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VKM Report 2023: 24

Scoping review of research on gastrointestinal effects of selected emulsifiers, stabilisers, and thickeners

Eva Denison, Monica Andreassen, Ellen Bruzell, Monica Hauger Carlsen, Tove Gulbrandsen Devold, Berit Granum, Gro Haarklou Mathisen, Camilla Svendsen, Josef Daniel Rasinger, Trine Husøy

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food and Environment

The Norwegian Scientific Committee for Food and Environment has prepared an overview of the current available research on gastrointestinal effects of agar, carrageenan, gellan gum, guar gum, processed Eucheuma seaweed, sodium alginate, sodium carboxymethyl cellulose, and xanthan gum.

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Preparation of the report

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the report. The project group consisted of VKM members and VKM staff. Two referees reviewed the draft report. The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics assessed and approved the final report.

Members of the project group and authors of the report

The authors have contributed to the report in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics.

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Contents

Summary

This scoping review was commissioned by the Norwegian Food Safety Authority. The aim was to map the scientific literature investigating effects on the gastrointestinal (GI) tract after intake of emulsifiers, stabilisers, and thickeners (ESTs). The background for the assignment was that certain published studies indicated that the ESTs carrageenan and sodium carboxymethyl cellulose may have negative effects on the GI tract. Eight ESTs are included in this scoping review: carrageenan (E 407) and sodium carboxymethyl cellulose (E 466), and six ESTs that may be used as their substitutes, namely sodium alginate (E 401), agar (E 406), processed Eucheuma seaweed (E 407a), guar gum (E 412), xanthan gum (E 415), and gellan gum (E 418).

Comprehensive literature searches were performed to map the scientific literature investigating GI tract effects after intake of the included ESTs. The eligibility criteria used for the study selection were included in the previously published protocol (VKM, 2023), and are as follows:

- Population: Humans, other mammals, as well as *ex vivo* GI tract model systems.
- Exposure: Oral intake of the included ESTs. The substances tested must fulfill the criteria for being used as food additives.
- Comparison: Placebo, no treatment, or dose comparison.
- Outcomes: Any GI tract effect.
- Study design: Controlled human studies, controlled animal studies, *ex vivo* GI tract model studies, and systematic reviews.
- Language: English, Norwegian, Danish, and Swedish.

There were no restrictions on publication year or country.

Fourteen studies of which one was a study on humans and 13 were studies on animals, fulfilled the eligibility criteria. The studies were conducted between 1977 and 2022. VKM evaluated whether the design and conduct of the included studies prevented bias (systematic errors), as bias may cause misleading results and wrong conclusions. Ten of the included studies had high risk of bias and none had low risk of bias.

An additional 214 studies fulfilled all eligibility criteria except the criterion that the substance tested must be in accordance with the regulations for food additives in the EU and Norway. Whenever the same name was used for similar substances having different chemical and biological properties, only the substance(s) approved for use as food additive was included in this scoping review. This applies to e.g. carrageenan, for which the size of the molecule is among the properties contributing to adverse effects caused by the substance. Low molecular weight (weight average of 20–40 kDa) carrageenan, also called degraded carrageenan, may cause e.g. cancer in animals (EFSA et al., 2018c). The degraded carrageenan is not approved as a food additive according to EU regulations. The regulations specify a limitation of no more than 5% of the carrageenan having a molecular weight below 50 kDa. In contrast, no such molecular weight limitation is set for carrageenan in specifications defined by the Joint

FAO/WHO Expert Committee on Food Additives (JECFA). This lack of specification may explain why the molecular weight of carrageenan often is omitted in reports of toxicological studies performed outside Europe. Nevertheless, in the EU, information about molecular weight of carrageenan tested in studies is crucial for evaluating its use as a food additive (EFSA et al., 2018c). In the 214 studies mentioned above, the substance being tested was not described well enough, and it was unknown whether it was approved as an additive.

None of the included studies investigated GI tract effects of sodium alginate (E 401) or gellan gum (E 418). GI tract effects were investigated in one animal study of agar (E 406) and Eucheuma seaweed (E 407a), in one human and one animal study of xanthan gum (E 415), two animal studies of sodium carboxymethyl cellulose (E 466), in four animal studies of guar gum (E 412), and in seven animal studies of carrageenan (E 407).

The outcomes addressed in the included studies were as follows (number of studies in parentheses):

- Changes in the gut microbiota composition and/or the microbiota numbers (5).
- Enzymatic activity (microbial or colonic mucosa; 6).
- Faecal or caecal content, weight, colour, consistency, and/or viscosity (8).
- Gastric transit time and stool frequency (1).
- Inflammation (colon or markers measured in faeces; 4).
- Intestinal permeability (markers measured in serum; 2).
- Intestinal utilisation and fermentation of nutrients (3).
- Macroscopic changes (stomach, small intestine and/or large intestine; 2).
- Microscopic changes (stomach, small intestine and/or large intestine; 10).
- Mucosal weight and/or protein content (colon; 2).
- Presence of mucus or blood in the faeces (1).
- Tumour development (small intestine and/or colon; 2).
- Weight and/or length (stomach, small intestine and/or large intestine; 6).

The number of studies addressing GI tract effects of substitution ESTs for carrageenan was limited, and none of these substances were included in the studies addressing gut inflammation or gut permeability. The outcomes were distributed between studies and substances as follows:

- Inflammation (colon or markers measured in the faeces) was investigated in four animal studies of which two were on carrageenan and two on sodium carboxymethyl cellulose.
- Intestinal permeability was investigated in two animal studies of which one was on carrageenan and one on sodium carboxymethyl cellulose.
- Changes in the gut microbiota composition and/or the microbiota numbers (i.e. the number of bacteria, virus, etc.) were investigated in five animal studies of which one each was on agar, carrageenan, and guar gum, and two were on sodium carboxymethyl cellulose.

Chronic exposures were not addressed in the included studies. Five out of the six animal studies on gut inflammation and gut permeability, as well as all five studies on

microbiota composition and/or numbers, had a high risk of bias. Rodents such as mice and rats are commonly used to study negative health effects in humans. Although the GI tract in rodents and humans are mostly similar, rodents have a forestomach that is absent in humans. Adverse health effects such as inflammation is known to be affected by the microbiome. The microbiome in rodents and humans share only 4% of the genes, indicating that the microbiome is different in rodents and humans and that rodents are not an appropriate model to study inflammation and microbiome changes in humans (Hugenholtz and de Vos, 2018; Ward et al., 2020).

Conclusion

GI tract effects of ESTs were addressed in 14 eligible studies. GI tract effects were not investigated for two of the ESTs included in the scoping review. GI tract effects were investigated in studies of agar (E 406), sodium alginate (E 401), carrageenan (E 407), processed Eucheuma seaweed (E 407a), sodium carboxymethyl cellulose (E 466), gellan gum (E 418), guar gum (E 412), and xanthan gum (E 415). None of the studies addressed chronic exposures. Animal models were used in 13 of the included studies, and the risk of bias was high in ten studies. Thus, the available research literature on GI tract effects, according to our inclusion criteria, is limited in quantity and has limited relevance for long-term exposure in humans and is encumbered with high risk of bias. These weaknesses limit the use of the results of the scoping review in a future risk assessment.

Key words: Agar, carrageenan, gellan gum, guar gum, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food and Environment, processed Eucheuma seaweed, sodium alginate, scoping review, sodium carboxymethyl cellulose, VKM, xanthan gum

Sammendrag på norsk

Denne kartleggingen er gjort på oppdrag for Mattilsynet. Målet var å kartlegge forsking som har studert konsistensmidlers effekter på fordøyelseskanalen. Bakgrunnen for oppdraget er at det er publisert studier som rapporterer negative effekter av konsistensmidlene karragenan og karboksymetylcellulose på fordøyelseskanalen. Åtte konsistensmidler er inkludert i kartleggingen; karragenan (E 407) og karboksymetylcellulose (E 466), samt seks andre konsistensmidler som kan bli brukt som erstatning for karragenan og karboksymetylcellulose. Disse er natriumalginat (E 401), agar (E 406), bearbeidet Eucheuma-tang (E 407a), guarkjernemel (E 412), xantangummi (E 415) og gellangummi (E 418).

VKM har gjort omfattende litteratursøk for å finne all forskning av effekter på fordøyelseskanalen av disse åtte konsistensmidlene. Alle studier som oppfylte følgende kriterier (VKM, 2023), ble inkludert:

- populasjon: Mennesker og andre pattedyr i tillegg til *ex vivo* modeller for fordøyelseskanalen.
- eksponering: Oralt inntak av de inkluderte konsistensmidlene. Disse måtte oppfylle kriteriene for bruk som tilsetningsstoff.
- sammenligning: Placebo, ingen behandling, sammenligning av ulike doser.
- effekter: Alle effekter på fordøyelseskanalen.
- studiedesign: Kontrollerte studier (mennesker og dyr), studier med *ex vivo*-modeller for fordøyelseskanalen og systematiske kunnskapsoppsummeringer.
- språk: Engelsk, norsk, dansk og svensk.

Det var ingen restriksjoner på grunnlag av publiseringsår eller land.

Fjorten studier, hvorav én på mennesker og 13 på dyr, oppfylte kriteriene. Studiene ble publisert i tidsrommet 1977 til 2022. Ingen av studiene undersøkte effekter av kronisk eksponering. For hver av de inkluderte studiene vurderte VKM om det var systematiske skjevheter i gjennomføringen av dem. Systematiske skjevheter kan introduseres i en studie når det er svakheter ved studiemetodene som brukes. I studier med høy risiko for systematiske skjevheter er det stor usikkerhet knyttet til resultater og konklusjoner. Ti av de inkluderte studiene var beheftet med høy risiko og ingen med lav risiko for systematiske skjevheter.

Ytterligere 214 studier oppfylte alle inklusjonskriterier med unntak av at stoffet som ble testet måtte være godkjent for bruk som tilsetningsstoff. Dette kriteriet ble inkludert fordi et gitt navn på et stoff kan omfatte en gruppe stoffer med ulike biologiske og kjemiske egenskaper. Dette gjelder for eksempel karragenan, hvor størrelsen på molekylet har betydning for den biologiske virkningen av stoffet. Karragenan med lav molekylvekt (gjennomsnitt på 20–40 kDa), også kalt degradert karragenan, kan for eksempel føre til kreft hos dyr (EFSA et al., 2018c). Degradert karragenan er ikke godkjent som tilsetningsstoff i EU. Forskriften for tilsetningsstoffer spesifiserer en begrensning på ikke mer enn 5% av karragenanet med en molekylvekt lavere enn 50 kDa. Derimot er ingen slik molekylvektbegrensning satt for karragenan i spesifikasjoner definert av JECFA (The Joint FAO/WHO Expert Committee on Food Additives). Denne

forskjellen i spesifikasjoner kan forklare hvorfor informasjon om molekylvekt ikke alltid er beskrevet i studier fra land utenfor Europa. I EU er likevel informasjon om molekylvekten til karragenan som er testet i studier avgjørende for å vurdere effekten av bruken som tilsetningsstoff (EFSA et al., 2018c). I de 214 studiene nevnt ovenfor var ikke stoffet som ble testet godt nok beskrevet, og det var ukjent om det var godkjent som tilsetningsstoff.

Ingen av studiene undersøkte effekter av natriumalginat (E 401) eller gellangummi (E 418) på fordøyelseskanalen. En studie hver omhandlet effekter av agar (E 406), Eucheuma-tang (E 407a). Effekter av xantangummi (E 415) var undersøkt i én studie på mennesker og én på dyr. To studier undersøkte effekter av natriumkarboksymetylcellulose (E 466). I henholdsvis fire og sju studier var effekter av guarkjernemel (E 412) og karragenan (E 407) undersøkt.

Følgende effekter ble studert (antall studier i parentes):

- mikrobiota (endringer i sammensetning og/eller i antall; 5).
- enzymaktivitet (mikrobiell og/eller i tykktarmens slimhinne; 6).
- avføring (vekt, farge, konsistens og/eller viskositet; 8).
- tid for passasje igjennom fordøyelseskanalen og avføringsfrekvens (1).
- betennelse (tykktarm eller markører målt i avføring; 4).
- tarmens permeabilitet (markører målt i serum; 2).
- fordøyelse og utnyttelse av næringsstoffer (3).
- makroskopiske endringer (mage, tynntarm og/eller tykktarm; 2).
- mikroskopiske forandringer (mage, tynntarm og/eller tykktarm; 10).
- slimhinnens vekt og/eller innhold av protein (tykktarm; 2).
- slim eller blod i avføringen (1).
- tumorutvikling (tynntarm og/eller tykktarm; 2).
- vekt og/eller lengde (mage, tynntarm og/eller tykktarm; 6).

Det forelå få studier av effekter på fordøyelseskanalen av de konsistensmidlene som kan erstatte karragenan, og ingen av studiene av erstatningsstoffene rapporterte om tarmbetennelse eller permeabilitet. Utfallene var fordelt på studier og stoffer som følger:

- Betennelse i tarm ble undersøkt i fire dyrestudier, to hver av henholdsvis karragenan og karboksymetylcellulose.
- Permeabilitet av tarm ble undersøkt i to dyrestudier, én hver av henholdsvis karragenan og karboksymetylcellulose.
- Endringer i tarmens mikroflora ble undersøkt i fem dyrestudier, én hver av henholdsvis agar og karragenan og to av karboksymetylcellulose.

Langtidsvirkninger av konsistensmidlene ble ikke undersøkt i noen av de inkluderte studiene. Fem av de seks dyrestudiene av betennelse og permeabilitet og alle fem studier av tarmflora hadde høy risiko for systematiske skjevheter. Selv om fordøyelseskanalen hos gnagere og mennesker har store likheter, har gnagere en formage som ikke mennesker har. Det er kjent at negative helseeffekter som betennelse kan påvirkes av mikrobiomet. Mikrobiomet hos gnagere og mennesker deler bare 4 prosent av genene, noe som indikerer at det er forskjeller mellom artene og at

gnagere ikke er en egnet dyremodell til studier av betennelse og endringer i mikrobiomet hos mennesker (Hugenholtz og de Vos, 2018; Ward et al., 2020).

Konklusjon

Det ble funnet 14 studier hvor effekter på fordøyelseskanalen av de åtte inkluderte konsistensmidlene var undersøkt. For to av konsistensmidlene var det ingen studier som oppfylte inklusjonskriteriene. For de resterende ble det funnet fra én til sju studier. Effekter av kronisk eksponering ble ikke undersøkt i noen av de inkluderte studiene. I 13 av studiene ble det brukt dyremodeller, og ti av studiene hadde høy risiko for systematiske skjevheter. Den vitenskapelige litteraturen på de åtte konsistensmidlene og effekter på fordøyelseskanalen har begrenset omfang, er av begrenset relevans for eksponering over lang tid hos mennesker, og har høy risiko for systematiske skjevheter. Det vil derfor være begrenset nytte ved å utføre en systematisk risikovurdering på effekter av disse konsistensmidlene på fordøyelseskanalen, basert på kartleggingen av tilgjengelige vitenskapelige studier.

Abbreviations

ADI acceptable daily intake

EST emulsifiers, stabilisers, and thickeners

EU European Union

GI gastrointestinal

IBD inflammatory bowel disease

IBS irritable bowel syndrome

PECO population, exposure, comparator, outcome

sp/spp one (sp.) or two or more (spp.) unspecified species within a genus.

Glossary

Emulsifiers, stabilisers, and thickeners: Food additives that affect the texture of food.

Emulsifier: Food additives preventing liquids that normally do not mix, such as water and oil, from separating. Compounds used as emulsifiers are amphiphilic in nature. In food systems, emulsifiers are used to form stable lipid droplets in liquid systems, so called oil-in-water emulsions such as mayonnaise, or to keep water droplets stable in oil-in-water emulsions such as margarine.

Gastrointestinal tract: A tube that is specialized along its length for the sequential processing of food. It consists of a series of hollow organs stretching from the mouth to the anus, including mouth, oropharynx, oesophagus, stomach, duodenum, small and large intestines, rectum, and anus (Berne and Levy, 2000; Vander et al., 1990).

The digestive system: is the gastrointestinal tract and the several accessory glands and organs that add secretions to these hollow organs. Included organs and glands are the following: mouth, oropharynx, oesophagus, stomach, duodenum, small and large

intestines, salivary glands, pancreas, liver, gallbladder, rectum, and anus (Boron and Boulpaep, 2016).

Gastrointestinal tract effects: Include effects on digestion and absorption of food, gastrointestinal tract illness, effects on intestinal microbiota, effects on immune status, and gastrointestinal tract well-being (Bischoff, 2011).

Risk of bias: Systematic errors in the conduct of a study that can cause misleading results and conclusions.

Scoping review: A type of knowledge synthesis that follows a systematic approach to map evidence on a topic and which identifies main concepts, theories, sources, and knowledge gaps (Tricco et al., 2018b).

Stabiliser: Food additives that maintain the consistency, texture, and appearance by preventing separation such as creaming or settling of different ingredients in foods. In emulsions, stabilisers prevent the dispersed lipid droplets from rising upward and forming a cream layer. In other food systems stabilisers prevent settling of dispersed particles (e.g. settling of cocoa particles in chocolate milk). Stabilisers work similarly to thickeners by increasing the viscosity or gel-like properties of the product.

Thickener: Food additives that increase the viscosity or gel-like properties of the final product.

Background as provided by the Norwegian Food Safety Authority

Emulsifiers, stabilisers, and thickeners (EST) are additives that may affect the consistency of food in several different ways.

Typical EST and their uses are:

• emulsifiers facilitate the mixing of water and oil, e.g. when producing mayonnaise

• thickeners make food more viscous

• stabilisers prevent, for example, the precipitation of cocoa in cocoa milk

EST are used in several foodstuffs on the Norwegian market. Carrageenan and processed Eucheuma seaweed are natural carbohydrates extracted from red algae. Typical use as a stabilizer is in cocoa milk to prevent the cocoa particles from clumping and precipitation. Sodium carboxymethyl cellulose is the partial sodium salt of a carboxymethyl ether of cellulose, the cellulose being obtained directly from strains of fibrous plant material. This substance can be used to retain moisture and prevent sugar from crystallising.

Individual studies have been published which indicate that carrageenan and sodium carboxymethyl cellulose can have negative effects on the digestive tract. This, together with concern amongst consumers, is the reason for food manufacturers in Norway to replace carrageenan with other food additives.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority requests VKM to do the following:

1. To map the hypotheses on association between effects in the digestive tract and the following EST: alginate (E 401), agar (E 406), carrageenan (E 407), processed Eucheuma seaweed (E 407a), guar gum (E 412), xanthan gum (E 415), gellan gum (E 418), and sodium carboxymethyl cellulose (E 466).
2. To map possible variants of the additives which have been studied, including:
    - Whether the ingredients are authorised as food additives.
    - Whether the oral intake is as a substance alone or as an ingredient in the food.
    - Which doses have been used in the studies.
    - Which effects on the digestive tract have been studied.
3. Assess risk of bias (i.e. internal validity) in the included studies.

1 Introduction

Emulsifiers, stabilisers, and thickeners (ESTs) are food additives that affect the consistency of food, and which are used in several food products on the Norwegian market. Emulsifiers facilitate the mixing of water and oil, thickeners increase viscosity or gel-like properties, and stabilisers prevent separation of food constituents due to gravity (precipitation of particles or creaming of lipid droplets in emulsions).

The European Food Safety Authority (EFSA) has established acceptable daily intakes (ADI) for some ESTs while for others, EFSA concludes that no numerical ADI is needed as there is no safety concern at the reported uses and use levels (see Section 1.4).

Following publications of studies reporting negative effects of some ESTs on the gastrointestinal (GI) tract (Bhattacharyya et al., 2017; Chassaing et al., 2022), public concerns have been raised regarding the use of these substances. This concern led some Norwegian food manufacturers to reduce the use of certain ESTs, including carrageenan (E 407), processed Eucheuma seaweed (E 407a), and sodium carboxymethyl cellulose (E 466). Possible replacements are agar (E 406), sodium alginate (E 401), gellan gum (E 418), guar gum (E 412), and xanthan gum (E 415). All the above-mentioned food additives are authorised as food additives in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives (Regulation (EC) No 1333/2008).

1.1 Research questions and aim

The aim of this scoping review performed by the Norwegian Scientific Committee for Food and Environment (VKM), is to map literature addressing GI tract effects investigated after intake of the ESTs agar (E 406), sodium alginate (E 401), carrageenan (E 407), processed Eucheuma seaweed (E 407a), sodium carboxymethyl cellulose (E 466), gellan gum (E 418), guar gum (E 412), and xanthan gum (E 415).

The research questions were as follows:

- Which study hypotheses have been tested?
- Which are the aims of the studies?
- How are studies on the ESTs designed?
- Which populations are included?
- Which doses have been investigated in the studies?
- Which health outcomes are addressed?
- Which intake/treatment/exposure comparisons are used?
- Is it likely that the design and conduct of the studies have prevented bias (systematic errors)?

1.2 ESTs included in the scoping review

The ESTs included in this scoping review are used as either emulsifiers, stabilisers, thickeners, or a combination thereof, in food products.

1.2.1 Carrageenan (E 407)

Carrageenan is available in a variety of commercial preparations for use in food (Regulation (EU) No 231/2012). A selection of the information on carrageenan (E 407) in Regulation (EU) No 231/2012 is given in Table 1.2.1-1. For more information, see Appendix I.

Table 1.2.1-1. Characteristics of carrageenan (E 407).

Synonyms	Products of commerce are sold under different names such as: Irish moss gelose; Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); Carrageenan (from *Chondrus* and *Gigartina* spp.)
Definition	The wording carrageenan is reserved for the non-hydrolysed or otherwise chemically degraded polymer. Carrageenan is obtained by extraction with water or dilute aqueous alkali of strains of seaweeds of *Gigartinaceae, Solieriaceae, Hypneaceae* and *Furcellariaceae*, families of the class *Rhodophyceae* (red seaweeds). Carrageenan consists chiefly of the potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharide. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The prevalent polysaccharides in carrageenan are designated as kappa (κ), iota (ι), lambda (λ) depending on the number of sulphates by repeating unit (i.e. 1,2,3 sulphate). Between κ and ι there is a continuum of intermediate compositions differing in number of sulphates per repeat units between 1 and 2. EINECS 232-524-2

1.2.2 Sodium carboxymethyl cellulose (E 466)

An overview of selected information on sodium carboxymethyl cellulose (E 466) in Regulation (EU) No 231/2012 is given in Table 1.2.2-1. For more information, see Appendix I.

Table 1.2.2-1. Characteristics of sodium carboxymethyl cellulose (E 466).

Synonyms	NaCMC; Sodium CMC
Definition	Sodium carboxy methyl cellulose is the partial sodium salt of a carboxymethyl ether of cellulose, the cellulose being obtained directly from strains of fibrous plant material. EINECS: Not included

1.2.3 Processed Eucheuma seaweed (E 407a)

According to EFSA et al. (2018c), processed Eucheuma seaweed (E 407a) and carrageenan (E 407) are closely related based on structural evaluation, and the main component of processed Eucheuma seaweed (E 407a) is carrageenan.

Processed Eucheuma seaweed (E 407a) differs from carrageenan (E 407) in composition, purity (content of carrageenan), the type of carrageenan present, the method used for extraction and the source (strains of seaweeds).

Selected information on processed Eucheuma seaweed (E 407a) in Regulation (EU) No 231/2012 is given in Table 1.2.3-1. For more information, see Appendix I.

Table 1.2.3-1. Characteristics of processed Eucheuma seaweed (E 407a).

Synonyms	PES (acronym for processed Eucheuma seaweed). The PES obtained from Euchema cottonii is generally called kappa (κ) PES and the PES from Euchema spinosum iota (ι) PES.
Definition	The wording processed Eucheuma seaweed is reserved to the non-hydrolysed or otherwise chemically degraded polymer. Processed Eucheuma seaweed is obtained by aqueous alkaline treatment at high temperature of the strains of seaweeds *Eucheuma cottonii* and *Eucheuma spinosum*, of the class *Rhodophyceae* (red seaweeds) followed by fresh water washing to remove impurities and drying to obtain the product. The product consists chiefly of the potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose polysaccharide. Up to 15% algal cellulose is also present in the product. EINECS: Not reported

1.2.4 Agar (E 406)

Selected information on agar (E 406) in Regulation (EU) No 231/2012 is given in Table 1.2.4-1. For more information, see Appendix I.

Table 1.2.4-1. Characteristics of agar (E 406).

Synonyms	Gelose; Kanten, Bengal, Ceylon, Chinese or Japanese isinglass; Layor Carang
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 sulphuric acid which is neutralised by calcium, magnesium, potassium or sodium. It is extracted from certain strains of marine algae of the families *Gelidiacea*e and *Gracilariaceae* and relevant red algae of the class *Rhodophyceae* EINECS: 232-658-1

1.2.5 Sodium alginate (E 400 – E 404)

No information on synonyms and no definition were available for sodium alginate (E 401) Regulation (EU) No 231/2012. For more information, see Appendix I.

1.2.6 Gellan gum (E 418)

Selected information on gellan gum (E 418) in Regulation (EU) No 231/2012 is given in Table 1.2.6-1. For more information, see Appendix I.

Table 1.2.6-1. Characteristics of gellan gum (E 418).

Synonyms	Not reported
Definition	EINECS: 275-117-5

1.2.7 Guar gum (E 412)

Selected information on guar gum (E 412) in Regulation (EU) No 231/2012, including purity criteria, is given in Table 1.2.7-1. For more information, see Appendix I.

Table 1.2.7-1. Characteristics of guar gum (E 412).

Synonyms	Gum cyamopsis; Guar flour
Definition	Guar gum is the ground endosperm of the seeds of strains of the guar plant, *Cyamopsis tetragonolobus* (L.) Taub. (family *Leguminosae*). Consists mainly of a high molecular weight hydrocolloidal polysaccharide composed of galactopyranose and mannopyranose units combined through glycosidic linkages, which may be described chemically as galactomannan. The gum may be partially hydrolysed by either heat treatment, mild acid or alkaline oxidative treatment for viscosity adjustment. EINECS: 232-536-0

1.2.8 Xanthan gum (E 415)

Selected information on xanthan gum (E 415) in Regulation (EU) No 231/2012, including purity criteria, is given in Table 1.2.8-1. For more information, see Appendix I.

Table 1.2.8-1. Characteristics of xanthan gum (E 415).

Synonyms	Not reported

Definition	Xanthan gum is a high molecular weight polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with strains of Xanthomonas campestris, purified by recovery with ethanol or propan-2-ol, dried and milled. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid, and is prepared as the sodium, potassium or calcium salt. Its solutions are neutral. EINECS: 234-394-2

1.3 Effects on the gastrointenstinal (GI) tract

GI tract effects are diverse in type and extent. Examples are diagnosed chronic diseases such as coeliac disease, inflammatory bowel disease and cancer, effects related to digestion and absorption, effects on the intestinal microbiota, and immune effects such as tolerance to specific foods or food ingredients. Effects also include absence of disease, such as a feeling of wellbeing (Bischoff, 2011) as well as reduction in disease incidence (Bischoff, 2011). For more details, please refer to Table 2.1-1.

1.4 Risk assessments by EFSA

Carrageenan (E 407), sodium carboxymethyl cellulose (E 466), processed Eucheuma seaweed (E 407a), agar (E 406), sodium alginate (E 401), gellan gum (E 418), guar gum (E 412), and xanthan gum (E 415) were evaluated as food additives by the European Food Safety Authority (EFSA) between 2016 and 2022 (EFSA et al., 2016; EFSA et al., 2017b; EFSA et al., 2018a; EFSA et al., 2018b; EFSA et al., 2017c; EFSA et al., 2018c; EFSA et al., 2022; EFSA et al., 2018d; EFSA et al., 2017d). An acceptable daily intake (ADI) was not set for any of the ESTs listed above except for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) which received a temporary group ADI.

In their re-evaluation of alginic acid and its sodium, potassium, ammonium and calcium salts (E 400–E 404) as food additives, EFSA concluded that there was no need for a numerical ADI for sodium alginate (E 401), and that there was no safety concern at the level of the refined exposure assessments (EFSA et al., 2017c).

No toxicological effects were observed for agar (E 406) (EFSA et al., 2016). EFSA concluded that no numerical acceptable daily intake (ADI) was needed, since there was no safety concern for the general population at the reported use and use levels.

For carrageenan (E 407) and processed Eucheuma seaweed (E 407a), EFSA concluded that the existing group acceptable daily intake (ADI) of 75 mg/kg bw per day should be considered temporary (EFSA et al., 2018c). This ADI was based on the no adverse effect at the highest dose tested from a study published in 1959. The ADI was made temporary due to lack of adequate data and uncertainty in the existing database. The following uncertainties were noted: there was limited or no description of the characterisation of the carrageenan material tested; there were no studies available on the low molecular weight (approx. 200 kDa) variant of carrageenan; there were limited data on chronic, reproductive, and developmental toxicity. EFSA also concluded that

the database should be improved within 5 years after publication of the 2018 opinion (EFSA et al., 2018c).

EFSA concluded that there is no need for a numerical ADI for guar gum (E 412), and there is no safety concern for the general population at the refined exposure assessment of guar gum as a food additive (EFSA et al., 2017b). For uses of guar gum in foods intended for infants and young children, EFSA concluded that the occurrence of abdominal discomfort should be monitored. If such effects are observed, doses should be identified as a basis for further risk assessment.

EFSA concluded that there is no need for a numerical ADI for xanthan gum (E 415), and that there is no safety concern for the general population at the refined exposure assessment of xanthan gum as food additive (EFSA et al., 2017d).

EFSA concluded that there is no need for a numerical acceptable daily intake (ADI) for gellan gum (E 418), and that there is no safety concern at the refined exposure assessment for the reported uses and use levels of gellan gum as a food additive (EFSA et al., 2018d).

Sodium carboxymethyl cellulose (E 466) was associated with local effects on the GI tract, such as effects on caecal size, gut microbiota and inflammation, but the effects were considered not adverse or of unknown adversity (EFSA et al., 2018b). EFSA concluded that no numerical ADI was needed, since there was no safety concern for the general population at the reported use and use level. Recently, EFSA concluded that they could not assess the safety of sodium carboxymethyl cellulose in food for infants and young children due to lack of toxicological data (EFSA et al., 2022).

2 Methods

This scoping review was conducted using scientific, systematic, and transparent methods, and the evidence was mapped in a systematic way, and main concepts, theories, sources, and knowledge gaps were identified (Colquhoun et al., 2014; Levac et al., 2010; Tricco et al., 2018).

A study protocol following the PRISMA Extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018) was completed before start of the review. The study protocol is published at vkm.no (VKM et al., 2023). Deviations from the protocol are described in Appendix IV.

2.1 Literature search and study selection

Literature searches were performed to retrieve studies relevant for answering the research questions (Section 1.1).

A research librarian performed literature searches in the electronic databases from MEDLINE (Ovid), Embase (Ovid), and Web of Science from inception to search date (March 1, 2023). The search terms and strategy are included in Appendix II. In addition, the website of EFSA was searched for Opinions on the included ESTs.

The study selection was based on the predefined eligibility criteria (Table 2.1-1) described in the protocol (VKM et al., 2023). The identified records were imported into EndNote (Thomson Reuters, version X9), duplicates were removed, and the records were imported into Rayyan (Ouzzani et al., 2016) for screening of title and abstracts. Screening of records for relevance was performed independently by pairs of reviewers. To ensure between-reviewer calibration, all reviewers screened 100 of the retrieved titles and abstracts, and conflicts were discussed and clarified in a calibration meeting. Records selected for full text assessment were evaluated independently using the software EPPI-Reviewer (Thomas et al., 2022) by pairs of reviewers. To ensure between-reviewer calibration, all reviewers evaluated 10 full text publications and discussed and clarified the application of the eligibility criteria in a following calibration meeting.

Table 2.1-1. Eligibility criteria for studies on GI tract effects.

Population	• Humans of all age groups, males, and females • Mammals • *Ex vivo* GI tract model systems (human faecal samples)

Exposure	• Separately tested, oral intake of agar (E 406), sodium alginate (E 401), gellan gum (E 418), guar gum (E 412), xanthan gum (E 415), carrageenan (E 407), Eucheuma seaweed (E 407a), and sodium carboxymethyl cellulose (E 466) • Dietary sources containing agar (E 406), sodium alginate (E 401), gellan gum (E 418), guar gum (E 412), xanthan gum (E 415), carrageenan (E 407), Eucheuma seaweed (E 407a), and sodium carboxymethyl cellulose (E 466) • The substance tested must be approved for use as food additive in certain foods in Norway/EU* (sub-set of criteria described below)
Comparison	• Placebo • No treatment • Dose comparison
Outcomes	Any GI tract effects including, but not restricted to: • Diagnosed chronic diseases and disorders, such as colorectal cancer, coeliac disease, food allergy, food intolerance e.g. lactose intolerance, and inflammatory bowel disease (IBD), i.e. Crohn's and ulcerative colitis • GI tract effects and symptoms, often reversible and without a defined diagnosis, such as nausea, vomiting, diarrhoea and abdominal pain. One or more of these symptoms also include irritable bowel syndrome (IBS) • (Non-symptomatic) GI alterations such as changes in the microbiota, mechanical barriers, immunity, or faecal biochemical composition • Other effects
Study design	• Human controlled studies • Animal experimental studies • Ex vivo GI tract model studies • Systematic reviews**
Publication year	No restriction
Country	No restriction
Language	Danish, English, Norwegian and Swedish

*The criteria used are specified in Table 2.1-2.

**A publication qualifies as a systematic review if 1) it describes a specific research question and the specific criteria used for selecting studies, 2) the authors have performed a systematic literature search, and 3) it includes a quality assessment of the selected studies (Cochrane Glossary, 2020).

A separate sub-set of eligibility criteria was developed for the exposure eligibility criterion "The substance tested must be approved for use as food additive in certain foods in Norway/EU", based on the regulatory specifications for food additives ((Regulation (EU) No 231/2012) required information about degree of substitution and description of hydrolysation and/or chemical degradation for specific food additives. If the E number of the food additive under investigation was mentioned in the method section of the paper, reviewers anticipated that the food additive adhered to the

specification described in the regulation. In this case, no further description was needed. The sub-set of eligibility criteria for the food additives is presented in Table 2.1-2.

Table 2.1-2. The sub-set of eligibility criteria for substances.

Food additive	Criteria applied to evaluate whether the investigated substance was approved for use as food additive in certain foods in Norway/EU
Agar (E 406) Gellan gum (E 418) Sodium alginate (E 401) Xanthan gum (E 415) Guar gum (E 412)	The following information must be described: • E number OR • Either of the terms "food additives" or "food grade" are used in the description of the substance in the method section *Note that for guar gum, "partially hydrolysed guar gum" is acceptable and is included.*
Carrageenan (E 407) Eucheuma seaweed (E407a)	The following information must be described: • E number OR • Either of the terms "food additives" or "food grade" are used in the description of the substance in the method section AND • The substance is not hydrolysed or chemically degraded AND • MW is described and the MW fraction < 50 kDa is no more than 5%
Sodium carboxymethyl cellulose (E 466)	The following information must be described: • E number OR • Either of the terms "food additives" or "food grade" are used in the description of the substance in the method section AND • Substitution is described and degree of substitution is not less than 0.2 and not more than 1.5 carboxymethyl groups ($-CH_2COOH$) per anhydroglucose unit

2.2 Data extraction

To ensure between-reviewer calibration, all reviewers extracted data from one full text publication. Reviewers discussed and clarified aspects of the data extraction (e.g., coding of data items) in a following harmonisation meeting. After revision of codes, three reviewers each extracted data from an allocated set of full text publications. Two reviewers independently verified the extracted data against the original publications, each reviewer assessing half the number of publications.

Codes for the following data items were created in EPPI-Reviewer (Thomas et al., 2022) to extract data from the included studies:

- Study ID (author, publication year)
- Publication type
- Reported conflict of interest
- Main objective(s)
- Any stated hypotheses regarding GI tract effects
- Population
- Exposure
- Comparison
- Outcomes addressed

Following data extraction, the outcomes addressed were sorted into broader categories, e.g. changes in gut microbiota number and changes in gut microbiota composition were both sorted under changes in gut microbiota composition and/or number. These broader categories were used for the evidence maps.

Codes for the following data items were created in EPPI-Reviewer to extract data from studies that were excluded because of insufficient description of the substance tested:

- Study ID (author, publication year)
- Population
- Exposure
- Outcomes addressed

2.3 Evaluation of risk of bias

Risk of bias (RoB) in the included primary human and animal studies was evaluated using the Handbook for conducting a literature-based health assessment using the National Toxicology Program (NTP), Office of Health Assessment and Translation (OHAT) approach for systematic review and evidence integration (NTP OHAT, 2015; NTP OHAT, 2019). The project group amended the RoB criteria for exposure assessment (Appendix III). An overview of the questions used to evaluate RoB, and the questions defined as key questions, is given in Table 2.3-1.

Table 2.3-1. Types of bias distributed between eight and nine RoB questions applied to human and animal studies, respectively. X: RoB-question asked for the study type indicated; *: key question; N.A.: not applicable

Type of bias	RoB question	Human study	Animal study
Selection	Was administered dose or exposure level adequately randomised?	X*	X*
	Was allocation to study groups adequately concealed?	X*	X

Type of bias	RoB question	Human study	Animal study
Performance	Were the research personnel (and human subjects) blinded to the study group during the study?	X*	X
	Were experimental conditions identical across study groups?	N.A.	X*
Attrition	Were outcome data complete without attrition or exclusion from analysis?	X	X
Detection	Can we be confident in the exposure characterisation?	X*	X*
	Can we be confident in the outcome assessment?	X*	X*
Selective reporting	Were all measured outcomes reported?	X*	X
Other bias	Were there no other potential threats to internal validity?	X	X

According to OHAT (NTP OHAT, 2015; NTP OHAT, 2019), the rating of all questions was integrated to classify the studies into tiers of overall RoB for each outcome in a study (modified from EFSA et al. (2017a) as shown in Table 2.3-2. Tiers 1, 2 and 3 represent low, moderate, and high RoB, respectively (the written expressions are not explicitly defined by OHAT).

Table 2.3-2. Classification of studies into tiers according to overall RoB for each outcome/study. Definitely low risk of bias (++); probably low risk of bias (+); probably high risk of bias (-); definitely high risk of bias (--).

	Tier 1 Low RoB	Tier 2 Moderate RoB	Tier 3 High RoB
Criteria for classification	All key questions are scored +/++ AND No more than one non-key question is scored – AND No non-key question is scored – –	All combinations not falling under tier 1 or 3	Any key or non-key question is scored – – OR More than one key question is scored –

To ensure between-reviewer calibration, five reviewers assessed RoB in one full text publication and discussed and clarified the aspects of the evaluation in a following harmonisation meeting. The reviewers carried out the remaining RoB assessments independently, in pairs of two. Disagreements were resolved by discussion and

consensus or, if consensus was not reached, by consulting a third reviewer. One author participated in all consensus meetings to further ensure between-reviewer calibration across pairs.

2.4 Data synthesis

The following extracted data in the included studies on GI tract effects of the selected ESTs were summarised in text, tables, figures, and interactive evidence and gap maps:

- The aims and hypotheses in each study, as stated by the authors.
- The characteristics of the studies, including populations, exposures, comparisons, and outcomes/endpoints studied within each study design.
- The risk of bias assessed in the included studies.
- The distribution of studies investigating GI tract effects of ESTs across publication years.
- The ESTs investigated by population.
- The outcomes addressed and overall risk of bias in each of the studies of ESTs.

3 Results

3.1 Literature search and study selection

The study selection of systematic reviews and single studies are presented in Figures 3.1-1 and 3.1-2, respectively. No systematic reviews were included. Fourteen primary studies from 14 separate publications fulfilled the eligibility criteria. An overview of all reports assessed for eligibility is available in Supplementary materials 1. There we list a) all studies that were assessed for eligibility, and b) the excluded studies with reasons for exclusion.

A total of 214 records fulfilled all eligibility criteria except the substance specific requirements. These publications did not describe the studied EST(s) sufficiently to determine whether the substance(s) fulfilled the food grade criteria. An overview of these publications including population and type of EST(s) is available in Supplementary materials 1.

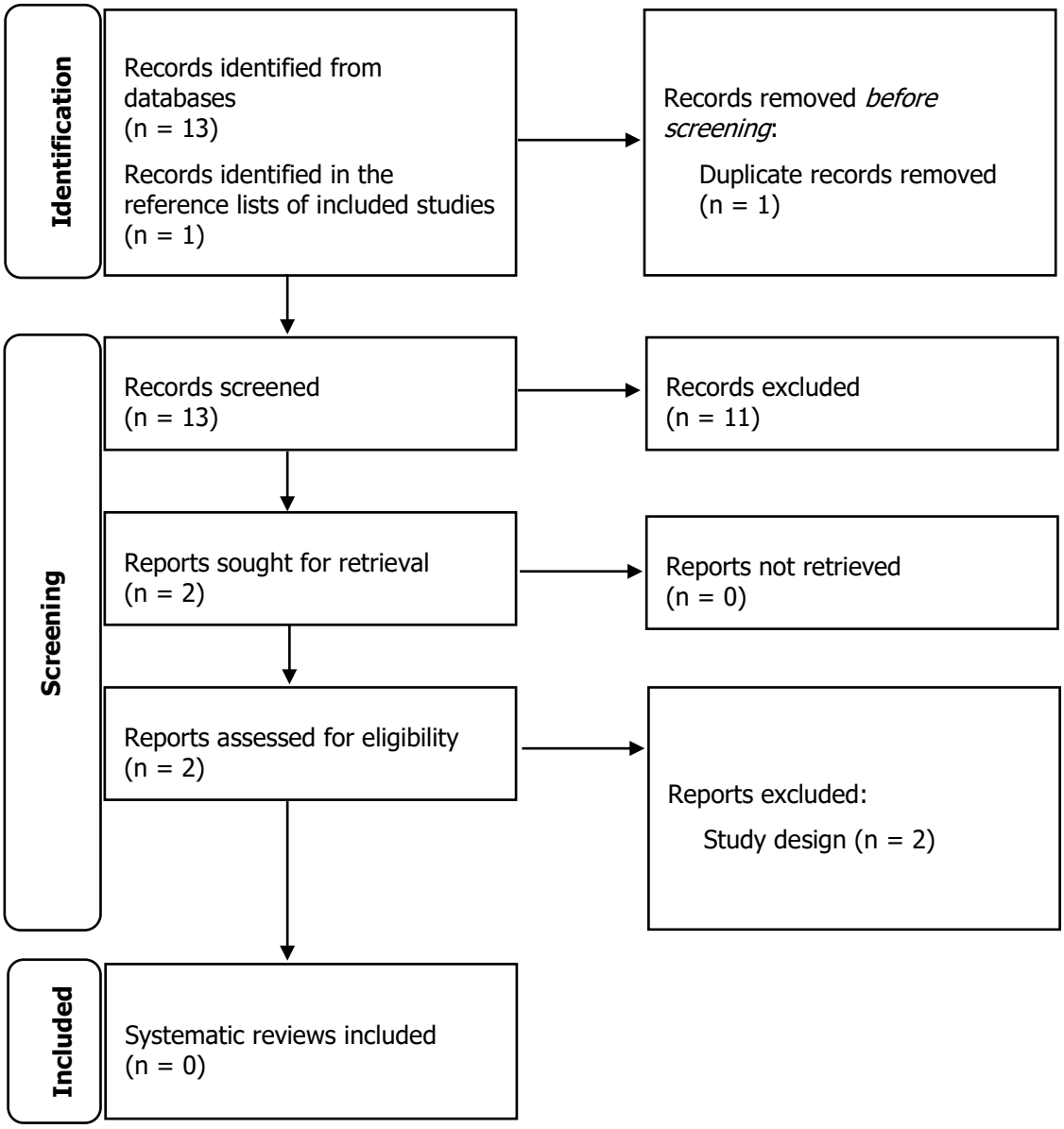

Figure 3.1-1. PRISMA flowchart for the selection of systematic reviews (from Moher et al. (2009).

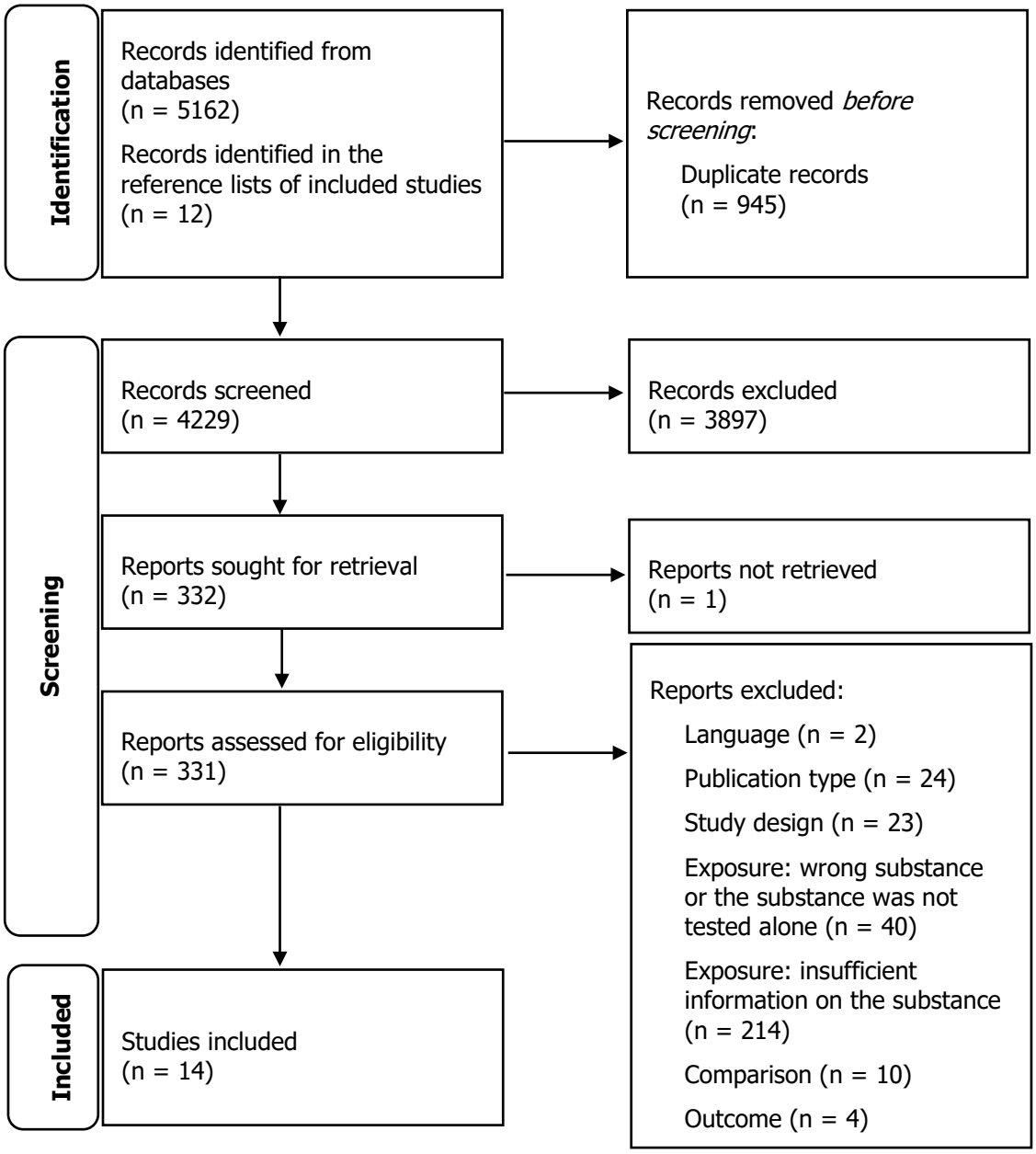

Figure 3.1-2. PRISMA Flowchart for the selection of human and animal experimental studies, and *ex vivo* GI tract model system studies (from Moher et al. (2009).

3.2 Results and data synthesis according to the research questions

3.2.1 Aims and hypotheses

The aims and hypotheses in the 14 included studies are presented in Table 3.2.1-1. Two of the publications presented a hypothesis to be tested in addition to an aim of the study.

Table 3.2.1-1. Aims and hypotheses in the included studies, as stated by the study authors.

Reference	Aim of the study	Hypothesis tested
Calvert and Reicks (1988)	To examine the relationship between colonic thymidine kinase enzyme activity and mucin histochemistry and the reported effects of various dietary fibers on chemically induced colon carcinogenesis.	Not reported
Calvert and Satchithanandam (1992)	To examine the effect on colonic cell proliferation of feeding high-molecular-weight carrageenan.	No reported
Cameron-Smith et al. (1994)	The aims were to determine the effect that the GI tract has on the viscosity of meals containing different soluble fibers, and to determine whether the glycaemic response of a meal (containing the soluble fiber) was predicted by the viscosity of the digesta in the small intestine.	Not reported
Gao et al. (2022)	To explore the risk of κ-carrageenan induced colitis under high-sucrose or high-salt diet in mice.	Not reported
Mallett et al. (1984)	To study the effect of feeding a number of hydrocolloid materials (agar, carboxymethylcellulose, carrageenan, guar gum, gum acacia, locust-bean gum and pectin) on a range of caecal microbial enzyme activities that are of toxicological importance to the host animal.	Not reported
McGill et al. (1977)	To test infant formulas made with and without carrageenan in a nonhuman primate infant. To detect deleterious effects of native carrageenan on overall	Not reported

Reference	Aim of the study	Hypothesis tested
	growth and development as well as on the alimentary tract and other tissues.	
Pogozhykh et al. (2021)	To assess the local and systemic toxic effects of the common food additive E407a in rats orally exposed to it for two weeks.	Not reported
Rideout et al. (2008)	To examine the influence of different resistant starch varieties and conventional fibers on the efficiency of nutrient utilisation and intestinal fermentation in pigs.	Not reported
Tomlin and Read (1988)	To investigate whether the degradation of viscous polysaccharides by colonic bacteria determines their effects on colonic function.	Not reported
Viennois et al. (2017)	To test whether regular consumption of dietary emulsifiers carboxymethylcellulose or polysorbate-80 exacerbate tumor development	In the present study, we hypothesized that emulsifiers could be involved in colorectal cancer development through the promotion of low-grade intestinal inflammation and alterations of the intestinal microbiota.
Viennois and Chassaing (2021)	To investigate the impact of dietary emulsifiers consumption on cancer initiation and progression in a genetical model of intestinal adenomas.	In the present study, we hypothesized that dietary emulsifier consumption could aggravate initiation and development of genetically driven colorectal cancer (CRC).
Weiner et al. (2007)	To evaluate food-grade carrageenan that has been characterized for the low molecular weight fraction.	The present subchronic dietary toxicity study was conducted in rats to test the hypothesis that kappa carrageenan containing a high percentage of the Low Molecular Weight Tail (LMT) below 50 kDa is safe for food use.
Weiner et al. (2015)	To evaluate (1) the potential absorption of carrageenan (CGN) in the gastrointestinal	Not reported

Reference	Aim of the study	Hypothesis tested
	tract, (2) the presence of CGN in serum following ingestion of swine-adapted infant formula containing CGN via toxicokinetic analysis and (3) to assess the impact of CGN on the developing immune system.	
Wilcox et al. (1992)	The effect on colonic cell proliferation of poligeenan, a nongenotoxic polysaccharide that induces colon tumors in rats, was compared with guar gum and carrageenan.	Not reported

3.2.2 Study characteristics

An overview of selected study characteristics of the 14 included studies, including design, population, substance and dose(s) tested, comparison, and outcome addressed, is shown in Table 3.2.2-2. The

Some of the 14 publications reported on more than one experiment/study, however only one study per publication did fulfill the eligibility criteria (e.g., sufficient information about the substance or addressing GI-related outcomes) were included. Two of the included studies assessed two ESTs that fulfilled the substance specific criteria.

Only one human study was included and no *ex vivo* studies that fulfilled the eligibility criteria were identified. The remaining 13 studies were experimental animal studies. The animal species used in the studies were baboons, pigs, mice, and rats.

The human study had a latin-square design (the arrangement of t treatments, each one repeated t times, in such a way that each treatment appears exactly one time in each row and each column in the design). The study had seven participants who received the substance in drink for a period of one week.

Randomisation was applied in eight of 13 animal experiments. The substances were administered in the feed (seven studies), drinking water (four studies), and infant formula (two studies). Four of the animal experiments were subchronic studies (exposure ≥13 weeks) and the remaining studies were subacute studies (≤12 weeks). Eight of the studies had an exposure duration of 4 weeks or less. No studies could be categorised as chronic.

All animal studies included a control group that did not receive the EST under investigation. Four studies reported the external dose in mg/kg bw/day and none of the studies reported internal dose. We have calculated the doses for the remaining nine studies, using information reported in the publication and/or using default values (EFSA, 2012). The calculations are available in Supplementary materials 1. External doses of carrageenan ranged from 52 to 6000 mg/kg bw/day. Doses for guar gum ranged from 4500 to 8400 mg/kg bw/day.

Most studies tested only one dose of the EST. Four of the studies included more than one dose (not including the zero dose of the control group) of which two studies included two doses and two studies included three doses.

Age was reported in eight of thirteen animal studies, whereas sex of the animals was reported in nine studies. Only males were used in five of the studies, whereas males and females were used in four studies.

Table 3.2.2-2. Characteristics of the included studies.

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
Tomlin et al (1988)	Latin-square design Cross-over	Human 6 males, 1 female Age not reported Healthy	Xanthan gum Given as drink together with self-selected diet restricted in fibre. 3 times daily for 1 week Dose: 15 g/day Dose [estimated]: 214 mg/kg bw per day	No treatment	Faecal weight Gastric transit time Stool frequency
McGill et al (1977)	Randomised experimental study	Baboon N=24, 3 groups, 3 males/group, 5 females/group Newborn	Carrageenan 0; 300; 1500 mg/L in infant formula 5 times/day first 14 days 4 times/day next 14 days 3 times/day next 56 days	No treatment	Faecal colour, weight, and consistency Microscopic changes (digestive tract) Macroscopic changes (small intestine and colon) Presence of mucus or blood in faeces

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
			2 times/day next 28 days until 112 days old Total formula consumed (g, mean) for concentration levels (mg/L): 0: 35 949 g 300: 34 252 g 1500: 38 899 g Mean daily doses (mg/kg bw per day) for each sex [estimated]: Males (mean): 0; 67; 353 Females (mean): 0; 71; 400		
Mallett et al (1984)	Randomised experimental study	Rat: Sprague-Dawley N=48, 8 groups, 6 males/group 3 weeks old at arrival (age at start of	Agar Guar gum 0; 50 g/kg in feed, ad libitum	No treatment	Changes in gut microbiota number (caecum) Microbial enzyme activity (caecum) Caecal content weight Concentration of ammonia (caecum)

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
		exposure: not reported)	Subacute, 4 weeks Dose [estimated]: 6000 mg/kg bw/day		
Calvert et al (1988)	Non-randomised experimental study	Rat: Fischer 344 N=32, 4 groups, 8 males/group 9-10 weeks at start of exposure	Carrageenan 0; 5% in feed, ad libitum Subacute, 4 weeks Dose [estimated]: 6000 mg/kg bw/day	No treatment	Enzymatic activity (colonic mucosa) Macroscopic changes (stomach, colon) Microscopic changes (colon) Mucosal weight (colon) Mucosal protein content (colon)
Wilcox et al (1992)	Non-randomised experimental study	Rat: Fischer 344 N=144, 4 diets, 9 timepoint groups/diet, 4 males/timepoint group 11 weeks at start of exposure	Guar gum 0; 5% in feed Subchronic, up to 91 days Dose [estimated]: 4500 mg/kg bw/day	No treatment	Enzymatic activity (colon) Cell proliferation (colon))
Cameron-Smith et al (1994)	Randomised experimental study	Rat: Sprague-Dawley N=20, 4 groups, 5 males/group Age not reported	Guar gum Xanthan gum	No treatment	Faecal viscosity (stomach and small intestine)

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
			0; 70 g/kg in feed, ad libitum Subacute, 2 weeks Dose [estimated]: 8400 mg/kg bw/day		
Rideout et al (2008)	Randomised experimental study	Pig: Yorkshire N=36, 6 groups, 4-6 pigs/group (guar gum n=5), sex not reported Age not reported	Guar gum 0; 10% in feed, ad libitum Subacute, 30 days Dose [estimated]: 5200 mg/kg bw/day	No treatment	Intestinal utilisation and fermentation of nutrients
Weiner et al (2007)	Randomised experimental study	Rat: Fischer 344 N=120, 3 groups/sex 20/sex/group 50 days at start of exposure	Carrageenan 0; 25 000; 50 000 ppm in feed, ad libitum Subchronic, 90 days Doses (mg/kg bw/day): 25 000 ppm: males 1656; females, 1872	No treatment	Faeces consistency Microscopic changes (gastrointestinal tract)

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
			50 000 ppm: males, 3394; females, 3867		
Weiner et al (2015)	Randomised experimental study	Pig: Yorkshire N=72, 4 groups/sex, 9/sex/group 4 days at start of exposure	Carrageenan 0; 0.5; 3.0; 10.0 g/L/day in infant formula 6 times/day (~83.33 mL/kg bw per dose) Subacute, 28 days Doses (mg/kg bw/day, ±SD in parentheses): 0.5: males 51.71 (4.06); females 55.57 (6.88) 3.0: males 192.86 (18.38); females 202.53 (12.72) 10.0: males 430.27 (67.33); females 448.25 (59.98)	No treatment	Microscopic changes (stomach, small intestine and large intestine) Immunohistochemical changes, TNF-alpha and IL-8 (colon) Weight of stomach, small intestine and large intestine
Pogozhykh et al (2021)	Randomised experimental study	Rat: WAG	Eucheuma seaweed	No treatment	Microscopic changes (small intestine and large intestine)

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
		N=16, 2 groups, 8/group, Sex not reported Adults	0; 1% PES solution in drinking water Dose: 140 mg/kg bw/day Subacute, 2 weeks		
Viennois et al. (2017)	Non-randomised experimental study	Mouse: C57BL/6; colitis-induced colorectal cancer model N varies depending on outcome 5-10/group, sex not reported 4 w at start of exposure	Sodium carboxymethyl cellulose 0; 1% (w/v) in drinking water Dose [estimated]: 1500 mg/kg bw/day Subchronic, 127 days over a period of 141 days ~ 18 w	No treatment	Colon length and weight Tumour development (colon) Myeloperioxidase activity (colonic tissue) Changes in gut microbiota composition Cell proliferation (colon) Faecal lipcalin-2 Lipopolysaccharide and D-lactic acid (serum)
Viennois et al (2021)	Randomised experimental study	Mouse: C57BL/6J wild-type and APC^{min} N varies depending on outcome 3-13/group	Sodium carboxymethyl cellulose 0; 1% (w/v) in drinking water	No treatment	Changes in gut microbiota composition Colon length and weight Faecal lipcalin-2 and macroscopic examination of inflammation parameters

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
		Both males and females 7 w at start of exposure	Dose [estimated]: 1500 mg/kg bw/day Subchronic, 15 weeks		Tumour development (small intestine and colon)
Calvert et al (1992)	Non-randomised experimental study	Rat: Fischer 344 N=28, 4 groups, 7 males/group Age not reported	Carrageenan 0; 0.65; 1.31; 2.61% in feed, ad libitum Subacute, 4 weeks Doses, week 1 (mg/kg bw/day, ±SE in parentheses): 0.65%: 467.8 (11.3) 1.31%: 947.5 (22.8) 2.61%: 1943.9 (106.2)	No treatment	Enzymatic activity (colonic mucosa) Faecal weight Microscopic changes (colon) Mucosal protein content (colon)
Gao et al (2022)	Randomised experimental study	Mouse: C57BL/6J N=40, 4 groups, 10 mice/group, sex not reported	Carrageenan 0; 0.5% in drinking water Subacute, 9 weeks	No treatment	Changes in gut microbiota composition Microscopic changes (colon) Lipopolysaccharide and D-lactic acid (serum)

Reference	Study design	Population	Substance and dose(s) tested	Comparison	Outcome(s)
		11 w at start of exposure	Dose [estimated]: 900 mg/kg bw/day		Myeloperoxidase activity (colonic tissue) Colon length

3.2.3 Risk of bias assessment

The RoB rating for the included studies is shown in Tables 3.2.3-1 (human study) and 3.2.3-2 (animal studies). An overview of the reasonings for the RoB rating is available in the Supplementary Materials 2. The human study was assessed to be tier 3. Four animal studies were assessed to be tier 2, and eight to be tier 3. None of the studies were assessed to be tier 1. All 14 studies received "probably high risk of bias" ratings for question 2 on concealment of the allocation. According to OHAT (NTP OHAT, 2015; NTP OHAT, 2019): "Allocation concealment prior to assigning the exposure level or treatment group … helps to assure that treatment is not given selectively based on potential differences in human subjects or non-human experimental animals."

All animal studies received "probably high risk of bias" ratings for question 4 on attrition and exclusion of data.

Table 3.2.3-1. RoB rating and classification into tier for the human study. *Key question. Definitely low risk of bias (++); probably low risk of bias (+); probably high risk of bias (-); definitely high risk of bias (--).

Reference	**1**.* Was administered dose or exposure level adequately randomised?	**2**.* Was allocation to study groups adequately concealed?	**3**.* Were the research personnel and human subjects blinded to the study group during the study?	**4**. Were outcome data complete without attrition or exclusion from analysis?	**5**.* Can we be confident in the exposure characterisation?	**6**.* Can we be confident in the outcome assessment?	**7**.* Were all measured outcomes reported?	**8**. Were there no other potential threats to internal validity?	**Tier**
Tomlin and Read (1988)	-	-	-	++	-	++	++	-	3

Table 3.2.3-2. RoB rating and classification into tiers of animal studies. *Key question. Definitely low risk of bias (++); probably low risk of bias (+); probably high risk of bias (-); definitely high risk of bias (--).

Reference	**1**.* Was administered dose or exposure level adequately randomised?	**2.** Was allocation to study groups adequately concealed?	**3**.* Were experimental conditions identical across study groups?	**4**. Were the research personnel blinded to the study group during the study?	**5**. Were outcome data complete without attrition or exclusion from analysis?	**6**.* Can we be confident in the exposure characterisation?	**7**.* Can we be confident in the outcome assessment?	**8**. Were all measured outcomes reported?	**9**. Were there no other potential threats to internal validity?	**Tier**
Calvert and Reicks (1988)	--	-	++	-	++	+	-	++	+	3

Reference	**1**.* Was administered dose or exposure level adequately randomised?	**2.** Was allocation to study groups adequately concealed?	**3**.* Were experimental conditions identical across study groups?	**4**. Were the research personnel blinded to the study group during the study?	**5**. Were outcome data complete without attrition or exclusion from analysis?	**6**.* Can we be confident in the exposure characterisation?	**7**.* Can we be confident in the outcome assessment?	**8**. Were all measured outcomes reported?	**9**. Were there no other potential threats to internal validity?	**Tier**
Calvert and Satchithanandam (1992)	-	-	++	-	++	-	+	++	+	3
Cameron-Smith et al. (1994)	-	-	+	-	+	-	+	++	-	3
Gao et al. (2022)	+	-	++	-	-	-	-	++	+	3
Mallett et al. (1984)	+	-	+	-	++	--	-	++	+	3
McGill et al. (1977)	+	-	+	-	+	+	+	+	-	2
Pogozhykh et al. (2021)	+	-	+	-	+	-	-	++	+	3
Rideout et al. (2008)	++	-	++	-	+	-	++	++	+	2
Viennois et al. (2017)	-	-	--	-	-	+	++	++	-	3
Viennois and Chassaing (2021)	-	-	-	-	-	-	+	+	-	3
Weiner et al. (2007)	++	-	++	-	+	++	-	++	-	2

Reference	**1**.* Was administered dose or exposure level adequately randomised?	**2.** Was allocation to study groups adequately concealed?	**3**.* Were experimental conditions identical across study groups?	**4**. Were the research personnel blinded to the study group during the study?	**5**. Were outcome data complete without attrition or exclusion from analysis?	**6**.* Can we be confident in the exposure characterisation?	**7**.* Can we be confident in the outcome assessment?	**8**. Were all measured outcomes reported?	**9**. Were there no other potential threats to internal validity?	**Tier**
Weiner et al. (2015)	+	-	+	-	+	++	-	++	-	2
Wilcox et al. (1992)	+	-	-	-	-	--	-	++	-	3

3.2.4 Data synthesis

An overview of the year of publication and the number of included studies per substance is shown in Figure 3.2.4-1. Approximately half of the included studies were published more than 30 years ago, and five of the 14 included studies were published in the last ten years.

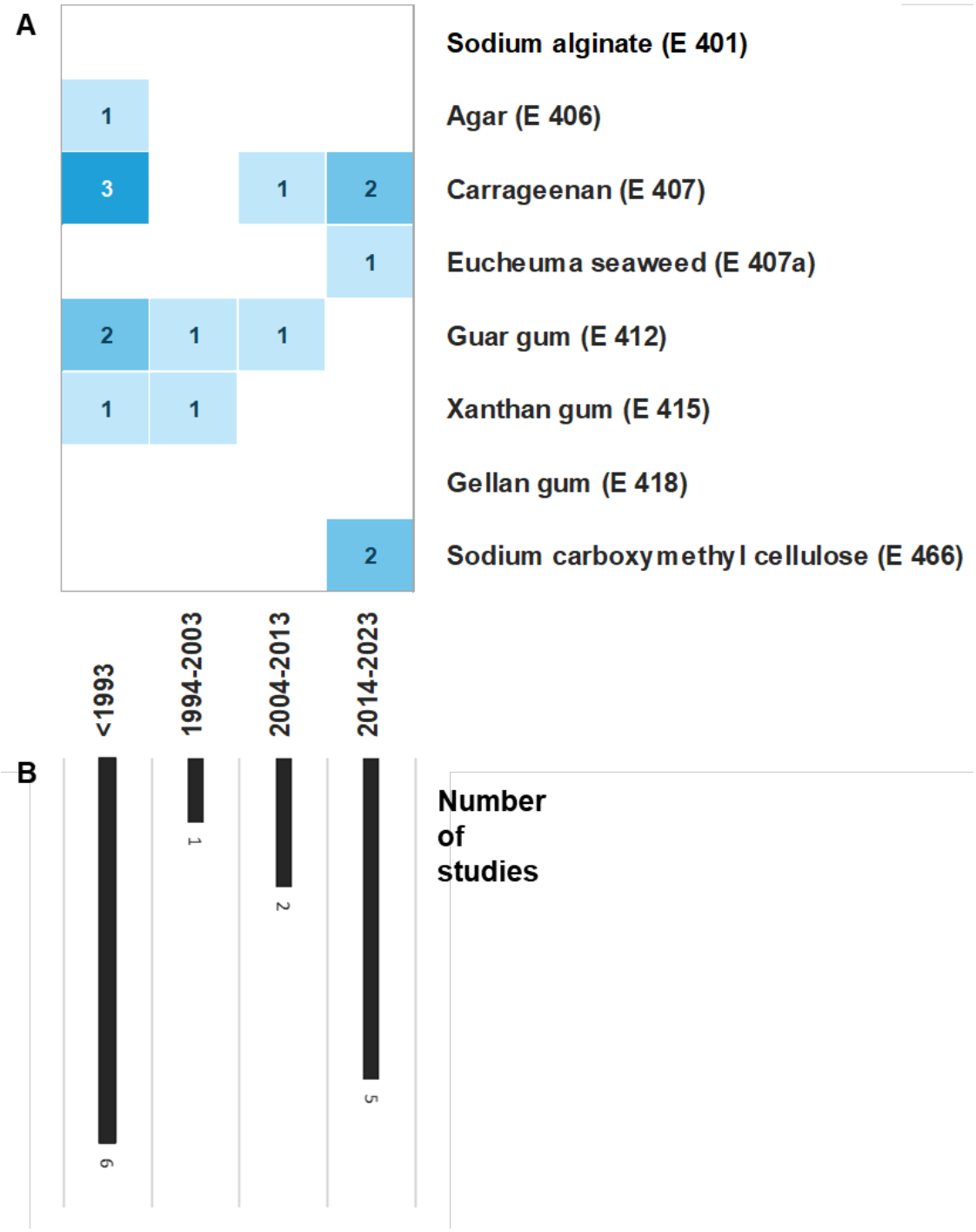

Figure 3.2.4-1. Heat map showing the number of studies on each substance (A) and bar graph showing publication year (B) up to March 1, 2023. A: Heat map: Number of studies for each publication year category for each substance. White squares indicate no included studies.

Note that two of the studies investigated two substances, making a total of 16 data points. B Bar graph: Bar labels indicate number of studies per year.

Rat was the most frequently used experimental species in the 13 animal studies and was investigated in seven of these studies. Other species used included mouse, pig, and baboon (Figure 3.2.4-2).

A wide range of GI-related outcomes were studied (Figure 3.2.4-3). Microscopic changes were the most studied outcome, followed by weight or length of the small or large intestine or both. Carrageenan and guar gum were the two most studied EST with six and four studies, respectively. We identified no studies that investigated sodium alginate or gellan gum with respect to GI tract effects. For the remaining ESTs we identified 1-3 studies. The extracted outcomes and their coding to broader outcome categories are presented in supplementary materials S1.

None of the identified studies were assessed to have low risk of bias. Most of the studies were assessed to have high risk of bias.

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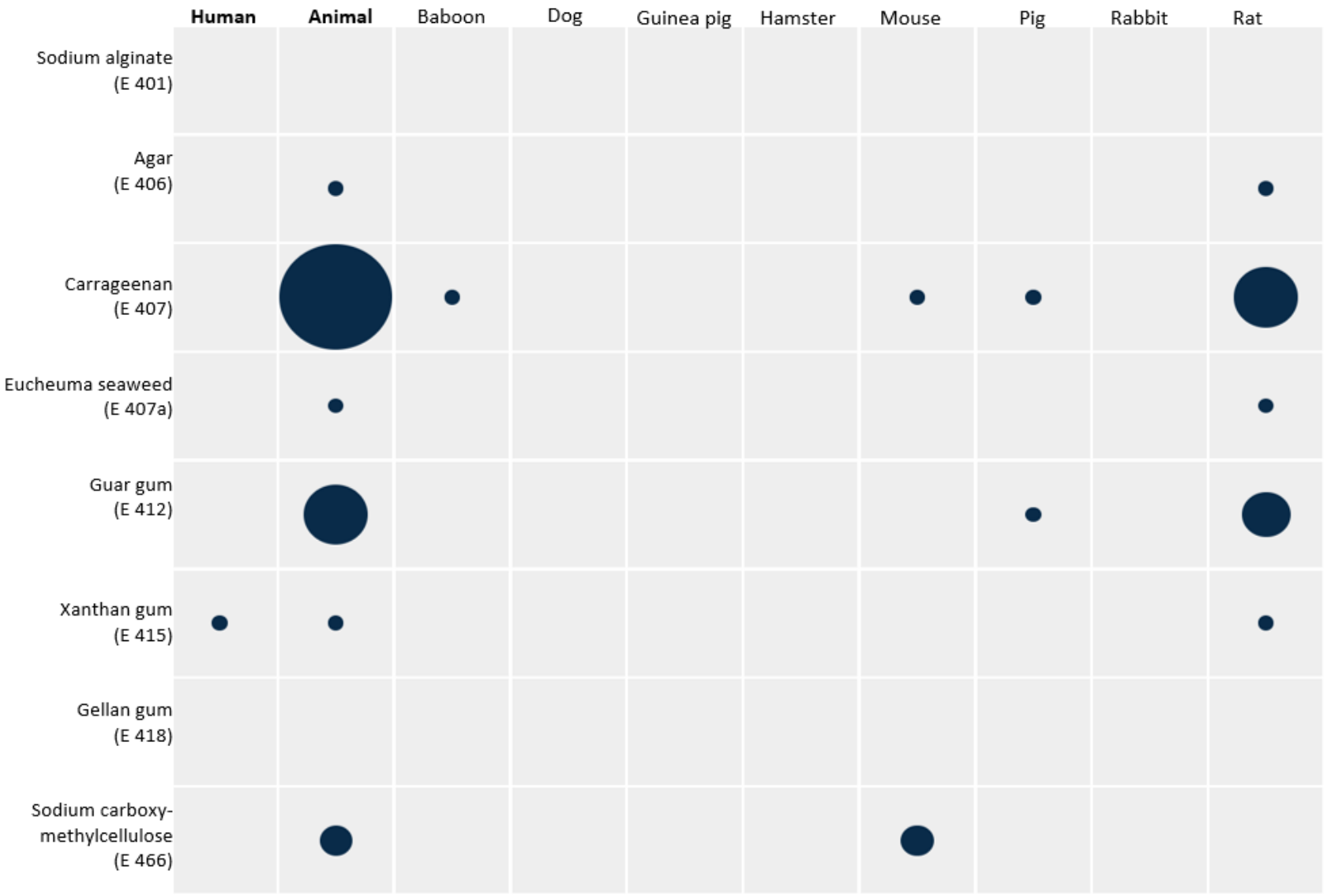

Figure 3.2.4-2. Evidence map of the emulsifiers, stabilisers, and thickeners investigated in the different populations. The sizes of the circles correspond to the number of studies in each category.

Interactive evidence map here:  FIREFO~1.HTM .

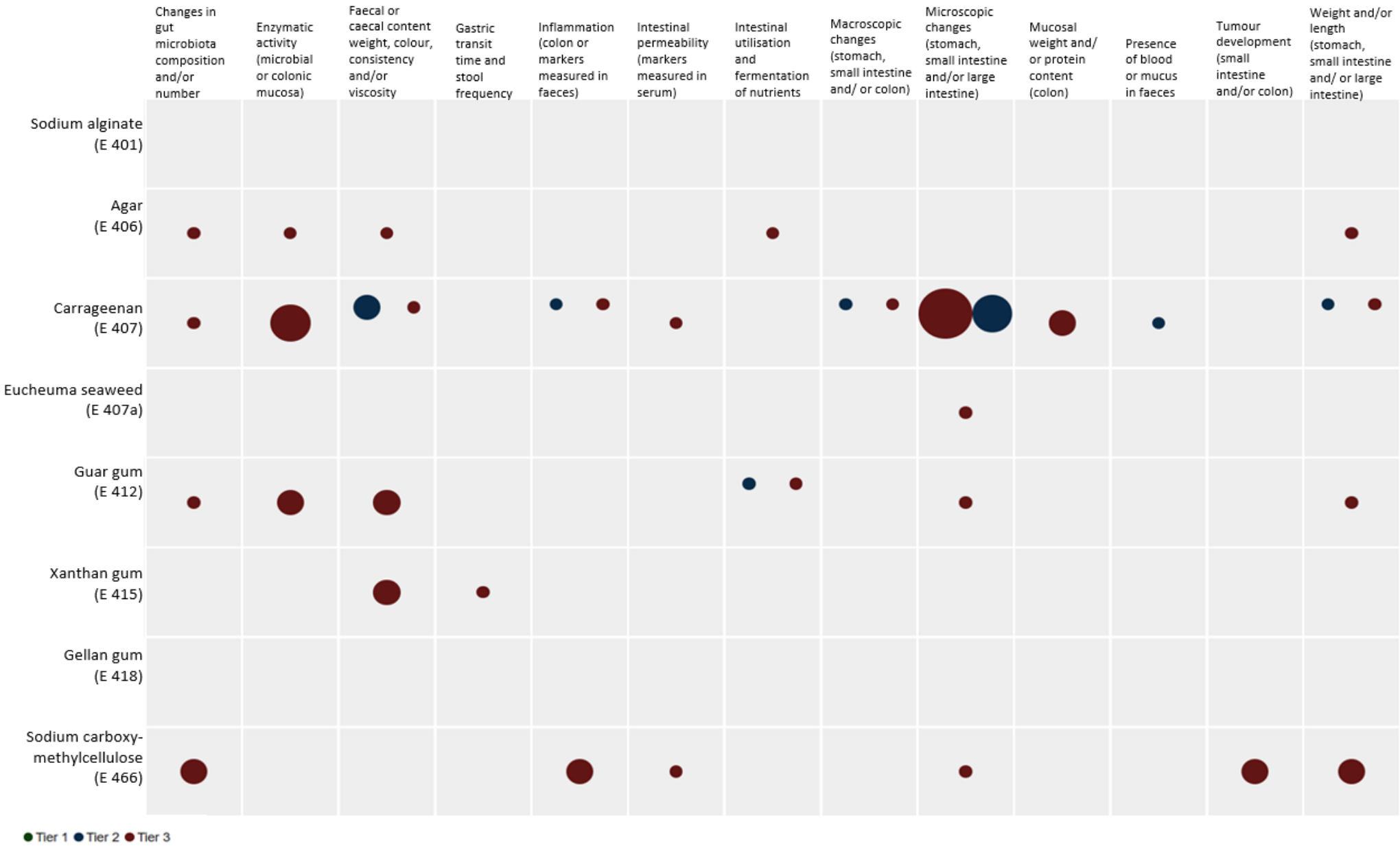
Changes in gut microbiota composition and/or number
Enzymatic activity (microbial or colonic mucosa)
Faecal or caecal content weight, colour, consistency and/or viscosity
Gastric transit time and stool frequency
Inflammation (colon or markers measured in faeces)
Intestinal permeability (markers measured in serum)
Intestinal utilisation and fermentation of nutrients
Macroscopic changes (stomach, small intestine and/ or colon)
Microscopic changes (stomach, small intestine and/or large intestine)
Mucosal weight and/ or protein content (colon)
Presence of blood or mucus in faeces
Tumour development (small intestine and/or colon)
Weight and/or length (stomach, small intestine and/ or large intestine)
Sodium alginate (E 401)
Agar (E 406)
Carrageenan (E 407)
Eucheuma seaweed (E 407a)
Guar gum (E 412)
Xanthan gum (E 415)
Gellan gum (E 418)
Sodium carboxy-methylcellulose (E 466)
Tier 1
Tier 2
Tier 3

Figure 3.2.4-3. Evidence map of the outcomes addressed and the overall risk of bias for each of the emulsifiers, stabilisers, and thickeners investigated. Tiers 1, 2 and 3 represent low, moderate, and high risk of bias, respectively. The sizes of the circles correspond to the number of studies in each category.

Interactive evidence map here: 11067E~1.HTM

4 Discussion and conclusions

This scoping review was commissioned by the Norwegian Food Safety Authority. The aim was to map the scientific literature investigating effects on the gastrointestinal (GI) tract after intake of emulsifiers, stabilisers, and thickeners (ESTs). The background for the assignment was that certain published studies indicated that the ESTs carrageenan and sodium carboxymethyl cellulose may have negative effects on the GI tract. Eight ESTs are included in this scoping review: carrageenan (E 407) and sodium carboxymethyl cellulose (E 466), and six ESTs that may be used as their substitutes, namely sodium alginate (E 401), agar (E 406), processed Eucheuma seaweed (E 407a), guar gum (E 412), xanthan gum (E 415), and gellan gum (E 418). More than 60 ESTs are approved used as food additives, thus, only a selection is included in this scoping review.

In the course of our work, more detailed criteria for systematic scoping reviews and mapping reviews were published (Campbell et al., 2023; Khalil and Tricco, 2022). Following these criteria, our scoping review would now rather be termed a mapping review. However, the checklist we followed (PRISMA-ScR; (Tricco et al., 2018)) was suggested for both types of review (Campbell et al., 2023), and thus we are confident that we have adhered to the recommended conduct of the review.

An additional 214 studies fulfilled all eligibility criteria except the criterion that the substance tested must be in accordance with the regulations for food additives in Europe and Norway. In a group of chemicals with different chemical and biological properties, but with the same name, only the chemical(s) approved for use as food additive was included in this scoping review. This applies to e.g. carrageenan, for which the size of the molecule is among the properties contributing to adverse effects caused by the substance. Low molecular weight (weight-average of 20–40 kDa) carrageenan, also called degraded carrageenan, may cause e.g. cancer in animals (EFSA et al., 2018c). The degraded carrageenan is not approved as a food additive in Europe according to EU regulations. The regulations specify a limitation of no more than 5% of the carrageenan having a a molecular weight below 50 kDa. In contrast, no such molecular weight limitation is set for carrageenan in specifications defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This lack of specification may explain why the molecular weight of carrageenan is omitted in toxicological studies performed outside Europe. Nevertheless, in Europe, the knowledge of molecular weight of carrageenan tested in studies is crucial for evaluating its effects (EFSA et al., 2018c).

Rodents such as the mouse and rat are among the most commonly used animal species used to study negative health effect in humans. Although the GI tract in rodents and humans are similar, rodents have a forestomach that is absent in humans. Negative health effects such as inflammation is known to be affected by the microbiome. The microbiome in rodents and humans share only 4% of the genes, indicating that the microbiome is different in rodents and humans and that rodents may not be the most appropriate model for studies of inflammation and microbiome changes in humans (Hugenholtz and de Vos, 2018; Ward et al., 2020). This difference does not disregard using rodents to study other negative health effects in humans, for

which rodents and humans share the same traits. However, whether negative health effects observed in rodents can be expected to apply to humans must be evaluated on a case-by-case basis.

Gut permeability and changes in the gut microflora are two outcomes that have been reported to be related to carrageenan and sodium carboxymethyl cellulose (Zinöcker and Lindseth, 2019). Due to the concern related to carrageenan and negative GI tract effects, the use of carrageenan in some food products in Norway has been reduced. It should be noted that the number of studies addressing GI tract effects of the ESTs that may be used as substitution for carrageenan was limited.

Limitations

Although we have conducted systematic searches in several electronic databases, searched the reference lists both of included studies and the website of EFSA for Opinions on the included ESTs, we have not searched other so called "grey literature" information sources. Furthermore, our results are up to date as of March 2023 and new studies may have been published in the period following the search and publication date for this review.

Conclusions

GI tract effects of ESTs were addressed in 14 eligible studies. GI tract effects were not investigated for two of the ESTs included in the scoping review. GI tract effects were investigated in studies of agar (E 406), sodium alginate (E 401), carrageenan (E 407), processed Eucheuma seaweed (E 407a), sodium carboxymethyl cellulose (E 466), gellan gum (E 418), guar gum (E 412), and xanthan gum (E 415). None of the studies addressed chronic exposures. Animal models were used in 13 of the included studies, and the risk of bias was high in ten studies. Thus, the available research literature on GI tract effects, according to our inclusion criteria, is limited in quantity and has limited relevance for long-term exposure in humans and is encumbered with high risk of bias. These weaknesses limit the use of the results of the scoping review in a future risk assessment.

5 Data gaps

There is currently not enough data available to evaluate whether ESTs may induce negative effects on the GI tract. Well-designed studies are needed that include sufficient description of the substance tested to know that it fulfils the criteria for being used as a food additive in addition to having a sufficient study size.

Studies addressing chronic effects are needed, because it is likely that most of the population are exposed to ESTs during the entire lifetime.

6 References

Bhattacharyya S., Shumard T., Xie H., Dodda A., Varady K.A., Feferman L., Halline A.G., Goldstein J.L., Hanauer S.B., Tobacman J.K. (2017) A randomized trial of the effects of the no-carrageenan diet on ulcerative colitis disease activity. Nutr Healthy Aging 4:181-192. DOI: 10.3233/nha-170023.

Bischoff S.C. (2011) 'Gut health': a new objective in medicine? BMC Medicine 9:24. DOI: 10.1186/1741-7015-9-24.

Calvert R.J., Reicks M. (1988) Alterations in colonic thymidine kinase enzyme activity induced by consumption of various dietary fibers. Proceedings of the Society for Experimental Biology & Medicine 189:45-51.

Calvert R.J., Satchithanandam S. (1992) Effects of graded levels of high-molecular-weight carrageenan on colonic mucosal thymidine kinase activity. Nutrition 8:252-7.

Cameron-Smith D., Collier G.R., O'Dea K. (1994) Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. British Journal of Nutrition 71:563-71.

Campbell F., Tricco A.C., Munn Z., Pollock D., Saran A., Sutton A., White H., Khalil H. (2023) Mapping reviews, scoping reviews, and evidence and gap maps (EGMs): the same but different— the "Big Picture" review family. Systematic Reviews 12:45. DOI: 10.1186/s13643-023-02178-5.

Chassaing B., Compher C., Bonhomme B., Liu Q., Tian Y., Walters W., Nessel L., Delaroque C., Hao F., Gershuni V., Chau L., Ni J., Bewtra M., Albenberg L., Bretin A., McKeever L., Ley R.E., Patterson A.D., Wu G.D., Gewirtz A.T., Lewis J.D. (2022) Randomized Controlled-Feeding Study of Dietary Emulsifier Carboxymethylcellulose Reveals Detrimental Impacts on the Gut Microbiota and Metabolome. Gastroenterology 162:743-756. DOI: 10.1053/j.gastro.2021.11.006.

Cochrane Glossary. (2020) https://community.cochrane.org/glossary#letter-S.

Colquhoun H.L., Levac D., O'Brien K.K., Straus S., Tricco A.C., Perrier L., Kastner M., Moher D. (2014) Scoping reviews: time for clarity in definition, methods, and reporting. J Clin Epidemiol 67:1291-4. DOI: 10.1016/j.jclinepi.2014.03.013.

EFSA. (2012) Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 10:2579. DOI: https://doi.org/10.2903/j.efsa.2012.2579.

EFSA, Gundert - Remy U, Bodin J, Bosetti C, FitzGerald R E, Hanberg A, Hass U, Hooijmans C, Rooney A A, Rousselle C, van Loveren H, Wölfle D, Barizzone F, Croera C, Putzu C, A C. (2017a) Bisphenol A (BPA) hazard assessment protocol. EFSA supporting publication 2017:EN-1354. DOI: 10.2903/sp.efsa.2017.EN-1354.

EFSA, Mortensen A., Aguilar F., Crebelli R., Di Domenico A., Frutos M.J., Galtier P., Gott D., Gundert-Remy U., Lambré C., Leblanc J.-C., Lindtner O., Moldeus P., Mosesso P., Oskarsson A., Parent-Massin D., Stankovic I., Waalkens-Berendsen I., Woutersen R.A., Wright M., Younes M., Brimer L., Peters P., Wiesner J., Christodoulidou A., Lodi F., Tard A., Dusemund B. (2016) Re-evaluation of agar

(E 406) as a food additive. EFSA Journal 14:e04645. DOI: https://doi.org/10.2903/j.efsa.2016.4645.

EFSA, Mortensen A., Aguilar F., Crebelli R., Di Domenico A., Frutos M.J., Galtier P., Gott D., Gundert-Remy U., Lambré C., Leblanc J.-C., Lindtner O., Moldeus P., Mosesso P., Oskarsson A., Parent-Massin D., Stankovic I., Waalkens-Berendsen I., Woutersen R.A., Wright M., Younes M., Brimer L., Peters P., Wiesner J., Christodoulidou A., Lodi F., Tard A., Dusemund B. (2017b) Re-evaluation of guar gum (E 412) as a food additive. EFSA Journal 15:e04669. DOI: https://doi.org/10.2903/j.efsa.2017.4669.

EFSA, Silano V., Bolognesi C., Castle L., Chipman K., Cravedi J.-P., Engel K.-H., Fowler P., Franz R., Grob K., Gürtler R., Husøy T., Kärenlampi S., Milana M.R., Pfaff K., Riviere G., Srinivasan J., Tavares Poças M.d.F., Tlustos C., Wölfle D., Zorn H., Beckman Sundh U., Benigni R., Binderup M.-L., Brimer L., Marcon F., Marzin D., Mosesso P., Mulder G., Oskarsson A., Svendsen C., Van Benthem J., Anastassiadou M., Carfì M., Mennes W. (2018a) Scientific Opinion of Flavouring Group Evaluation 406 (FGE.406): (S)-1-(3-(((4-amino-2,2-dioxido-1H-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one. EFSA Journal 16:e05120. DOI: https://doi.org/10.2903/j.efsa.2018.5120.

EFSA, Younes M., Aggett P., Aguilar F., Crebelli R., Di Domenico A., Dusemund B., Filipič M., Jose Frutos M., Galtier P., Gott D., Gundert-Remy U., Georg Kuhnle G., Lambré C., Leblanc J.-C., Lillegaard I.T., Moldeus P., Mortensen A., Oskarsson A., Stankovic I., Tobback P., Waalkens-Berendsen I., Wright M., Tard A., Tasiopoulou S., Woutersen R.A. (2018b) Re-evaluation of celluloses E 460(i), E 460(ii), E 461, E 462, E 463, E 464, E 465, E 466, E 468 and E 469 as food additives. EFSA Journal 16:e05047. DOI: https://doi.org/10.2903/j.efsa.2018.5047.

EFSA, Younes M., Aggett P., Aguilar F., Crebelli R., Filipič M., Frutos M.J., Galtier P., Gott D., Gundert-Remy U., Kuhnle G.G., Lambré C., Leblanc J.-C., Lillegaard I.T., Moldeus P., Mortensen A., Oskarsson A., Stankovic I., Waalkens-Berendsen I., Woutersen R.A., Wright M., Brimer L., Lindtner O., Mosesso P., Christodoulidou A., Horváth Z., Lodi F., Dusemund B. (2017c) Re-evaluation of alginic acid and its sodium, potassium, ammonium and calcium salts (E 400–E 404) as food additives. EFSA Journal 15:e05049. DOI: https://doi.org/10.2903/j.efsa.2017.5049.

EFSA, Younes M., Aggett P., Aguilar F., Crebelli R., Filipič M., Frutos M.J., Galtier P., Gott D., Gundert-Remy U., Kuhnle G.G., Lambré C., Leblanc J.-C., Lillegaard I.T., Moldeus P., Mortensen A., Oskarsson A., Stankovic I., Waalkens-Berendsen I., Woutersen R.A., Wright M., Brimer L., Lindtner O., Mosesso P., Christodoulidou A., Ioannidou S., Lodi F., Dusemund B. (2018c) Re-evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives. EFSA Journal 16:e05238. DOI: https://doi.org/10.2903/j.efsa.2018.5238.

EFSA, Younes M., Aquilina G., Castle L., Degen G., Engel K.-H., Fowler P.J., Frutos Fernandez M.J., Fürst P., Gürtler R., Husøy T., Manco M., Mennes W., Moldeus P., Passamonti S., Shah R., Waalkens-Berendsen I., Wright M., Dusemund B., Mortensen A., Turck D., Wölfle D., Barmaz S., Mech A., Rincon A.M., Tard A., Vianello G., Gundert-Remy U. (2022) Opinion on the re-evaluation of sodium carboxy methyl cellulose (E 466) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as food additive for uses in

foods for all population groups. EFSA Journal 20:e07665. DOI: https://doi.org/10.2903/j.efsa.2022.7665.

EFSA M., Aggett P., Aguilar F., Crebelli R., Filipic M., Frutos M.J., Galtier P., Gott D., Gundert-Remy U., Kuhnle G.G., Lambré C., Leblanc J.-C., Lillegaard I.T., Moldeus P., Mortensen A., Oskarsson A., Stankovic I., Waalkens-Berendsen I., Woutersen R.A., Wright M., Brimer L., Mosesso P., Christodoulidou A., Cascio C., Tard A., Lodi F., Dusemund B. (2018d) Re-evaluation of gellan gum (E 418) as food additive. EFSA Journal 16:e05296. DOI: https://doi.org/10.2903/j.efsa.2018.5296.

EFSA M., Alicja, Aguilar F., Crebelli R., Di Domenico A., Frutos M.J., Galtier P., Gott D., Gundert-Remy U., Lambré C., Leblanc J.-C., Lindtner O., Moldeus P., Mosesso P., Oskarsson A., Parent-Massin D., Stankovic I., Waalkens-Berendsen I., Woutersen R.A., Wright M., Younes M., Brimer L., Christodoulidou A., Lodi F., Gelgelova P., Dusemund B. (2017d) Re-evaluation of xanthan gum (E 415) as a food additive. EFSA Journal 15:e04909. DOI: https://doi.org/10.2903/j.efsa.2017.4909.

Gao Y., Wu A., Li Y., Chang Y., Xue C., Tang Q. (2022) The risk of carrageenan-induced colitis is exacerbated under high-sucrose/high-salt diet. International Journal of Biological Macromolecules 210:475-482. DOI: https://dx.doi.org/10.1016/j.ijbiomac.2022.04.158.

Hugenholtz F., de Vos W.M. (2018) Mouse models for human intestinal microbiota research: a critical evaluation. Cell Mol Life Sci 75:149-160. DOI: 10.1007/s00018-017-2693-8.

Khalil H., Tricco A.C. (2022) Differentiating between mapping reviews and scoping reviews in the evidence synthesis ecosystem. J Clin Epidemiol 149:175-182. DOI: 10.1016/j.jclinepi.2022.05.012.

Levac D., Colquhoun H., O'Brien K.K. (2010) Scoping studies: advancing the methodology. Implement Sci 5:69. DOI: 10.1186/1748-5908-5-69.

Mallett A.K., Wise A., Rowland I.R. (1984) Hydrocolloid food additives and rat caecal microbial enzyme activities. Food & Chemical Toxicology 22:415-8.

McGill H.C., Jr., McMahan C.A., Wigodsky H.S., Sprinz H. (1977) Carrageenan in formula and infant baboon development. Gastroenterology 73:512-7.

Moher D., Liberati A., Tetzlaff J., Altman D.G., The PRISMA Group. (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. Plos Medicine 6. DOI: ARTN e1000097 10.1371/journal.pmed.1000097.

NTP OHAT. (2015) OHAT Risk of Bias Rating Tool for Human and Animal Studies, Office of Health Assessment and Translation (OHAT), Division of the National Toxicology Program, National Institute of Environmental Health Sciences, https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf.

NTP OHAT. (2019) Handbook for Conducting a Literature Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration, Office of Health Assessment and Translation (OHAT), Division of the National Toxicology Program, National Institute of Environmental Health Sciences, https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookmarch2019_508.pdf.

Ouzzani M., Hammady H., Fedorowicz Z., Elmagarmid A. (2016) Rayyan-a web and mobile app for systematic reviews. Systematic Reviews 5:210. DOI: 10.1186/s13643-016-0384-4.

Pogozhykh D., Posokhov Y., Myasoedov V., Gubina-Vakulyck G., Chumachenko T., Knigavko O., Polikarpova H., Kalashnyk-Vakulenko Y., Sharashydze K., Nakonechna O., Prokopyuk V., Onishchenko A., Tkachenko A. (2021) Experimental Evaluation of Food-Grade Semi-Refined Carrageenan Toxicity. International Journal of Molecular Sciences 22:16. DOI: https://dx.doi.org/10.3390/ijms222011178.

Regulation (EC) No 1333/2008. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. European Union, https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32008R1333.

Regulation (EU) No 231/2012. 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. European Union, https://op.europa.eu/en/publication-detail/-/publication/a42dd9b2-b63f-438b-a790-1fa5995b7d41.

Rideout T.C., Liu Q., Wood P., Fan M.Z. (2008) Nutrient utilisation and intestinal fermentation are differentially affected by the consumption of resistant starch varieties and conventional fibres in pigs. British Journal of Nutrition 99:984-92.

Thomas J., Graziosi S., Brunton J., Ghouze Z., O'Driscoll P., Bond M., A. K. (2022) EPPI-Reviewer: advanced software for systematic reviews, maps and evidence synthesis, version 4.13.0.2, EPPI-Centre, UCL Social Research Institute, University College London., https://eppi.ioe.ac.uk/cms/Default.aspx?tabid=2914.

Tomlin J., Read N.W. (1988) The relation between bacterial degradation of viscous polysaccharides and stool output in human beings. British Journal of Nutrition 60:467-75.

Tricco A.C., Lillie E., Zarin W., O'Brien K.K., Colquhoun H., Levac D., Moher D., Peters M.D.J., Horsley T., Weeks L., Hempel S., Akl E.A., Chang C., McGowan J., Stewart L., Hartling L., Aldcroft A., Wilson M.G., Garritty C., Lewin S., Godfrey C.M., Macdonald M.T., Langlois E.V., Soares-Weiser K., Moriarty J., Clifford T., Tunçalp Ö., Straus S.E. (2018) PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med 169:467-473. DOI: 10.7326/m18-0850.

Viennois E., Chassaing B. (2021) Consumption of Select Dietary Emulsifiers Exacerbates the Development of Spontaneous Intestinal Adenoma. International Journal of Molecular Sciences 22:05.

Viennois E., Merlin D., Gewirtz A.T., Chassaing B. (2017) Dietary Emulsifier-Induced Low-Grade Inflammation Promotes Colon Carcinogenesis. Cancer Res 77:27-40. DOI: 10.1158/0008-5472.Can-16-1359.

VKM. (2018) Routine for approval of risk assessments (in Norwegian), Norwegian Scientific Committee for Food and Environment, https://vkm.no/download/18.433c8e05166edbef03bbda5f/1543579222271/Rutine%20for%20godkjenning%20av%20risikovurderinger.pdf.

VKM. (2019) Criteria for authorship and scientific responsibility in VKM's statements (in Norwegian), Norwegian Scientific Committee for Food and Environment, https://vkm.no/download/18.48566e5316b6a4910fc2dbd6/1561035075341/VKMs%20forfatterskapskriterier_revidert%20versjon%2020.06.2019.pdf.

VKM, Denison E., Andreassen M., Bruzell E., Carlsen M.H., Devold T.G., El Yamani N., Granum B., Mathisen G.H., Svendsen C., Rasinger J.D., Husøy T. (2023) Protocol for a scoping review of research on gastrointestinal effects of selected emulsifiers, stabilisers, and thickeners, Norwegian Scientific Committee for Food and Environment, https://vkm.no/download/18.5348de361864a581c39b3273/1677057768892/Protocol%20for%20a%20scoping%20review%20of%20research%20on%20gastrointestinal%20effects%20of%20selected%20emulsifiers,%20stabilisers,%20and%20thickeners_22.02.2023_final.pdf.

Ward R.E., Benninghoff A.D., Hintze K.J. (2020) Food matrix and the microbiome: considerations for preclinical chronic disease studies. Nutr Res 78:1-10. DOI: 10.1016/j.nutres.2020.02.012.

Weiner M.L., Ferguson H.E., Thorsrud B.A., Nelson K.G., Blakemore W.R., Zeigler B., Cameron M.J., Brant A., Cochrane L., Pellerin M., Mahadevan B. (2015) An infant formula toxicity and toxicokinetic feeding study on carrageenan in preweaning piglets with special attention to the immune system and gastrointestinal tract. Food and Chemical Toxicology 77:120-131. DOI: 10.1016/j.fct.2014.12.022.

Weiner M.L., Nuber D., Blakemore W.R., Harriman J.F., Cohen S.M. (2007) A 90-day dietary study on kappa carrageenan with emphasis on the gastrointestinal tract. Food and Chemical Toxicology 45:98-106. DOI: 10.1016/j.fct.2006.07.033.

Wilcox D.K., Higgins J., Bertram T.A. (1992) Colonic epithelial cell proliferation in a rat model of nongenotoxin-induced colonic neoplasia. Laboratory Investigation 67:405-11.

Zinöcker M.K., Lindseth I.A. (2019) Tilsetningsstoffer – er vi føre var? Tidsskriftet den norske legeforening 12.

7 Appendix I: Information on the included ESTs

A selection of the information on the included ESTs in Regulation (EU) No 231/2012 is given in Tables 7-1 to 7-8.

Table 7-1. Characteristics of carrageenan (E 407) (Regulation (EU) No 231/2012).

Definition	Chemical formula: Not included Molecular weight: Not included
Purity	Solvent residues: Not more than 0.1% of methanol, ethanol, propan-2-ol, singly or in combination Viscosity: Not less than 5 mPa•s (1,5% solution at 75 °C) Loss on drying: Not more than 12% (105 °C, 4 hours) Sulphates: Not less than 15% and not more than 40% on the dried basis (as SO4) Ash: Not less than 15% and not more than 40% determined on the dried basis at 550 °C Acid-insoluble ash: Not more than 1% on the dried basis (insoluble in 10% hydrochloric acid) Acid-insoluble matter: Not more than 2% on the dried basis (insoluble in 1% v/v sulphuric acid) Low molecular weight carrageenan (Molecular weight fraction below 50 kDa): Not more than 5% Arsenic: Not more than 3 mg/kg Lead: Not more than 5 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 2 mg/kg

Table 7-2. Characteristics of sodium carboxymethyl cellulose (E 466) (Regulation (EU) No 231/2012).

Definition	Chemical name: Sodium salt of the carboxymethyl ether of cellulose Chemical formula: The polymers contain substituted anhydroglucose units with the following general formula: C6H7O2(OR1)(OR2)(OR3), where R1, R2, R3 each may be one of the following: - H - CH2COONa

	- CH2COOH Molecular weight: Higher than approximately 17 000 (degree of polymerisation approximately 100)
Purity	Degree of substitution: Not less than 0,2 and not more than 1,5 carboxymethyl groups (-CH2COOH) per anhydroglucose unit Loss on drying: Not more than 12% (105 °C to constant weight) Arsenic: Not more than 3 mg/kg Lead: Not more than 2 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 1 mg/kg Total glycolate: Not more than 0,4%, calculated as sodium glycolate on the anhydrous basis Sodium: Not more than 12,4% on the anhydrous basis

Table 7-3. Characteristics of processed Eucheuma seaweed (E 407a) (Regulation (EU) No 231/2012)

Definition	Chemical name: Not reported Chemical formula: Not reported Molecular weight: Not reported
Purity	Solvent residues: Not more than 0,1% of methanol, ethanol, propan-2-ol, singly or in combination Viscosity: Not less than 5 mPa•s (1,5% solution at 75 °C) Loss on drying: Not more than 12% (105 °C, 4 hours) Sulphate: Not less than 15% and not more than 40% on the dried basis (as SO4) Ash: Not less than 15% and not more than 40% determined on the dried basis at 550 °C Acid-insoluble ash: Not more than 1% on the dried basis (insoluble in 10% hydrochloric acid) Acid-insoluble matter: Not less than 8% and not more than 15% on the dried basis (insoluble in 1% v/v sulphuric acid) Low molecular weight carrageenan (Molecular weight fraction below 50 kDa): Not more than 5% Arsenic: Not more than 3 mg/kg

	Lead: Not more than 5 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 2 mg/kg

Table 7-4. Characteristics of agar (E 406) (Regulation (EU) No 231/2012).

Definition	Chemical name: Not reported Chemical formula: Not reported Molecular weight: Not reported
Purity	Loss on drying: Not more than 22% (105 °C, 5 hours) Ash: Not more than 6,5% on the anhydrous basis determined at 550 °C Acid-insoluble ash (insoluble in approximately 3N Hydrochloric acid): Not more than 0,5% determined at 550 °C on the anhydrous basis Insoluble matter (after stirring for 10 minutes in hot water): Not more than 1,0% Starch: 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 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 minutes 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 hours. 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 Arsenic: Not more than 3 mg/kg Lead: Not more than 5 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 1 mg/kg

Table 7-5. Characteristics of sodium alginate (E 401)(Regulation (EU) No 231/2012).

Definition	Chemical name: Sodium salt of alginic acid Chemical formula: (C6H7NaO6)n Molecular weight: 10 000-600 000 (typical average)

Purity	Loss on drying: Not more than 15% (105 °C, 4 hours) Water insoluble matter: Not more than 2% on the anhydrous basis Formaldehyde: Not more than 50 mg/kg Arsenic: Not more than 3 mg/kg Lead: Not more than 5 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 1 mg/kg

Table 7-6. Characteristics of gellan gum (E 418) (Regulation (EU) No 231/2012).

Definition	Chemical name: Not reported Chemical formula: Not reported Molecular weight: Approximately 500 000
Purity	Loss on drying: Not more than 15% after drying (105 °C, 2,5 hours) Nitrogen: Not more than 3% Propan-2-ol: Not more than 750 mg/kg Arsenic: Not more than 3 mg/kg Lead: Not more than 2 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 1 mg/kg

Table 7-7. Characteristics of guar gum (E 412) (Regulation (EU) No 231/2012).

Definition	Chemical name: Not reported Chemical formula: Not reported Molecular weight: 50 000-8 000 000
Purity	Loss on drying: Not more than 15% (105 °C, 5 hours) Ash: Not more than 5,5% determined at 800 °C Acid-insoluble matter: Not more than 7% Protein: Not more than 10% (factor N x 6,25) Starch: 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) Organic peroxides: Not more than 0,7 meq active oxygen/kg sample

	Furfural: Not more than 1 mg/kg Pentachlorophenol: Not more than 0,01 mg/kg Arsenic: Not more than 3 mg/kg Lead: Not more than 2 mg/kg Mercury: Not more than 1 mg/kg Cadmium: Not more than 1 mg/kg

Table 7-8. Characteristics of xanthan gum (E 415) (Regulation (EU) No 231/2012).

Definition	Chemical name: Not reported Chemical formula: Not reported Molecular weight: Approximately 1 000 000
Purity	Loss on drying: Not more than 15% (105 °C, 2,5 hours) Total ash: Not more than 16% on the anhydrous basis determined at 650 °C after drying at 105 °C for four hours Pyruvic acid: Not less than 1,5% Nitrogen: Not more than 1,5% Ethanol and propan-2-ol: Not more than 500 mg/kg singly or in combination Lead: Not more than 2 mg/kg

8 Appendix II: Literature search

Contact person	Gro Haarklou Mathisen
Drafting the search strategy and performing the search	Bente Foss
Critical review of the search strategy	Trude Anine Muggerud
Duplications (EndNote)	Before removal of duplicates: 5162 studies and 13 systematic reviews Result after removal of duplicates: 4127 studies and 12 systematic reviews

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to February 28, 2023>
Date: 01.03.2023
Result: 3171 studies and 3 systematic reviews

1	Agar/ or Carrageenan/ or Carboxymethylcellulose Sodium/ or Alginates/	36211
2	(agar or "9002-18-0" or "E406" or carrageenan* or "9000-07-1" or "E407" or "carboxymethyl cellulose" or carboxymethylcellulose or K679OBS311 or "9004-32-4" or "E466" or "E407a" or processed Eucheuma seaweed? or sodium alginate? or "28961−37−7" or "E412" or gellan gum? or "E418" or guar gum? or xanthan gum? or "E415").tw,kf.	97051
3	1 or 2	113648
4	exp Gastrointestinal Tract/	688861
5	((GI or gastrointestinal or "gastro intestinal" or digestive or alimentary or aliment or gastrointestine or intestine) adj (tract? or tractus or canal?)).tw,kf.	105820
6	4 or 5	761906
7	3 and 6	3964
8	limit 7 to "therapy (maximizes sensitivity)"	1464
9	("randomi controlled trial" or "controlled clinical trial").pt. or (edised or randomly or rct or placebo or trial or groups).tw,kf,bt.	3690327
10	8 or (7 and 9)	1695
11	exp Rodentia/ or Mice/ or Animals/ or Rats/ or Rabbits/ or Dogs/ or Haplorhini/ or Swine/ or Guinea Pigs/	7265948
12	("ex vivo" or "exvivo" or cell? or "in vivo" or invivo or mouse or mice or animal? or rat? or rabbit? or dog? or pig? or monkey? or rodent* or leporidae? or haplorhini).tw,kf.	10293495
13	11 or 12	12637623
14	7 and 13	2790
15	10 or 14	3173
16	limit 15 to "reviews (maximizes specificity)"	3

17	Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	491064
18	16 or (15 and 17)	3
19	15 not 18	3170

Database: Embase <1974 to 2023 February 28>
Date: 01.03.2023
Result: 1258 studies and 8 systematic reviews

1	agar/ or carrageenan/ or carboxymethylcellulose/ or alginic acid/ or gellan/ or guar/ or guar gum/ or xanthan/	77805
2	(agar or "9002-18-0" or "E406" or carrageen* or "9000-07-1" or "E407" or "carboxymethyl cellulose" or carboxymethylcellulose or K679OBS311 or "9004-32-4" or "E466" or "E407a" or processed Eucheuma seaweed? or sodium alginate? or "28961−37−7" or "E412" or gellan gum? or "E418" or guar gum? or xanthan gum? or "E415").tw,kf.	118085
3	1 or 2	151251
4	exp gastrointestinal tract/	77715
5	((GI or gastrointestinal or "gastro intestinal" or digestive or alimentary or aliment or gastrointestine or intestine) adj (tract? or tractus or canal?)).tw,kf.	144093
6	4 or 5	185716
7	3 and 6	1880
8	limit 7 to "therapy (maximizes sensitivity)"	195
9	("randomised controlled trial" or "controlled clinical trial").pt. or (randomised or randomly or rct or placebo or trial or groups).tw,kf,bt.	5054652
10	8 or (7 and 9)	332
11	exp rodent/ or mouse/ or animal/ or rat/ or leporidae/ or dog/ or haplorhini/ or pig/ or guinea pig/	5331199
12	("ex vivo" or "exvivo" or cell? or "in vivo" or invivo or mouse or mice or animal? or rat? or rabbit? or dog? or pig? or monkey? or rodent* or leporidae? or haplorhini).tw,kf.	12814639
13	11 or 12	13837641
14	7 and 13	1136
15	10 or 14	1267
16	limit 15 to "reviews (maximizes specificity)"	3
17	exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	738846
18	16 or (15 and 17)	8
19	15 not 18	1259

Database: Web of Science

Date: 02.03.2023
Result: 721 studies and 2 systematic reviews

#	Search Query	Results
1	TS=("Agar" or "9002-18-0" or "E406" or "carrageen*" or "carragen*" or "carrhagen*" or "carragheen*" or "carrogeen*" or "carboxymethyl cellulose" or "carboxymethylcellulose" or "K679OBS311" or "9004-32-4" or "E466" or "E407a" or "processed Eucheuma seaweed$" or "sodium alginate$" or "28961−37−7" or "E412" or "gellan gum$" or "E418" or "guar gum$" or "xanthan gum$" or "E415")	120308
2	TS=(("GI" or "gastrointestinal" or "gastro intestinal" or "digestive" or "alimentary" or "aliment" or "gastrointestine" or "intestine") NEAR/0 ("tract$" or "tractus" or "canal$"))	103088
3	#1 AND #2	1057
4	TS=("randomised" or "randomised" or "randomly" or "rct" or "placebo" or "trial" or "groups")	4566087
5	#3 AND #4	143
6	TS=("ex vivo" or "exvivo" or cell$ or "in vivo" or "invivo" or "mouse" or "mice" or "animal$" or "rat$" or "rabbit$" or "dog$" or "pig$" or "monkey$" or "rodent*" or "Leporidae$" or "haplorhini")	12883337
7	#3 AND #6	671
8	#5 OR #7	723
9	TS=(("systematic*" NEAR/1 "review*") or ("review" and (("structured" or "database*" or "systematic*") NEAR/1 "search*")) or "integrative review*" or ("evidence" NEAR/1 "review*")) OR TI=("metaanal*" or "meta anal*") OR AB=("metaanal*" or "meta anal*")	539560
10	#9 AND #8	2
11	#8 NOT #10	721

9 Appendix III: Studies with insufficient information on the substance tested

In total, 214 publications containing one or more relevant studies were excluded because of insufficient information on the substance tested, and it was therefore not possible to evaluate if the criteria for being used as food additive were fulfilled. These 214 studies fulfilled all other eligibility criteria. The number of studies on the different ESTs ranged from three (gellan gum) to 113 (guar gum) (Table 9-1). Twenty studies included human participants, 186 were animal studies, and 11 were *ex vivo* model studies. The references for these studies, and the reason for exclusion, are included in the Supplementary materials 1.

Table 9-1. Studies excluded after full-text assessment.

	Human studies	Animal studies	*Ex vivo* studies	**Sum**
Agar	1	7		8
Sodium carboxymethyl cellulose		27	2	29
Carrageenan	1	32	1	34
Gellan gum		3		3
Guar gum	14	93	6	113
Sodium alginate	3	15	2	20
Xanthan gum	1	9		10
Sum	20	186	11	

10 Appendix IV: Deviations from the protocol

VKM decided to use the software Rayyan for the screening on title and abstract instead of EPPI-Reviewer since most reviewers were familiar with this tool and thus, this would be timesaving.

VKM decided to present selected characteristics of the studies that were excluded because of insufficient information on the substance tested. This is available in Supplementary materials 1.

Addition to the OHAT RoB criterion for the key question: "Can we be confident in the exposure characterisation?"

- **Definitely low risk of bias (++)**: …AND there is direct evidence that the substance(s) in question is (are) stable or homogenenously distributed or dissolved in the diet AND that the amount of intake of the substance is reported.
- **Probably low risk of bias (+)**: ...AND there is indirect evidence that the substance(s) in question is (are) stable or homogenenously distributed or dissolved in the diet AND that the amount of intake of the substance is reported OR it is deemed that stability or homogeneity will not appreciably bias results.
- **Probably high risk of bias (-)**: ...OR there is indirect evidence that stability or homogeneity OR amount of intake is not reported.
- **Definitely high risk of bias (--)**: There is direct evidence that stability or homogeneity OR amount of intake is not reported.

11 Appendix V: Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE #
TITLE			
Title	1	Identify the report as a scoping review.	Title page
ABSTRACT			
Structured summary	2	Provide a structured summary that includes (as applicable): background, objectives, eligibility criteria, sources of evidence, charting methods, results, and conclusions that relate to the review questions and objectives.	8-10
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. Explain why the review questions/objectives lend themselves to a scoping review approach.	18
Objectives	4	Provide an explicit statement of the questions and objectives being addressed with reference to their key elements (e.g., population or participants, concepts, and context) or other relevant key elements used to conceptualize the review questions and/or objectives.	18
METHODS			
Protocol and registration	5	Indicate whether a review protocol exists; state if and where it can be accessed (e.g., a Web address); and if available, provide registration information, including the registration number.	24
Eligibility criteria	6	Specify characteristics of the sources of evidence used as eligibility criteria (e.g., years considered, language, and publication status), and provide a rationale.	24-26
Information sources*	7	Describe all information sources in the search (e.g., databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed.	24
Search	8	Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	66-68
Selection of sources of evidence†	9	State the process for selecting sources of evidence (i.e., screening and eligibility) included in the scoping review.	24

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE #
Data charting process‡	10	Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was done independently or in duplicate) and any processes for obtaining and confirming data from investigators.	26
Data items	11	List and define all variables for which data were sought and any assumptions and simplifications made.	27
Critical appraisal of individual sources of evidence§	12	If done, provide a rationale for conducting a critical appraisal of included sources of evidence; describe the methods used and how this information was used in any data synthesis (if appropriate).	27-28
Synthesis of results	13	Describe the methods of handling and summarizing the data that were charted.	29
RESULTS			
Selection of sources of evidence	14	Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram.	30-31
Characteristics of sources of evidence	15	For each source of evidence, present characteristics for which data were charted and provide the citations.	32-35
Critical appraisal within sources of evidence	16	If done, present data on critical appraisal of included sources of evidence (see item 12).	42-44
Results of individual sources of evidence	17	For each included source of evidence, present the relevant data that were charted that relate to the review questions and objectives.	36-42
Synthesis of results	18	Summarize and/or present the charting results as they relate to the review questions and objectives.	46-51
DISCUSSION			
Summary of evidence	19	Summarize the main results (including an overview of concepts, themes, and types of evidence available), link to the review questions and objectives, and consider the relevance to key groups.	52-53
Limitations	20	Discuss the limitations of the scoping review process.	53
Conclusions	21	Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps.	53

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE #
FUNDING			
Funding	22	Describe sources of funding for the included sources of evidence, as well as sources of funding for the scoping review. Describe the role of the funders of the scoping review.	Click here to enter text.

JBI = Joanna Briggs Institute; PRISMA-ScR = Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews.

* Where *sources of evidence* (see second footnote) are compiled from, such as bibliographic databases, social media platforms, and Web sites.

† A more inclusive/heterogeneous term used to account for the different types of evidence or data sources (e.g., quantitative and/or qualitative research, expert opinion, and policy documents) that may be eligible in a scoping review as opposed to only studies. This is not to be confused with *information sources* (see first footnote).

‡ The frameworks by Arksey and O'Malley (6) and Levac and colleagues (7) and the JBI guidance (4, 5) refer to the process of data extraction in a scoping review as data charting.

§ The process of systematically examining research evidence to assess its validity, results, and relevance before using it to inform a decision. This term is used for items 12 and 19 instead of "risk of bias" (which is more applicable to systematic reviews of interventions) to include and acknowledge the various sources of evidence that may be used in a scoping review (e.g., quantitative and/or qualitative research, expert opinion, and policy document).