 mg/kg bw per day) in freeze-dried or gel form. There was no post-observation period. The dosing caused no signs of illness and there were no significant differences with respect to final body weights and feed conversion (weight gain per gram dry feed consumed). No further parameters were tested (Morrissey and Norred, 1984).

3.5.3. Short-term and subchronic toxicity

3.5.3.1. Short-term toxicity

In a 14-day oral study, groups of five F344 rats and five B6C3F1 mice of either sex were dosed via the diet with 6,300, 12,500, 25,000, 50,000 or 100,000 mg/kg diet (in rats equivalent to 756, 1,500, 3,000, 6,000 or 12,000 mg/kg bw per day; and in mice equivalent to 1,260, 2,500, 5,000, 10,000 or 20,000 mg/kg bw per day). No differences in weight and mortality were observed. At necropsy on day 15, no substance-related effects were noted. No further study details were provided (Doc. provided to EFSA n.8).

JECFA described a short-term toxicity study not available as following:

'Six weanling male rats fed a diet containing 25% of agar for four weeks showed growth retardation during the third week. The dry weight of the cleaned stomach and caecum was found moderately increased. Colon and rectum were more than twice as heavy as in the controls (Fischer, 1957)'.

Glauert et al. (1983) performed a 6-week feeding study in Sprague–Dawley rats. Ten male animals were randomly assigned to a group receiving a control diet, or an agar diet consisting of the control diet plus 8% added agar (equivalent to 9,600 mg/kg bw per day). The dosing caused no significant differences concerning diet intake (956 or 993 g for control diet or diet with 8% agar, respectively) and weight gain (116 or 119 g for control diet or diet with 8% agar, respectively). While in rats fed the control diet, the colon mucosa was flat with circular or slit-like crypt openings occurring regularly, surface irregularities (deep, coarse folds appearing as discontinuities in the epithelial lining observed with an electron microscope scanning) developed in rats fed agar. The authors discussed that the disruptions in the colon surface might allow an increased absorption of toxins into the epithelial cells and also may cause an increase in cell proliferation in the crypts to replace damaged cells on the colon surface. The Panel noted that the high concentrations at which agar was administered in the diet may have led to damage of the lining epithelium of the colon. The Panel further noted that the observed effects appeared only 6 weeks after treatment and that other rat strains exposed to agar for longer periods and at higher concentrations in subchronic toxicity studies did not show similar effects.

In a study performed by Booth et al. (1963), one group of six weanling male rats was dosed with 15% of agar (equivalent to 1,350 mg/kg bw per day) via diet over 62 days.

The weight gain of the dosed animals was comparable to the controls (190 $\pm$ 11.9 g vs 199 $\pm$ 10.4 g in controls), but the food efficiency (weight gain/food intake) was significantly lower ($-$18%). Other parameters were not investigated. The Panel noted that the high concentrations in which agar has been administered in the diet may have caused nutritional imbalance.

3.5.3.2. Subchronic toxicity

JECFA described in 1974 a subchronic toxicity study not available to the Panel as follows:

'Four groups of six rats received diets containing 5%, 10%, 20% and 30% of agar, respectively, for 10 weeks. The group receiving the 10% diet gained weight about 20% faster than the controls; the other groups gained weight at the same rate as the controls. The rats fed diets containing 20% and 30% of agar required significantly more feed and water per gram of weight gain than the control group (Nilson and Schaller, 1941)'.

In a 13-week oral dose range-finding study, groups of 10 animals per sex (F344 rats and 10 B6C3F1 mice) were dosed via the diet with 0, 3,100, 6,300, 12,500, 25,000 or 50,000 mg/kg of diet (equivalent to 0, 279, 567, 1,125, 2,250 or 4,500 mg/kg bw per day in rat and 0, 629, 1,260, 2,500, 5,000 or 10,000 mg/kg bw per day in mice). Animals were observed twice daily, weighed weekly, and at study termination on day 91, all survivors were killed and necropsied. There were two deaths, one male control rat and one male control mouse. The dosing caused no changes in body weights and there were no substance-related gross or histopathology effects. No further information was provided (abstract in NTP (1982), Doc. provided to EFSA n.8). The Panel noted that the NTP studies did not include haematology, urinalysis and clinical chemistry.

3.5.4. Genotoxicity

3.5.4.1. *In vitro* studies

In the study by Litton Bionetics (Doc. provided to EFSA n.6), agar was assessed for its mutagenicity in the reverse mutation assay using *Salmonella* Typhimurium strains TA 1530 and G-46 and for induction of mitotic gene conversion in *Saccharomyces cerevisiae* (strain D3) and no genotoxicity was observed. However, the Panel noted that the results from the bacteria gene mutation assay are limited due to the inadequate number of *S.* Typhimurium tester strains used and missing information on the use of metabolic activation and concentrations of test substance employed. In addition, the Panel noted that the gene conversion assay with *S. cerevisiae* has not been validated and it is no longer employed for risk assessment.

Agar was also assessed for its capability to induce chromosomal aberrations in anaphase in human embryonic lung cells (WI-38) at dose levels of 10, 100 and 1,000 μg/mL without S9 mix. No

cytogenetic effects were reported by authors (Doc. provided to EFSA n.6). However, the Panel noted that this assay, though scientifically sound, did not receive further validation.

3.5.4.2. *In vivo* studies

In a host-mediated assay, groups of five male IBR mice were administered agar by oral gavage at 7.15, 71.5 and 715.0 mg/kg either once (acutely) or on five consecutive days 24 h apart. Negative and positive control animal groups were also included. The highest dose level of agar (715 mg/kg) corresponded to the calculated LD5. The indicator organisms used in this study were: (i) two histidine auxotrophs (his G-46, TA 1530) of *S.* Typhimurium for induction of reverse mutation and (ii) a diploid strain (D-3) of *S. cerevisiae* for the induction of mitotic gene conversion. Both for the acute and subacute studies, within 30 min from the last administration, all animals received 2 mL of the indicator organism, intraperitoneally, each mL containing 3.0×10^8 cells for *Salmonella* and 5.0×10^8 cells for *Saccharomyces*. Three hours after the injection of the indicator organism, the mice were sacrificed and the host cells were aseptically washed out of the peritoneum of each animal and plated under standard conditions. A second experiment was additionally performed under the same experimental conditions with only one dose level of agar but at 5,000 mg/kg. Results obtained in both experiments indicated that agar did not show genotoxic activity in any of the indicator organisms and dose levels employed (Doc. provided to EFSA n.6). However, the Panel noted that the host-mediated assay has not received further validation and it is no longer employed for risk assessment.

In an *in vivo* cytogenetic assay, the induction by agar of chromosomal aberrations in bone marrow cells of rats was investigated. Groups of five male albino rats were administered with test substance by oral gavage acutely at 7.15, 71.5 and 715 mg/kg bw or subacutely on five consecutive days, 24 h apart, at the same dose levels employed for the acute treatment. Negative and positive control animal groups were also included. For the acute treatment, sampling of bone marrow cells was performed at 6, 24 and 48 h from the last administration, while in the subacute study sampling was only performed at 6 h from the last administration. A second experiment was additionally performed under the same experimental conditions with only one dose level of agar but at 5,000 mg/kg. Results obtained indicated that agar induced no increases in the incidence of chromosomal aberrations in the bone marrow cells following both acute and subacute administration, at any of dose level employed. The Panel noted that this study, essentially complies with the OECD Guideline 475 requirements, although it was performed much earlier in the 1974 (Doc. provided to EFSA n.6).

In a dominant lethal assay, agar was administered by oral gavage to groups of 10 male albino rats acutely at 7.15, 71.5 and 715 mg/kg bw or subacutely on five consecutive days, 24 h apart, at the same dose levels employed for the acute treatment. Negative and positive control animal groups were also included. Following treatment, the males were sequentially mated with two females per week for 8 weeks (7 weeks in the subacute study) and housed separately until sacrifice. A second experiment was additionally performed under the same experimental conditions with only one dose level of agar but at 5,000 mg/kg. Total implants (live fetuses plus early and late fetal deaths), total dead (early and late fetal deaths), dead implants per total implants and preimplantation loss (calculated as the difference between the total corpora lutea and total implant counts) were evaluated. Results obtained indicated that agar induced some increases compared to the negative control in average implantations and average resorptions in the low dose group at week 8 in the acute treatment and some differences at various weeks throughout the parameters in the subacute treatment. However, these effects revealed no dose–response or time-trend pattern. No effects were observed at 5,000 mg/kg both for the acute and subacute treatment. The author concluded that agar does not induce dominant lethal mutations under the reported experimental condition (Doc. provided to EFSA n.6). The Panel agreed with this conclusion and further noted that the number of animals employed for this study was low.

Overall, the Panel noted that, although the available *in vitro* and *in vivo* studies are limited, no genotoxic activity has been observed for agar. Moreover, agar is routinely used as a substrate in *in vitro* gene mutation assays in bacteria and mammalian cells. In addition, considering the chemical structure of agar and its negligible absorption, the Panel considered that there is no concern with respect to the genotoxicity of agar (E 406).

3.5.5. Chronic toxicity and carcinogenicity

No chronic toxicity studies according to OECD guidelines or equivalent have been identified by the Panel.

Mice

A carcinogenicity study performed by NTP in 1982 (Doc. provided to EFSA n.8) and published by Melnick et al., 1983 was performed in groups of 50 B6C3F1 mice of either sex exposed to agar via diet (mixed to the feed, no solvent) at concentrations of 0, 25,000 or 50,000 mg agar/kg diet (equivalent to 0, 2,250 or 4,500 mg agar/kg bw per day in mice) for 103 weeks followed by 2 weeks on basal diet before study termination (Doc. provided to EFSA n.8, Melnick et al., 1983). Animals were observed twice daily, individual weights and clinical signs of toxicity were recorded monthly. Moribund animals and those that survived to the end of the study were killed. Gross and microscopic examinations were performed on all major organs and tissues, while parameters such as haematology, clinical chemistry and urinalysis were not investigated. In female mice, reduced mean bodyweights (−20% and −16% for low- and high-dosed animals at study termination, respectively), in comparison with controls, were seen after week 20 and remained lower throughout the study. The treatment caused no adverse effects concerning clinical signs of toxicity, survival rate feed consumption and non-neoplastic changes and there was no indication of a substance-related increased tumour incidence in both species.

Rats

A carcinogenicity study performed by NTP in 1982 (Doc. provided to EFSA n.8) and published by Melnick et al., 1983 was performed in groups of 50 F344 rats of either sex exposed to agar via diet (mixed to the feed, no solvent) at concentrations of 0, 25,000 or 50,000 mg agar/kg diet (equivalent to 0, 1,250 or 2,500 mg agar/kg bw per day in rat) for 103 weeks according to the same design than the mice study above. The treatment caused no adverse effects concerning clinical signs of toxicity, survival rate, feed consumption and non-neoplastic changes, and there was no indication of a substance-related increased tumour incidence in both species. Therefore, the Panel considered that there was no indication for a carcinogenic potential and that no adverse effects were observed at 2,500 mg agar/kg bw per day, the highest dose tested.

Initiation–promotion studies

The Panel noted a study which had investigated potential enhancement of agar on the development of cancer in animal models treated with an initiating agent (Glauert et al., 1981). The Panel did not consider this study relevant to the risk assessment of agar as a food additive.

3.5.6. Reproductive and developmental toxicity

3.5.6.1. Reproductive toxicity studies

No studies available.

3.5.6.2. Developmental toxicity studies

In all studies performed by the Food and Drug Research Laboratories (Doc. provided to EFSA n.3) described below, body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. Animals were administered by gavage (1.0 mL/kg bw) different doses of agar (not specified) suspended in anhydrous corn oil; the control groups were vehicle-treated. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups 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).

Mice

CD-1 mice were treated by oral gavage once daily from gestation day (GD) 6 to 15 with doses of 0, 15.7, 72.9, 329 or 1,570 mg/kg bw per day agar in corn oil (23, 24, 25, 25, and 48 mated females/group, respectively). This resulted in groups with 22, 20, 23, 24 and 20 pregnancies, which all survived to term, except those in the highest dose group (1,570 mg/kg bw per day) (Doc. provided to EFSA n.3). At necropsy on GD 17, the surviving dams appeared to be completely normal and the number of implantations and live fetuses was comparable to the control group, except in the highest dose group of 1,570 mg/kg bw per day. In this treatment group, a significantly increased mortality in dams (9 out of 48 animals) and a decrease in the pregnancy rate (42%) of survivors were observed. At doses up to 329 mg agar/kg bw per day, no effects were found on implantation, maternal and fetal survival and on the numbers of live or dead fetuses, resorptions, and on fetal weights. However, a marked increase in the number of resorptions and a significant growth retardation of live fetuses (with decreased fetal weights

and incomplete ossification) were observed at the highest dose (1,570 mg/kg bw per day). The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal examination of the agar-treated groups did not differ from the vehicle-treated control group (Doc. provided to EFSA n.3). The Panel noted that the no-observed-adverse-effect-level (NOAEL) in this study was 329 mg agar/kg bw per day for both maternal and developmental toxicity.

Rats

Pregnant Wistar rats (25/group) were treated by oral gavage once daily from GD 6 to 15 with doses of 0, 11.4, 53, 247 or 1,140 mg/kg bw per day of agar in corn oil (Doc. provided to EFSA n.3). At necropsy on GD 20, the dams treated with doses up to 1,140 mg agar/kg bw per day appeared to be completely normal and had no effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implantations and fetal weights did not differ amongst the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the agar-treated groups, did not differ from the number occurring in the vehicle-treated dams of the control group. Even at the highest dose level of 1,140 mg/kg bw per day, no adverse effects on both dams and fetuses were noted (Doc. provided to EFSA n.3). The Panel noted that the NOAEL for both maternal and developmental toxicity was 1,140 mg agar/kg bw per day, the highest dose tested.

Hamsters

Pregnant Golden hamsters (25–30 animals/group) were treated by oral gavage once daily from GD 6 to 10 of gestation with doses of 0, 6.5, 30.2, 140.3 or 650 mg agar/kg bw per day in corn oil (Doc. provided to EFSA n.3). At necropsy on GD 14, the dams treated with doses up to 650 mg agar/kg bw per day appeared to be completely normal and showed no effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implant sites or fetal weights did not differ amongst the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the agar-treated groups, did not differ from the number occurring in vehicle-treated dams of the control group (Doc. provided to EFSA n.3). The Panel noted that the NOAEL for both maternal and developmental toxicity was 650 mg agar/kg bw per day, the highest dose tested.

Rabbits

Artificially inseminated Dutch-belted rabbits were treated by oral gavage once daily from GD 6 to 18 with doses of 0, 5.5, 25.6, 118.5 or 400 mg/kg bw per day of agar in corn oil (21, 15, 15, 15 or 25 mated females/group, respectively) (Doc. provided to EFSA n.3). At term (GD 29), the mortality of the dams was 3/21, 3/17, 1/15, 0/15 and 9/25 dosed with 0, 5.5, 25.6, 118.5 and 400 mg/kg bw per day agar, respectively. The authors concluded that there was an evident maternal lethality in the highest dose group. Those dams, surviving, appeared normal throughout the observation period. No effect was observed on the number of implantations. At caesarean section, the pregnant rabbits appeared to have normal fetuses (Doc. provided to EFSA n.3). The Panel noted that the NOAEL for maternal lethality/toxicity was 118.5 mg agar/kg bw per day, and that the NOAEL for developmental toxicity was 400 mg agar/kg agar bw per day, the highest doses tested.

The Panel noted that the mortality observed in female mice at 1,570 mg/kg bw per day and in female rabbits at 400 mg/kg bw per day was not in line with the findings from acute toxicity studies (Bailey and Morgareidge (1976). This effect was not observed in other species. The Panel noted that the mortality could be caused by the difficulty of dosing mice and rabbits with a viscous solution. Therefore, the Panel considered the maternal toxicity in these two species as not relevant for the risk assessment.

Overall, no data on fertility effects were available. The Panel considered the available prenatal developmental toxicity studies in mice and rabbits not suitable for the risk assessment due to high maternal toxicity (mortality). In the prenatal developmental toxicity studies in rats and hamsters, no maternal and developmental effects were observed up to the highest dose tested (1,140 and 650 mg agar/kg bw per day, respectively) (Doc. provided to EFSA n.3).

3.5.7. Other studies including hypersensitivity, allergenicity and food intolerance

3.5.7.1. Animal studies

The effect of polysaccharides, including agar, fed at the 10% level in a semisynthetic 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 was studied over a period of 8 days in five groups of 12 weanling male rats each and compared to a control group (Harmuth-Hoene and Schelenz, 1980). Agar reduced significantly the absorption of all minerals tested. When agar was fed continuously over 21 weeks (same dose), interference with the absorption of iron and copper was most pronounced during the initial phase of rapid animal growth. In the case of zinc, chromium and cobalt absorption was reduced significantly during the entire study. In these animals, the increased faecal losses of minerals caused by ingestion of agar were not reflected in total mineral content of carcasses at the completion of the 21-week feeding trial.

3.5.7.2. Human data

Food uses

Case reports

A 64-year-old woman with a gastric bypass for morbid obesity experienced nausea, vomiting and dysphagia over 72 h after ingestion of an agar-based dessert. A gastric agar bezoar was detected 2 weeks later and removed (Gero et al., 2015).

A 70-year-old Japanese woman was presented to the emergency room with abdominal pain, nausea and vomiting. Forty-eight hours prior to admission, she had eaten a large amount of highly concentrated agar dissolved in boiling water. Bezoars, considered to have formed from the agar, were found impacted in the distal jejunum and removed by surgery. The woman had been diagnosed with type-2 diabetes 6 years earlier and reported to take dietary agar sometimes (Osada et al., 2008). Two additional cases of bezoar formation after drinking agar solutions have been reported (Sugiyama et al., 2009).

The Panel considered these cases as not relevant for the safety evaluation of the food additive use of agar (E 406) in food for the general population, since agar was either consumed for dietary purposes in large amounts (Osada et al., 2008; Sugiyama et al., 2009) or under the special medical conditions of a patient with a gastric bypass (Gero et al., 2015).

Clinical studies

Maeda et al. (2005) evaluated the efficacy of an agar diet in combination with a conventional diet (traditional Japanese food) for obese patients with impaired glucose tolerance and type-2 diabetes. After a 4-week run-in period on their habitual diets, 76 patients were randomly assigned to have conventional diet or conventional diet with agar. The agar-diet subjects received 180 g of jelly-like preparation containing 4.5 g agar, seasoned with condiments, each day 15 min before the evening meal. Both groups were on these diets for 12 weeks. Body weight, body mass index (BMI), glycaemic control, blood pressure, insulin resistance, total body fat, fat distribution and lipids were assessed before and after the experimental period. After 12 weeks, mean changes of body weight, BMI values and total cholesterol were significantly greater in the agar-diet group than in the conventional diet group. The agar diet resulted in marked weight loss due to the maintenance of reduced calorie intake and to an improvement in metabolic parameters. According to the authors, the 38 participants in the agar-diet group completed the 12-week experiment without noticeable side effects.

Pharmaceutical uses

The action of agar is described as a mild laxative, due to its swelling properties. The intake of agar is thought to cause an increase in the bulk of the content of the intestine which stimulates the intestinal muscles, thereby aiding peristalsis. No adverse effects are described and the posology is given with three times daily 1–2 teaspoons (a teaspoon of 5 mL corresponds to 2.75 g agar resulting in a daily dose of up to 16.5 g) together with liquids (Heber, 2007).

From data provided by European Medicines Agency (EMA), information about the current medicinal usage of agar and the usage as an excipient in medicinal products was retrieved (Doc. Provided to EFSA n.1). For agar as an active ingredient, only few authorised medicinal products exist (or existed) within the EU (as combination with other active ingredients). In those combination products, the daily dosage of agar is claimed with up to 900 mg (up to three times daily 300 mg) as an adjuvant in treatment of obesity. Intestinal stenosis is mentioned as a contraindication. The patient is furthermore warned that prudence in case of megacolon due to altered colic motility confined to bed (faecalome risk). As side effect, meteorism especially in the beginning of the treatment is mentioned. Usage in

pregnancy and lactation is possible if medically needed (it is claimed that no absorption occurs). The usage in children is not foreseen.[24]

Also in literature, bowel obstruction and abnormal oesophageal or intestinal narrowing are mentioned as contraindication for agar as a mild laxative (Duke, 2002; Gardner and McGuffin, 2013).

The US Food and Drug Administration (FDA) concluded that for over-the-counter (OTC) drug products for human use, containing agar or another water-soluble gum, hydrophilic gum, or hydrophilic mucilloid as an active ingredient, when marketed in a dry or incompletely hydrated form (e.g. capsules, granules, powders, tablets, wafers) the following labelling is needed to prevent oesophageal obstruction or asphyxiation: 'Taking this product without adequate fluid may cause it to swell and block your throat or esophagus and may cause choking. Do not take this product if you have difficulty in swallowing. If you experience chest pain, vomiting, or difficulty in swallowing or breathing after taking this product, seek immediate medical attention' (FDA, 2016).

The results of literature research did not reveal any published case reports of oesophageal obstruction or asphyxiation associated with the intake of agar in solid/dehydrated forms.

4. Discussion

Agar is extracted from certain strains of marine algae of the families Gelidiaceae and Gracilariaceae and relevant red algae of the class Rhodophyceae (Commission Regulation (EU) No 231/2012). Agar has the CAS Registry Number 9002-18-0, and the EINECS No 232-658-1.

Agar (E 406) is defined as a hydrophilic colloidal polysaccharide consisting mainly of galactose units with a regular alternation of L- and D-isomeric forms. These hexoses are alternately linked with alpha-1,3 and beta-1,4 bonds in the copolymer. On about every tenth D-galactopyranose unit, one of the hydroxyl groups is esterified with sulfuric acid, which is neutralised by calcium, magnesium, potassium or sodium.

Degradation of agar has been investigated in an *in vitro* human model. This study demonstrated that agar would be partially fermented during its passage through the large intestine by the action of the intestinal tract microflora. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that agar is unlikely to be absorbed unchanged, and that the limited extent of fermentation of agar would lead to products such as SCFA. Based on the available knowledge on the role of SCFA as end products of the fermentation of dietary fibres by the anaerobic intestinal microflora (Topping and Clifton, 2001; Den Besten et al., 2013), the Panel considered that their potential formation as fermentation products from agar does not raise any concern.

The acute toxicity of agar in mice, rats and rabbits is low.

Two dose range-finding subchronic toxicity studies have been performed by NTP in 1982 (Doc. provided to EFSA n.8) in mice (0, 629, 1,260, 2,500, 5,000 or 10,000 mg/kg bw per day) and rats (0, 279, 567, 1,125, 2,250 or 4,500 mg/kg bw per day). No adverse effects have been identified up to 10,000 in mice and 4,500 mg/kg bw per day in rat, the highest dose tested. The Panel noted that the NTP studies did not include haematology, urinalysis and clinical chemistry.

No genotoxic activity was observed in the available *in vitro* and *in vivo* genotoxicity assays with agar. The Panel noted that the package of available studies was limited, but also that agar is routinely used as substrate in *in vitro* gene mutation assays in bacteria and mammalian cells. Overall, considering its chemical structure and negligible absorption, the Panel concluded that there is no concern with respect to the genotoxicity of agar (E 406).

No chronic toxicity studies according to OECD guidelines (452) or equivalent have been identified by the Panel.

In carcinogenicity studies by NTP (Doc. provided to EFSA n.8; Melnick et al., 1983), no indication of a substance-related increased tumour incidence was reported. The authors stated that under the experimental conditions used, agar was not carcinogenic to mice and rats at the highest doses tested (up to 4,500 or 2,500 mg/kg bw per day, respectively). The Panel agreed with the authors.

No data on fertility effects were available. In the prenatal developmental toxicity studies in rats and hamsters, no maternal and developmental effects were observed up to the highest dose tested (1,140 and 650 mg agar/kg bw per day, respectively) (Doc. provided to EFSA n.3).

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA, 2014), the Panel considered that

[24] Information about the product is available online: http://www.medisite.fr/dictionnaire-des-medicaments-pseudophage-granule-pour-solution-buvable-en-sachet-dose.778863.8028.html

sufficient toxicity studies were available showing no adverse effects at the highest doses tested. Therefore, the Panel considered that there is no need to allocate a numerical ADI for agar (E 406).

In literature, there is also information about the usage of agar as an adjuvant in the treatment of diabetes and/or obesity (Maeda et al., 2005). This study did not show noticeable side effects in participants receiving a daily dose of 4.5 g agar for 12 weeks.

No adverse effects were described for agar as a mild laxative in single doses from 2.75 to 5.5 g or in daily doses from 4 to 16.5 g administered with sufficient fluids, but intestinal stenosis was given as a contraindication (Heber, 2007).

Agar (E 406) is authorised in a wide range of foods but reported by industry to be used in a limited number of food categories (9/70). According to the Panel, it is not expected that brand loyalty will result in higher exposure in the general population, except in specific populations consuming foods belonging to the food categories 13.2, 13.3 and 13.4 (see Table 2). The Panel therefore selected the non-brand-loyal refined scenario as the most relevant exposure scenario for this additive.

In the maximum level and refined scenarios, only seven food categories (out of 70 in which agar is authorised in the Regulation (EC) No 1333/2008) for which data have been reported by industry, were included. The Panel considered that the uncertainties identified would result in an overestimation of exposure for these seven food categories.

Considering information from the Mintel GNPD, only approximately 50% of the food products labelled with agar (E 406) belonged to food subcategories for which reported use levels were available, and consequently used in the exposure assessment. The Panel noted that given the information from the Mintel GNPD it may be assumed that agar is used in food categories for which no data have been provided by food industry. If this is confirmed, the present assessment would therefore result in an underestimation of the exposure.

The Panel further noted that the exposure to agar from its use according the Annex III (Part 1, 2, 3, 4 and 5 section A) was not considered in the exposure assessment.

The Panel also noted that the refined exposure estimates are based on information provided on the reported level of use of agar (E 406). If actual practice changes, this refined estimates may no longer be representative and should be updated.

5. Conclusions

According to the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA, 2014) and given that:

- the safety assessment carried out by the Panel is limited to the use and use levels in seven food categories for which data were considered in this opinion out of the 70 food categories in which agar (E 406) is authorised;
- an indicative high refined exposure assessment up to 26 mg/kg bw per day has been calculated in toddlers at the 95th percentile (non-brand-loyal scenario);
- agar is unlikely to be absorbed unchanged and is slightly fermented by intestinal microbiota;
- sufficient toxicity data were available;
- there is no concern with respect to the genotoxicity of agar (E 406);
- no carcinogenic effects were reported in carcinogenicity studies in mice and rats at the doses of 4,500 mg/kg bw per day and 2,500 mg/kg bw per day, respectively, the highest doses tested;
- oral intake of agar (4,500 mg/person corresponding to 64 mg/kg bw per day) was tolerated in humans for 12 weeks without noticeable side effects,

the Panel concluded that there is no need for a numerical ADI for agar, and that there is no safety concern for the general population at the refined exposure assessment for the reported uses of agar as food additive.

6. Recommendations

The Panel recommended that the maximum limits for the impurities of toxic elements (lead, mercury, cadmium and arsenic) in the EU specification for agar (E 406) should be revised in order to ensure that agar (E 406) as a food additive will not be a significant source of exposure to those toxic elements in food, in particular for infants and children.

The Panel recommended that information on the possible use of formaldehyde should be provided since no such information was made available following the call for data.[12]

Due to the discrepancies observed between the data reported from industry and Mintel database, where agar is labelled in more products than in food categories for which data were reported from industry, the Panel recommended collection of data of usage and use levels of agar (E 406) in order to perform a more realistic exposure assessment.

Documentation provided to EFSA

1) European Medicines Agency (EMA) communication to EFSA request in 4 May 2015, for information on a certain group of substances used as food additives, June 2015.
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4) Hispanagar S.A., 2010. Reply to EFSA: Call for data on emulsifier, stabiliser and gelling agents. Information on chemistry and specification, manufacturing process, present usage, and other studies. Submitted to EFSA in 2010.
5) Informatics Inc., prepared for Food and Drug Administration (FDA), 1972. GRAS (Generally Recognized as Safe) Food Ingredients – Agar-Agar. Submitted by Marinalg in 2010.
6) Litton Bionetic Inc., prepared for Food and Drug Administration (FDA), 1974. Mutagenic evaluation of compound FDA 71-53, powdered agar. Mutagenic test results in 3 systems-Host mediated Assay in vitro and in vivo; Cytogenetics in vitro and in vivo; Dominant lethal in vivo. Submitted by Hispanagar and Marinalg in 2010.
7) Marinalg International., 2010. Reply to EFSA: Call for data on emulsifier, stabiliser and gelling agents. Information on chemistry and specification, ADME, subchronic toxicity, genotoxicity, chronic toxicity and carcinogenicity, reproduction and developmental toxicity, other studies. Submitted to EFSA in 2010.
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Abbreviations

ADI	acceptable daily intake
AFC	EFSA Former Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
BMI	body mass index
BPC	British Pharmaceutical Codex
CAS	Chemical Abstracts Service
DEAE	diethylaminoethyl cellulose
EMA	European Medicines Agency
EINECS	European Inventory of Existing Commercial Chemical Substances
FCS	Food Classification System
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
GD	gestation day
GNPD	Global New Products Database
ICGA	International Chewing Gum Association
IPCS	International Programme on Chemical Safety
IR	infrared (spectroscopy)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	liquid chromatography
LD_{50}	lethal dose

MPL	maximum permitted level
NOAEL	no-observed-adverse-effect-level
NTP	National Toxicology Program
OTC	over-the-counter
QS	*quantum satis*
SCF	Scientific Committee for Food
SmPC	summary of product characteristics
TDI	tolerable daily intake
TMDI	theoretical maximum daily intake

Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of agar (E 406) provided by industry

Food Category number	Food category name	E-Number/ Group	MPL	Restrictions	Nr	Usage level typical			Usage level max	Provider Name	Comments
						Mean	Min	Max			
01.4	Flavoured fermented milk products including heat treated products	Group I	QS		1	340.0			356.0	FDE	
03	Edible ices	Group I	QS		1	90.0*			150.0*	Interested party 1	Original level provided for semifinish product. This semiproduct are added 100 g inside 1 kg of finished product
03	Edible ices	Group I	QS		5	94.0	0.0	470.0	1162.0	FDE	
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I	QS		1	87.5			150.0	FDE	Specifically reported for 'fruit filling for pastry application'
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Group I	QS	Only energy-reduced or with no added sugar	1	141.0			2881.0	FDE	
05.3	Chewing gum	Group I	QS		1	8800.0			18000.0	ICGA	
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	Group I	QS		1	16000.0			20000.0	Interested party 1	
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	Group I	QS		3	140.1	25.0	370.0	720.0	FDE	Usage level as expressed in the pastry application, in the cream filling
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I	QS		1	16000.0			16000.0	BABBI Confectionery Industry	Levels provided 'sweet sauces for desserts'
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I	QS		1 NP	0.3			0.3	Biovegan GmbH	Levels provided for 'instant powder for different kind of glazes, vegan, organic'

Food Category number	Food category name	E-Number/ Group	MPL	Restrictions	Nr	Usage level typical			Usage level max	Provider Name	Comments
						Mean	Min	Max			
08.3.1	Meat preparations as defined by Regulation (EC) No 853/ 2004				1	40.0*			237.5*	FDE	Initial levels provided for 'the seasoning or extracts as consumed for example in a sausage, will be much lower as only a small percentage (between 2.5-10%)'
16	Desserts excluding products covered in category 1, 3 and 4	Group I	QS		2	2079.1	103.0	4055.1	4055.1	FDE	
18		Group I	QS		2 NP	0.525	0.05	1.0	1.0	Biovegan GmbH	Levels provided as 'pure agar'/'gelling agent for different kinds of products: aspic, confectionery, fruit spread'

MPL: maximum permitted level; FDE: FoodDrinkEurope; ICGA: International Chewing Gum Association.
*Application of a factor 10 on the initial levels provided.

Appendix B – Number and percentage of food products labelled with agar (E 406) out of the total number of food products present in Mintel GNPD per food subcategory between 2011 and 2016[25]

Mintel subcategory	Total number of products	Products labelled with agar (E 406)	
		Number	%
Mixed assortments	271	14	5.2
Chilled desserts	5,582	208	3.7
Non-individually wrapped chocolate pieces	4,687	137	2.9
Caramel & cream spreads	243	7	2.9
Pastilles, gums, jellies & chews	3,346	81	2.4
Other frozen desserts	1,419	32	2.3
Shelf-stable desserts	2,945	50	1.7
Soft cheese desserts	1,365	21	1.5
Curd & quark	849	13	1.5
Liquorice	690	9	1.3
Processed cheese	1,876	23	1.2
Meat pastes & pates	2,776	33	1.2
Cakes, pastries & sweet goods	11,854	136	1.1
Confiture & fruit spreads	4,266	46	1.1
Cream	1,454	14	1.0
Seasonal chocolate	4,962	47	0.9
Marshmallows	431	4	0.9
Chocolate countlines	2,059	19	0.9
Medicated confectionery	891	8	0.9
Soy-based drinks	609	5	0.8
Dairy-based frozen products	7,001	49	0.7
Salads	2,334	16	0.7
Sandwich fillers/spreads	901	6	0.7
Individually wrapped chocolate pieces	2,296	15	0.7
Water-based frozen desserts	1,072	7	0.7
Baking ingredients & mixes	8,031	44	0.5
Snack/cereal/energy bars	4,232	21	0.5
Meat substitutes	1,908	9	0.5
Growing up milk (1-4 years)	223	1	0.4
Dips	1,282	5	0.4
Standard & power mints	787	3	0.4
Other chocolate confectionery	263	1	0.4
Other sugar confectionery	950	3	0.3
Poultry products	5,483	17	0.3
Rice snacks	351	1	0.3
Soy yogurt	355	1	0.3
Meat products	13,984	34	0.2
Other sauces & seasonings	851	2	0.2
Chocolate spreads	979	2	0.2
Fresh cheese & cream cheese	2,457	5	0.2
Instant noodles	995	2	0.2
Hors d'oeuvres/canapes	3,630	7	0.2

[25] http://www.gnpd.com/sinatra/home/ accessed on 25/10/2016.

Mintel subcategory	Total number of products	Products labelled with agar (E 406)	
		Number	%
Dessert toppings	573	1	0.2
Other natural sweeteners	591	1	0.2
Prepared meals	9,894	16	0.2
Flavoured milk	1,272	2	0.2
Nut spreads	645	1	0.2
Chocolate tablets	7,344	11	0.1
Savoury vegetable pastes/spreads	1,416	2	0.1
RTD (iced) coffee	768	1	0.1
Mayonnaise	802	1	0.1
Sandwiches/wraps	2,406	3	0.1
Table sauces	5,376	6	0.1
Fruit snacks	2,892	3	0.1
Sweet biscuits/cookies	15,483	16	0.1
Pasta	8,874	8	0.1
Soft cheese & semi-soft cheese	4,995	4	0.1
Fish products	10,920	7	0.1
Pickled condiments	5,003	3	0.1
Wheat & other grain-based snacks	1,690	1	0.1
Meal kits	1,809	1	0.1
Stuffing, polenta & other side dishes	1,999	1	0.1
Cold cereals	5,472	2	0.0
Drinking yogurt & liquid cultured milk	2,886	1	0.0
Hard cheese & semi-hard cheese	5,902	2	0.0
Dressings & vinegar	3,035	1	0.0
Vegetables	9,283	3	0.0
Wet soup	3,751	1	0.0
Pizzas	3,886	1	0.0
Cooking sauces	4,446	1	0.0
Spoonable yogurt	8,753	1	0.0
Bread & bread products	8,926	1	0.0
Total Sample	**254,032**	**1,261**	**0.5**

Appendix C – Concentration levels of agar (E 406) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

Food category number	Food categories name	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
01.3	Unflavoured fermented milk products, heat-treated after fermentation		QS	–	–	Not taken into account (no concentration data)
01.4	Flavoured fermented milk products including heat treated products		QS	340	356	Reported use levels
01.6.2	Unflavoured live fermented cream products and substitute products with a fat content of less than 20%		QS	–	–	Not taken into account (no concentration data)
01.6.3	Other creams		QS	–	–	Not taken into account (no concentration data and no FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16	Except mozzarella	QS	–	–	Not taken into account (no concentration data)
01.7.5	Processed cheese		QS	–	–	Not taken into account (no concentration data)
01.7.6	Cheese products (excluding products falling in category 16)		QS	–	–	Not taken into account (no concentration data)
01.8	Dairy analogues, including beverage whiteners		QS	–	–	Not taken into account (no concentration data)
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions		QS	–	–	Not taken into account (no concentration data)
02.3	Vegetable oil pan spray		QS	–	–	Not taken into account (no concentration data and no FoodEx code)
03	Edible ices		QS	92	1,162	Reported use levels

Food category number	Food categories name	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
04.2.1	Dried fruit and vegetables		QS	–	–	Not taken into account (no concentration data)
04.2.2	Fruit and vegetables in vinegar, oil, or brine		QS	–	–	Not taken into account (no concentration data)
04.2.4.1	Fruit and vegetable preparations excluding compote		QS	–	–	Not taken into account (no FoodEx code for 'fruit filling')
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	(b)	10 000	–	–	Not taken into account (no concentration data)
04.2.5.3	Other similar fruit or vegetable spreads	(b)	10 000	–	–	Not taken into account (no concentration data)
04.2.5.4	Nut butters and nut spreads		QS	–	–	Not taken into account (no concentration data)
04.2.6	Processed potato products		QS	–	–	Not taken into account (no concentration data)
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	Only energy-reduced or with no added sugar	QS	141	2,881	Reported use levels
05.2	Other confectionery including breath refreshening microsweets	E 406 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	QS	–	–	Not taken into account (no concentration data)
05.3	Chewing gum		QS	8800	18000	Reported use levels
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4		QS	–	–	Not taken into account (no FoodEx code)

Food category number	Food categories name	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
06.2.2	Starches		QS	–	–	Not taken into account (no concentration data)
06.3	Breakfast cereals		QS	–	–	Not taken into account (no concentration data)
06.4.2	Dry pasta	Only gluten free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS	–	–	Not taken into account (no concentration data)
06.4.4	Potato Gnocchi	Except fresh refrigerated potato gnocchi	QS	–	–	Not taken into account (no concentration data)
06.4.5	Fillings of stuffed pasta (ravioli and similar)		QS	–	–	Not taken into account (no concentration data)
06.5	Noodles		QS	–	–	Not taken into account (no concentration data)
06.6	Batters		QS	–	–	Not taken into account (no concentration data and no FoodEx code)
06.7	Pre-cooked or processed cereals		QS	–	–	Not taken into account (no concentration data and no FoodEx code)
07.1	Bread and rolls	Except products in 7.1.1 and 7.1.2	QS	–	–	Not taken into account (no concentration data)
07.2	Fine bakery wares		QS	–	–	Not taken into account (no concentration data)
08.3.1	Non-heat-treated meat products		QS	40	237.5	Reported use levels for the FC 08.3.1, used for both categories of meat products
08.3.2	Heat-treated meat products	Except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	QS			

Food category number	Food categories name	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
08.3.3	Casings and coatings and decorations for meat		QS	–	–	Not taken into account (no concentration data and no FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans		QS	–	–	Not taken into account (no concentration data)
09.3	Fish roe	Only processed fish roe	QS	–	–	Not taken into account (no concentration data)
10.2	Processed eggs and egg products		QS	–	–	Not taken into account (no concentration data)
11.2	Other sugars and syrups		QS	–	–	Not taken into account (no concentration data)
12.1.2	Salt substitutes		QS	–	–	Not taken into account (no concentration data)
12.2.2	Seasonings and condiments		QS	–	–	Not taken into account (no concentration data)
12.3	Vinegars		QS	–	–	Not taken into account (no concentration data)
12.4	Mustard		QS	–	–	Not taken into account (no concentration data)
12.5	Soups and broths		QS	–	–	Not taken into account (no concentration data)
12.6	Sauces		QS	–	–	Not taken into account (no concentration data)
12.7	Salads and savoury based sandwich spreads		QS	–	–	Not taken into account (no concentration data)
12.8	Yeast and yeast products		QS	–	–	Not taken into account (no concentration data)
12.9	Protein products, excluding products covered in category 1.8		QS	–	–	Not taken into account (no concentration data)

Food category number	Food categories name	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Only foods in tablet and coated tablet format	QS	–	–	Not taken into account (no concentration data)
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		QS	–	–	Not taken into account (no concentration data)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Including dry pasta	QS	–	–	Not taken into account (no concentration data)
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Only vegetable juices	QS	–	–	Not taken into account (no concentration data)
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Only vegetable nectars	QS	–	–	Not taken into account (no concentration data)
14.1.4	Flavoured drinks		QS	–	–	Not taken into account (no concentration data)
14.1.5.2	Other	Excluding unflavoured leaf tea; including flavoured instant coffee	QS	–	–	Not taken into account (no concentration data)
14.2.3	Cider and perry		QS	–	–	Not taken into account (no concentration data)
14.2.4	Fruit wine and made wine		QS	–	–	Not taken into account (no concentration data)
14.2.5	Mead		QS	–	–	Not taken into account (no concentration data)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Except whisky or whiskey	QS	–	–	Not taken into account (no concentration data)
14.2.7.1	Aromatised wines		QS	–	–	Not taken into account (no concentration data)
14.2.7.2	Aromatised wine-based drinks		QS	–	–	Not taken into account (no concentration data)

Food category number	Food categories name	Restrictions/exceptions	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
14.2.7.3	Aromatised wine-product cocktails		QS	–	–	Not taken into account (no concentration data)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		QS	–	–	Not taken into account (no concentration data)
15.1	Potato-, cereal-, flour- or starch-based snacks		QS	–	–	Not taken into account (no concentration data)
15.2	Processed nuts		QS	–	–	Not taken into account (no concentration data)
16	Desserts excluding products covered in category 1, 3 and 4		QS	2079	4,055	Reported use levels
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		QS	–	–	Not taken into account (no concentration data)
17.2	Food supplements supplied in a liquid form		QS	–	–	Not taken into account (no concentration data)
17.3	Food supplements supplied in a syrup-type or chewable form		QS	–	–	Not taken into account (no concentration data)
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children		QS	–	–	Not taken into account (no usable concentration data)

MPL: maximum permitted level; QS: *quantum satis*.

Appendix D – Summary of total estimated exposure of agar (E 406) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and high level (mg/kg bw per day)

	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Infants							
Bulgaria (NUTRICHILD)	659	0.1	0.6	0.1	0.6	0.1	0.1
Germany (VELS)	159	1.2	7.7	1.1	6.8	0.6	3.6
Denmark (IAT 2006_07)	826	2.6	13.4	2.4	12.0	1.5	8.0
Finland (DIPP_2001_2009)	500	0.01	0.0	0.01	0.0	0.01	0.0
United Kingdom (DNSIYC_2011)	1,366	3.8	20.9	3.6	19.6	2.0	10.5
Italy (INRAN_SCAI_2005_06)	12	0.5	–	0.5	–	0.1	–
Toddlers							
Belgium (Regional_Flanders)	36	11.9	–	9.4	–	4.7	–
Bulgaria (NUTRICHILD)	428	1.0	5.6	0.9	5.1	0.2	1.5
Germany (VELS)	348	7.7	24.3	6.3	21.4	3.3	11.5
Denmark (IAT 2006_07)	917	4.8	14.3	3.6	11.4	2.0	7.4
Spain (enKid)	17	9.9	–	8.4	–	4.9	–
Finland (DIPP_2001_2009)	500	1.7	6.7	1.5	6.2	1.3	5.4
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	6.4	24.8	5.7	22.2	2.8	11.8
United Kingdom (DNSIYC_2011)	1,314	6.2	26.3	5.7	24.9	3.0	13.5
Italy (INRAN_SCAI_2005_06)	36	2.1	–	1.8	–	1.0	–
Netherlands (VCP_kids)	322	17.0	48.7	14.8	44.5	8.4	26.2
Children							
Austria (ASNS_Children)	128	3.1	8.5	2.6	6.8	0.6	3.0
Belgium (Regional_Flanders)	625	10.2	26.7	8.2	22.5	4.5	14.2
Bulgaria (NUTRICHILD)	433	1.9	7.3	1.7	6.8	0.2	0.6
Czech Republic (SISP04)	389	5.4	16.6	4.3	13.9	1.6	7.0
Germany (EsKiMo)	835	4.4	12.4	3.6	10.1	1.1	4.7
Germany (VELS)	293	8.6	23.0	6.5	19.7	3.3	11.0
Denmark (DANSDA 2005-08)	298	3.7	9.2	2.6	6.6	0.9	3.6
Spain (enKid)	156	6.7	23.0	5.5	19.6	2.6	10.1
Spain (NUT_INK05)	399	4.3	15.1	3.6	14.5	1.5	7.2
Finland (DIPP_2001_2009)	750	4.2	8.6	3.1	6.6	1.5	4.4
France (INCA2)	482	8.2	21.9	6.5	18.8	3.2	10.0
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	4.9	15.0	4.0	12.4	1.7	6.4
Greece (Regional_Crete)	838	2.2	7.6	1.9	6.6	0.6	2.6
Italy (INRAN_SCAI_2005_06)	193	2.3	7.3	1.9	5.5	0.4	1.7
Latvia (EFSA_TEST)	187	3.6	13.5	3.0	11.3	0.9	4.2
Netherlands (VCP_kids)	957	13.9	37.6	11.6	34.2	6.6	19.6
Netherlands (VCPBasis_AVL2007_2010)	447	9.8	28.0	8.1	24.3	4.3	13.9
Sweden (NFA)	1,473	4.5	11.2	3.5	9.0	1.7	4.8
Adolescents							
Austria (ASNS_Children)	237	1.2	4.5	1.0	3.5	0.2	0.8
Belgium (Diet_National_2004)	576	2.0	6.3	1.8	5.1	0.4	2.5

	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Cyprus (Childhealth)	303	0.9	3.2	0.8	2.7	0.1	0.5
Czech Republic (SISP04)	298	2.5	8.1	2.0	6.4	0.7	2.3
Germany (National_Nutrition_Survey_II)	1,011	1.5	6.4	1.3	5.5	0.5	2.7
Germany (EsKiMo)	393	2.8	7.7	2.3	6.1	0.5	2.5
Denmark (DANSDA 2005-08)	377	2.0	6.2	1.5	4.4	0.5	1.8
Spain (AESAN_FIAB)	86	1.4	4.4	1.1	3.9	0.3	1.7
Spain (enKid)	209	3.1	9.7	2.4	6.8	1.0	3.9
Spain (NUT_INK05)	651	2.0	6.6	1.7	5.9	0.6	2.7
Finland (NWSSP07_08)	306	1.9	4.7	1.5	3.6	0.8	2.0
France (INCA2)	973	3.6	10.0	2.8	8.5	1.3	4.4
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	2.0	6.8	1.6	5.5	0.6	2.7
Italy (INRAN_SCAI_2005_06)	247	1.2	4.1	1.0	3.5	0.2	0.8
Latvia (EFSA_TEST)	453	2.2	8.3	1.8	7.0	0.4	2.6
Netherlands (VCPBasis_AVL2007_2010)	1,142	5.1	15.8	4.2	14.1	2.1	7.7
Sweden (NFA)	1,018	2.4	7.5	1.9	5.6	0.8	2.8
Adults							
Austria (ASNS_Adults)	308	1.1	5.7	1.0	5.0	0.4	2.3
Belgium (Diet_National_2004)	1,292	1.4	5.4	1.2	4.6	0.4	2.1
Czech Republic (SISP04)	1,666	0.8	2.7	0.7	2.3	0.3	1.0
Germany (National_Nutrition_Survey_II)	10,419	1.1	4.6	1.0	4.0	0.4	1.9
Denmark (DANSDA 2005-08)	1,739	1.0	3.4	0.8	2.6	0.3	1.2
Spain (AESAN)	410	0.9	4.0	0.8	3.1	0.2	1.5
Spain (AESAN_FIAB)	981	0.9	3.5	0.7	3.1	0.2	1.4
Finland (FINDIET2012)	1,295	1.5	6.0	1.3	5.2	0.7	2.9
France (INCA2)	2,276	1.7	6.0	1.4	5.2	0.7	2.7
United Kingdom (NDNS-RollingProgrammeYears1-3)	1,266	0.9	3.4	0.8	3.1	0.3	1.5
Hungary (National_Repr_Surv)	1,074	0.5	1.7	0.4	1.5	0.1	0.4
Ireland (NANS_2012)	1,274	0.7	2.5	0.6	2.1	0.2	0.9
Italy (INRAN_SCAI_2005_06)	2,313	0.5	1.8	0.4	1.5	0.1	0.6
Latvia (EFSA_TEST)	1,271	0.8	4.0	0.7	3.3	0.2	1.2
Netherlands (VCPBasis_AVL2007_2010)	2,057	2.6	10.0	2.3	8.8	1.1	4.8
Romania (Dieta_Pilot_Adults)	1,254	0.3	1.3	0.3	1.0	0.0	0.2
Sweden (Riksmaten 2010)	1,430	0.9	3.0	0.7	2.5	0.3	1.4
The elderly							
Austria (ASNS_Adults)	92	0.7	3.3	0.6	3.2	0.2	1.7
Belgium (Diet_National_2004)	1,215	1.3	6.4	1.2	6.0	0.5	3.2
Germany (National_Nutrition_Survey_II)	2,496	0.9	3.8	0.8	3.5	0.3	1.8
Denmark (DANSDA 2005-08)	286	0.7	2.0	0.5	1.6	0.2	0.7
Finland (FINDIET2012)	413	0.8	3.1	0.7	2.7	0.4	1.8
France (INCA2)	348	1.2	4.1	1.1	3.7	0.5	2.1
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	1.4	5.1	1.2	4.6	0.6	2.5
Hungary (National_Repr_Surv)	286	0.3	0.8	0.2	0.6	0.1	0.3
Ireland (NANS_2012)	226	0.8	3.7	0.8	3.7	0.4	1.9
Italy (INRAN_SCAI_2005_06)	518	0.2	1.1	0.2	1.1	0.1	0.4
Netherlands (VCPBasis_AVL2007_2010)	173	2.1	8.8	1.9	8.5	1.0	4.4

	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Netherlands (VCP-Elderly)	739	2.6	9.8	2.4	9.3	1.2	4.9
Romania (Dieta_Pilot_Adults)	128	0.2	0.7	0.1	0.5	0.0	0.1
Sweden (Riksmaten 2010)	367	0.9	2.9	0.7	2.6	0.3	1.5

MPL: maximum permitted level; p95: 95th percentile.
–: p95 of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a).