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Opinion on the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as food additives in foods for infants below 16 weeks of age and follow-up of their re-evaluation as food additives for uses in foods for all population groups

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright, Birgit Dusemund, Alicja Mortensen, Dominique Turck, Stefania Barmaz, Alexandra Tard, Giorgia Vianello and Ursula Gundert-Remy

Abstract

Pectin (E 440i) and amidated pectin (E 440ii) were re-evaluated in 2017 by the former EFSA Panel on Food Additives and Nutrient sources added to Food (ANS). As a follow-up to this assessment, the Panel on Food Additives and Flavourings (FAF) was requested to assess the safety of pectins (E 440i,ii) for their uses as food additives in food for infants below 16 weeks of age. In addition, the FAF Panel was requested to address the issues already identified during the re-evaluation of the same food additive. The process involved the publication of a call for data to allow the interested business operators to provide the requested information to complete the risk assessment. Based on the information submitted in response to the call for data, the FAF Panel considered it feasible to amend the current specifications, in particular for the toxic elements arsenic, lead, cadmium, mercury and for sulfur dioxide and to introduce new specifications for aluminium and microbiological criteria. Studies on neonatal piglets, clinical studies and post-marketing data were made available during the call for data. Due to the low internal validity of the clinical studies, the Panel concluded that a reference point could not be derived from them, but the results of the adequate piglet study could serve to derive a reference point. When calculating the margin of safety for pectins exposure, this was below 1 for some scenarios. At the maximum permitted levels (MPLs), an internal methanol dose would be produced that could lead to adverse health effects in infants below 16 weeks of age. The FAF Panel recommended a reduction of the MPL of pectin (E 440i) and amidated pectin (E 440ii) in food categories 13.1.5.1 and 13.1.5.2, in order to reduce the exposure to both the additives themselves and to methanol.

Keywords: Pectin, amidated pectin, E 440i, E 440ii, food additive, infants

Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

Panel members: Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle and Matthew Wright.

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Summary

In accordance with Regulation (EU) No 257/2010, the European Food Safety Authority (EFSA) is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. The risk assessment approach followed in the re-evaluation has not covered the use of food additives in food for infants below 12 weeks of age. Additionally, while re-evaluating the safety of food additives referred to above, EFSA identified some concerns, namely (1) data gaps that have triggered recommendations in the published scientific opinions; and/or (2) data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the Panel from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additive used in food for infants below this age. The age up to 16 weeks was selected in the guidance because infants are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before.

As follow-up of the above, this Opinion addresses the data gaps previously identified during the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) including the risk assessment of pectin (E 440i) and amidated pectin (E 440ii) for the use as food additive in food according to food category (FC) 13.1.5.1 (dietary foods for infants for special medical purposes and special formulae for infants) and FC 13.1.5.2 (dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) in infants above 16 weeks of age and young children up to 3 years and the safety in the special subpopulation of infants below 16 weeks of age. The process followed involved the publication of a dedicated call for data allowing all interested parties to provide the requested information for completing the assessment and to confirm that the additive is present in food categories 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants). The data submitted in response to the call for data on pectin (E 440i) and amidated pectin (E 440ii) comprised technical information and biological and toxicological data i.e. studies on neonatal animals, literature studies including clinical studies on gastrointestinal effects in adults and post-marketing surveillance reports.

According to Commission Regulation (EU) No 231/2012, pectin (E 440i) consists mainly of the partial methyl esters of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of strains of appropriate edible plant material, usually citrus fruits or apples. Amidated pectin (E 440ii) consists mainly of the partial methyl esters and amides of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of appropriate strains of edible plant material, usually citrus fruits or apples and treatment with ammonia under alkaline conditions. Specifications for pectin (E 440i) and amidated pectin (E 440ii) have been defined in Commission Regulation (EU) No 231/2012.

The Panel considered feasible to amend the EU specifications based on the information submitted in response to the call for data. This refers to lowering existing limits for toxic elements (arsenic, lead, cadmium, mercury) and sulfur dioxide and to include limits for aluminium and microbiological criteria (including *Cronobacter* (Enterobacter) *sakazakii*) for the food additive.

Dietary exposure to pectins (E 440) from their uses as food additives was assessed based on (1) MPLs set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario).

For infants below 16 weeks of age, both scenarios are based on the recommended consumption levels from the relevant EFSA Scientific Committee guidance which recommends values of 200 and 260 mL formula/kg body weight (bw) per day as conservative mean and high-level consumption values for 14- to 27-day-old infants. For infants below 16 weeks of age consuming food for special medical purpose (FC 13.1.5.1), mean exposure to pectins (E 440) in the regulatory maximum level exposure assessment scenario was estimated at 2,000 mg/kg bw per day while at the high level was estimated at 2,600 mg kg bw per day. Using the maximum level reported by industry, exposure estimates for pectins (E 440) were estimated at 834 mg/kg bw per day at the mean and at 1,084 mg/kg bw per day at the high level. For the scenario using the mean of the reported use levels from industry, exposure estimates for pectins (E 440) were of 693 mg/kg bw per day at the mean and 901 mg/kg bw per day at the high level of consumption. For infants above 16 weeks of age and toddlers consumers of foods for special medical purposes, mean dietary exposure to pectins (E 440) ranged from 9 mg/kg bw per

day in toddlers to 434 mg/kg bw per day in infants above 16 weeks of age. At the high level (95th percentile), dietary exposure to pectins (E 440) ranged from 30 mg/kg bw per day up to 1,263 mg/kg bw per day in infants above 16 weeks of age.

No new data were provided concerning ADME, acute toxicity, short-term and subchronic toxicity, genotoxicity, chronic toxicity and carcinogenicity and reproductive and developmental toxicity.

Two 21-day feeding studies in neonatal piglets available to the Panel were allocated to tier 1 in the risk of bias (RoB) assessment (low risk of bias). In these studies, the piglets received a milk replacer formula containing pectins in concentrations of 0, 0.5, 3 and 10 g/L or 0, 2 and 10 g/L, respectively. No adverse effects on body weight, feed intake clinical parameters (haematology, clinical chemistry and urinalysis) and post-mortem organ weights and gross and histopathology up to 3 g pectins/L in the first study and on body weight and feed intake at 2 g pectins/L in the second study were observed. These concentrations corresponded to 1,069 and 704 mg pectins/kg bw per day, respectively. The data were not suitable to perform benchmark dose modelling. Due to the dose spacing and the broader selection of toxicologically relevant endpoints, the Panel considered that the no observed adverse effect level (NOAEL) for pectin was 1,069 mg /kg bw per day.

Seven publications of clinical studies were submitted by the interested business operator. The composition of the tested formulas was not available neither from the publications nor provided from the interested business operators. Only the content of pectins was provided and in addition the information on the presence of other thickeners in the formulas. The Panel noted that none of the formulas did contain pectins as the only source for thickening but additional locust bean gum (five formulas) or xanthan gum (three formulas). The clinical studies were assessed with respect to their RoB and all were allocated to tier 3 (high risk of bias). The Panel notes the methodological limitations of the clinical studies, in particular the absence of randomisation and the fact that in none of the studies pectin and amidated pectins (E 440i, E 440ii) were the only thickeners used so that the results cannot be attributed to these food additives. Furthermore, most of the studies are studies without a control group precluding any conclusions to be drawn. Because of the limitations the Panel considers that the studies cannot support the assessment of the safety of pectin and amidated pectin (E 440i, E 440ii). Furthermore, publications with pectin-derived acidic oligosaccharides were reviewed by the Panel and assigned a tier 3 (high risk of bias). These studies did not provide any relevant information for RA of pectins as a food additive.

The Panel considered that the post-marketing surveillance data do not show specific alerts except for the very rare symptoms of 'allergic reaction/intolerance'.

The Panel decided to base its safety assessment on the MOS calculated for the different age groups and different scenarios. Due to the low internal validity of the clinical studies, the Panel concluded that a reference point could not be derived from them, but the results of the adequate piglet study could serve to derive a reference point. When calculating the MOS for pectins exposure, this was below 1 for some scenarios. The Panel concluded that an MOS below 1 is too low.

According to the literature, consumption of pectin (75% methylated) induced a significant increase in methanol in the breath and, by inference, in the blood. At the dose of 10 g, the lowest amount of methanol released is 400 mg per adult person (5.7 mg/kg bw). The extrapolation of these results to the assessment of exposure via foods for infants below 16 weeks of age under FC 13.1.5.1 would result in an exposure of 79.7 mg/kg bw per day (for pectin exposure of 2,000 mg/kg bw per day) and of 103.6 mg/kg bw per day (for pectin exposure of 2,600 mg/kg bw per day) towards methanol released from pectin in infants below 16 weeks of age. Although the flora of gastrointestinal tract is not fully comparable in the first days of life to that of the adult, after birth the microbiome of the newborn infants becomes similar to that of the mother. The interested business operators reported a degree of methylation between 50% and 90% for pectin (E 440i) and between 20% and 50% for amidated pectin (E 440ii). When performing the calculation with a degree of methylation of 90% (instead of 75%), the resulting values would be 15% higher (91.6 mg/kg bw per day and 119.4 mg/kg bw per day). This exposure could lead to adverse health effects. Blindness in human may occur, as reported, at doses as low as 214 mg/kg bw as a single acute dose and 260 mg/kg by the ECHA Committee for risk assessment in 2015. Metabolic acidosis is another health impairment which becomes relevant at doses between 100 and 150 mg/kg bw per day. A derived no effects level (DNEL) of 88 mg/kg bw (based on methanol ocular toxicity, i.e. blindness in humans) was set by the ECHA Committee for risk assessment (RAC) in 2015 for adults. When the maximum use level provided by industry are considered, the exposure to methanol via foods for infants below 16 weeks of age under FC 13.1.5.1 would result in an exposure of 43.2 mg/kg bw per day (for pectin exposure of 1,084 mg/kg bw per day, 95th percentile) towards methanol released from pectin. The Panel considered this as a

conservative approach also noting that the intake of pectins will not be as a single bolus dose but in divided doses over the day.

On the basis of the above, the Panel recommended the European Commission to consider revising the current EU specifications and to consider lowering the MPL of pectin (E 440i) and amidated pectin (E 440ii) in FCs 13.1.5.1 and 13.1.5.2, in order to reduce the exposure to both the additives themselves and to methanol.

Table of contents

1. Introduction

The present opinion deals with:

- the risk assessment of pectin (E 440i) and amidated pectin (E 440ii) in food for infants below 16 weeks of age in the food categories (FC) 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) according to Annex II to the Regulation (EC) No 1333/2008[1] on food additives.
- the follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as a food additive (EFSA ANS Panel, 2017) including the risk assessment of pectin (E 440i) and amidated pectin (E 440ii) for the use as food additive in food according to FC 13.1.5.1 (dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) and FC 13.1.5.2 (dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) in infants above 16 weeks of age and young children up to 3 years.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The composition of food intended for infants and young children, as defined by Regulation (EU) No 609/2013[2], is regulated at EU level and such rules include requirements concerning the use of substances as food additives.

The use of food additives is regulated by Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II and III to that Regulation, may be placed on the market and used in food under the conditions of use specified therein.

In accordance with Regulation (EU) No 257/2010[3], EFSA is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. However, the risk assessment approach followed until now has not covered the use of food additives in food for infants below 12 weeks of age. Consequently, EFSA published several scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 but not addressing their use in food for infants below 12 weeks of age.

In addition, in these opinions EFSA identified some concerns, namely (1) Data gaps that have triggered recommendations in the (to be) published scientific opinions, and/or; (2) Data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the EFSA from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance document (EFSA Scientific Committee, 2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additives used in food for infants below 12 weeks of age.[4] Now EFSA is expected to launch dedicated calls for data to be able to perform such risk assessments.

The EC considers it is more effective that EFSA, in the context of these dedicated calls for data, also addresses all the issues and data gaps already identified in the relevant (to be) published scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1.

In accordance with the current EC approach for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified, a specific call for data would be published by the EC on DG SANTE's website[5] on food additives and additional (missing) information would then be provided by interested business operators to the EC.

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[2] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.
[3] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[4] See Section 1.1.3.
[5] https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en

However, for those scientific opinions on the re-evaluation of the safety of permitted food additives in food category 13.1 for which the risk assessment does not address their uses in food for infants below 12 weeks of age and for which some concerns have been identified by EFSA, the EC considers that for the sake of efficiency it would be appropriate to streamline the approach as described above.

Therefore, the EC requests EFSA to address all the issues and data gaps already identified in the relevant published scientific opinions of those food additives (or groups of additives that can be addressed simultaneously) as part of the upcoming work on the safety assessment of food additives uses in food for infants below 12 weeks of age.

This follow-up aims at completing the re-evaluation of the food additives in question for all food categories, and includes calls for data covering the actual use and usage levels of food additives in food for both infants below 12 or 16 weeks of age as well as for older infants, young children and other groups of the population for which EFSA has already finalised its assessment.

The future evaluations of EFSA should systematically address the safety of use of food additives for all age groups, including the infants below 12 or 16 weeks of age.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[6], and as part of EFSA's work in completing its risk assessments concerning the use of food additives in food for infants below 12 weeks of age[5], covered by the re-evaluation programme and its terms of reference, the European Commission requests the European Food Safety Authority to address all the data gaps specified in the recommendations made in this scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 (food for infants and young children) of annex II to Regulation (EC) No 1333/2008.

1.1.3. Interpretation of Terms of reference

Before the publication of the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017), EFSA has taken 12 weeks as a cut off age for the applicability of the safety assessment. However, according to EFSA Scientific Committee (2017), the assessment will include infants up to 16 weeks of age because they are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before this age (see EFSA Scientific Committee, 2017).

This assessment refers exclusively to the uses of pectin (E 440i) and amidated pectin (E 440ii) as food additives in food, including food supplements, and does not include a safety assessment of other uses of pectins.

1.2. Previous evaluations of pectin (E 440i) and amidated pectin (E 440ii) for use in foods for infants

The Scientific Committee on Food (SCF) in 1978 (SCF, 1978) endorsed the acceptable daily intake (ADI) 'not specified' already established by JECFA (1974) for non-amidated pectins.

In JECFA (1981), assessed additional long-term feeding studies in rats, as well as multi-generation studies, showing no toxicological differences between pectins and amidated pectins. This evaluation was endorsed by the SCF (1985) who concluded that the database was sufficient, and established a group ADI 'not specified' for non-amidated and amidated pectin.

In view of limited information on their potential effects in infants, the SCF (2003) recommended that pectins should not be used in infant formulae and follow-on formulae. The SCF had no objections against the continued use of pectins up to a maximum level of 10 g/L in dietary foods for special medical purposes for infants to be used under medical supervision.

In 2014, JECFA evaluated the safety of non-amidated pectin for uses in infant formula and formula for special medical purposes intended for infants (JECFA, 2015) and stated that *'the Committee was made aware that a further pectin product is available on the market. This product, known as pectin-derived acidic oligosaccharides (pAOS), is produced by enzymatic hydrolysis of pectin. pAOS has not*

[6] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

been evaluated by the Committee and is not covered by the existing specifications for pectins.' On the basis of the observed decreased food intake and body weight gain in a neonatal pig study, JECFA also concluded that the use of non-amidated pectin in infant formulae at the maximum proposed use levels (0.5%) is of concern.

In an updated safety evaluation (JECFA, 2016a, 2017), JECFA calculated the margins of exposure (MOE) for the use of pectin in infant formula at a maximum proposed use level of 0.2% using as reference point the no-observed-effect level (NOAEL) of 1,049 mg/kg bw per day identified from a neonatal study in piglets. The obtained margins of exposure for average and high consumption were 2.9 and 2.4, respectively. Overall, on the basis of a number of considerations (low toxicity of pectin, NOAEL derived from a relevant study in neonatal piglets, relation of adverse effects in piglet study to viscosity at the concentration of 1%, support of clinical studies for tolerance of infants to pectin up to the concentration of 0.2% and conservative exposure estimates), the Committee concluded that the MOE indicate a low risk for the health of infants aged 0–12 weeks. JECFA further stated that '*there is variability in medical conditions among infants requiring formula for special medical purposes and that these infants would be normally under medical supervision'*.

In its evaluation, the Nordic Council of Ministers (TemaNord, 2002) stated that pectin has been a natural component of human diet throughout evolution and there is no indication of toxic effects induced by pectin or amidated pectin.

The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) addressed the scientific substantiation of health claims in relation to pectins and reduction of post-prandial glycaemic responses, maintenance of normal blood cholesterol concentrations and increase in satiety leading to a reduction in energy intake (EFSA NDA Panel, 2010). A cause and effect relationship between the consumption of pectins and a reduction of post-prandial glycaemic responses and maintenance of normal blood cholesterol concentrations was established.

1.3. Summary of the previous EFSA re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) for uses in food for all population groups except for infants below 12 weeks of age[7]

Under the frame of Regulation (EC) No 257/2010, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) has re-evaluated the safety of pectin (E 440i) and amidated pectin (E 440ii) when used as a food additive (EFSA ANS Panel, 2017).

In its scientific opinion, the ANS Panel reviewed available technical, biological and toxicological data on of pectin (E 440i) and amidated pectin (E 440ii).

The ANS Panel concluded that pectin and amidated pectin are not absorbed intact, but extensively fermented by intestinal microbiota in animals and humans. Although the available data were limited, the ANS Panel concluded that there was no indication of genotoxicity for pectin and amidated pectin. In a chronic toxicity study in rats at levels up to 5,000 mg pectin/kg bw per day, the highest dose tested, adverse effects were not reported. In a dietary one-generation reproductive toxicity study with pAOS in rats at up to 6,200 mg/kg body weight (bw) per day, the highest dose tested, no treatment-related effects were observed.

The ANS Panel did not consider E 440i and E 440ii as having allergenic potential.

In humans (adults), a dose of 36 g/day (equivalent to 515 mg/kg bw per day) for 6 weeks was without adverse effects.

The ANS Panel concluded that there is no safety concern for the use of pectin (E 440i) and amidated pectin (E 440ii) as food additives for the general population and that there is no need for a numerical ADI.

The ANS Panel, however, considered that the conclusions reached on the re-evaluation of the food additive were not applicable to the use of pectin (E 440i) and amidated pectin (E 440ii) in food for infants under the age of 12 weeks. They considered that these uses would require a specific risk assessment.

Concerning the use of pectins (E 440) in 'dietary foods for special medical purposes and special formulae for infants' (FC 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (FC 13.1.5.2), the ANS Panel concluded that the available data do not allow for an adequate assessment of the safety of use of pectins (E 440) in

[7] According to the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017), this opinion will include infants up to 16 weeks of age.

infants and young children consuming these foods for special medical purposes at the presently permitted maximum use levels of 1%.

In addition, the following recommendations relevant for the current assessment were made:

- the European Commission considers lowering the maximum limits for the impurities of toxic elements arsenic, lead, mercury and cadmium in the EU specifications for pectin (E 440i) and amidated pectin (E 440ii) in order to ensure that pectin (E 440i) and amidated pectin (E 440ii) as food additives will not be a significant source of exposure to those toxic elements in food; special requirements might be defined in the specifications for pectin (E 440i) and amidated pectin (E 440ii) to be used in formulae or food for infants, toddlers and other young children
- limits for aluminium should be considered for inclusion in the EU specifications, as aluminium can be used in the manufacturing process
- the European Commission considers harmonising the microbiological specifications for polysaccharidic thickening agents, such as pectins, and including criteria for the absence of *Salmonella* spp. and *Escherichia coli*, for total aerobic microbial count (TAMC) and for total combined yeasts and moulds count (TYMC) in the EU specifications for pectin (E 440i) and amidated pectin (E 440ii)
- additional clinical data should be generated to assess the safety of pectins (E 440) when used in 'dietary foods for special medical purposes and special formulae for infants' (FC 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (FC 13.1.5.2)

2. Data and methodologies

2.1. Data

EFSA launched a public call for data[8] to collect relevant information from interested business operators.

The Panel based its assessment on information submitted to EFSA following the public call for data, information from previous evaluations and additional available literature up to 1 December 2020.

To verify the use of the food additive pectin (E 440i) and amidated pectin (E 440ii) in food products, the Mintel's Global New Products Database (GNPD) was used. This database is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3.4 million food and beverage products of which more than 1,300,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries, and Norway and UK presented in the Mintel GNPD.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee and in particular the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017).

In order to conclude on the safety of pectin (E 440i) and amidated pectin (E 440ii) for all population groups and to address the data gaps identified during the re-evaluation, the FAF Panel assessed the information provided:

- for the follow-up on issues that have been raised in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as a food additive (EFSA ANS Panel, 2017) including the risk assessment of pectin (E 440i) and amidated pectin (E 440ii) for the use as food additive in food according to food category (FC) 13.1.5.1 (dietary foods for infants for special medical purposes and special formulae for infants) and FC 13.1.5.2 (dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) in infants above 16 weeks of age and young children up to 3 years; and

[8] Call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) for uses as food additives in foods for all population groups including infants below 16 weeks of age. Published: 18 July 2018. Available online: http://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-pectin-e440i-and-amidated

- for the risk assessment of pectin (E 440i) and amidated pectin (E 440ii) in food for infants below 16 weeks of age in the FC 13.1.5.1 (Dietary foods for special medical purposes and special formulae for infants).

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

The studies in neonatal piglets and the clinical trials were assessed for their risk of bias (RoB) by two reviewers (members of the FAF Panel Working Group) applying an assessment tool modified from the OHAT RoB tool (NTP-OHAT, 2015, 2019). The elements considered for the appraisal are described in the Appendix B to this opinion, as well as the decision rule for assigning the studies to Tiers of reliability.

Dietary exposure to pectin (E 440i) and amidated pectin (E 440ii) from their use as a food additive in foods for infants below 16 weeks of age was estimated combining the mean and high-level consumption values reported for the period of 14–27 days of life which corresponds to 200 and 260 mL/kg bw per day (EFSA Scientific Committee, 2017), respectively, with the maximum levels according to Annex II and Annex III, Part 5 Section B to Regulation (EC) No 1333/2008 and reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.4.1). Uncertainties on the exposure assessment were identified and discussed.

As pectin (E 440i) and amidated pectin (E 440ii) are also authorised in the food category 13.1.5.2, an exposure assessment considering FC 13.1.5.1 and FC 13.1.5.2 was performed to estimate the exposure of infants (above 16 weeks) and toddlers who may eat and drink these foods for special medical purposes (FSMP). The consumption of these foods is not reported in the EFSA Comprehensive database. To consider potential exposure to pectin (E 440i) and amidated pectin (E 440ii) via these foods, the Panel assumes that the amount of FSMP consumed by infants and toddlers resembles that of comparable foods in infants and toddlers from the general population. Thus, the consumption of FSMP categorised as FC 13.1.5 was assumed equal to that of formulae and food products categorised as FCs 13.1.1, 13.1.2, 13.1.3 and 13.1.4.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012[9], pectin (E 440i) consists mainly of the partial methyl esters of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of strains of appropriate edible plant material, usually citrus fruits or apples.

Amidated pectin (E 440ii) consists mainly of the partial methyl esters and amides of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of appropriate strains of edible plant material, usually citrus fruits or apples and treatment with ammonia under alkaline conditions.

Commission Regulation No 231/2012 differentiates between pectin (E 440i) and amidated pectin (E 440ii) whilst JECFA (JECFA, 2016b) included both types of pectins (pectin and amidated pectin) under the same food additive designation (INS 440).

[9] 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 Text with EEA relevance. OJ L 83, 22.3.2012, p. 1–295.

Commercial pectins are divided into low-methoxy pectins (LM-pectins), where less than 50% (typically 20–40%) of the carboxyl groups are methylated, whereas in high-methoxy pectins (HM-pectins), more than 50% (typically 55–75%) are methylated (cite re-eval). In Lindinger et al. (1997), it is stated that 'natural pectin consists of joined galacturonic acid units, some of which are esterified with methyl alcohol ranging from 30% in grapes typically to 75% in apples'.

The Panel noted that according to the interested business operators (documentation provided to EFSA n. 5), the typical degree of methylation used in infant food is [redacted] (50%–90%) for pectin (E 440i) and [redacted] (20%–50%) for amidated pectin (E 440ii).

For more information on the physical properties and the chemical composition and structure of pectin (E 440i) and amidated pectin (E 440ii) the reader is referred to the ANS Panel opinion (EFSA ANS Panel, 2017).

3.1.2. Specifications

The specifications for pectin (E 440i) and amidated pectin (E 440ii) as defined in the Commission Regulation (EU) No 231/2012 are listed in Table 1.

Table 1: Specifications for pectins (E 440i,ii) according to Commission Regulation (EU) No 231/2012

	Commission Regulation (EU) No 231/2012		JECFA (2016b)
	Pectin (E 440i)	**Amidated pectin (E 440ii)**	**Pectins (INS 440)**
Definition	Pectin consists mainly of the partial methyl esters of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of strains of appropriate edible plant material, usually citrus fruits or apples. No organic precipitant shall be used other than methanol, ethanol and propan-2-ol	Amidated pectin consists mainly of the partial methyl esters and amides of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of appropriate strains of edible plant material, usually citrus fruits or apples and treatment with ammonia under alkaline conditions. No organic precipitant shall be used other than methanol, ethanol and propan-2-ol	Consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts; obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples; no organic precipitants shall be used other than methanol, ethanol and isopropanol; in some types, a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. Sulfur dioxide may be added as a preservative
Assay	Content not less than 65% of galacturonic acid on the ash-free and anhydrous basis after washing with acid and alcohol	Content not less than 65% of galacturonic acid on the ash-free and anhydrous basis after washing with acid and alcohol	Not less than 65% of galacturonic acid calculated on the ash-free and dried basis
Description	White, light yellow, light grey or light brown powder	White, light yellow, light greyish or light brownish powder	White, yellowish, light greyish or light brownish powder
Identification			
Solubility	Soluble in water forming a colloidal, opalescent solution. Insoluble in ethanol	Soluble in water forming a colloidal, opalescent solution. Insoluble in ethanol	–
Test for pectins	–	–	Passes test[(a)]
Test for amide group	–	–	Passes test (amidated pectins only)[(a)]
Purity			
Loss on drying	Not more than 12% (105°C, 2 h)	Not more than 12% (105°C, 2 h)	Not more than 12% (105°C, 2 h)

	Commission Regulation (EU) No 231/2012		JECFA (2016b)
	Pectin (E 440i)	**Amidated pectin (E 440ii)**	**Pectins (INS 440)**
Acid insoluble ash	Not more than 1% (insoluble in approximately 3N hydrochloric acid)	Not more than 1% (insoluble in approximately 3N hydrochloric acid)	Not more than 1%
Degree of amidation	–	Not more than 25% of total carboxyl groups	Not more than 25% of total carboxyl groups of pectin
Sulfur dioxide	Not more than 50 mg/kg on the anhydrous basis	Not more than 50 mg/kg on the anhydrous basis	Not more than 50 mg/kg
Nitrogen content	Not more than 1.0% after washing with acid and ethanol	Not more than 2.5% after washing with acid and ethanol	Not more than 2.5% after washing with acid and ethanol
Total insoluble	Not more than 3%	Not more than 3%	Not more than 3%
Solvent residues	Not more than 1% of free methanol, ethanol and propan-2-ol, singly or in combination, on the volatile matter-free basis	Not more than 1% of free methanol, ethanol and propan-2-ol, singly or in combination, on the volatile matter-free basis	Not more than 1% methanol, ethanol and isopropanol, singly or in combination
Arsenic	Not more than 3 mg/kg	Not more than 3 mg/kg	–
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg	Not more than 2 mg/kg for general use and 0.5 mg/kg for use in infant formula
Mercury	Not more than 1 mg/kg	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	Not more than 1 mg/kg	–

(a): For description, see JECFA (2016b).

The Panel noted that no microbiological specifications are currently set for E440i and E440ii according to Commission Regulation (EU) No 231/2012.

The revisions of the existing EU specifications proposed by the Panel are provided under section 3.5.

3.1.2.1. Analytical data from commercial samples of the food additive

3.1.2.1.1. Toxic elements

The call for data, requested:

- analytical data on current levels of arsenic, lead, cadmium, mercury and aluminium in commercial samples of the food additives;
- the lowest technologically achievable level for lead, mercury, cadmium, arsenic and aluminium in order to adequately define their maximum limits in the specifications;

Analytical data were provided by one interested business operator for levels of arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg) and aluminium (Al) in commercial pectin (E 440i) and amidated pectin (E 440ii) samples regarding the follow-up of the conclusions and the recommendations of the EFSA ANS Panel opinion on the safety of pectin (E 440i) and amidated pectin (E 440ii) as food additives of the EFSA call of data and this includes all age groups(documentation provided to EFSA n. 1).

Data on 15 independent batches were provided for toxic elements in **pectin (E 440i)**. For arsenic, one result was reported as 0.04 mg/kg and the remaining 14 batches were all reported as < 0.04 mg/kg. For cadmium, one result was given as 0.01 mg/kg and 14 batches as < 0.01 mg/kg. All results for mercury were reported as < 0.01 mg/kg. The lead levels ranged between < 0.015 and 0.431 mg/kg (median: 0.036, mean: 0.097, P90: 0.26 mg/kg). All analyses were performed with inductively coupled plasma-mass spectrometry (ICP-MS) with limits of quantification (LOQs) of 0.04 (As), 0.015 (Pb) and 0.01 (Cd, Hg) mg/kg, respectively. The determination of aluminium was performed with two methods, ICP-MS (LOQ: 0.50 mg/kg) and ICP-Optical Emission Spectrometry (ICP-OES) (LOQ: 2.0 mg/kg). The results maintained by ICP-MS and ICP-OES ranged from 0.81 to 99.0 (median: 26.7, mean: 40.0, P90: 87.2) mg/kg, and < 2.0–91.7 mg (median: 28.1, mean: 37.5, P90: 79.3) mg/kg, respectively.

For **amidated pectin (E 440ii)**, analytical data for toxic elements in 14 independent batches were provided. All results for As and Hg were given as < 0.04 and < 0.01 mg/kg, respectively. For Cd, one batch contained 0.01 mg/kg, all other batches were < 0.01 mg/kg. The lead levels ranged between < 0.015 and 0.350 mg/kg (median: 0.042, mean: 0.095, P90: 0.26 mg/kg). All analyses were performed by ICP-MS with LOQs of 0.04 (As), 0.015 (Pb) and 0.01 (Cd, Hg) mg/kg, respectively. The determination of Al was performed with two methods, ICP-MS (LOQ: 0.50 mg/kg) and ICP-OES (LOQ: 2.0 mg/kg). The results maintained by ICP-MS and ICP-OES ranged from 0.59 to 201 (median: 16.9, mean: 37.9, P90: 117.2) mg/kg, and < 2.0–178 mg (median: 16.8, mean: 35.2, P90: 107.5) mg/kg, respectively.

On the question regarding the lowest technically achievable levels in pectin (E 440i) and amidated pectin (E 440ii), the interested business operator stated: '*Due to the ability of pectin molecules to bind strongly with di-valent and tri-valent metal kations which may stem from raw materials or added as processing aids (Calcium and Aluminium), IPPA considers the lowest technically achievable levels to be as follows',* see Table 2.

Table 2: Lowest technically achievable levels of the toxic elements As, Pb, Cd, Hg and Al in commercial pectin products, as proposed by one interested business operator (documentation provided to EFSA n.1)

Arsenic	Lead	Cadmium	Mercury	Aluminium
1 mg/kg	2 mg/kg	0.5 mg/kg	0.5 mg/kg	200 mg/kg

The Panel noted that the analytical data on the toxic metals provided for the 29 independent commercial E 440i and E 440ii samples are substantially lower than the lowest technically achievable levels proposed by the business operator.

3.1.2.1.2. Microbiological criteria

The call for data, requested:

- Because of both the botanical origin and the polysaccharidic nature of pectin, they can be a substrate of microbiological contamination. Data should be provided demonstrating the absence of *Salmonella* spp. and *Escherichia coli* and on the lowest total aerobic microbial count (TAMC) and total combined yeast and mould count (TYMC) that can be reached.

Microbiological data were provided for seven batches of pectin (E 440i) and six batches for amidated pectin (E 440ii) (documentation provided to EFSA n. 1). In all samples, the total viable count was < 10 CFU/g, the yeasts (incl. osmophilic yeasts) < 10 CFU/g, moulds (incl. xerophilic moulds) < 10 CFU/g, *E. coli* negative in 10 g and *Salmonella* spp. negative in 25 g. The analyses were performed with Deutsches Institut für Normung (DIN) and/or ISO methods.

Due to the limited risk of contamination linked to the processing and packaging of commercial pectin products, the business operator considers the lowest technically achievable microbial data to be as follows:

- *Salmonella* spp.: negative by test in 25 g; *E. coli*: negative by test in 10 g; Total Aerobic plate count (30°C): less than 500 CFU/g, and Total yeast and mould count (25°C): less than 100 CFU/g.

The business operator stated that the '*Pectin production involves aqueous extraction of the raw materials at elevated temperatures and acidic pH for a long time. Gram negative bacteria sensitive to pasteurization – like E. coli and Salmonella* spp – *are thus eliminated at this stage. Further processing involves precipitation and/or wash with alcohol followed by drying and milling in closed systems. Accordingly microbial counts on pectin products are relatively low*'. The same statement is given by the business operator for the potential occurrence of *Cronobacter sakazakii* in E 440i and E 440ii.

The Panel noted that the analytical data submitted for the total aerobic plate count and the total yeast and mould count are substantially lower than the respective proposed lowest technically achievable values. Furthermore, the Panel noted that the statement on *Cronobacter sakazakii* (documentation provided to EFSA n. 3,4) is not supported by analytical data, as no results nor lowest technically achievable values were provided.

3.1.3. Information on particular specifications in the additive for use in infant formulae

The call for data, requested:

- Information on particular specification requirements for identity and purity of pectin (E 440i) and amidated pectin (E 440ii) (e.g. content of toxic elements, methanol, ethanol, propan-2-ol, sulfur oxide) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1).
- Analytical data on impurities in the final special formulae for infants below 16 weeks of age need to be provided when no legal limit has been established.

In addition, data were requested demonstrating the absence of *Cronobacter (Enterobacter) sakazakii.*

One interested business operator provided information on residual alcohols and sulfur dioxide in pectins (documentation provided to EFSA n. 3). According to their information, they currently only use isopropanol as precipitation agent. The company claims that routine testing ensures that the content of isopropanol never exceeds the legal limit, and the average content is reported as approximately 0.3%. Neither methanol nor ethanol is used in the production process of pectins. No information is given on the frequency of the analyses nor on the total number of batches analysed. Following a request for clarification, the interested business operator provided some information in relation to the pectin material used in the study on neonatal piglets (Dilger, 2015; documentation provided to EFSA n. 3, 4). The Panel noted that the EU specification is met.

The same business operator informs that sulfur dioxide is not used in their manufacturing process of pectins and that *'past testing has indicated a level of SO_2 at below 1 mg/kg (applied level of detection)'.* This demonstrates that pectins (E 440i,ii) can be produced without the use of sulfur dioxide and, therefore, the limit value of 50 mg/kg in the EU specifications could be revised downward.

The Panel noted that the information provided by the business operators on the above impurities in pectin (E 440i) and amidated pectin (E 440ii) in the final special formulae for infants below 16 weeks of age is scarce.

Analytical data on arsenic and mercury in the final product for infants below 16 weeks of age were provided for two companies by one interested business operator (documentation provided to EFSA n. 3,4). One company reported results in five batches of finished special medical purpose formula for addition to human milk (utilising different/mixed vendor lots of pectins (E 440i)). In all five samples analysed by ICP-MS, the results for arsenic and mercury were below the LOQ of 0.01 and 0.005 mg/kg, respectively.

The second company submitted data on 66 batches of products in powder form containing pectins. For mercury, all results were reported as 'ND' at an LOQ of 0.01 mg/kg. For arsenic, 16 samples had concentrations between 0.02 and 0.05 mg/kg, the 50 remaining samples were below LOQ of 0.01 mg/kg. Upon request the interested business operator submitted information on the method of analysis applied in the analytical measurements of the second company and the procedure to establish the limit of detection (LOD) and LOQ. The interested business operator informed that an in-house method based on NF EN 15763 and NF EN 13805 was developed applying ICP-MS following digestion with HNO_3/HCL/H_2O_2.

The Panel noted that analytical data on toxic elements in the finished products for infants below 16 weeks of age were submitted for arsenic and mercury for which legal maximum limits are not set in Commission Regulation (EC) No 1881/2006[10]. This interested business operator did not provide data on toxic elements in pectin (E 440i) and amidated pectin (E 440ii) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1). However, following a clarification request they stated that *'SNE, representing manufacturers of infant formula, does not possess such information and we rely to the International Pectin Producers' Association (IPPA) for this request as they are in better position to provide those clarifications*'. The Panel further noted that the limit for lead in pectins in the specifications in the JECFA monograph (JECFA, 2016b) was reduced from 5 mg/kg to 2 mg/kg for general use, and a limit of 0.5 mg/kg was included for use in infant formula, see Table 1 and Section 3.5.

Information on the typical degree of methylation for pectin (E 440i) and for amidated pectin (E 440ii) when used in infant food was provided, see Section 3.1.1.

[10] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). OJ L 364, 20.12.2006, p. 5–24.

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

The call for data, requested:

- information on the fate and the reaction products of pectin (E 440i) and amidated pectin (E 440ii) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1).

Two companies provided information on fate and reaction products of pectins in the special formulae for infants below 16 weeks of age under special medical conditions (documentation provided to EFSA n. 3,4). One company determined the impact of processing conditions on pectin in [redacted] by analysing total uronic acid. Sample matrices analysed were two batches of 'pectin raw material' from one vendor, [redacted] before the sterilisation step (two batches, produced using the pectin raw material), and [redacted] after the final sterilisation step.

The company summarises the results of the analyses as follows:

'*The total uronic acid analysis showed the* [redacted] *and pectin raw material contained analogous amounts of total pectin, which demonstrates the total measured pectin amount in the HMF matches the pectin amount added. The scope of this project was intended to include total uronic acid data in* [redacted] *both before and after the final manufacturing sterilization step (Steps 2 and 3 in Sample Matrices section), however due to unforeseen circumstances (specifically, a fire at the* [laboratory][11] *site) the total uronic acid result in the* [redacted] *after sterilization is not available. Nevertheless, in food systems pectin depolymerization is a result of either acid hydrolysis or* β*-elimination. At low pH values (pH < 3) acid hydrolysis is the predominant mechanism. At pH values (pH 5–7),* β*-elimination becomes the predominant mechanism. The pH range for* [redacted] *is pH 4.2–4.5 and was developed to avoid pectin depolymerization during the UHT sterilization step and therefore is not expected to impact the total uronic acid content of the finished product'.* The Panel noted that no data are provided for [redacted] after sterilisation and therefore a conclusion on the impact of the sterilisation process on pectins cannot be drawn. Furthermore, it concluded that the analysis of total uronic acid is not very specific as the [redacted] matrix contains additional UV active compounds which influence the analytical results of total uronic acids.

The second company reports that they use pectin in powdered FSMP (containing less than 2% humidity), packed under a protective atmosphere and claim that pectins are stable in the finished product. During the process, pectins are solubilised in water before being blend with the other ingredients of the infant formula. [redacted]

In addition, the company reports on possible deesterification and depolymerisation of pectin based on two publications (Sriamornsak, 2003 and Diaz et al., 2007):

'*Theorically, pectins, in solution, can go through 2 types of reactions, which compete with each other:*

- *Deesterification, which occurs at low pH and low temperature*
- *Depolymerization, either by hydrolysis or by* β*-elimination. It occurs at a high temperature (> 75°C) and at neutral or alkaline pH. It leads to an increase of both reducing sugars and unsaturated uronides. However, the high level of carbohydrates in our products tend to limit this reaction.*

Once pectins are degradated, they lose their ability to form gel and to develop viscosity, so that it will be visible in the finished product'.

Following a request for clarification, the interested business operators reported (documentation provided to EFSA n. 5 that '*during the production process of infant formulae, pectins are solubilized in water before being blend with the other ingredients.* [redacted] *During the process, the pH is higher than the one tested by Diaz* et al. *of pH 4.5 and it has been described that lower pH favors deesterification. Moreover, the temperature used during the process are lower than the lowest one tested by Diaz of,*[12] *at which the*

[11] Edited; the name of the laboratory site was removed.
[12] The lowest temperature tested was 75°C.

accumulation of methanol after 2 h (corresponding to the maximum process time) is very close to zero (see Figure 1 in Diaz et al.*)'.*

Therefore, based on the above, the interested business operators consider that the deesterification of pectins during the process is not relevant.

The Panel noted that the information on fate and reaction products of pectins in finished products are very general and not supported by analytical data.

3.2. Authorised uses and use levels

Maximum levels of pectin (E 440i) and amidated pectin (E 440ii) in foods for infants below 16 weeks of age are defined in Regulation (EC) No 1333/2008 on food additives, as amended. In this opinion, these levels are termed maximum permitted levels (MPLs).

According to Regulation (EC) No 1333/2008 pectin (E 440i) and amidated pectin (E 440ii) are authorised in 'dietary foods for infants for special medical purposes and special formulae for infants' (FC 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes' as defined in Directive 1999/21/EC' (FC 13.1.5.2) at a maximum level of 10,000 mg/L, see Table 3.

Table 3: MPLs of pectin (E 440i) and amidated pectin (E 440ii) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 440	From birth onwards in products used in case of gastro- intestinal disorders	10,000
13.1.5.2	Dietary foods for babies and young children for special medical purposes' as defined in Directive 1999/21/EC	E 440	From birth onwards in products used in case of gastro-intestinal disorders	10,000

MPL: maximum permitted level.

Pectin (E 440i) and amidated pectin (E 440ii) are also authorised for uses in nutrient preparations according to section B of Part 5 of Annex III to Regulation (EC) No 1333/2008, see Table 4.

Table 4: MPLs of pectin (E 440i) and amidated pectin (E 440ii) in foods for infants and young children according to Annex III to Regulation (EC) No 1333/2008

E number	Name of the food additive	Maximum permitted level	Nutrient to which the food additive may be added	Food category
E 440	Pectins	For uses in nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded	All nutrients	Follow-on formulae and processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC

3.3. Exposure data

Some food additives are authorised in the EU in infants' formulae as defined by Commission Directive 2006/141/EC (FC 13.1.1) and in dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual use levels are required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[13] for technical and toxicological data on pectins (E 440) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. In response to this public call, information on the actual use levels of pectins (E 440) in foods was made available to EFSA by industry. No analytical data on the concentration of pectins (E 440) in foods were made available by the Member States.

3.3.1. Reported use levels in food category 13.1.5.1

Industry provided EFSA with use levels (n = 10) of pectins (E 440) (documentation provided to EFSA n. 3 and 4). The levels were provided by two companies. Levels of pectins were provided for either for E 440i or E 440ii or also for both pectins. Industry indicated that these additives (E 440i and E 440ii) are also used in combination with other thickening agents (xanthan gum (E 415) or locust bean gum (E 410)) in the food category 13.1.5.1.

When levels were provided for both pectins, the two levels were added, and the sum was used as occurrence level in the current exposure assessment (see Table 5).

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

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3.4 million food and beverage products of which more than 1,300,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries; Norway and UK presented in the Mintel GNPD.[14]

For the purpose of this Scientific Opinion, Mintel's GNPD[15] was used for checking the labelling of food and beverage products and food supplements for pectins (E 440) within the EU's food market as the database contains the compulsory ingredient information on the label.

No products intended for use in infants below 16 weeks were found in Mintel's GNPD as labelled with pectins (E 440). The additive is authorised for direct use according to Annex II to Regulation N°1333/2008, in dietary foods for infants for special medical purposes and special formulae for infants, therefore, for special formulae for infants described in Mintel as e.g. formulae for infants suffering from allergies, regurgitation, … or for prematurely born infants. Both these types of formulae are not fully covered in the Mintel GNPD.

3.4. Exposure estimates for infants and young children

Exposure to pectins (E 440) from their uses as food additives in formulae for infants below 16 weeks was estimated. This scenario is based on the recommended consumption levels from SC Guidance (EFSA Scientific Committee, 2017). This guidance 'recommends values of 200 and 260 mL formula[16]/kg bw per day as conservative mean and high level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age'. These recommended consumption levels correspond to 14- to 27-day-old infants consumption. For the regulatory maximum level exposure assessment scenario, MPL for special infant formulae (10,000 mg/kg for FC 13.1.5.1) was used. For the refined scenario, reported use levels (maximum and mean) was considered.

3.4.1. Dietary exposure to pectins (E 440i, ii) from infant formulae

Table 5 summarises the estimated exposure to pectins (E 440i, ii) from their uses as food additives in FC 13.1.5.1 for infants below 16 weeks of age.

[13] Call for technical and toxicological data on pectins (E 440i,ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Available online: http://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-pectin-e440i-and-amidated

[14] Missing Cyprus, Luxembourg and Malta.

[15] http://www.gnpd.com/sinatra/home/ accessed on 15/9/2020.

[16] The term 'formula' had been added.

Table 5: Dietary exposure to pectins (E 440i, ii) in foods for infants below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
Regulatory maximum level exposure assessment scenario (10,000 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	2,000
• High-level consumption (95th percentile, 260 mL/kg bw per day)	2,600
Refined estimated exposure assessment scenario	
Scenario using maximum use level reported by industry (4,170 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	834
• High-level consumption (95th percentile, 260 mL/kg bw per day)	1,084
Scenario using mean use level reported by industry (3,466 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	693
• High-level consumption (95th percentile, 260 mL/kg bw per day)	901

The refined estimated exposure assessment scenario using the maximum use level reported by industry was used in the assessment. The mean occurrence scenario is reported and indicates that there are products on the market with giving lower exposure levels.

3.4.2. Exposure estimates for infants above 16 weeks of age and toddlers consuming FSMP

As pectins (E 440i, ii) are authorised in the food categories 13.1.5.1 and 13.1.5.2, an additional exposure assessment scenario considering these two food categories was performed to estimate the exposure of infants (above 16 weeks) and toddlers (classified as young children in Commission Delegated Regulation (EU) 2016/127, age of 1–3 years) who may eat and drink these FSMP. The consumption of these foods is not reported in the EFSA Comprehensive database. To consider potential exposure to pectins (E 440i, ii) via these foods, the Panel assumes that the amount of FSMP consumed by infants and toddlers resembles that of comparable foods in infants and toddlers from the general population. Thus, the consumption of FSMP categorised as FC 13.1.5 was assumed equal to that of formulae and food products categorised as FCs 13.1.1, 13.1.2, 13.1.3 and 13.1.4. Levels for only one sample of FC 13.1.5.2 was received for pectins (E 440i, ii); it was provided by pectins producer which is then considered as recommended level and not as use level (see EFSA ANS Panel, 2017). The maximum level equals the MPL.

This scenario was estimated as follows:

– Consumers only of FSMP were assumed to be exposed to pectins (E 440i, ii) present at the maximum reported use level on a daily basis via consumption of FC 13.1.5.1 and at the MPL for FC 13.1.5.2. For the remaining food categories, the mean of the typical reported use levels was used.

Table 6: Dietary exposure to pectins (E 440i, ii) in foods for infants above 16 weeks of age and toddlers according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (16 weeks–11 months)	Toddlers (12–35 months)
FSMP consumers only scenario		
• Mean	10–434	9–273
• 95th percentile	30–1,263	42–672

In the Food for Special Medical Purposes consumers only scenario, mean dietary exposure to pectins (E 440) ranged from 9 mg/kg bw per day in toddlers to 434 mg/kg bw per day in infants above 16 weeks of age. At the high level (95th percentile), dietary exposure to pectins (E 440) ranged from 30 mg/kg bw per day up to 1,263 mg/kg bw per day in infants above 16 weeks of age.

For both infants and toddlers, the main contributing food category was Dietary foods for babies and young children for special medical purposes as defined in directive 1999/21/EC (FC 13.1.5.2).

3.4.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainty have been considered and summarised in Table 7.

Table 7: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: – one reference point only to estimate exposure during the period of up to 16 weeks of age – consumption data: different methodologies/representativeness/underreporting/misreporting/ no portion size standard for subjects above 16 weeks of age	 +/– +/–
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days for subjects above 16 weeks of age	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Database: uncertainties to which types of food the levels refer	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Infants below 16 weeks of age: Regulatory maximum level exposure assessment scenario: – exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/ 2008	 +
Infants below 16 weeks of age: Refined exposure assessment scenarios: – exposure calculations based on the maximum levels (reported use from industry) – exposure calculations based on the mean levels (reported use from industry)	 +/– +/–
Infants above 16 weeks of age and toddlers: – exposure calculations based on the maximum or mean levels (reported use from industry)	 +/–

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

Pectins (E 440) is authorised in dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) and in dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC (FC 13.1.5.2) according to Annex II to Regulation (EC) No 1333/2008 as well as in follow-on formulae and processed cereal based foods and baby foods for infants and young children as defined by Directive 2006/125/EC according to Annex III to Regulation (EC) No 1333/2008.

Based on the assumption that carers of children with allergy or any other medical disorders would be brand-loyal to an infant formula for special medical purposes (FC 13.1.5.1) that suits his medical disorder, the refined scenario using maximum use level reported by industry (Table 5) would in general result in a reliable estimation of exposure for infants below 16 weeks of age with medical disorders.

The Panel noted that information from the Mintel GNPD indicated that no FSMP products for infant and young children were labelled with pectins (E 440). These types of formulae are not fully covered in the Mintel GNPD.

Considering that the maximum reported levels were used for foods under FC 13.1.5.1 and 13.1.5.2 while mean reported use levels were used for the rest of the diet, the Panel considered that the dietary exposure to pectins (E 440) would result in a realistic estimation of the exposure to pectins (E 440) from their uses as food additives according to Annex II for infants above 16 weeks of age and toddlers.

It should be noted that the use according to Annex III to Regulation N°1333/2008 was taken into account in the regulatory maximum level exposure assessment scenario. The maximum level authorised according to the Annex III is 'For uses in nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded'.

3.4.4. Exposure to methanol and formaldehyde

Please refer to Sections 3.6.2.5 and 3.6.2.6 for an assessment of the exposure to methanol and formaldehyde following their release from pectins (E 440i,ii) in infants and young children.

3.5. Proposed revision to existing EU Specifications for pectin (E 440i) and amidated pectin (E 440ii)

The Panel noted that the occurrence data on toxic elements submitted by the interested business operators are substantially lower than the current limits in the EU specifications (documentation provided to EFSA n. 1). The Panel emphasises that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. If the EC decides to revise the current limits in the EU specifications, the estimates of toxic elements intake as below could be considered.

All analytical results submitted by the interested business operators for As, Cd and Hg were either at the LOQ or below the corresponding LOQ. The Panel considered that the LOQs can be taken as a starting point to establish maximum limits for the specifications of these toxic elements in E 440i and E 440ii. The LOQs of the methods of analysis (ICP-MS) used are the same for Cd and Hg, at 0.01 mg/kg and at 0.04 mg/kg for As, respectively. Multiplying these values by a factor of 10 to account for representativeness, homogeneity and analytical measurement uncertainty the maximum limit values for the revision of the EU specifications could be derived as 0.1, 0.1, and 0.4 mg/kg, for Cd, Hg and As, respectively.

The Pb concentrations reported by the interested business operator for commercial samples of E440i and E440ii ranged between < 0.015 and 0.431 mg/kg (P90: 0.26 mg/kg). Based on IPC-MS analysis, the Al levels in E440i and E440ii ranged from 0.81 to 99.0 (P90: 99.0) and 0.59 to 201 (P90: 117.2) mg/kg, respectively. The Panel considered the P90 value for Pb both in E440i and E440ii, and the P90 for Al in E440ii rounded to 0.30 mg/kg and 120 mg/kg, respectively, as starting points to establish maximum limits for these two elements.

The Panel emphasises that the choice of the factor and the percentiles, as well as other considerations, such as on multiple sources of exposure to conclude on the maximum limits for toxic elements in the specifications is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic elements as presented below.

The potential exposure to the toxic elements from the use of the food additives E 440i and E 440ii can be calculated by assuming contamination of the additive may be up to the specifications limit values and then by calculation pro-rata to the estimates of exposure to the food additive itself. With regard to the dietary exposure to the food additive for infants below 16 weeks of age, the Panel considered values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values, and the scenarios based on the regulatory maximum level exposure assessment, and the reported use levels (refined scenario, Table 4). The group of infants above 16 weeks of age to 11 months of age has the highest exposure level among all population groups above 16 weeks.

The above-mentioned 'modulated' maximum limits combined with the estimated intakes of pectins (E 440) could result in an exposure which can be compared with the following reference points or health-based guidance values for the five elements; a $BMDL_{01}$ of 0.3–8 μg/kg bw per day for arsenic (EFSA CONTAM Panel, 2009a), a $BMDL_{01}$ of 0.5 μg/kg bw per day for lead (EFSA CONTAM Panel, 2010), a TWI of 2.5 μg/kg bw per day for cadmium (EFSA CONTAM Panel, 2009b), a TWI of 4 μg/kg bw for mercury (EFSA CONTAM Panel, 2012) and a TWI of 1 mg/kg bw for aluminium (EFSA, 2008).

The outcome of such an exercise (Table 8) illustrates the health impact that would result if the calculated maximum limits for toxic elements were to be used.

Table 8: Risk assessment for toxic elements based on the analytical data submitted by interested business operator, and 'modulated' by the Panel, in pectins (E 440i and E 440ii) for use in food for all age groups (Documentation provided to EFSA n. 1)

Exposure to E 440 (mg/kg bw per day)[a],[e]	MOS/MOE for As at 0.4 mg/kg	MOS/MOE for Pb at 0.30 mg/kg	% of the TWI for Cd at 0.1 mg/kg	% of the TWI for Hg at 0.1 mg/kg	% of the TWI for Al at 120 mg/kg
2,000[b]	0.38–10	0.83	56	35	168
2,600[b]	0.29–7.7	0.64	73	46	218
834[c]	0.90–24	2.0	23	15	70
1,084[c]	0.69–19	1.5	30	19	91
693[d]	1.1–29	2.4	19	12	58
901[d]	0.8–22	1.9	25	16	76
434[e]	1.7–46	3.8	12	7.6	36
1,263[e]	0.6–16	1.3	35	22	107

(a): Data from Table 5 (Section 3.4.1).
(b): Regulatory maximum level exposure assessment scenario.
(c): Refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (4,170 mg/kg).
(d): Refined estimated exposure assessment scenario – Scenario using mean use level reported by industry (3,466 mg/kg).
(e): Highest exposure level for the population above 16 weeks of age (FSMP consumers only scenario – 95th percentile; data from Table 6 (Section 3.4.2).

The same interested business operator provided lowest technically achievable levels for As (1.0 mg/kg), Pb (2.0 mg/kg), Cd (0.5 mg/kg), Hg (0.5 mg/kg) and Al (200 mg/kg) in commercial pectins (E 440i and E 440ii) (documentation provided to EFSA, for the purpose of defining appropriate specifications).

The health impact that would result if the maximum limits proposed by the interested business operator are combined with the above-mentioned estimated intakes of pectins (E 440) is illustrated in Table 9.

Table 9: Risk assessment for toxic elements based on the lowest technically achievable limits in pectins (E 440i and E 440ii) for use in food for all age groups as proposed by the interested business operator (documentation provided to EFSA n. 1)

Exposure to E 440 (mg/kg bw per day)[a],[e]	MOS/MOE for As at 1 mg/kg	MOS/MOE for Pb at 2.0 mg/kg	% of the TWI for Cd at 0. 5 mg/kg	% of the TWI for Hg at 0.5 mg/kg	% of the TWI for Al at 200 mg/kg
2,000[b]	0.2–4	0.1	280	175	280
2,600[b]	0.1–3	0.1	364	228	364
834[c]	0.4–10	0.3	117	73	117
1,084[c]	0.3–7	0.2	152	95	152
693[d]	0.4–12	0.4	97	61	97
901[d]	0.3–9	0.3	126	79	126
434[e]	0.7–18	0.6	61	38	61
1,263[e]	0.2–6	0.2	177	110	177

(a): Data from Table 5 (Section 3.4.1).
(b): Regulatory maximum level exposure assessment scenario.
(c): Refined estimated exposure assessment scenario (Scenario using maximum use level reported by industry – 4,170 mg/kg).
(d): Refined estimated exposure assessment scenario (Scenario using mean use level reported by industry – 3,466 mg/kg).
(e): Highest exposure level for the population above 16 weeks of age (FSMP consumers only scenario – 95th percentile; data from Table 6 (Section 3.4.2).

The resulting figures in both scenarios show that the exposure to toxic elements from the consumption of E 440 is substantial. The Panel noted that the MOS/MOE for arsenic and lead are very low. For arsenic the reference point is based on carcinogenicity for which the MOS/MOE should be at least 10,000 (EFSA, 2005).

For lead, the reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. In the opinion on lead (EFSA CONTAM Panel, 2010), it is mentioned that a 1 point reduction in IQ is related to a 4.5%

increase in the risk of failure to graduate from high school and that a 1 point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOS/MOE lower than 1).

The MOS/MOE is calculated by dividing the reference point through the exposure estimate. The assessment of the uncertainty in the exposure showed no potential to for overestimation of exposure for the different scenarios with the exception of the regulatory maximum level exposure assessment scenario (see Table 7). Hence, the MOS/MOE estimates are not underestimated with the exception of that based on the MPLs. Even considering that the MOS/MOE might be underestimated using the exposure estimates at the MPL, the order of magnitude between the MOS/MOE for arsenic and for lead compared to the MOS/MOE requested (greater than 10,000 or greater than 1, respectively) exceeds by far the possibility of being explained by an overestimation of the exposure.

Using the existing specifications for As (3 mg/kg), Pb (5 mg/kg), Cd (1 mg/kg) and Hg (1 mg/kg) in E 440i and E 440ii, the exposure to toxic elements would be considerably higher and thus the resulting MOS/MOE for As and Pb explicitly lower, and the exceedances of the TWIs for Cd and Hg distinctly higher as illustrated in Table 10. Specifications for aluminium in pectins (E 440i and E 440ii) are currently not set in Commission Regulation (EU) No 231/2012.

Table 10: Risk assessment for toxic elements based on the current limits for toxic elements in pectins (E 440) for use in food for all age groups (Documentation provided to EFSA n. 1)

Exposure to E 440 (mg/kg bw per day)[a],[e]	MOS/MOE for As at 3 mg/kg	MOS/MOE for Pb at 5.0 mg/kg	% of the TWI for Cd at 1 mg/kg	% of the TWI for Hg at 1 mg/kg
2,000[b]	0.05 – 1	0.05	560	350
2,600[b]	0.04 – 1	0.04	728	455
834[c]	0.1 – 3	0.12	234	146
1,084[c]	0.1 – 2	0.09	304	190
693[d]	0.1 – 4	0.14	194	121
901[d]	0.1 – 3	0.11	252	158
434[e]	0.23-6	0.23	122	76
1,263[e]	0.1-2	0.08	354	221

(a): Data from Table 5 (Section 3.4.2).
(b): Regulatory maximum level exposure assessment scenario.
(c): Refined estimated exposure assessment scenario (Scenario using maximum use level reported by industry (4,170 mg/kg)).
(d): Refined estimated exposure assessment scenario (Scenario using mean use level reported by industry (3,466 mg/kg)).
(e): Highest exposure level for the population above 16 weeks of age (FSMP consumers only scenario – 95th percentile; data from Table 6 (Section 3.4.2).

The Panel noted that the calculations clearly indicate the need to decrease the current maximum limits for arsenic, lead, cadmium and mercury, and to set limit values for aluminium in pectins (E 440i and E 440ii), considering also other sources of exposure to these toxic elements. Furthermore, the Panel noted that maximum level for Pb and Cd in infant formula is set by Reg. 1881/2006; therefore, the Panel calculated the impact of the concentration of the toxic elements Pb and Cd in the food additive on the final product and compared that with the legal limits for elements in the final formula (see Appendix D).

Of note, at its 82nd meeting JECFA (2016b) reduced the limit for lead in pectins from 5 mg/kg to 2 mg/kg for general use, and a limit of 0.5 mg/kg was included for use in infant formula (JECFA, 2016a,b).

With regard to the maximum limit for sulfur dioxide (SO_2), the business operator informed that this compound is not used in the manufacturing process of pectin and that the past testing indicated a level of SO_2 at below the limit of detection of 1 mg/kg. This demonstrates that pectins (E 440i,ii) can be produced without the use of sulfur dioxide and, therefore, the limit value of 50 mg/kg in the EU specifications could be revised downwards.

With regard to maximum limits for solvent residuals, one interested business operator provided information on residual alcohols in pectins (documentation provided to EFSA n. 3). According to their information, they currently only use isopropanol as precipitation agent. The company claims that routine testing ensures that the content of isopropanol never exceeds the legal limit, and the average content is reported as approximately 0.3%. Neither methanol nor ethanol is used in the production process of pectins. No information is given on the frequency of the analyses nor on the total number of batches analysed. Following a request for clarification, the interested business operator provided some information in relation to the pectin material used in the study on neonatal piglets (Dilger, 2015;

documentation provided to EFSA n. 3, 4). For the particular pectin used in this study, the Panel noted that the EU specification is met.

According to the interested business operators (documentation provided to EFSA n. 5), the typical degree of methylation used in infant food is [redacted] (50–90%) for Pectin (E 440i) and [redacted] (20–50%) for amidated pectin (E 440ii).

Because of both the botanical origin and the polysaccharidic nature of pectins, they can be a substrate prone to microbiological contamination. Therefore, the Panel noted that microbiological specifications, including also *Cronobacter (Enterobacter) sakazakii*, should be set on the basis of the information provided.

Overall, based on the analytical data provided by the interested business operators in response to the EFSA call for data[17] (documentation provided to EFSA n. 1, 3 and 4) and the above considerations, the Panel recommends the following revisions of the existing EU specifications for pectins (E 440i and E 440ii) as listed in Table 11.

Table 11: Proposal for a revised version of the existing EU Specifications for pectins (E 440i and E 440ii)

	Commission Regulation (EU) No 231/2012	**Comment/justification for revision**
Definition	See Table 1	Unchanged
Assay	See Table 1	Unchanged
Description	See Table 1	Unchanged
Identification	See Table 1	Unchanged
Solubility	See Table 1	Unchanged
Purity	See Table 1	Unchanged
Loss on drying	See Table 1	Unchanged
Acid insoluble ash	See Table 1	Unchanged
Degree of amidation	See Table 1	Unchanged
Sulfur dioxide	See Table 1	Lowered on the basis of the information provided
Nitrogen content	See Table 1	Unchanged
Total insoluble	See Table 1	Unchanged
Solvent residues	See Table 1	Unchanged
Arsenic	Not more than 3 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Lead	Not more than 5 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Mercury	Not more than 1 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Cadmium	Not more than 1 mg/kg	Lowered on the basis of the information provided and on the considerations of the Panel
Aluminium	Not presently specified	To be included on the basis of the information provided and the considerations of the Panel
Aerobic plate count	Not presently specified	Microbiological criteria should be included on the basis of the information provided
Total yeasts and moulds	Not presently specified	Microbiological criteria should be included on the basis of the information provided
E. coli	Not presently specified	Microbiological criteria should be included on the basis of the information provided
Salmonella spp.	Not presently specified	Microbiological criteria should be included on the basis of the information provided
Cronobacter (Enterobacter) sakazakii	Not presently specified	Microbiological criteria should be included on the basis of the information provided

[17] Call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. Published: 18 July 2018. Available from: https://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-pectin-e440i-and-amidated.

3.6. Biological and toxicological data

3.6.1. Previous evaluation by ANS Panel (2017)

The following text (in italics) is from the opinion published in 2017 (EFSA ANS Panel, 2017). New information and assessments related to the specific age group below 16 weeks of age are added in the following paragraph.

Absorption, distribution, metabolism and excretion

Data on in vitro degradation of pectins and amidated pectins indicated that their digestibility was low in the upper parts of the digestive tract, but they would be fermented during their passage through the large intestine. These in vitro data are in agreement with in vivo studies demonstrating the absence of degradation of pectins in germ-free rats by comparison to conventional animals. (…) Knaup et al. (2008) investigated the human gastrointestinal metabolism of amidated pectin. (…) Methanol liberation was observed (…). As demonstrated in ileostomy patients, the main end products of this colonic anaerobic digestive process are SCFA, such as acetic, propionic and butyric acids, which are absorbed from the colon and considered of no safety concern by the Panel. These data indicated that pectins and amidated pectins would not be absorbed intact but extensively fermented by intestinal microbiota in animals and humans.

Acute, subchronic, genotoxicity, chronic, developmental and reproductivity studies

The acute oral toxicity of pectin is low. Data on amidated pectin were not available, but a low acute oral toxicity is expected based on the structural similarity to pectin.

In subchronic studies, after oral exposure (in the diet or drinking water) to non-amidated and/or amidated pectins, the NOAEL ranged from 3,366 mg/kg bw per day (Takagi et al., 1997) to 13,500 mg/kg bw per day (Til et al., 1972). In subchronic studies with pAOS in the diet, the NOAEL ranged from 1,700 to 3,400 mg/kg bw per day (Garthoff et al., 2010).

Although the available data were limited, there was no indication of genotoxicity for pectins. This conclusion was also supported by the negative results obtained with manufactured pAOS (Garthoff et al., 2010). Data on amidated pectin were not available, but considering its chemical structure and its negligible absorption, the Panel considered that there is no concern with respect to genotoxicity for amidated pectin.

A feeding study on chronic toxicity of pectin or amidated pectin in rats was available with sufficient information for the evaluation of this endpoint, including data on a concurrent control (Palmer et al., 1974; cited in Borzelleca et al., 1996). The NOAEL was 10% in the diet, equivalent to 5,000 mg/kg bw per day.

Two reproductive toxicity studies and one developmental toxicity study with pectin, which were considered inadequate for risk assessment, were available. In a dietary one-generation reproductive toxicity study with pAOS in rats, a NOAEL of 6,200 mg/kg bw per day, the highest dose tested, was identified.

Other studies

Five groups of pigs (n = 10, body weight 8.6 ± 1.4 kg, 4 weeks of age) were fed a low-fibre (LF) (raw wheat and barley flour), mid-fibre (MFH) and high-fibre (HFH) diets by adding barley hulls, or mid- (MFP) and high- (HFP) fibre diets containing pectin (Genu pectin type B, 75% methylation) for 9 days (Hedemann et al., 2006). The pectin diets contained 71 g/kg pectin; the HFP diet contained, in addition, 96 g/kg barley hulls. After 9 days, the animals were sacrificed and the entire gastrointestinal tract was removed. The feed intake in the pectin-fed groups was decreased. The mucosal-enzyme activity was affected by the fibre content of the diets in the MFH, MFP, HFH and HFP diet groups. The villi and the crypts were shorter in the pectin groups, but the villous height/crypt ratio was unaltered. The area of mucins in the crypts was decreased, suggesting that pigs fed pectin-containing diets are more susceptible to pathogenic bacteria.

Hypersensitivity, allergenicity and food intolerance

Overall, in view of the available data, the Panel considered that there is no indication that the reported immune-modulatory properties of pectin may lead to an adverse response, the data being rather indicative of an effect which would limit the hypersensitivity response. Therefore, the Panel did

not consider the food additives pectin (E 440i) and amidated pectin (E 440ii) as having an allergenic potential.

3.6.2. Newly available data

3.6.2.1. Absorption, distribution, metabolism and excretion

No new data were submitted by the interested business operators or found in the provided literature search (documentation provided to EFSA n. 1 - 4).

3.6.2.2. Toxicological data

Studies on neonatal piglets

In the former evaluation (EFSA ANS Panel, 2017), the summary of a 3-week dietary toxicity study on pectins (E 440i and E 440ii) in farm piglets (MPI, 2013 as referred to in JECFA, 2015) was available. This study and an additional study (Dilger, 2015) were briefly described. However, the study reports were not available. The Panel received the full study reports (Documentation provided to EFSA n. 3,4). The results of Dilger (2015) were also published by Fleming et al., 2020.

The two studies on neonatal piglets were assessed by means of a risk of bias (RoB) scoring scheme (see Appendix B); both were allocated to tier 1 (low risk of bias).

The FAF Panel was provided with report and an amended version of the MPI study (**MPI,** 2013, 2014; documentation provided to EFSA n.3). This study followed the FDA (2006), EMA (2009) and ICH (2010) guidelines. The study was performed according to Good Laboratory Practice (GLP). This 21-day feeding study evaluated the impact of pectin in pre-weaning farm piglets (Yorkshire-bred, age 2 days) on growth and development, including the gastrointestinal system (changes in gut microbiota, short chain fatty acids and inflammatory findings). A milk replacer containing 0, 0.5, 3.0 or 10 g pectin/L (GENU Pectin Type YM-100-L, 72% esterified) was given six times per day to groups of six males and six female piglets, at a dose volume of 500 mL/kg bw per day (doses equal to 0, 130, 1,069 or 4,069 mg/kg bw per day). No treatment-related clinical signs were observed. Statistically significant decreases were observed in male piglets of the high-dose group for the mean body weight at Day 21 (19.3%), mean food consumption at days 19–21 (up to 30%) and food efficiency during the entire study period. In female piglets of the high-dose group a not statistically significant decrease (5%) in mean body weight was observed starting on day 15. No changes in body weights were observed in the mid- and low-dose groups (0.5 and 3.0 g/L) of females and males compared to controls. Re-analysis of the data was performed using RMANOVA with sex included in the model. On days 17, 19 and 21, mean body weight of the high-dose group was found to be statistically decreased (12, 13 and 14% lower, respectively) as compared to controls based on the least square means. The decrease in mean body weight in the high-dose group correlated with statistically significant decreases in food efficiency. Statistically significant haematology findings were reported only in males, i.e. a reduction in absolute reticulocytes at the high dose on day 14, which resolved by day 21, a reduction in platelets in all treated males on day 21 and an increase in neutrophils on day 21 suggesting according to the authors a slight elevation of an inflammatory response. Some statistically significant clinical chemistry findings were also reported, i.e. a mild reduction in alkaline phosphatase (days 14 and 21) and creatinine (day 14) in mid- and high-dose males, in aspartate aminotransferase (AST) and total bilirubin on day 14 in high-dose males and in albumin (day 21) levels in the mid-dose males. The authors concluded that the findings at a 10.0 g/L may be the result of the altered effects on growth at this concentration. Some statistically significant effects were also observed in females, i.e. reductions in total bilirubin (mid and high dose) and in Gamma-glutamyl transferase (GGT) (high dose) as well as increases in globulin (high dose, on day 14) and total protein levels (mid dose). The Panel noted that the findings in haematology and clinical chemistry were not consistent between sexes, not clearly dose-related or resolved at day 21 and therefore considered that they may not be toxicologically relevant. Also, none of the fluctuations in urinalysis were considered toxicologically meaningful. Analysis of short-chain fatty acid (e.g. acetic, propionic and butyric acids) in the caecum and colon showed an increase in the short-chain fatty acids (SCFA) in large bowel contents with increasing pectin concentrations in the diet. There was a decrease (trend) in pH level of the caecum and colon contents, which may be related according to the authors to the production of SCFA by endogenous microbiota. The authors considered that the evaluation of the intestinal microbiota in piglets indicate that based on the large inhomogeneities between individuals, there may be a strong host-dependent composition of the bacterial microbiota regardless of dietary treatments. There were no statistically significant

changes in absolute and relative organ weights – except for increases in caecum and colon weights in the mid- and high-dose groups of females and males. No relevant macroscopic findings were reported. The microscopic evaluation indicated a slight increase of subacute inflammation (minimal to mild) in the caecum and colon of females and males of the high-dose group compared to controls. However, no systemic inflammation was assumed by the authors due to the absence of changes in blood cytokines (IL-1 beta, IL-6, IL-8 and TNF-alpha). According to the authors, the NOAEL for this study was the mid-dose, 1,069 mg/kg bw per day, based on decreased body weight in piglets irrespective of sex at the highest dose (4,069 mg/kg bw per day, males). The Panel agreed with this conclusion.

The FAF Panel was provided with the original report of a non-GLP study described in the EFSA Panel opinion (EFSA ANS Panel et al., 2017) as (**Dilger,** 2015; documentation provided to EFSA n. 3,4) and the publication of this study (Fleming et al., 2020). The study was conducted to evaluate the impact of pectin in pre-weaning farm piglets (Domestic Yorkshire Crossbred Swine Yorkshire-bred, age 2 days) on growth and digestibility. Pectin was derived from citrus peel (GENU® pectin type YM-100-L, 72% esterified). The pectin-containing ingredient was analysed to contain 45.1% pectin, with sucrose as the majority of the remaining product. Six piglets/sex per treatment group (controls: 5 males[18] and 7 females) were administered pectin in milk replacer as their sole source of nutrition for 3 weeks from postnatal day 2 at a target concentration of 2 or 10 g/L (equal to 704 and 4,461 mg/kg bw per day, respectively, for males and females combined). Faeces were collected and weighed on study days 12–14 (phase 1) and 19–21 (phase 2). On day 21, pigs were euthanised and digesta from the middle of the ileum (defined as the distal 20% of the small intestine proximal to the ileocecal junction) were collected. Quantification of the digestibility was measured by incorporation of ytterbium chloride as an indigestible liquid marker from day 10–21. Apparent ileal digestibility (AID) and apparent tract total digestibility (ATTD)[19] of nutrients for dry matter and crude protein and energy were measured. The differences in the analyses of the diets of the organic matter, crude protein and gross energy were minimal. Diets of the control and the 2 g/L group exhibited similar viscosity. The 10 g/L group showed a higher viscosity and was 'thick' and slow flowing compared to the control and the 2 g/L group. All pigs exhibited loose stool from days 2–4; this is considered as a common effect for artificial reared pigs. No treatment-related clinical signs were noted. Consumption of milk replacer and growth (from day 11) and feed efficiency were significantly reduced in the 10 g/L group. In the high-dose group final body weight, body weight gain, feed intake and feed efficiency were lower than in the control group (27%, 38%, 19% and 23%, respectively); no such differences from controls were observed in the lower dose group. In addition, a significantly reduced digestibility (AID and ATTD) in the 10 g pectin/L group was reported. According to the authors, the NOAEL for this study was 2 g pectin/L (704 mg pectin/kg bw per day) based on these findings. The Panel agreed with this conclusion.

Other studies from the literature

A study investigating protective effect of Banana Pectin Extract on aluminium absorption and its impairment of cognitive function was conducted in mice (**Zeng et al.,** 2018; documentation provided to EFSA n. 2). IRC male mice (7 weeks old at the start of the treatment, n = 12/group) received intra-gastrically (by gavage) 0.9% NaCl (vehicle control), 35 mg aluminium/kg bw per day, or 100 mg banana pectin and 35 mg aluminium/kg bw per day for 42 days. The banana pectin contained 79.56 ± 1.81% of galacturonic acid and 2.38 ± 0.10% of protein and the degree of esterification was 64.73 ± 1.37%. The percentage of galacturonic acid complies with the specifications of pectin (E 440i). At the end of the treatment, body weight gain was similar in the control group and the banana pectin group (97% of the control). The Panel noted that daily administration by gavage of 100 mg banana pectin/kg bw for 42 days had no adverse effect on body weight gain of mice as compared to the controls.

A literature study investigating effects of animal or plant origin protein and indigestible carbohydrates on concentration of short-chain fatty acids in the large intestine in growing pigs was provided (**Taciak et al.,** 2017; documentation provided to EFSA n. 2). Due to the poor reporting of toxicological relevant endpoints including body weight results, this study does not provide information relevant for the risk assessment of pectins (E 440i, ii) as food additive.

[18] A total of 17 intact males and 19 females were delivered to the laboratory).

[19] AID is a measure of the energy and nutrients absorbed by the host within the gastrointestinal tract prior to the distal end of the ileum, and ATTD is a measure of the nutrients and the energy that are both absorbed by the host and fermented/metabolised by the colonic microbiota. While both AID and ATTD are well-accepted and standard methods to measure nutrient and energy digestibility, AID can be considered to better represent nutrient and energy available to the pig, as energy and other nutrients have not been affected by bacterial fermentation to an appreciable extent prior to the caecum.

3.6.2.3. Clinical data

Studies with pectins

The following seven references of clinical studies were submitted by the interested business operators in response to the call for data launched by EFSA: Dupont et al., 2015; Vandenplas et al., 2016; Dupont et al., 2016a; Vandenplas et al., 2014; Rossetti et al., 2019; Dupont and Vandenplas, 2016b, 2019 (Documentation provided to EFSA n.3).

The Panel noted that according to the publications, it was not the primary aim of the studies to investigate the influence of pectins and amidated pectins (E 440i; E 440 ii) on the development of weight or on the tolerability in infants with the exception of the study of Vandenplas et al., 2016. The publications do not contain information on the composition of the formulas tested. However, the interested business operator provided information on the content of pectins (E 440i) and amidated pectins (E 440ii) (Documentation provided to EFSA n.3). The Panel noted that the formulae used in the clinical studies contained a combination of pectin (E 440i) and amidated pectin (E 440ii) with locust bean gum or xanthan gum. The Panel noted that thickening agents have similar properties.

The studies were assessed by means of a risk of bias (RoB) scoring scheme. The reviewers gave identical RoB scores for all studies (see Appendix C for further details). Only minor inconsistencies on the scoring for some of the questions/elements were noted and clarified. All studies were allocated to tier 3 (high risk of bias).

In the study of **Dupont et al.,** 2015, infants suffering from cow's milk protein allergy were allocated to two formulas. The composition of the formulas is not given, but just mentioned that the test formula '*had a similar nitrogen content (1.9 g/100 mL*[20]*) and differed mostly by the presence of a patented thickening mixture including fibres (0.5 g/100 mL), mainly composed of pectin, which thickens at gastric pH compared to the control formula*'. No anthropometric data are given. The age is given as < 18 months. No data on growth development are given. Concerning the safety, the authors state '*the most common adverse events were gastrointestinal tract affections and infections and were not related to the study product and that the incidence of adverse events was not different between groups*'. Because of lacking information, this study does not contribute to the assessment of the safety of pectins (E 440i) and amidated pectins (E 440ii). The study was allocated to tier 3 in the RoB assessment.

The study of **Vandenplas et al.** (2016) is an extension of a study with a duration of 1 month (Vandenplas et al., 2014) and reports on the outcome after 3 months and 6 months. Infants with cow milk allergy and regurgitations or frequent vomiting below 6 months were recruited for this study. The primary aim was to report on anthropometric data after 6 months. The study has several major flaws. The number of participants was in the beginning 36 infants in the group allocated to the formula with thickener and 41 infants in the group allocated to the formula not containing the thickener. At 6 months, the number of participants was 20 in the group treated with thickener containing formula and 20 in the group treated with formula containing no thickener. This corresponds to more than 20% of drop out and by this invalidates the results of the study (Genaidy et al., 2007). The detailed composition of the formulae is not reported. For the thickener containing formula the publication reports that the formula contains 3.6 g fibres/100 g of powder and 1.0 g starch/100 g of powder which is different from the composition the interested business operator has reported for this study after which the formula does not contain starch but xanthan gum (E 415) in addition to pectins and amidated pectins (E 440i and E 440ii). No information is given as to whether infants in both groups received complementary feeding. The anthropometric data were compared with non-randomised comparisons (i.e. comparisons to historical controls, such as comparisons to growth reference charts the data of which are of observational nature. Because of lacking information, this study does not contribute to the assessment of the safety of pectins (E 440i) and amidated pectins (E 440ii). The study was allocated to tier 3 in the RoB assessment.

The study of **Dupont et al.** (2016a) was a non-randomised, non-controlled prospective and multicenter study in 30 infants with a mean age of 4.8 ± 3 months diagnosed with cow milk allergy. The authors describe the tested formula given for 4 months as '*Allernova AR®, Novalac, United Pharmaceuticals, France contains an extensively casein-based hydrolysate as protein source and is thickened with a patented complex containing fibres (0.5 g/100 mL), mainly composed of pectin, to reduce regurgitation but also to help intestinal transit regulation.*' According to the information of the interested business operator the formula contains xanthan gum (E 415) in addition to pectins and

[20] Assumed by the Panel as 1.9 g protein/100 mL.

amidated pectins (E 440i and E 440ii). The study was aimed at demonstrating beneficial effects of the formula and no specific section addresses the safety. The outcomes in terms of weight gain or height development cannot be interpreted with respect to the effect of pectin (E 440i) and amidated pectin (440ii) as the study had no control group. Because of lacking information and methodological limitations (absence of randomisation), this study does not contribute to the assessment of the safety of pectins (E 440i) and amidated pectins (E 440ii). The study was allocated to tier 3 in the RoB assessment.

The study of **Vandenplas et al.** (2014) was a non-randomised, non-controlled study in 40 infants with a mean age of 3.4 ± 1.5 months diagnosed with cow milk allergy. The authors describe the tested formulae given for 6 months as containing 0.5 g fibre/100 mL or 0.5 g fibre/100 mL and 0.1 g starch/100 mL which is different from the composition the interested business operator has reported for this study after which the formula does not contain starch but xanthan gum (E 415) in addition to pectins and amidated pectins (E 440i and 440ii).The study was aimed at demonstrating beneficial effects of the formula and no specific section addresses the safety. The authors described 79 adverse events and 5 severe adverse events (infections) and state that they were not related to the study formula with no further comments. The absence of a control group does not allow to draw any conclusions. The outcomes in terms of weight gain or height development cannot be interpreted with respect to the effect of pectin (E 440i) and amidated pectin (440ii) as the study had no control group. Because of lacking information and methodological limitations (absence of randomisation), this study does not contribute to the assessment of the safety of pectins (E 440i) and amidated pectins (E 440ii). The study was allocated to tier 3 in the RoB assessment.

The aim of the study of **Rossetti et al.** (2019) is described as *'the primary objective of this study was therefore to evaluate the hypoallergenicity of a new thickened extensively hydrolyzed casein-based formula (TeHCF) in children with CMA [cow milk allergy*]'. The authors describe the tested formula as 'Allernova, new thickener (Novalac, United Pharmaceuticals, Paris, France)] had an energy density of 67 kcal/100 mL and contained 1.6, 3.5, 6.9 and 0.5 g of proteins, lipids (including arachidonic acid and docosahexaenoic acid), carbohydrates and fibre per 100 mL, respectively. The new thickener was a patented mix of fibres, including *pectin and locust bean gum'* which is close to the composition the interested business operator has reported for this study. The study was a non-randomised, non-controlled study in 29 infants with a mean age of 8.03 ± 7.43 months treated over 90 days. The outcomes in terms of weight gain or height development cannot be interpreted with respect to the effect of pectin (E 440i) and amidated pectin (E 440ii) as the study had no control group. Because of lacking information and methodological limitations (absence of randomisation), this study does not contribute to the assessment of the safety of pectins (E 440i) and amidated pectins (E 440ii). The study was allocated to tier 3 in the RoB assessment.

The aim of the study of **Dupont and Vandenplas (2016b)** is described as: *'This prospective international open pilot multicentre clinical trial was conducted to evaluate the efficacy of a new formula (Novalac, Paris, France) on regurgitation and defecation*.' One hundred patients less than 5 months old with regurgitations entered the study, after 14 days 90 patients remained in the study and were treated for 90 days. During that period 11 patients dropped out and were lost to follow up. Weight was followed up but no specific information was reported in the publication concerning safety. The outcomes in terms of weight gain or height development cannot be interpreted with respect to the effect of pectin (E 440i) and amidated pectin (440ii) as the study had no control group. Because of lacking information and methodological limitations (absence of randomisation), this study does not contribute to the assessment of the safety of pectins (E 440i) and amidated pectins (E 440ii). The study was allocated to tier 3 in the RoB assessment.

The publication of **Dupont and Vandenplas** (2019) is a review reporting retrospectively on four open label, interventional, single-group, multi-centric clinical trials (including Dupont and Vandenplas, 2016b). The test formulae (United Pharmaceuticals SAS/Novalac, Paris, France) were cows' milk based and contained a thickening complex composed of pectin, locust bean gum and either tapioca or corn starch. The exact composition of the thickening complex varied between the test formulae. Weight was followed up but no specific information was reported in the publication concerning safety. The outcomes in terms of weight gain or height development cannot be interpreted with respect to the effect of pectin (E 440i) and amidated pectin (E 440ii) as the studies had no control group. The adverse events are described in the safety population. The authors stated that 141 adverse events have been reported in the four studies without mentioning the measures taken to collect adverse events. They further report that '*Seven of them were serious adverse events, of which none were considered related to the study formula*' without giving details on the method to judge on the

relationship between event and study formula. The events concerned respiratory and ear, nose and throat infections, gastrointestinal symptoms including gastroenteritis, distress and crying, allergic reactions and other troubles including pyrexia, roseola and fungal infection. Twenty-nine non-serious adverse events were considered to be related to the study formula by the investigators, as reported by the authors. Most of these events were mild to moderate in terms of severity and related to the gastrointestinal tract: eight had constipation, five diarrhoea, seven colic/abdominal pain and five worsening of regurgitations. The review cannot be assessed with the RoB tool as relevant details are lacking. Because of the lack of a control group, the single studies would have to be allocated to tier 3 in the RoB assessment.

In the former EFSA opinion a multicenter, third-party-blinded, randomised-controlled, prospective study, in preterm infants is described (**Moya et al.,** 2012). In this study, the infants were fed with human milk to which either a pectin-containing (0.085% pectin[21]) so-called fortifier or another fortifier (control) was added. The publication does not contain information on the content of pectin in the tested so-called fortifiers. From 75 infants recruited for the study, 74 consumed the tested fortifier and 51 completed the study whereas in the control group 72 consumed the fortifier and 55 completed the study. Hence, in both arms, the attrition rate is greater than 20% and by this the results of the study are invalidated (Genaidy et al., 2007). At 14 days with 66 infants in the control group and 65 infants in the tested group not differences were seen between the two groups, whereas at 28 days, the infants fed the tested so-called fortifier milk achieved a higher linear growth rate and greater increase in weight and length but no difference in head circumference compared with the control formula. In the study only specific life threatening side effects were explicitly collected but no other side effects. Because of the many limitations, the study was allocated to Tier 3.

The Panel notes the methodological limitations of several clinical studies (absence of randomisation; no control group). The Panel also notes that all studies had a high risk of bias. The Panel considers that no conclusions can be drawn from the clinical studies with respect to the assessment of the safety of pectins and amidated pectins.

Studies with pectin-derived acidic oligosaccharides

The following references of clinical studies performed with pectin-derived acidic oligosaccharides were submitted by the interested business operators in response to the call for data launched by EFSA: Fanaro et al., 2005; Harvey et al., 2014; Burks et al., 2015; Harvey et al., 2017; Magne et al., 2008; Piemontese et al., 2011; Westerbeek et al., 2008, 2010 (Documentation provided to EFSA n.3). JECFA (2015) concluded that studies using pAOS were relevant for the evaluation of pectins in infant formulae. The ANS Panel agreed with this conclusion (EFSA ANS Panel, 2017).

The studies were assessed by means of a risk of bias (RoB) scoring scheme. The reviewers gave identical RoB scores for all studies (see Appendix C for further details). Only minor inconsistencies on the scoring for some of the questions/elements were noted and clarified. All studies were allocated to tier 3 (high risk of bias).

In the study of **Fanaro et al.** (2005), a mixture of 80% neutral oligosaccharides form long-chain galacto (GOS)- and long-chain fructooligosaccharides (FOS) with 20% acidic oligosaccharides (AOS) was investigated in a three armed prospective, randomised (method not mentioned) study in healthy children (age not given, most probably newborn). A standard formula was fed to which (1) maltose (0.8 g/100 mL) (control group), or (2) 0.2 g/100 mL acidic oligosaccharides (+ acidic OS) plus 0.6 g maltose/100 mL or (3) 0.2 g/100 mL acidic oligosaccharides plus 0.6 g/100 mL neutral oligosaccharides (+ acidic+ neutral OS) was added. The composition of the formulas was not disclosed. The study lasted for 6 weeks and measurements were performed at day 1 and at the end of the study. The primary endpoint was the intestinal flora; further endpoints were stool characteristics, pH of stool and tolerance. Incidence of crying, regurgitation and vomiting was to be recorded by the mothers. Five of the infants enrolled did not complete the study; however, it is not indicated from which study arm the infants dropped out. No information is given on the amount per day consumed. Weight gain and length gain did not differ between the groups and the stool became softer in the study groups (n = 16 + acidic OS and n = 15 + acidic + neutral OS) compared to the control group (n = 15). No information was given on the tolerability but at the end of the discussion the authors claim that '*pectin hydrolysates . . . were well tolerated as a supplement to an infant formula*'. In the abstract, the authors claimed that the study was double blind but, in the methods section, there is no indication that the

[21] The content of pectin was reported in JECFA. . ..

study was blinded. Because of methodological flaws, the study was allocated to tier 3 and does not provide relevant information on the safety of pectins in infants.

The publication of **Harvey et al.** (2014) reports on two clinical studies, the first in 115 healthy infants with a duration of 16 weeks, the second in 30 infants and children with cow milk allergy with a duration of 7 days. Because of the short duration the latter study is not included into this assessment. The first study was a randomised (method not mentioned), blinded (method not mentioned) study with a control group (n = 56) receiving a commercially available Neocate Infant DHA and ARA whereas the test group (n = 59) received Neocate Infant DHA and ARA with synbiotics (combination of *Bifidobacterium breve* M-16V as probiotic and a combination of neutral fructooligosaccharides and pectin-derived acidic oligosaccharides as the prebiotic). The composition of the two preparations is not given in detail. According to the abstract, the primary outcome measures were weight, height and head circumference. Secondary endpoints were stool characteristics and gastrointestinal symptoms. In the abstract, the authors also state that clinical examinations, dietary intake, clinical laboratory results and adverse events were recorded. Investigations were made in week 2, 4, 12 and 16. The drop out from the study was high with 115 infants entering the study; 54.2% of the infants completed the study in the test group, which had started with 59 infants and 67.9% in the control group which had started with 56 infants. Statistical procedures are not fully described (intent to treat without describing the method how the values of the drop outs were handled) and the numbers of infants in the study by week 16 given for gastrointestinal side effects differ from the number of in infants which completed the study (gastrointestinal side effects n = 29 for the control and reported as completed 38; n = 27 for the test and reported as competed n = 32). Therefore, all the reported results may have been heavily biased and can only be assessed with low confidence. According to the authors, there is no difference between the two groups in the course of the study. Because of the methodological flaws, the study was allocated to tier 3 and does provide no relevant information on the safety of pectins in infants.

The study of **Burks et al.** (2015) was a randomised (method not mentioned), blinded (method not mentioned) study with a control group (n = 56) receiving a commercially available Neocate Infant DHA and ARA) whereas the test group (n = 54) received Neocate Infant DHA and ARA with synbiotics (combination of *Bifidumbacterium breve* M-16V as probiotic and a combination of chicory-derived neutral oligofructoses, long-chain inulin and pectin-derived acidic oligosaccharides as the prebiotic). The composition of the two preparations is not given in detail, but reference is made to the publication of Harvey et al. (2014) (see above). The infants, aged 0.6–8.9 months, had to have a confirmed cow milk allergy. The primary outcome parameter was weight, length and head circumference. Secondary outcome parameters were a scale for allergic skin lesions, allergic symptoms, stool parameters, formula intake faecal microbiota, faecal pH and short- chain fatty acids. Furthermore, safety parameters were recorded (adverse events, medication use, blood parameters and clinical chemistry). From the 110 infants in the study 90 completed the study. Weight, length and head circumference were not statistically different. Formula intake was not reported and difference between groups could not been ruled out. As more than 50% participants had an age above 4 months complimentary feeding is to be expected which had not recorded. Therefore, these findings are hard to interpret with respect to the influence of the tested formula. A statistically significant difference was noted for the number of adverse event with the symptom diarrhoea (more frequent in the test group compared to control group) and the symptom infection (more frequent in the control group compared to the test group). Drugs for functional gastrointestinal disorders and antibacterial drugs for systemic use were more often needed in the test group compared to the control group. These differences cannot be attributed to the composition of the formulas with the reasons given above. Because of many methodological flaws, the study was allocated to tier 3 and does not provide relevant information on the safety of pectins in infants.

The study of **Harvey et al.** (2017) aimed at assessing the mineral status (calcium, phosphorus, chloride, sodium, potassium, magnesium and iron) of infants receiving an amino-acid-based formula. The participants of this evaluation were the identical with the infants in the Burks et al. (2015) study, described above. The results are not split up into control and test group but are given for all participants in the Burks et al. (2015) study. Hence, the study results cannot be attributed to the group having been fed with pectin-derived acidic oligosaccharides. Therefore, the study does not provide relevant information on the safety of pectins in infants.

The aim of the study of **Piemontese et al.** (2011) was *'to assess the tolerance and safety of a formula containing an innovative mixture of oligosaccharides in early infancy'*. The study was a multi-centre, randomised, double-blind, placebo-controlled trial performed in healthy, term infants, with a normal birth weight. A total of 830 infants were randomised before the age of 8 weeks to feeding with

a formula containing 6,800 mg/L of neutral short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS), ratio 9:1 and 1,200 mg/L pectin derived acidic-oligosaccharides (study group, n = 414), or with a formula without oligosaccharides (control group, n = 416). A total of 300 non-randomised breast-fed infants was used as a reference group. Anthropometric parameters (weight, length, head circumference, mid-upper arm circumference, triceps and subscapular skinfolds), gastrointestinal tolerance (regurgitations, vomiting, stool frequency and consistency) and adverse events were assessed at 8, 16, 24 and 52 weeks of age. There was no difference in the dropouts between both groups. No differences were reported in anthropometric parameters, gastrointestinal tolerance and adverse events between infants fed the formula containing oligosaccharides and infants fed the control formula. Stool consistency was significantly lower in the study group at 8, 16 and 24 weeks compared to the control group and closer to that of the breastfeeding group. Because of the presence in the study formula of not only pectin-derived acidic oligosaccharides but also scGOS and lcFOS, this study does not contribute to the assessment of the safety of pectins. The study was allocated to tier 3 in the RoB assessment.

The aim of the study of **Magne et al.** (2008) was *'to test the safety and effect on faecal microbiota of a formula with prebiotic oligosaccharides alone or in combination with acidic oligosaccharides in infants at the age of partial formula feeding'*. The study was a double-blind, placebo-controlled, randomised trial performed in healthy, full-term, partially breast-fed, 1 week to 3 months old, infants. A total of 82 infants were randomised to feeding with a whey formula (control group, n = 28), a whey formula with scGOS and lcFOS (scGOS/lcFOS group, n = 27), or a whey formula with a combination of scGOS/lcFOS and pectin-derived acidic oligosaccharides (scGOS/lcFOS/pAOS group, n = 27). The content of sGOS, lcFOS and pAOS was 0.54, 0.06 and 0.20 g/L, respectively. Infants were studied for the duration of the partial formula feeding period and every 2 weeks for 2 months after the cessation of breastfeeding. Outcome parameters were assessed every 2 weeks. There was no difference in the dropouts between the three groups. Primary outcomes included total bacteria count and proportion of seven bacterial families. Secondary outcomes included weight gain, complaints and illnesses, number and consistency of stools. There were no differences in weight gain, stool characteristics and adverse effects between the three groups. The total bacterial count did not alter with time or type of feeding. Compared with the control group, there was a significant increase of the *Bifidobacterium* genus and a significant decrease of proportions from the *Bacteroides* group and the *Clostridium coccoides* group in both oligosaccharidic groups. The proportion of bifidobacteria was significantly higher in the scGOS/lcFOS/pAOs group compared with the scGOS/lcFOS group. Because all the infants included in the study were partially breastfed during the first part of the study period, this study does not contribute to the assessment of the safety of pectins. The study was allocated to tier 3 in the RoB assessment.

The aim of the study of **Westerbeek et al.** (2010) was *'to determine the effect of enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides (scGOS/lcFOS) and acidic oligosaccharides (AOS) on serious infectious morbidity in preterm infants'*. The design and the methodology of the study are described in the paper of **Westerbeek et al.** (2008). The study was a randomised controlled trial performed in preterm infants (gestational age < 3 weeks and/or birth weight < 1,500 g). A total of 114 preterm infants were randomised to enteral supplementation of 80% scGOS/lcFOs and 20% AOS (1.5 g/kg per day) or placebo (maltodextrin) between days 3 and 30 of life. The presence of pectins in the AOS is not mentioned explicitly in the Methods section of the 2008 and the 2010 papers from Westerbeek et al. Primary outcome was serious infectious morbidity defined as a culture positive for sepsis, meningitis, pyelonephritis or pneumonia. Secondary outcomes during the 80 days follow-up were feeding tolerance, days of no enteral feeding, occurrence of Bell stage II and III necrotising enterocolitis, growth, need for mechanical ventilation, bronchopulmonary dysplasia (BPD), retinopathy of prematurity and death. Enteral supplementation of scGOS/lcFOS/AOS did not significantly reduce the risk of serious infectious morbidity in preterm infants. With regard to secondary outcomes, the only significant difference between both groups was a lower incidence of mild BPD in the supplemented group. Because of lacking information, this study does not contribute to the assessment of the safety of pectins. The study was allocated to tier 3 in the RoB assessment.

The Panel notes the methodological limitations of above clinical studies. The Panel also notes that all studies had a high risk of bias. The Panel considers that no conclusions can be drawn from these clinical studies with respect to the assessment of the safety of pectins and amidated pectins.

Other studies from the literature

The interested business operators submitted a literature study (van den Berg et al., 2016; documentation provided to EFSA n. 2) on the effect of short-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides/pectin-derived acidic oligosaccharides (scGOS/lcFOS/pAOS) on neurodevelopmental outcomes in infants at 24 months. The Panel considered that this study does not contribute to the assessment of pectins and was not further considered.

3.6.2.4. Post-marketing surveillance data

Post-marketing data were obtained from one interested business operator in the form of a summary table of adverse events of two different companies (documentation provided to EFSA n. 3). The first company provided data collected between January 2016 and September 2019 on pectin containing formulae available on the market in the Bahrein, Balcans, France, Greece, KSA, South Africa, Spain, Mexico, Italy, during which period few millions of individual items were sold. From 14 reported adverse events, the terms most often mentioned were liquid and/or frequent stools: gas (7 events) and cutaneous reactions (3 events). The second company provided data collected from January 2015 to September 2019 on pectin-containing products. Among several millions of individual items sold, 114 adverse events were reported in total. Acidosis (64 events), allergic reaction (19 events), gastrointestinal symptoms (12 events) were the most used terms to describe the events.

The Panel considered that the relationship between the events and the intake of pectin (E 440i) and amidated pectin (E 440ii) is not confirmed and that the post-marketing surveillance data do not show specific alerts except for the very rare symptoms of 'allergic reaction/intolerance'.

No additional cases of adverse reactions were found by a literature search submitted by the interested business operators (Documentation provided to EFSA n.3).

3.6.2.5. Assessment of methanol exposure

In human volunteers, consumption of 10 g (143 mg/kg bw) isolated pectin (75% methylated) induced a significant increase in methanol in the breath and, by inference, in the blood (Lindinger et al., 1997). Lindinger et al. (1997) calculated the amount of methanol released to be 400 mg per adult person (5.7 mg/kg bw). In another study in human volunteers, a daily dose of 36,000 mg pectin (equivalent to 515 mg/kg bw per day) for 6 weeks in humans was associated with only abdominal distension and increasing flatus in some individuals. No clinically relevant health impact was found and the level of gamma-glutamyl transferase as a marker for liver toxicity remained unchanged (Cummings et al., 1979). In this study the dose, applying the results from the study of Lindinger et al. (1997), would produce 20.5 mg/kg bw per day of methanol.

The extrapolation of the results from the study of Lindinger et al. (1997), assuming a degree of methylation of 75%, to the assessment of exposure via food for infants below 16 weeks under FC 13.1.5.1 would result in an exposure of 79.7 mg/kg bw per day (for pectin exposure of 2,000 mg/kg bw per day) and of 103.6 mg/kg bw per day (for pectin exposure of 2,600 mg/kg bw per day) towards methanol released from pectin for infants below 16 weeks of age. The interested business operator reported a degree of methylation between 50% and 90% for pectin (E 440i) and between 20% and 50% for amidated pectin (E 440ii). When performing the calculation with a degree of methylation of 90% (instead of 75%), the resulting values would be 15% higher (91.6 mg/kg bw per day and 119.1 mg/kg bw per day). This exposure could lead to adverse health effects. Blindness in human may occur, as reported, at doses as low as 214 mg/kg bw as a single acute dose (US EPA – Integrated Risk Information System[22]) and 260 mg/kg by the ECHA Committee for risk assessment in 2015. Metabolic acidosis is another health impairment which becomes relevant at doses between 100 and 150 mg/kg bw per day (Ashurst and Nappe, 2020). A derived no effects level (DNEL) of 88 mg/kg bw (based on methanol ocular toxicity, i.e. blindness in humans) was set by the ECHA Committee for risk assessment (RAC) in 2015 for adults (ECHA Committee for risk assessment, 2015). The Panel noted that the intake of pectins will not be as a single bolus dose but in divided doses over the day. However, because of the lack of data on the formation and the kinetics of methanol and also on the effect duration, the Panel has to take the bolus dose in adult as the reference point.

[22] https://web.archive.org/web/20121205004930/http://www.epa.gov/iris/subst/0305.htm, accessed on 28/10/2020.

3.6.2.6. Assessment of formaldehyde exposure

The Panel assumed that all methanol was converted into formaldehyde, which then formed a formaldehyde acetal with water. Concerning the metabolic conversion by aldehyde dehydrogenase, there are data in infants showing that the enzyme activity is the same as in adult (Schippan et al., 1975). The increase in formaldehyde acetal associated with 91.6 mg/kg per day methanol given as a single dose (the Panel noted that this was an unlikely scenario for dietary pectin) would result in an increase of nearly 70% (for the steady state level) of the normal intracellular endogenous formaldehyde level (EFSA ANS Panel, 2013).

This can be used to make an estimate of the additional burden of formaldehyde acetal associated with the intake of pectin. Such an additional burden should be evaluated in the light of the naturally occurring interspecies and intraspecies variation in the internal level of formaldehyde and formaldehyde acetal. Kleinnijenhuis et al. (2013) using a sensitive and specific method have measured a formaldehyde concentration in blood of 2.25 ± 0.67 (mean ± SD) mg/L in rats. This corresponds to a coefficient of variation of 30% in endogenous formaldehyde blood levels in rats (EFSA ANS Panel, 2013). Hence, the additional burden of formaldehyde due to methanol formed by release from pectin with a degree of methylation of 90% in infants below 16 weeks of age is above the upper end of the natural range.

3.7. Discussion

Analytical data on toxic elements were provided by one interested business operator for levels of arsenic, lead, cadmium, mercury and aluminium in commercial pectin (E 440i) and amidated pectin (E 440ii) samples in response to the call of data (documentation provided to EFSA n. 1). The Panel noted that the analytical data on the toxic metals provided for the 29 independent commercial E440i and E440ii samples are substantially lower than the lowest technically achievable levels proposed by the business operator.

Microbiological data on viable count, yeasts including osmophilic yeasts, moulds including xerophilic moulds, *E. coli* and *Salmonella spp.* were provided for 7 batches of (E 440i) and 6 batches for (E 440ii) (documentation provided to EFSA n. 1). The Panel noted that the analytical data submitted for the total aerobic plate count and the total yeast and mould count are substantially lower than the respective proposed lowest technically achievable values by the business operator. Furthermore, the Panel noted that the statement of the business operator whereupon *Cronobacter sakazakii* (documentation provided to EFSA n. 3,4) are eliminated during the production process is not supported by analytical data, as no results nor lowest technically achievable values were provided. The information on fate and reaction products of pectins in finished products provided by the business operators is very general and not supported by representative analytical data.

Concerning the toxic elements, the potential exposure from the consumption of E 440 could be substantial. In the case of lead and cadmium in infant formulae, the food additive specification values are not consistent with Reg. (EC) No 1881/2006[23] (see Appendix D). The Panel noted that the MOS/MOE for arsenic and lead are very low. For arsenic the reference point is based on carcinogenicity for which the MOS/MOE should be at least 10,000 (EFSA, 2005). For lead, the reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. In the Opinion on lead (EFSA CONTAM Panel, 2010), it is mentioned that a 1 point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a decrease of 1 IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the BMDL01 (MOS/MOE lower than 1).

The Panel considered feasible to amend the EU specifications based on the information submitted in response to the call for data. This refers to lowering existing limits for toxic elements (arsenic, lead, cadmium, mercury) and sulfur dioxide and to include limits for aluminium and microbiological criteria (including *Cronobacter (Enterobacter) sakazakii*) for the food additive (see Table 11).

No new data were provided concerning ADME, acute toxicity, short-term and subchronic toxicity, genotoxicity, chronic toxicity and carcinogenicity and reproductive and developmental toxicity.

Two 21-day feeding studies in neonatal piglets available to the Panel were allocated to tier 1 in the risk of bias (RoB) assessment (low risk of bias). In these studies, the piglets received a milk replacer formula containing pectins in concentrations of 0, 0.5, 3 and 10 g/L or 0, 2 and 10 g/L, respectively.

[23] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance) OJ L 364, 20.12.2006, p. 5–24.

No adverse effects on body weight, feed intake clinical parameters (haematology, clinical chemistry and urinalysis) and *post mortem* organ weights and gross and histopathology up to 3 g pectins/L in the first study and on body weight and feed intake at 2 g pectins/L in the second study were observed. These concentrations corresponded to 1,069 and 704 mg pectins/kg bw per day, respectively. The data were not suitable to perform BMD modelling. Due to the dose spacing and the broader selection of toxicologically relevant endpoints, the Panel considered that the NOAEL for pectin was 1,069 mg /kg bw per day.

Seven publications of clinical studies were submitted by the interested business operator. The composition of the tested formulas was not available neither from the publications nor provided from the interested business operators. Only the content of pectins was provided and in addition the information on the presence of other thickeners in the formulas. The Panel noted that none of the formulas did contain pectins as the only source for thickening but additional locust bean gum (5 formulas) or xanthan gum (3 formulas). The clinical studies were assessed with respect to their RoB and all were allocated to tier 3 (high risk of bias) see Appendix C. The Panel notes the methodological limitations of the clinical studies, in particular the absence of randomisation and the fact that in none of the studies pectin and amidated pectins (E 440i, E 440ii) were the only thickeners used so that the results cannot be attributed to these food additives. Furthermore, most of the studies are studies without a control group precluding any conclusions to be drawn. Because of the limitations the Panel considers that the studies cannot support the assessment of the safety of pectin and amidated pectin (E 440i, E 440ii).

Furthermore, publications with pectin-derived acidic oligosaccharides were reviewed by the Panel and assigned a tier 3 (high risk of bias). These studies did not provide any relevant information for the risk assessment of pectins as a food additive.

The Panel considered that the post-marketing surveillance data do not show specific alerts except for the very rare symptoms of 'allergic reaction/intolerance'.

Dietary exposure to pectins (E 440) from their uses as food additives was assessed based on (1) MPLs set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the reported use levels (defined as the refined exposure assessment scenario).

For infants below 16 weeks of age, both scenarios are based on the recommended consumption levels from SC Guidance (EFSA Scientific Committee, 2017) which recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values for 14- to 27-day-old infants. For infants below 16 weeks of age consuming FSMP (FC 13.1.5.1), mean exposure to pectins (E 440) in the regulatory maximum level exposure assessment scenario was estimated at 2,000 mg/kg bw per day while at the high level was estimated at 2,600 mg/kg bw per day. Using the maximum level reported by industry, exposure estimates for pectins (E 440) were estimated at 834 mg/kg bw per day at the mean and at 1,084 mg/kg bw per day at the high level. For the scenario using the mean of the reported use levels from industry, exposure estimates for pectins (E 440) were of 693 mg/kg bw per day at the mean and 901 mg/kg bw per day at the high level of consumption.

For infants above 16 weeks of age and toddlers consumers of foods for special medical purposes, mean dietary exposure to pectins (E 440) ranged from 9 mg/kg bw per day in toddlers to 434 mg/kg bw per day in infants above 16 weeks of age. At the high level (95th percentile), dietary exposure to pectins (E 440) ranged from 30 mg/kg bw per day up to 1,263 mg/kg bw per day in infants above 16 weeks of age.

The Panel decided to base its safety assessment on the MOS calculated for the different age groups and different scenarios. Due to the low internal validity of the clinical studies and further flaws, the Panel concluded that a reference point could not be derived from them. The Panel noted that the results of the piglet study with pectin could serve to derive a reference point for pectins (E 440i, ii). Which would be the NOAEL of 1,069 mg pectins/kg bw per day.

With a NOAEL of 1,069 mg pectin/kg bw per day and an exposure in the regulatory maximum level exposure assessment scenario for pectins (E 440) of 2,000 mg/kg bw per day (mean consumption) and 2,600 mg/kg bw per day (high level consumption) the MOS is below 1. The MOS is above 1 (1.3) when maximum use level reported by industry of 834 mg/kg bw per day (mean consumption) and below 1 when maximum use level reported by industry of 1,084 mg/kg bw per day (high level consumption) are used as estimates for the exposure. The MOS is higher than 1 with mean use level reported by industry of 693 mg/kg bw per day (mean consumption) and with high-level consumption of 901 mg/kg bw per day (1.5 and 1.2, respectively). This assessment is valid for infants below 16 weeks consuming FSMP (FC 13.1.5.1).

For infants above 16 weeks of age consuming FSMP according to FC 13.1.5.1. and FC 13.1.5.2, the MOS calculations gave the following results:

- for toddlers (12–35 months) the MOS is between 119 and 4 using the mean dietary exposure to pectins (E 440i,ii) and 26 and 1.6 at the high level (95th percentile) of dietary exposure to pectins (E 440i,ii), respectively;
- for infants (16 weeks to 11 months) the MOS ranged from 107 and 2.5 using the mean dietary exposure to pectins (E 440i,ii) and between 36 and 0.8 at the high level (95th percentile) of dietary exposure to pectins (E 440i,ii).

The Panel concluded that an MOS below 1 is too low. It is noted that the exposure estimates are considered not an overestimation but realistic (see Section 3.4.3). JECFA (JECFA, 2015) considered that an MOS in the region of 1–10 could be interpreted as low risk for the health of infants aged 0–12 weeks for a thickener like starch.

The maximum use levels proposed by industry to JECFA in 2016 (JECFA, 2016a, 2017) for uses in infant formula and formula for special medical purposes intended for infants of 0.2% would result in an MOS above 1 for all exposure scenarios. Because a neonatal piglet model has been used avoiding the need for an additional inter species uncertainty factor, the proposed maximum use level of 0.2% would not be of safety concern.

The Panel further noted that for combinations of pectins (E 440) with other thickeners which seem to be on the market no studies in an appropriate animal model are available. The clinical studies presented by interested business operators have a low internal validity; most of them are uncontrolled studies.

Consumption of pectin (75% methylated) induced a significant increase in methanol in the breath and, by inference, in the blood (Lindinger et al., 1997). At the dose of 10 g, the lowest amount of methanol released is 400 mg per adult person (5.7 mg/kg bw). The extrapolation of these results to the assessment of exposure via foods for infants below 16 weeks of age under FC 13.1.5.1 (see Section 3.4.1) would result in an exposure of 79.7 mg/kg bw per day (for pectin exposure of 2,000 mg/kg bw per day) and of 103.6 mg/kg bw per day (for pectin exposure of 2,600 mg/kg bw per day) towards methanol released from pectin in infants below 16 weeks of age. Although the flora of gastrointestinal tract is not fully comparable in the first days of life to that of the adult, after birth the microbiome of the newborn infants becomes similar to that of the mother (Bäckhed et al., 2015). The interested business operators reported a degree of methylation between 50% and 90% for pectin (E 440i) and between 20% and 50% for amidated pectin (E 440ii). When performing the calculation with a degree of methylation of 90% (instead of 75%), the resulting values would be 15% higher (91.6 mg/kg bw per day and 119.4 mg/kg bw per day). This exposure could lead to adverse health effects. Blindness in human may occur, as reported, at doses as low as 214 mg/kg bw as a single acute dose (US EPA- Integrated Risk Information System) and 260 mg/kg by the ECHA Committee for risk assessment in 2015. Metabolic acidosis is another health impairment which becomes relevant at doses between 100 and 150 mg/kg bw per day (Ashurst and Nappe, 2020). A derived no effects level (DNEL) of 88 mg/kg bw (based on methanol ocular toxicity, i.e. blindness in humans) was set by the ECHA Committee for risk assessment (RAC) in 2015 for adults (ECHA Committee for risk assessment, 2015). When the maximum use level provided by industry are considered, the exposure to methanol via foods for infants below 16 weeks of age under FC 13.1.5.1 would result in an exposure of 43.2 mg/kg bw per day (for pectin exposure of 1,084 mg/kg bw per day, 95th percentile) towards methanol released from pectin. The Panel considered this as a conservative approach also noting that the intake of pectins will not be as a single bolus dose but in divided doses over the day.

For infants above 16 weeks of age, the exposure towards methanol is estimated to be roughly 50% of that for the infants below 16 weeks of age.

4. Conclusions

- Due to the low internal validity of the clinical studies, the Panel concluded that a reference point could not be derived from them, but the results of the adequate piglet study could serve to derive a reference point. When calculating the MOS for pectins exposure, this was below 1 for some scenarios. The Panel concluded that an MOS below 1 is too low.
- Under the current exposure estimates at the MPLs, an internal methanol exposure (in the range of methylation given for pectin (E 440i)) would result that could lead to adverse health effects in infants below 16 weeks of age.

- The Panel concluded that the risk assessment for toxic elements clearly indicates the need to decrease the current maximum limits for arsenic, lead, cadmium, mercury and sulfur dioxide and to set a maximum limit for aluminium in pectins (E 440i and E 440ii), considering also other sources of exposure to these toxic elements.
- Because of both the botanical origin and the polysaccharidic nature of pectins (E 440i, ii), they can be a substrate of microbiological contamination. Therefore, the Panel noted that microbiological specifications (including *C. sakazakii*) should be set on the basis of the information provided.

5. Recommendation

The Panel recommends:

- The European Commission to consider revising the current specifications for the food additive pectin (E 440i) and amidated pectin (E 440ii) in line with the proposals made on the basis of the information provided and based on the considerations of the Panel, see Table 11.
- The European Commission to consider lowering the MPL of pectin (E 440i) and amidated pectin (E 440ii) in FCs 13.1.5.1 and 13.1.5.2, in order to reduce the exposure to both the additives themselves and to methanol.

6. Documentation as provided to EFSA

1) International Pectin Producers Association (IPPA), 2019. Submission of data in response to the call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on December 2019.
2) International Pectin Producers Association (IPPA), 2020. Clarification on the submission of data in response to the call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on August 2020.
3) Specialised Nutrition Europe, 2019. Submission of data in response to the call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on December 2019.
4) Specialised Nutrition Europe, 2020. Clarifications on the submission of data in response to the call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on September 2020.
5) Specialised Nutrition Europe, 2020. Clarifications on the submission of data in response to the call for technical and toxicological data on pectin (E 440i) and amidated pectin (E 440ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on November 2020.

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstract Service
CFU	Colony-forming unit
FAF Panel	Panel on Food Additives and Flavourings
FAO/WHO	Food and Drug Organisation/World Health Organisation
FC	Food category
FSMP	Food for special medical purposes
JECFA	Joint FAO/WHO Expert Committee on Food Additives
Mintel GNPD	Mintel's Global New Products Database
MOE	margin of exposure
MOS	margin of safety
MPL	maximum permitted levels
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
SC	Scientific Committee of EFSA
SCF	Scientific Committee on Food
TAMC	total anaerobic microbial count
TYMC	total combined yeast and mould count

Appendix A – Data requested in the call for data (Call for technical and toxicological data on pectins (E 440i,ii) for uses as a food additive in foods for all population groups including infants below 16 weeks of age[24]

Kind of data	Data requested in the call for data	Responses from interested business operators	Comment
A. Information regarding the follow-up of the conclusions and the recommendations of the EFSA ANS Panel opinion on the safety of pectins (E 440i,ii) as food additive			
1. Technical data	• Analytical data on current levels of arsenic, lead, cadmium, mercury and aluminium in commercial samples of the food additives; • the lowest technologically achievable level for lead, mercury, cadmium, arsenic and aluminium in order to adequately define their maximum limits in the specifications; • Because of both the botanical origin and the polysaccharidic nature of pectin, they can be a substrate of microbiological contamination. Data should be provided demonstrating the absence of Salmonella spp. and Escherichia coli and on the lowest total aerobic microbial count (TAMC) and total combined yeast and mould count (TYMC) that can be reached.	Received	Assessed, no further follow-up
2. Toxicological data	According to the conclusions and recommendations in the Scientific opinion on the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as a food additives by the EFSA ANS Panel published in 2017, the generation of additional data to assess the potential health effects of pectin (E 440i) and amidated pectin (E 440ii) when used as a food additive in 'dietary foods for infants for special medical purposes and special formulae for infants' (Food category 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (Food category 13.1.5.2) was recommended. These requirements could be addressed as outlined in Section B.2	Not specific data provided	No further follow-up
3. Literature searches	Literature searches should be conducted relevant for the safety evaluation of pectin (E 440i) and amidated pectin (E 440ii) for all uses in foods for all population groups from 12/10/2016 up to the date of the data submission, as described in the Guidance for submission for food additive evaluations (see its Section 5.3)	Received	Assessed, no further follow-up

[24] Available online: https://www.efsa.europa.eu/en/consultations/call/call-technical-and-toxicological-data-pectin-e440i-and-amidatedand responses from interested business operators.

Kind of data	Data requested in the call for data	Responses from interested business operators	Comment
B. Information required for the risk assessment of pectins (E 440i,ii) as food additive for use in foods for infants below 16 weeks of age			
1. Technical data	• Information on the usage levels of pectin (E 440i) and amidated pectin (E 440ii), alone or in combination with other thickening agents (indication of food additive name and level of use) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1); • Information on the fate and the reaction products pectin (E 440i) and amidated pectin (E 440ii) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1); • Information on particular specification requirements for identity and purity of pectin (E 440i) and amidated pectin (E 440ii) (e.g. content of toxic elements. methanol, ethanol, propan-2-ol, sulfur oxide) in the special formulae for infants below 16 weeks of age under special medical conditions (FC 13.1.5.1). Analytical data on impurities in the final special formulae for infants below 16 weeks of age need to be provided when no legal limit has been established. In addition, data should be provided demonstrating the absence of *Cronobacter (Enterobacter) sakazakii*.	Received	Assessed, no further follow-up
2. Toxicological data	• The full reports of the repeated dose studies in neonatal piglets (Dilger, 2015; MPI, 2013); • clinical data focusing on gastrointestinal effects to assess the safety of pectin (E 440i) and amidated pectin (E 440ii) as food additives when used in 'dietary foods for special medical purposes and special formulae for infants' (FC 13.1.5.1); • post-marketing surveillance reports on undesired and adverse reactions, indicating the ages and other relevant data of the exposed infants and young children and the use levels of pectin (E 440i) and amidated pectin (E 440ii) in the marketed products; • published and unpublished case reports (e.g. available nutrivigilance data) on undesired and adverse effects, including e.g. flatulence, gastrointestinal discomfort, changes of stool-frequencies and -consistency, diarrhoea and allergic reactions, associated with the oral administration of pectin (E 440i) and amidated pectin (E 440ii) in any form, to infants and young children.	Received	Assessed, no further follow-up
3. Literature searches	Literature searches should be conducted relevant for the safety evaluation of pectin (E 440i) and amidated pectin (E 440ii) when used in foods for infants below 16 weeks of age up to the date of the data submission, as described in the Guidance for submission for food additive evaluations (Section 5.3).	Received	Assessed, no further follow-up

Appendix B – Risk of bias/Internal validity for Experimental Animal Studies (modified from to NTP-OHAT, 2015, 2019)

The ratings of the key and non-key questions (++, +, –, ––) will be integrated to classify the studies in tiers from 1 to 3 corresponding to decreasing levels of internal validity.

Tier 1:

- All the key questions are scored +/++

  AND

- No more than one non-key question is scored –

  AND

- No non-key question is scored –

Tier 2:

- All the other combinations not falling under tier 1 or 3

Tier 3:

- Any question is scored – –

  OR

- More than one key question is scored –

Number	Question	Domain of bias	Rating (++, +, –, ––)
1*	**Was administered dose or exposure level adequately randomised? (please apply the question also on F1 and F2 generation)** **Key question**	Selection	++ if the method is described and it is adequate + if the authors only indicate that randomisation was done but do not describe the method – no mentioning of randomisation –– direct evidence of no randomisation
2	**Was allocation to study groups adequately concealed?**	Selection	++ properly concealed and described how concealment was performed + mentioning that concealment was performed; + is also appropriate if non-concealment does not influence the outcome – if non-concealment does influence the outcome (measurements with a subjective part (e.g. preparation of fat pads, observation of behaviour) –– if non-concealment does influence the outcome to a very important part (subjective measurements)
3*	**Were experimental conditions identical across study groups?** **Key question**	Performance	++ experimental conditions described and identical across study groups (feeding, water supply, bedding, day/night cycle; temperature; humidity) + incomplete description of experimental conditions; + is also appropriate if lack of information does not influence the outcome – if lack of information does influence the outcome –– if factors clearly indicate that treatment conditions were different does influence the outcome to a very important part

Number	Question	Domain of bias	Rating (++, +, –, ––)
4*	**Was the research personnel blinded to the study group? Key question**	Performance	++ if there is direct evidence that the research personnel did not know what group animals were allocated to, and it is unlikely that they could have broken the blinding of allocation
			+ if not reported and lack of adequate allocation concealment would not appreciably affect the allocation of animals to different study groups (e.g. methods used which do not have a subjective component)
			– if not reported and lack of adequate allocation concealment would appreciably affect the allocation of animals to different study groups (e.g. methods used which have a subjective component)
			–– if there is direct evidence that it was possible for the research personnel to know what group animals were allocated to, or it is likely that they could have broken the blinding of allocation
5	**Were outcome data complete without attrition or exclusion from analysis?**	Attrition/exclusion	++ There is direct evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study. OR Missing data have been imputed using appropriate methods (ensuring that characteristics of animals are not significantly different from animals retained in the analysis).
			+ There is indirect evidence that loss of animals was adequately addressed, and reasons were documented when animals were removed from a study. OR It is deemed that the proportion lost would not appreciably bias results. This would include reports of no statistical differences in characteristics of animals removed from the study from those remaining in the study. OR There is insufficient information provided about loss of animals (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.
			– There is indirect evidence that loss of animals was unacceptably large and not adequately addressed (e.g. if unexplained loss is equal or more than 25%). OR There is insufficient information provided about loss of animals (record 'NR' as basis for answer) and it is suspected that this would have an impact on the validity of the study. Note: Unexplained inconsistencies between materials and methods and results sections (e.g. inconsistencies in the numbers of animals in different groups) could be an example of indirect evidence.
			–– There is direct evidence that loss of animals was unacceptably large and not adequately addressed.

Number	Question	Domain of bias	Rating (++, +, –, ––)
6*	**Can we be confident in the exposure characterisation?** **Key question**	Detection	++ There is direct evidence that the substance was sufficiently described and consistently administered (e.g. with the same method and timeframe) across treatment groups.
			+ There is indirect evidence that the substance was sufficiently described and consistently administered (i.e. with the same method and time-frame) across treatment groups. OR There is insufficient information provided about description and administration of the substance (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.
			– There is indirect evidence that the substance was not sufficiently described and was not consistently administered (e.g. with the same method and timeframes) across groups. OR There is insufficient information provided about description and administration of the substance (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study.
			–– There is direct evidence that the substance was not sufficiently described and/or was not consistently administered (e.g. with the same method and timeframes) across groups.
7*	**Can we be confident in the outcome assessment?** **Key question**	Detection	
		Element 1 Was the outcome assessed at the same length of time (i.e. day and/or time of day) after initial exposure in all study groups? (remember to take into consideration the endpoints assignments) **Element 2** Was a reliable and sensitive animal model used for investigating the test compound and selected endpoints? **Element 3** Was the number of animals per dose group appropriate?	

Number	Question	Domain of bias	Rating (++, +, –, ––)
		Element 4 Was the number of animals per sex in each cage appropriate for the study type and animal model? **Element 5** Was the timing and duration of administration of the test compound appropriate? **Element 6** Were reliable and sensitive test methods used for investigating the selected endpoints? **Element 7** Were the measurements collected at suitable time points in order to generate sensitive, valid and reliable data?	
8	**Were all outcomes measured according to the methodology section reported?**	Selective reporting	++ There is direct evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
			+ There is indirect evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have been reported. This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not). OR Analyses that had not been planned in advance (i.e. retrospective unplanned subgroup analyses) are clearly indicated as such and it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g. appropriate analyses of an unexpected effect). OR There is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.

Number	Question	Domain of bias	Rating (++, +, –, ––)
			– There is indirect evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract and/or introduction that are relevant for the evaluation have not been reported. OR There is indirect evidence that unplanned analyses were included that may appreciably bias results. OR There is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study. Note: Unexplained inconsistencies between materials and methods and results/abstract or summary sections (e.g. inconsistencies in the numbers of animals in different groups) could be an example of indirect evidence.
			–– There is direct evidence that not all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract, and/or introduction that are relevant for the evaluation have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.
9	**Were statistical methods appropriate?**	Other sources of bias	++ There is direct evidence that the statistical methods seem appropriate and were clearly reported (adequate treatment of multiple testing).
			+ Statistical methods were not clearly reported but it may be inferred from other information that they were appropriate. OR There is insufficient information provided about statistical methods (record 'NR' as basis for answer), but it is considered that this does not have an impact on the validity of the study.
			– Statistical methods were not clearly reported, but it may be inferred from other information that they were not appropriate. OR There is insufficient information provided about statistical methods (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study.
			–– There is direct evidence that the statistical methods applied were inappropriate.

Appendix C – Risk of bias/Internal validity for the clinical studies (modified from to NTP-OHAT, 2015, 2019)

C.1. Decision rules

The ratings of the key and non-key questions (++, +, –, ––) will be integrated to classify the studies in tiers from 1 to 3 corresponding to decreasing levels of internal validity.

Tier 1:

- All the key questions are scored +/++

  AND

- No more than one non-key question is scored –

  AND

- No non-key question is scored –

Tier 2:

- All the other combinations not falling under tier 1 or 3

Tier 3:

- Any question is scored ––

  OR

- More than one key question is scored –

Elements considered in the assessment

Question	Rating	Explanation for expert judgement
1. Was the administered dose or exposure level adequately randomised? **Key question**	++	There is direct evidence that subjects (or clusters) were allocated to any study group including controls using a method with a random component. Acceptable methods of randomisation include referring to a random number table, using a computer random number generator, coin tossing, shuffling cards or envelopes, throwing dice, or drawing of lots (Higgins and Green, 2011). Restricted randomisation (e.g. blocked randomisation) to ensure particular allocation ratios will be considered low risk of bias. Similarly, stratified randomisation and minimisation approaches that attempt to minimise imbalance between groups on significant prognostic factors (e.g. body weight) will be considered acceptable.
	+	There is indirect evidence that subjects (or clusters) were allocated to study groups using a method with a random component (i.e. authors state that allocation was random, without description of the method used). **OR** It is deemed that allocation without a clearly random component during the study would not appreciably bias results. For example, approaches such as biased coin or urn randomisation, replacement randomisation, mixed randomisation and maximal randomisation may require consultation with a statistician to determine risk-of-bias rating (Higgins and Green, 2011).
	NR	There is insufficient information provided about how subjects (or clusters) were allocated to study groups.
	–	There is indirect evidence that subjects (or clusters) were allocated to study groups using a method with a non-random component. *NOTE: Non-random allocation methods may be systematic but have the potential to allow participants or researchers to anticipate the allocation to study groups. Such 'quasi-random' methods include alternation, assignment based on date of birth, case record number, or date of presentation to study.*

Question	Rating	Explanation for expert judgement
	−−	There is direct evidence that subjects (or clusters) were allocated to study groups using a non-random method including judgement of the clinician, preference of the participant, the results of a laboratory test or a series of tests or availability of the intervention (Higgins and Green, 2011).
2. Was the allocation to study groups adequately concealed?	++	There is direct evidence that at the time of recruitment the research personnel and subjects did not know what study group subjects were allocated to, and it is unlikely that they could have broken the blinding of allocation until after assignment was complete and irrevocable. Acceptable methods used to ensure allocation concealment include central allocation (including telephone, web-based and pharmacy-controlled randomisation); sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes; or equivalent methods.
	+	There is indirect evidence that the research personnel and subjects did not know what study group subjects were allocated to and it is unlikely that they could have broken the blinding of allocation until after recruitment was complete and irrevocable. **OR** It is deemed that lack of adequate allocation concealment would not appreciably bias results (e.g. some crossover designs).
	NR	There is insufficient information provided about allocation to study groups.
	−	There is indirect evidence that at the time of recruitment it was possible for the research personnel and subjects to know what study group subjects were allocated to, or it is likely that they could have broken the blinding of allocation before assignment was complete and irrevocable. *NOTE: Inadequate methods include using an open random allocation schedule (e.g. a list of random numbers); assignment envelopes used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; or any other explicitly unconcealed procedure. For example, if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed.*
	−−	There is direct evidence that at the time of recruitment it was possible for the research personnel and subjects to know what study group subjects were allocated to, or it is likely that they could have broken the blinding of allocation before recruitment was complete.
3. Were the research personnel and human subjects blinded to the study group during the study?	++	There is direct evidence that the subjects and research personnel were adequately blinded to study group, **AND** it is unlikely that they could have broken the blinding during the study. Methods used to ensure blinding include central allocation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes; or equivalent methods.
	+	There is indirect evidence that the subjects and research personnel were adequately blinded to study group, AND it is unlikely that they could have broken the blinding during the study. **OR** There is direct evidence for no blinding during the study (including where it was not possible to implement) AND it is deemed that no blinding would appreciably bias results BUT bias minimising measures have been adequately implemented. **OR** It is deemed that lack of adequate blinding or no blinding during the study would not appreciably bias results (e.g. controls unlikely to behave differently for factors other than sodium intake) (e.g. cross-over).
	NR	There is insufficient information provided about blinding to study group during the study (including possible breaking and minimising measures).

Question	Rating	Explanation for expert judgement
	–	There is indirect evidence that it was possible for research personnel or subjects to infer the study group **AND** it is deemed that lack of adequate blinding or no blinding during the study would appreciably bias results (e.g. no comparable treatment of controls, including not comparable exposure to factors other than the interventions of interest; differential behaviour) **AND** no bias minimising measures have been adequately implemented.
	––	There is direct evidence for lack of adequate blinding of the study group (including no blinding or incomplete blinding) of research personnel and subjects **AND** it is deemed that lack of adequate blinding or no blinding during the study would appreciably bias results (e.g. no comparable treatment of controls, including not comparable exposure to factors other than the interventions of interest, differential behaviour) **AND** no bias minimising measures have been adequately implemented.
4. Were outcome data complete without attrition or exclusion from analysis? **Key question**	++	There is direct evidence that there was no loss of subjects during the study and outcome data were complete. **OR** Loss of subjects (i.e. incomplete outcome data) was adequately addressed and reasons were documented when human subjects were removed from a study or analyses. Review authors should be confident that the participants included in the analysis are exactly those who were randomised into the trial. Acceptable handling of subject attrition includes: very few missing outcome data (e.g. less than 10% in each group (Genaidy et al., 2007)) **AND** reasons for missing subjects unlikely to be related to outcome (for survival data, censoring unlikely to be introducing bias) **AND** missing outcome data balanced in numbers across study groups, with similar reasons for missing data across groups (i.e. unlikely to be related to exposure). **OR** Analyses (such as intention-to-treat analysis) in which missing data have been imputed using appropriate methods (ensuring that the characteristics of subjects lost to follow up or with unavailable records are described in identical way and are not significantly different from those of the study participants). *NOTE: Participants randomised but subsequently found not to be eligible need not always be considered as having missing outcome data) (Higgins and Green, 2011)*.
	+	There is indirect evidence that loss of subjects (i.e. incomplete outcome data) was adequately addressed and reasons were documented when human subjects were removed from a study. **OR** It is deemed that the proportion lost to follow-up would not appreciably bias results (e.g. less than 20% in each group in parallel studies (Genaidy et al., 2007)). This would include reports of no statistical differences in characteristics of subjects lost to follow up or with unavailable records from those of the study participants. Generally, the higher the ratio of participants with missing data to participants with events, the greater potential there is for bias. For studies with a long duration of follow-up, some withdrawals for such reasons are inevitable. *NB: For crossover designs, this may be less of an issue*.
	NR	There is insufficient information provided about numbers of subjects lost to follow-up.
	–	There is indirect evidence that loss of subjects (i.e. incomplete outcome data) was unacceptably large (e.g. greater than 20% in each group in parallel studies (Genaidy et al., 2007)) and not adequately addressed.

Question	Rating	Explanation for expert judgement
	−−	There is direct evidence that loss of subjects (i.e. incomplete outcome data) was unacceptably large and not adequately addressed (e.g. greater than 20% in each group in parallel studies (Genaidy et al., 2007)). Unacceptable handling of subject attrition includes: reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across study groups (i.e. likely to be related to the exposure); or potentially inappropriate application of imputation.
5. Can we be confident in the exposure characterisation? **Key question**	++	There is direct evidence that the exposure (including compliance with the treatment, if applicable) was independently characterised **AND** that exposure was consistently administered (i.e. with the same method and time-frame) across treatment groups.
	+	There is indirect evidence that the exposure (including compliance with the treatment, if applicable) was independently characterised **AND** there is indirect evidence that exposure was consistently administered (i.e. with the same method and time-frame) across treatment groups.
	NR	There is insufficient information provided to judge the exposure characterisation
	−	There is indirect evidence that the exposure (including compliance with the treatment, if applicable) was assessed using poorly validated methods (e.g. FFQs, spot urine etc.). **OR** There is indirect evidence that the exposure assessment was probably biased.
	−−	There is direct evidence that the exposure (including compliance with the treatment, if applicable) was assessed using poorly validated methods (e.g. FFQs, spot urine etc.). **OR** There is direct evidence that the exposure assessment was biased.
6. Can we be confident in the outcome assessment? **Key question**	++	There is direct evidence that the outcome was assessed using well-established methods (e.g. for office BP: according to a clearly described methodology, including e.g. repeated measurements per visit, trained technician, resting period before each measurement). **AND** There is direct evidence that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding prior to reporting outcomes.
	+	There is indirect evidence that the outcome was assessed using acceptable methods (i.e. deemed valid and reliable but not the gold standard) **OR** it is deemed that the outcome assessment methods used would not appreciably bias results. **AND** There is indirect evidence that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding before reporting outcomes **OR** it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results.
	NR	There is insufficient information provided about blinding of outcome assessors or the method of measurement.
	−	There is indirect evidence that the outcome assessment method is an unacceptable method. **OR** There is indirect evidence that it was possible for outcome assessors to infer the study group before reporting outcomes.

Question	Rating	Explanation for expert judgement
	−−	There is direct evidence that the outcome assessment method is an unacceptable method. **OR** There is direct evidence for lack of adequate blinding of outcome assessors (including study subjects if home BP is the outcome), including no blinding or incomplete blinding.
7. Were all measured outcomes reported?	++	There is direct evidence that all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract and/or introduction (that are relevant for the evaluation) have been reported.
	+	There is indirect evidence that all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract and/or introduction (that are relevant for the evaluation) have been reported. **OR** Analyses that had not been planned in advance (i.e. retrospective unplanned subgroup analyses) are clearly indicated as such and it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g. appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
	NR	There is insufficient information provided about selective outcome reporting.
	−	There is indirect evidence that all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract and/or introduction (that are relevant for the evaluation) have not been reported. **OR** There is indirect evidence that unplanned analyses were included that may appreciably bias result.
	−−	There is direct evidence that all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.
8. Were there no other potential threats to internal validity? **NOTE:** Baseline characteristics should be appraised **only if** Q1 (randomisation) was rated with ++/+ and Q2 (allocation concealment) was rated with ++/+/NR	++	There is evidence that variables, other than the exposure and outcome, did not differ between groups during the course of the intervention in a way that could bias results. **AND,** in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported': There is no evidence of differences in baseline characteristics. **OR** There is no information on both BUT no concern.

Question	Rating	Explanation for expert judgement
	+	1. There is evidence that variables, other than the exposure and outcome, differed between groups during the course of the intervention. **AND** it is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis). **AND**, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported': There is evidence that reported variables differed between groups at baseline. **AND** It is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis). **OR** 2. There is evidence that variables, other than the exposure and outcome, did not differ between groups during the course of the intervention in a way that could bias results. **AND**, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported': There is evidence that reported variables differed between groups at baseline. **AND** It is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis). **OR** 3. There is evidence that variables, other than the exposure and outcome, differed between groups during the course of the intervention. **AND** it is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis). **AND**, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported': There is no evidence of differences in baseline characteristics. **OR** There is no information BUT no concern.
	–	There is no information on baseline characteristics **AND/OR** there is no information about differences between groups during the course of the intervention. **AND** There is concern.
	––	There is evidence that variables, other than the exposure and outcome, differed between groups during the course of the intervention. **AND** It is deemed that these differences appreciably biased results (there is concern (e.g. not adequately addressed by analysis)) **OR**, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported': There is evidence that reported variables differed between groups at baseline. **AND** It is deemed that these differences appreciably biased results (there is concern (e.g. not adequately addressed by analysis)).

C.2. 'Risk of bias summary' for the clinical studies with pectins (modified from the Cochrane RoB tool)

	Random sequence generation (selectionbias)	Allocation concealment (selection bias)	Blinding of participants and personnel(performancebias)	Incomplete outcome data (attrition bias)	Confidence in exposure to the interventions	Confidencein outcome assessment	Selective reporting (reporting bias)	Other potential treats to internal validity	Tier
Dupont et al. (2015)	++	++	++	−−	−−	−	++	++	3
Vandenplas et al. (2016a)	++	++	++	−−	−−	−	++	++	3
Dupont et al. (2016a)	−−	−−	−−	++	−	−	++	NA	3
Vandenplas et al. (2014)	−−	−−	−−	+	−	−	+	NA	3
Rossetti et al. (2019)	−−	−−	−−	−	−	−	+	NA	3
Dupont and Vandenplas (2016b)	−−	−−	−−	+	−	−	++	NA	3
Moya et al. (2012)	+	+	+	−	−	+	++	+	3

Definitely low risk of bias (++), Probably low risk of bias (+), Probably high risk of bias (−), Definitely high risk of bias (−−), NA not applicable.

C.3. 'Risk of bias summary' for the clinical studies with pectin-derived acidic oligosaccharides (modified from the Cochrane RoB tool)

	Random sequence generation (selectionbias)	Allocation concealment (selection bias)	Blinding of participants and personnel(performancebias)	Incomplete outcome data (attrition bias)	Confidence in exposure to the interventions	Confidencein outcome assessment	Selective reporting (reporting bias)	Other potential treats to internal validity	Tier
Fanaro et al. (2005)	+	−	−−	+	−−	−	−	NA	3
Harvey et al. (2014)	+	−	−−	−	−−	−	−	NA	3
Burks et al. (2015)	+	−	−−	−	−−	−	−	−	3
Magne et al. (2008)	++	++	++	+	−−	+	++	++	3
Piemontese et al. (2011)	++	++	++	−−	−−	+	++	++	3
Westerbeek et al. (2010)	++	++	+	−−	−−	+	++	++	3

Definitely low risk of bias (++), Probably low risk of bias (+), Probably high risk of bias (−), Definitely high risk of bias (−−), NA not applicable.

Appendix D – Estimation of the fraction of the concentration of toxic elements in pectin (E 440i) and amidated pectin (E 440ii) with respect to the regulatory maximum levels in the final food product for which the additive is used

The Panel estimated the fraction (%) of the concentration of the toxic elements lead and cadmium in pectin (E 440i) and amidated pectin (E 440ii) with respect to the regulatory maximum levels in the final product (formulae) as sold as laid down in Regulation (EC) No 1881/2006[23] considering:

- The current specification for lead and cadmium for pectin (E 440i) and amidated pectin (E 440ii) according to Regulation (EU) No 231/2012, 5 and 1 mg/kg, respectively.
- The lowest technically achievable levels of lead and cadmium in commercial pectin products, 2 and 0.5 mg/kg, respectively, as proposed by one interested business operator (IBO; documentation provided to EFSA n. 1), see also Section 3.5.
- The 'modulated' limits calculated by the Panel considering the analytical data as provided by one IBO (documentation provided to EFSA n. 1), see also Section 3.5.
- The maximum permitted use level of pectins E 440i,ii in the final food of 10,000 mg/kg, the maximum use level reported by industry (4,170 mg/kg) and the mean use level reported by industry (3,466 mg/kg) for the uses in food for infants below 16 weeks of age, see Section 3.4
- The range of maximum levels (ML) for lead (0.01–0.05 mg/kg) and cadmium (0.005–0.02 mg/kg) in formulae for infants as laid down in Regulation (EC) No 1881/2006.[23]

The results of the calculations can be found in Tables D.1 and D.2 for lead and Tables D.3 and D.4 for cadmium.

Table D.1: Estimation of the fraction of the concentration of lead in pectin (E 440i) and amidated pectin (E 440ii) with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age)

Specification for toxic elements status	Lead (mg/kg additive)	MPL or Use level of food additive in the formula as consumed i.e. liquid (mg additive/kg food)	Resulting concentration of toxic element in final product (mg lead/kg food)	Maximum level in Reg. 1881/2006 (mg lead/kg liquid formulae)	Fraction of toxic element from FA as %age of final product ML (%)
Current EU specifications	5.0	10,000	0.0500	0.010	500.0
Current EU specifications	5.0	4,170	0.0209	0.010	208.5
Current EU specifications	5.0	3,466	0.0173	0.010	173.3
Proposal IBO	2.0	10,000	0.0200	0.010	200.0
Proposal IBO	2.0	4,170	0.0083	0.010	83.4
Proposal IBO	2.0	3,466	0.0069	0.010	69.3
Modulated*	0.3	10,000	0.0030	0.010	30.0
Modulated*	0.3	4,170	0.0013	0.010	12.5
Modulated*	0.3	3,466	0.0010	0.010	10.4

MPL: maximum permitted level.
*: Analytical values multiplied by a factor of 10 to account for representativeness, homogeneity and analytical measurement uncertainty.

Table D.2: Estimation of the fraction of the concentration of lead in pectin (E 440i) and amidated pectin (E 440ii) with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age)

Specification for toxic elements status	Lead (mg/kg additive)	MPL of food additive in the formula as consumed i.e. liquid (mg additive/kg food)	MPL for the formula in powder form (i.e. considering the dilution*)	Resulting concentration of toxic element in final product (mg lead/kg)	Maximum level in Reg. 1881/2006 (mg lead/kg powder formulae)	Fraction of toxic element from FA as % age of final product ML (%)
Current EU specifications	5.0	10,000	80,000	0.4000	0.050	800.0
Proposal IBO	2.0	10,000	80,000	0.1600	0.050	320.0
Modulated**	0.3	10,000	80,000	0.0240	0.050	48.0

MPL: maximum permitted level.
*: Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 30 May 2018 available online https://zenodo.org/record/1256085#.X89vU9hKiUk.
**: Analytical values multiplied by a factor of 10 to account for representativeness, homogeneity and analytical measurement uncertainty.

Table D.3: Estimation of the fraction of the concentration of cadmium in pectin (E 440i) and amidated pectin (E 440ii) with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age)

Specification for toxic elements status	Cadmium (mg/kg additive)	MPL or Use level of food additive in the formula as consumed i.e. liquid (mg additive/kg food)	Resulting concentration of toxic element in final product (mg cadmium/kg)	Maximum level in Reg. 1881/2006 (mg cadmium/kg liquid formulae)	Fraction of toxic element from FA as % age of final product ML (%)
Current EU specifications	1.0	10,000	0.010	0.005	200.0
Current EU specifications	1.0	4,170	0.004	0.005	83.4
Current EU specifications	1.0	3,466	0.003	0.005	69.3
Proposal IBO	0.5	10,000	0.005	0.005	100.0
Proposal IBO	0.5	4,170	0.002	0.005	41.7
Proposal IBO	0.5	3,466	0.002	0.005	34.7
Modulated*	0.1	10,000	0.001	0.005	20.0
Modulated*	0.1	4,170	0.000	0.005	8.3
Modulated*	0.1	3,466	0.000	0.005	6.9

MPL: maximum permitted level.
*: Analytical values multiplied by a factor of 10 to account for representativeness, homogeneity and analytical measurement uncertainty.

Table D.4: Estimation of the fraction of the concentration of cadmium in pectin (E 440i) and amidated pectin (E 440ii) with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age)

Specification for toxic elements status	Cadmium (mg/kg additive)	MPL of food additive in the formula as consumed i.e. liquid (mg additive/ kg food)	MPL for the formula in powder form (i.e. considering the dilution*)	Resulting concentration of toxic element in final product (mg cadmium/ kg)	Maximum level in Reg. 1881/2006 (mg cadmium/kg powder formulae)	Fraction of toxic element from FA as %age of final product ML (%)
Current EU specifications	1.0	10,000	80,000	0.080	0.020	400.0
Proposal IBO	0.5	10,000	80,000	0.040	0.020	200.0
Modulated**	0.1	10,000	80,000	0.008	0.020	40.0

MPL: maximum permitted level.
*: Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 30 May 2018 available online https://zenodo.org/record/1256085#.X89vU9hKiUk.
**: Analytical values multiplied by a factor of 10 to account for representativeness, homogeneity and analytical measurement uncertainty.

Considering the results of the above estimations and the fact that the food additive is not the only potential source of toxic elements in the final food for infants, the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.