FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Food additives safety assessment

Safety & technology Consultation paper
Proposal P1028—Review of the regulation of infant formula products

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Executive summary

Food Standards Australia New Zealand (FSANZ) has considered the safety of 22 food additives listed as acidity regulators either in the Codex Standard for Infant formula and formulas for special medical purposes intended for infants (CXS 72-1981 (Codex 2016)), or in EU regulations for infant formula and/or infant formula products for special dietary use (IFPSDU) type products. These 22 acidity regulators currently do not have specific permissions in the Code for use as food additives in infant formula.

The 22 acidity regulators can be broadly divided into three groups: (1) calcium carbonates, calcium citrates, and calcium hydroxide; (2) sodium carbonates, sodium hydroxide, potassium carbonates, and potassium hydroxide; and (3) phosphoric acid, sodium phosphates, potassium phosphates, and calcium phosphates.

Each of these food additives have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and with the exception of phosphoric acid are currently listed in the Code as permitted forms of minerals and electrolytes for addition to infant formula. FSANZ has concluded that the use of the proposed acidity regulators does not raise toxicological concerns, provided that:

- the levels of calcium content in infant formula are consistent with the required minimum and recommended maximum set out in S29—9 and S29—10 of the Code, respectively
- limits on sodium, potassium and phosphorus content in infant formula, prescribed in S29—9, are maintained, and
- the ratio of calcium to phosphorus in infant formula is no less than 1.2:1 and no more than 2:1.

FSANZ has also considered the safety of five other food additives with Codex and/or EU permissions for use in infant formula and/or IFPSDU type products. These were citric and fatty acid esters of glycerol (CITREM), used as an emulsifier, and starch sodium octenyl succinate, carob [locust] bean gum, xanthan gum, and pectins, used as emulsifiers or thickeners. JECFA has evaluated the safety of these substances for use in infant formula.

FSANZ concluded that there are no toxicological concerns for CITREM, starch sodium

octenyl succinate and xanthan gum at their proposed maximum levels as considered by JECFA. Clinical studies in formula-fed infants and/or appropriate toxicological studies in experimental animals, including studies in neonatal animals, were available to support the safety of these substances.

JECFA concluded that the available data were not sufficient for the evaluation of carob bean gum for use in infant formula at the proposed use level of 10,000 mg/L. While no serious adverse effects were observed in human infant feeding studies at concentrations of up to 6000 mg/L, these concentrations are lower than the proposed use level and the studies were not designed to evaluate effects on infant gut morphology or health. No studies in neonatal animals were available, therefore a margin of exposure (MOE) could not be calculated to support the safety of carob bean gum in infant formula. No toxicological studies with neonatal animals were found in an updated literature review by FSANZ that would change the conclusions of the JECFA evaluation.

For pectins, JECFA concluded that a maximum use level of 5000 mg/L was of concern, but a reduced maximum level of 2000 mg/L was of low risk for infants. No new information that would change these conclusions was identified by FSANZ.

Submitters to previous rounds of consultation expressed concerns regarding potential adverse effects of formulas thickened with gum-based thickeners. These concerns are based on case reports in the literature suggesting an association between the use of gum-based thickeners and gastrointestinal disorders in infants, including necrotising enterocolitis (NEC). The majority of case reports involve carob bean gum or xanthan gum. FSANZ has reviewed these case reports and other relevant information in the scientific literature. Based on the available data it is not possible to determine if there is a causal association between NEC in infants and xanthan gum, carob bean gum or other thickeners. JECFA has reached the same conclusion in considering these case reports. The US FDA has also noted that further study is needed to determine if there is an actual link between consumption of xanthan gum-based thickener and development of NEC. FSANZ considers that best clinical practice should be followed in feeding premature infants and/or infants with gastro-oesophageal reflux disease or other medical conditions, and that such infants should be under medical supervision.

In conclusion, there are no toxicological concerns associated with the use of the 22 acidity regulators assessed, or CITREM, starch sodium octenyl succinate, xanthan gum at the maximum levels assessed by JECFA. For pectins, there are no concerns at a maximum level of 2000 mg/L, but levels ≥ 5000 mg/L are of concern. The available data are not sufficient to support a conclusion of safety for carob bean gum in infant formula at the proposed use level of 10,000 mg/L.

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1 Introduction

FSANZ is considering whether to harmonise infant formula food additive provisions in the Australia New Zealand Food Standards Code (the Code) with those of Codex or the EU for:

- 22 food additives permitted as acidity regulators either in the Codex Standard for Infant formula and formulas for special medical purposes intended for infants (CXS 72-1981 (Codex 2016)), or in EU regulations for infant formula and/or infant formula products for special dietary use (IFPSDU), and
- five other food additives with Codex and/or EU permissions for use in infant formula and/or IFPSDU. Those were CITREM, used as an emulsifier and starch sodium octenyl succinate, carob [locust] bean gum, xanthan gum, and pectins, used as thickeners.

A safety assessment by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was available for each of the food additives being evaluated. FSANZ has used these JECFA assessments as the basis of its evaluation and conducted a literature review to determine whether there is any new information that would require a revision of the JECFA conclusions. FSANZ has also had regard to the already permitted forms of minerals and electrolytes for addition to infant formula listed in the Code, given that the acidity regulators are all sources of calcium, sodium, potassium or phosphoric acid.

2 Acidity regulators

There are 22 food additives listed as acidity regulators either in the Codex Standard for Infant formula and formulas for special medical purposes intended for infants (CODEX STAN 72-1981) (Codex 2016), or in EU regulations for infant formula and/or IFPSDU, which currently do not have specific permissions in the Code for use as food additives in infant formula. These substances are subject to other permissions in the Code which would allow their use in infant formula, however.

FSANZ is considering amendments to the Code to specifically list these substances as permitted food additives in infant formula or IFPSDU, in order to align with Codex and/or the EU.

The acidity regulators can be broadly split into three groups:

- Calcium carbonates, calcium citrates and calcium hydroxide;
- Sodium carbonates, sodium hydroxide, potassium carbonates and potassium hydroxide; and
- Phosphoric acid, sodium phosphates and potassium phosphates.

2.1 Calcium carbonates (INS 170), calcium citrates (INS 333) and calcium hydroxide (INS 526)

FSANZ is considering permitting calcium carbonates and calcium citrates as food additives (acidity regulators) in IFPSDU at good manufacturing process (GMP) levels to align with the EU, and to permit calcium hydroxide in all infant formula at a maximum permitted level (MPL) of 2000 mg/kg of the product ready for consumption, to align with Codex.

This assessment addresses the following substances: calcium carbonate (INS 170i) and calcium hydrogen carbonate (INS 170ii), monocalcium citrate (INS 333i), dicalcium citrate (INS 333ii), tricalcium citrate (INS 333iii) and calcium hydroxide (INS 526).

2.1.1 Existing permissions in the Food Standards Code

Calcium carbonates, calcium citrates and calcium hydroxide are already permitted forms of minerals for addition to infant formula products, food for infants and food for special medical purposes in Schedule 29—7 of the Code. Each of these substances is also a permitted food additive at GMP in Schedule 16—2 and may be used in infant formula products in Australia and New Zealand as processing aids, if a technological purpose for their use exists.

Calcium hydroxide is a permitted food additive in infant formula at GMP in Schedule 15.

The Code lists minimum and recommended maximum concentrations of calcium in infant formula to ensure appropriate levels of calcium in the diet. It also prescribes a calcium to phosphorus ratio in infant formula and follow-on formula of no less than 1.2:1 and no more than 2:1.

Details on existing permissions for these substances in the Code, Codex and the EU are summarised in Table 1.

Table 1 Current permissions for calcium carbonates, calcium citrates and calcium hydroxide in the Code, Codex and EU regulations

	Calcium carbonates	**Calcium citrates**	**Calcium hydroxide**
The Code			
Infant formula food additive permission and MPL (S 15)	Not listed	Not listed	Infant formula products: GMP
Additive permitted at GMP (S 16-2)*	Yes – listed as calcium carbonates	Yes – listed as calcium citrate	Yes
Generally permitted processing aid (S 18-2)**	Not listed	Not listed	Not listed
Permitted form of mineral/electrolyte in infant formula products, food for infants & food for special medical purposes (S 29-7)	Yes – listed as calcium carbonate	Yes – listed as calcium citrate	Yes
Additional comments	Limits for calcium in infant formula in S29-9 & S29-10: Minimum: 12 mg/100 kJ Recommended maximum: 33 mg/100 kJ Standard 2.9.1-12 states that the ratio of calcium to phosphorus in infant formula must be no less than 1.2:1 and no more than 2:1		
Codex Alimentarius			
Permitted food additive and MPL (CODEX STAN 72-1981)	No permission	No permission	Permitted as an acidity regulator in all types of infant formula (STAN 72-1981) 0.2 g/100 mL of product ready for

	Calcium carbonates	Calcium citrates	Calcium hydroxide
			consumption; singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula
Codex Advisory list of nutrient compounds for use in foods for infants and children (CAC/GL 10-1979)	Yes – listed as calcium carbonate	Yes – listed as Tricalcium dicitrate (Calcium citrate)	Yes
JECFA specification available	Specification available for calcium carbonate; not for calcium hydrogen carbonate	Yes	Yes
Additional comments	Calcium limits in STAN 72-1981: Minimum: 12 mg/100 kJ Guidance upper level: 35 mg/100 kJ Calcium: Phosphorus ratio: Min: 1:1; Max: 2:1		
EU			
Permitted food additive and MPL (Regulation [EC] No 133/2008)	13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. *Quantum satis* (i.e. GMP)	13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. *Quantum satis* (i.e. GMP)	13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. *Quantum satis* (i.e. GMP) *[NB: Only for pH adjustment]*
Permitted form of mineral in infant formula (Regulation [EC] No. 609/2013)	Yes – listed as calcium carbonate	Yes – listed as calcium salts of citric acid	Yes
European Commission specification available?	Specification available for calcium carbonate; not for calcium hydrogen carbonate	Yes	Yes
Additional comments	Calcium limits in EU Regulations: Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates, or soya protein isolates: Minimum: 12 mg/100 kJ Maximum: 33.5 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: Minimum: 12 mg/100 kJ Maximum: 60 mg/100 kJ		

	Calcium carbonates	Calcium citrates	Calcium hydroxide
	Calcium: available phosphorus ratio: Min: 1:1; Max: 2:1		

* An additive permitted at GMP may be used as a processing in aid in any food if it is used at a level necessary to achieve a technological purpose in the processing of that food.

** A substance listed as a generally permitted processing aid in S 18-2 may be used as a processing in aid in any food if it is used at a level necessary to achieve a technological purpose in the processing of that food.

2.1.2 Codex and EU permissions

Calcium carbonates, calcium citrates and calcium hydroxide are permitted forms of calcium in infant formula by Codex (Codex 2015).

Calcium hydroxide is permitted by Codex as an acidity regulator in all types of infant formula (at 0.2 g/100 mL [2000 mg/L] of product ready for consumption; singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium) (Codex 2016).

In the EU, calcium carbonates, calcium citrates and calcium hydroxide are all permitted as forms of calcium for addition to infant formula. They are also permitted to be used as food additives in dietary foods for infants for special medical purposes and special formulas for infants.

2.1.3 Health risk assessments for calcium carbonates, calcium citrates and calcium hydroxide by domestic and international agencies

National Health and Medical Research Council (NHMRC) and New Zealand Ministry of Health (MoH)

The Australian National Health and Medical Research Council (NHMRC) and New Zealand Ministry of Health (MoH) established an Acceptable Intake (AI) for calcium of 210 mg/day for infants aged 0-6 months, and 270 mg/day for infants aged 7-12 months (NHMRC and MoH 2006a). The AI for 0-6 months was set by multiplying together the average intake of breast milk (0.78 L/day) and the average concentration of calcium in breast milk (264 mg/L) from 10 studies. Formula-fed babies require additional intakes in the vicinity of 350 mg/day as calcium is less bioavailable in formula.

An Upper Level of Intake (UL) for calcium could not be established for infants aged 0-12 months. For all other age groups, a UL of 2500 mg/day has been set.

Calcium carbonates

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Calcium carbonate was evaluated for use as a food additive by JECFA at its ninth meeting, together with a range of other bases used for pH adjustment (WHO 1966). The Committee considered that the amounts and concentrations used are not likely to be of toxicological significance. No restrictions were placed on the food additive uses of the bases evaluated, provided that the contribution to the dietary load of calcium is assessed and considered acceptable. An acceptable daily intake (ADI) ‘not limited’ was established.

The 15th JECFA meeting, which included consideration of additives in baby foods (WHO 1972), found that a variety of chemical compounds are available that are suitable for use as pH regulators in foods for infants and young children. JECFA commented that calcium intake

must be considered in relation to the level of phosphates in the diet. JECFA noted there is a wide latitude for dietary variations in calcium content without toxicological effects, and that calcium from food additives is unlikely to make a substantial impact on total intake. It was agreed that the calcium:phosphorus ratio in a baby food should not be less than 1:1.2, and this provision should be made to apply at least to infant formulas and cereal-based baby foods.

Calcium hydrogen carbonate was evaluated along with several other anions and cations at the 29th JECFA meeting (WHO 1986). The Committee agreed with the previous evaluation in placing no restrictions on the use of calcium cations, provided that their contribution to the diet is assessed and considered acceptable. In the case of calcium salts, the desirability of maintaining nutritionally sound ratios of calcium and phosphorus in the diet was reinforced.

European Scientific Committee on Food (SCF)

The Scientific Committee on Food (SCF) assessed the safety of calcium carbonate as a food additive in an opinion on a range of acids, bases and their salts (SCF 1991). The Committee concluded that calcium and carbonate are natural constituents of man, animals and plants and therefore occur in foods. A group ADI 'not specified' was established. It was noted that no safety problems are likely to arise, provided the contributions from food intake do not disturb the homeostatic mechanisms controlling the electrolyte balance of the body.

European Food Safety Authority (EFSA)

The European Food Safety Authority (EFSA) re-evaluated calcium carbonate as a food additive in 2011 (EFSA 2011). This review did not include an evaluation of the risks for infants under 16 weeks of age. EFSA is currently undertaking a review of food additives approved for use in infant formula for babies under 16 weeks old, including calcium carbonate.

EFSA agreed with the ADI 'not specified' previously established by the European SCF in 1991 for a group of carbonates including calcium carbonate. It was noted that intakes of calcium should be below the European Tolerable Upper Intake Level (UL) of 2500 mg/day for calcium of all sources, which was determined by the SCF in 2003 (SCF 2003).

Calcium citrates

JECFA

JECFA evaluated calcium citrate as well as the potassium and sodium salts of citric acid at its 17th meeting (WHO 1974). The Committee noted that citric acid is an intermediary substance in oxidative metabolism, as part of the tricarboxylic acid cycle (also referred to as the Krebs cycle). Citric acid and citrates occur in many foods and are normal metabolites in the body. The Committee also observed that potassium and sodium citrate in doses of up to 4 g have been extensively used in medical practice for many years without adverse effects. Calcium citrate was considered likely to behave similarly in the body. It was concluded that calcium citrate, as well as the potassium and sodium salts of citric acid do not constitute a significant toxicological hazard to humans. An ADI 'not limited' was established.

At its 79th meeting, JECFA also considered the safety of dietary exposure to citric acid from infant formula as part of its evaluation of citric and fatty acid esters of glycerol (CITREM; containing approximately 13-50% citric acid) (WHO 2014, 2015). The evaluation considered available data relating to the potential for free citric acid in the gut to cause diarrhoea. No effects were observed in one study of 13 infants exposed to citric acid (added as citrate salts) in cows' milk formula at doses of approximately 500 mg/kg bw/day. In another study,

diarrhoea was observed in four of eight infants given free citric acid by gavage (vehicle unspecified) in divided doses over 24 hours at total doses (from food plus dosed citric acid) of approximately 400–700 mg/kg bw per day. JECFA noted that the diarrhoea in this study may have been due to osmolality and the gavage mode of administration, and that citrate salts have been used successfully in oral rehydration solutions for the treatment of diarrhoea in infants.

Based on typical and high maximum concentrations of CITREM added to formula (2.7 and 9 g/L reconstituted formula, respectively), the Committee concluded that a citric acid exposure of 440 mg/kg bw/day (estimated from a CITREM concentration of 2.7 g/L) in infants was unlikely to cause diarrhoea. Exposure to citrate of 1140 mg/kg bw/day (estimated from a CITREM concentration of 9 g/L) might result in diarrhoea in some infants, but the risk was considered to be low.

FSANZ notes that the concentrations of citric acid in infant formula from use of calcium citrate as a food additive at an MPL of GMP would be expected to be lower than the concentrations of citric acid from use of CITREM at 9 g/L (equivalent to up to 4.5 g/L citric acid), for which the risk of diarrhoea was considered, by JECFA, to be low.

European SCF

The SCF assessed the safety of calcium citrate as a food additive as part of an opinion on a range of acids, bases and their salts (SCF 1991). The Committee concluded that calcium and citrate are natural constituents of man, animals and plants and therefore occur in foods. A group ADI ‘not specified’ was established. It was noted that no safety problems are likely to arise, provided the contributions from food intake do not disturb the homeostatic mechanisms controlling the electrolyte balance of the body.

Calcium hydroxide

JECFA

Calcium hydroxide was evaluated by JECFA at the ninth meeting, together with a range of other bases used for pH adjustment (WHO 1966). JECFA noted that the amounts and concentrations used are unlikely to be of any toxicological significance. No restrictions were placed on the food additive uses of the bases evaluated, provided that the contribution to the dietary load of calcium is assessed and considered acceptable. An ADI ‘not limited’ was established.

European SCF

The SCF assessed the safety of calcium hydroxide as a food additive as part of an opinion on a range of acids, bases and their salts (SCF 1991). The Committee concluded that calcium is a natural constituent of man, animals and plants and therefore occurs in foods. A group ADI ‘not specified’ was established. It was noted that no safety problems are likely to arise, provided the contributions from food intake do not disturb the homeostatic mechanisms controlling the electrolyte balance of the body.

2.1.4 Discussion and conclusions

FSANZ considers that permitting calcium carbonates and calcium citrates as food additives (acidity regulators) in IFPSDU at GMP, and calcium hydroxide in all infant formula at an MPL of 2000 mg/kg, does not pose toxicological concerns. Calcium carbonates, calcium citrates and calcium hydroxide have been evaluated as food additives by JECFA. The Committee noted that there is a wide latitude for dietary variations in calcium content without

toxicological effects, and established an ADI of 'not specified' for all three food additives.

An ADI not specified is established for compounds of very low toxicity that are not considered to represent a risk to health based on current usage levels.

JECFA also considered the safety of dietary exposure to citric acid from infant formula as part of its evaluation of citric and fatty acid esters of glycerol. While there was some evidence of diarrhoea in infants at high doses, the risks are low at the levels at which calcium citrate will be used as an acidity regulator.

Calcium carbonates, calcium citrates and calcium hydroxide are also already permitted forms of minerals for addition of calcium to infant formula products, food for infants and food for special medical purposes in Schedule 29—7. Therefore their use as food additives does not raise additional toxicological concerns provided that acceptable total levels of calcium in the diet and nutritionally appropriate ratios of calcium to phosphorus ratio are maintained.

At the proposed MPL for calcium hydroxide of 2000 mg/kg, the recommended maximum level of calcium set out in S29—10 could be exceeded slightly (~108 mg/100 mL versus ~97 mg/100 mL, based on the proposed maximum energy content of 295 kJ/100 mL infant formula ready for consumption). It is not anticipated that this slight exceedance would be of toxicological significance.

2.2 Sodium carbonates (INS 500), sodium hydroxide (INS 524), potassium carbonates (INS 501) and potassium hydroxide (INS 525)

FSANZ is considering whether to permit the use of sodium carbonate (INS 500i), sodium hydrogen carbonate (INS 500ii), sodium hydroxide (INS 524), potassium carbonate (INS 501i), potassium hydrogen carbonate (INS 501ii) and potassium hydroxide (INS 525) as food additives (acidity regulators) in infant formula at an MPL of 2000 mg/kg of the product ready for consumption, to align with Codex.

Details on existing permissions for these substances in the Code, Codex and the EU are summarised in Table 2.

Table 2 Current permissions for sodium carbonates, sodium hydroxide, potassium carbonate and potassium hydroxide in the Code, Codex and EU regulations

	Sodium carbonates	**Sodium hydroxide**	**Potassium carbonates**	**Potassium hydroxide**
The Code				
Infant formula food additive permission and MPL (S 15)	Not listed	Not listed	Not listed	Not listed
Additive permitted at GMP (S 16-2)*	Yes	Not listed	Yes	Not listed
Generally permitted processing aid (S 18-2)**	Not listed	Yes	Not listed	Yes

	Sodium carbonates	**Sodium hydroxide**	**Potassium carbonates**	**Potassium hydroxide**
Permitted form of mineral/electrolyte in infant formula products, food for infants & food for special medical purposes (S 29-7)	Yes	Yes	Yes	Yes
Additional comments	Limits on sodium and potassium content in infant formula listed in S 29-9: Sodium: Minimum: 5 mg/100 kJ Maximum: 15 mg/100 kJ Potassium: Minimum: 20 mg/100 kJ Maximum: 50 mg/10 kJ			
Codex Alimentarius				
Permitted food additive and MPL (CODEX STAN 72-1981)	Permitted as acidity regulators in all types of infant formula (STAN 72-1981) 0.2 g/100 mL of product ready for consumption; singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula	Permitted as acidity regulators in all types of infant formula (STAN 72-1981) 0.2 g/100 mL of product ready for consumption; singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula	Permitted as acidity regulators in all types of infant formula (STAN 72-1981) 0.2 g/100 mL of product ready for consumption; singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula	Permitted as acidity regulators in all types of infant formula (STAN 72-1981) 0.2 g/100 mL of product ready for consumption; singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula
Codex Advisory list of nutrient compounds for use in foods for infants and children (CAC/GL 10-1979)	Yes – sodium carbonate and sodium hydrogen carbonate (sodium bicarbonate) listed	Yes	Yes – potassium carbonate and potassium hydrogen carbonate (potassium bicarbonate) listed	Yes
JECFA specification available	Yes	Yes	Yes	Yes

	Sodium carbonates	Sodium hydroxide	Potassium carbonates	Potassium hydroxide
Additional comments	Limits in STAN 72-1981: <u>Sodium</u> Minimum: 5 mg/100 kJ Maximum: 14 mg/100 kJ <u>Potassium</u> Minimum: 14 mg/100 kJ Maximum: 43 mg/100 kJ			
EU				
Permitted food additive and MPL (Regulation [EC] No 133/2008)	Not listed	13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. Quantum satis (GMP) *[NB: Only for pH adjustment]*	Not listed	13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. Quantum satis (GMP) *[NB: Only for pH adjustment]*
Permitted form of mineral in infant formula (Regulation [EC] No. 609/2013)	Yes – listed as sodium carbonate	Yes	Yes – listed as potassium carbonate	Yes
European Commission specification available	Yes	Yes	Yes	Yes
Additional comments	Limits in EU Regulations: <u>Sodium</u> Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates, or soya protein isolates: Minimum: 6 mg/100 kJ Maximum: 14.3 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: as above <u>Potassium</u> Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates, or soya protein isolates: Minimum: 19.1 mg/100 kJ Maximum: 38.2 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: as above			

* An additive permitted at GMP may be used as a processing in aid in any food if it is used at a level necessary to achieve a technological purpose in the processing of that food.

** A substance listed as a generally permitted processing aid in S 18-2 may be used as a processing in aid in any food if it is used at a level necessary to achieve a technological purpose in the processing of that food.

2.2.1 Existing permissions in the Food Standards Code

Sodium carbonates, sodium hydroxide, potassium carbonates and potassium hydroxide are all permitted forms of sodium or potassium for addition to infant formula products, food for infants and food for special medical purposes in Schedule 29—7 of the Code.

In addition, sodium carbonates and potassium carbonates are permitted food additives at GMP in Schedule 16—2, while sodium hydroxide and potassium hydroxide are generally permitted processing aids in Schedule 18—2. They may therefore be used as processing aids in infant formula products in Australia and New Zealand if a technological purpose for their use exists.

The Code lists minimum and maximum concentrations of sodium and potassium in infant formula in Schedule 29—9.

2.2.2 Codex and EU permissions

Sodium carbonates, sodium hydroxide, potassium carbonates and potassium hydroxide are permitted forms of sodium or potassium in infant formula by Codex (Codex 2015).

In addition, these substances are permitted by Codex for use as acidity regulators in infant formula at concentrations up to 0.2 g/100 mL (2000 mg/L) of product ready for consumption, singly or in combination with a range of other acidity regulators and within the limits for sodium, potassium and calcium (Codex 2016).

In the EU, sodium carbonate, sodium hydroxide, potassium carbonate and potassium hydroxide are permitted forms of sodium and potassium for addition to infant formula. In addition, sodium hydroxide and potassium hydroxide are permitted to be used as food additives in dietary foods for infants for special medical purposes and special formulas for infants.

2.2.3 Health risk assessments for sodium carbonates, sodium hydroxide, potassium carbonate and potassium hydroxide by domestic and international agencies

NHMRC and MoH

An AI for sodium of 120 mg/day has been established for infants aged 0-6 months by the NHMRC and New Zealand MoH, and an AI of 170 mg/day has been established for infants aged 7-12 months. A UL for sodium could not be established for infants, but it is noted that the source of intake for infants should be through breast milk, formula and food only. A UL of 1000 mg/day for sodium has been established for children aged 1-3 years (NHMRC and MoH 2017).

For potassium, AIs of 400 mg/day and 700 mg/day have been set for infants aged 0-6 months and infants aged 7-12 months, respectively. No ULs have been set for potassium from dietary sources for any age group, although it is recommended that infants' intake should be limited to potassium occurring in breast milk, formula and complementary foods (NHMRC and MoH 2006c).

JECFA

JECFA reviewed the safety of carbonates, hydrogen carbonates and hydroxides of sodium and potassium used for pH adjustment at its ninth meeting (WHO 1966). The Committee noted that the amounts and concentrations used are not likely to have any toxicological significance. No restrictions on the food additive uses of these substances were proposed, provided that the contribution made to the dietary load of sodium and potassium is assessed and considered acceptable. ADI's 'not limited' were established for these substances.

The 15th JECFA meeting included consideration of additives in baby foods, which noted that a variety of chemical compounds are available that are suitable for use as pH regulators in foods for infants and young children (WHO 1972). JECFA observed that sodium is a necessary nutrient in infant formula.

SCF

The SCF assessed the safety of sodium carbonate, sodium hydroxide, potassium carbonate and potassium hydroxide as food additives in an opinion on a range of acids, bases and their salts (SCF 1991). The Committee concluded that these substances are natural constituents of man, animals and plants and therefore occur in foods. A group ADI 'not specified' was established. It was noted that no safety problems are likely to arise, provided the contributions from food intake do not disturb the homeostatic mechanisms controlling the electrolyte balance of the body.

2.2.4 Discussion and conclusions

Sodium carbonates, sodium hydroxide, potassium carbonates and potassium hydroxide were assessed by JECFA at its ninth meeting. The Committee established ADIs 'not specified' for all of these additives.

Sodium carbonates, sodium hydroxide, potassium carbonates and potassium hydroxide are also already permitted forms of minerals for addition of sodium and potassium to infant formula products, food for infants and food for special medical purposes in Schedule 29—7. Therefore their use as food additives does not raise additional toxicological concerns provided that limits on sodium and potassium content in infant formula, prescribed in S29—9 are maintained.

FSANZ notes that at the proposed MPL for sodium carbonates and sodium hydroxide of 2000 mg/kg, it is possible that the maximum level of sodium set out in S29—9 could be exceeded. For example, based on the proposed maximum energy content of 295 kJ/100 mL in formula ready for consumption, use of sodium hydroxide at the MPL would be expected to result in a sodium concentration of approximately 114 mg/100 mL, compared with a permitted maximum of approximately 44 mg/100 mL. Maximum potassium levels would not be expected to be exceeded at the proposed levels.

2.3 Phosphoric acid (INS 338), sodium phosphates (INS 339), potassium phosphates (INS 340) and calcium phosphates (INS 341)

FSANZ is considering permitting the use of phosphoric acid, sodium phosphates, potassium phosphates and calcium phosphates as food additives (acidity regulators) in infant formula at a concentration of 450 mg/kg as phosphorus in the product ready for consumption. This would align with the EU permission for all of these substances and the Codex permissions for sodium phosphates and potassium phosphates.

The following substances are addressed in this section: phosphoric acid (INS 338), sodium

dihydrogen phosphate (INS 339i), disodium hydrogen phosphate (INS 339ii), trisodium phosphate (INS 339iii), potassium dihydrogen phosphate (INS 340i), dipotassium hydrogen phosphate (INS 340ii), tripotassium phosphate (INS 340iii), calcium dihydrogen phosphate (INS 341i), calcium hydrogen phosphate (INS 341ii) and tricalcium phosphate (INS 341iii).

Details on existing permissions for these substances in the Code, Codex and the EU are summarised in Table 3.

Table 3 Current permissions for phosphoric acid, sodium phosphates, potassium phosphates and calcium phosphates in the Code, Codex and EU regulations

	Phosphoric acid	**Sodium phosphates**	**Potassium phosphates**	**Calcium phosphates**
The Code				
Infant formula food additive permission and MPL (S 15)	Not listed	Not listed	Not listed	Not listed
Additive permitted at GMP (S 16-2)	Not listed	Yes – listed as sodium phosphates	Yes – listed as potassium phosphates	Yes – listed as calcium phosphates
Generally permitted processing aid (S 18-2)	Yes	Not listed	Not listed	Not listed
Permitted form of mineral/electrolyte in infant formula products, food for infants & food for special medical purposes (S 29-7)	Not listed	Yes – listed sources of phosphorus & sodium	Yes –listed sources of phosphorus & sodium	Yes – listed sources of phosphorus & calcium
Additional comments	Limits on phosphorus, sodium, potassium and calcium in infant formula listed in S 29-9: <u>Phosphorus</u> Minimum: 6 mg/100 kJ Maximum: 25 mg/100 kJ Limits on sodium and potassium content in infant formula listed in S 29-9: <u>Sodium</u> Minimum: 5 mg/100 kJ Maximum: 15 mg/100 kJ <u>Potassium</u> Minimum: 20 mg/100 kJ Maximum: 50 mg/100 kJ <u>Calcium</u> Minimum: 12 mg/100 kJ Recommended maximum: 33 mg/100 kJ			

	Phosphoric acid	Sodium phosphates	Potassium phosphates	Calcium phosphates
	Standard 2.9.1-12 states that the ratio of calcium to phosphorus in infant formula must be no less than 1.2:1 and no more than 2:1			
Codex Alimentarius				
Permitted food additive and MPL (CODEX STAN 72-1981)	No permission	Permitted as an acidity regulator in all types of infant formula (STAN 72-1981) 45 mg/100 mL of product ready for consumption as phosphorus, singly or in combination and within the limits for sodium, potassium and phosphorus in section 3.1.3 (e)	Permitted as an acidity regulator in all types of infant formula (STAN 72-1981) 45 mg/100 mL of product ready for consumption as phosphorus, singly or in combination and within the limits for sodium, potassium and phosphorus in section 3.1.3 (e)	No permission
Codex Advisory list of nutrient compounds for use in foods for infants and children (CAC/GL 10-1979)	Not listed (does not include sources of phosphorus)	Yes – listed sources of sodium	Yes – listed sources of potassium	Yes – listed sources of calcium
JECFA specification available	Yes	Yes	Yes	Yes
Additional comments	Limits in STAN 72-1981: <u>Phosphorus</u> Minimum: 6 mg/100 kJ Guidance upper level: 24 mg/100 kJ *[NB: this GUL should accommodate higher needs with soy formula]* <u>Sodium</u> Minimum: 5 mg/100 kJ Maximum: 14 mg/100 kJ <u>Potassium</u> limits Minimum: 14 mg/100 kJ Maximum: 43 mg/100 kJ <u>Calcium</u> Minimum: 12 mg/100 kJ Guidance upper level: 35 mg/100 kJ Calcium: Phosphorus ratio: Min: 1:1; Max: 2:1			

	Phosphoric acid	Sodium phosphates	Potassium phosphates	Calcium phosphates
EU				
Permitted food additive and MPL	13.1.1 Infant formula as defined by Directive 2006/141/EC 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. 1000 mg/L, expressed as P_2O_5 (equivalent to ~440 mg/L P) [NB: within limits for minerals set out in EU Regulations]	13.1.1 Infant formula as defined by Directive 2006/141/EC 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. 1000 mg/L, expressed as P_2O_5 [NB: singly or in combination within limits for minerals set out in EU Regulations]	13.1.1 Infant formula as defined by Directive 2006/141/EC 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. 1000 mg/L, expressed as P_2O_5 [NB: singly or in combination within limits for minerals set out in EU Regulations]	13.1.1 Infant formula as defined by Directive 2006/141/EC 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. 1000 mg/L, expressed as P_2O_5 [NB: singly or in combination within limits for minerals set out in EU Regulations]
Permitted form of mineral in infant formula (Regulation [EC] No. 609/2013)	Not listed (does not include sources of phosphorus)	Yes – listed as sodium salts of orthophosphoric acid (source of sodium)	Yes – listed as potassium salts of orthophosphoric acid (source of potassium)	Yes – listed as calcium salts of orthophosphoric acid (source of calcium)
European Commission specification available	Yes	Yes	Yes	Yes
Additional comments	Limits in EU Regulations: <u>Phosphorus</u> Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates: Minimum: 6 mg/100 kJ Maximum: 21.5 mg/100 kJ Infant formula or Follow-on formula based on soy: Minimum: 7.2 mg/100 kJ Maximum: 24 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: Minimum: 6 mg/100 kJ Maximum: 24 mg/100 kJ			

	Phosphoric acid	Sodium phosphates	Potassium phosphates	Calcium phosphates
	Calcium:available phosphorus ratio: Min: 1:1; Max: 2:1 <u>Sodium</u> Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates, or soya protein isolates: Minimum: 6 mg/100 kJ Maximum: 14.3 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: as above <u>Potassium</u> Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates, or soya protein isolates: Minimum: 19.1 mg/100 kJ Maximum: 38.2 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: as above <u>Calcium</u> Infant formula or Follow-on formula manufactured from cows' milk or goats' milk proteins or protein hydrolysates, or soya protein isolates: Minimum: 12 mg/100 kJ Maximum: 33.5 mg/100 kJ Food for special medical purposes developed to satisfy the nutritional requirements of infants: Minimum: 12 mg/100 kJ Maximum: 60 mg/100 kJ Calcium: available phosphorus ratio: Min: 1:1; Max: 2:1			

* An additive permitted at GMP may be used as a processing in aid in any food if it is used at a level necessary to achieve a technological purpose in the processing of that food.

** A substance listed as a generally permitted processing aid in S 18-2 may be used as a processing in aid in any food if it is used at a level necessary to achieve a technological purpose in the processing of that food.

2.3.1 Existing permissions in the Food Standards Code

Sodium phosphates, potassium phosphates and calcium phosphates are permitted forms of minerals and electrolytes for addition to infant formula products, food for infants and food for special medical purposes in Schedule 29—7 of the Code. Phosphoric acid is not a permitted form in the Code.

Sodium phosphates, potassium phosphates and calcium phosphates are permitted food additives at GMP in Schedule 16—2, and phosphoric acid is a generally permitted processing aid in Schedule 18—2. They may be used in infant formula products in Australia and New Zealand as processing aids if a technological purpose for their use exists.

The Code lists minimum and maximum concentrations of sodium, potassium and

phosphorus in infant formula, minimum and guideline maximum amounts of calcium and prescribes a calcium to phosphorus ratio in infant formula and follow-on formula.

2.3.2 Codex and EU permissions

Sodium phosphates, potassium phosphates and calcium phosphates are also permitted by Codex as forms of sodium, potassium and calcium in infant formula (Codex 2015). In addition, sodium phosphates and potassium phosphates are permitted by Codex as acidity regulators in all types of infant formula at 45 mg/100 mL (450 mg/L) of product ready for consumption as phosphorus; singly or in combination and within established limits for sodium, potassium and phosphorus (Codex 2016).

In the EU, sodium phosphates, potassium phosphates and calcium phosphates are permitted forms of sodium, potassium and calcium for addition to infant formula. Phosphoric acid, sodium phosphates, potassium phosphates and calcium phosphates are all permitted in the EU for use as food additives in infant formula and dietary foods for infants for special medical purposes and special formulas for infants. The MPL is 1000 mg/L, expressed as P_2O_5 (equivalent to ~440 mg/L phosphate), and within the limits for minerals set out in EU Regulations.

2.3.3 Health risk assessments for phosphoric acid, sodium phosphates, potassium phosphates and calcium phosphates by domestic and international agencies

NHMRC and MoH

AIs for phosphorus of 100 mg/day and 275 mg/day have been established for infants aged 0-6 months and 7-12 months, respectively. It was not possible to establish a UL for infants but it is noted that phosphorus intake for infants should be only be through naturally occurring food sources and formula. A UL of 3000 mg/day for phosphorus has been established for children aged 1-3 years (NHMRC and MoH 2006b).

As noted above, AIs for infants aged 0-6 and 7-12 months have been established for sodium, potassium and calcium, but no ULs have been identified for these age groups (NHMRC and MoH 2006c, 2017).

JECFA

Phosphoric acid and a range of phosphate salts including sodium phosphates, potassium phosphates and calcium phosphates were reviewed by JECFA at its 26th meeting (WHO 1982). The Committee noted that metabolically, phosphate salts provide a source of the various cations and the phosphate ion. Free monophosphate is the major form in which phosphorus is absorbed from the diet, and the amount and rate at which other phosphates are absorbed depends on their enzymatic hydrolysis to monophosphates.

Phosphate salts were not mutagenic in a number of test systems and teratogenic effects have not been observed in mammalian studies. Animal studies have shown that excessive dietary phosphorus causes an increase of plasma phosphorus and a decrease in serum calcium. The reduced calcium stimulates secretion of parathyroid hormone, which increases the rate of bone resorption and decreases calcium excretion.

These changes may result in bone loss and calcification of soft tissues, in particular the kidney, in animal studies, most of which were with rats. However, JECFA noted that the rat is very sensitive to calcification and hydronephrosis upon exposure to acids forming calcium chelates or complexes, and that the doses of phosphate producing nephrocalcinosis were not consistent among the available rat feeding studies. The lowest doses producing

nephrocalcinosis overlapped with the highest doses that did not induce this effect. JECFA commented that there is uncertainty over the optimum calcium:phosphorus ratio and whether this ratio is of any dietary significance in man.

JECFA established a maximum tolerable daily intake (MTDI) of 70 mg/kg bw/day for phosphates. The MTDI is expressed as phosphorus and applies to the sum of phosphates naturally present in food and from the food additives evaluated. The MTDI was based on the lowest level of phosphate that produced nephrocalcinosis in the rat (1% in the diet), extrapolated on the basis of a daily food intake of 2800 calories. This was considered the best estimate of the lowest level that might conceivably cause nephrocalcinosis in man. The Committee noted that the MTDI applies to diets nutritionally adequate in respect of calcium. If the calcium intake were high, the intake of phosphate could be proportionately higher, and vice versa.

JECFA discussed additives in baby foods at its 15th meeting and noted that a variety of chemical compounds are available that are suitable for use as pH regulators in foods for infants and young children (WHO 1972). JECFA noted that the calcium:phosphorus ratio in a baby food should not be less than 1:1.2, and this provision should be made to apply at least to infant formulas and cereal-based baby foods. It was noted that the contribution of the phosphate load from the carry-over of phosphates used as additives in certain ingredients of infant formulas should be allowed for when calculating the calcium:phosphorus ratio in infant foods.

At its 29th meeting, JECFA evaluated magnesium dihydrogen diphosphate as a food additive (WHO 2002). As part of its assessment, JECFA noted that information submitted to the Committee and in the scientific literature did not indicate that the MTDI for phosphates is insufficiently health protective. It was noted that the basis for its derivation, nephrocalcinosis in the rat, might not be relevant to humans as rats are particularly sensitive to mineralisation in the kidneys from an imbalance of dietary calcium and phosphate. JECFA concluded that the MTDI could be overly conservative and indicated a need to review the toxicological basis of the MTDI for phosphate salts expressed as phosphorus. To date the MTDI has not been reviewed.

EU SCF

The SCF reviewed phosphates as food additives in 1991 (SCF 1991). The Committee agreed with the JECFA MTDI of 70 mg/kg bw for man, calculated as phosphorus, as the sum of phosphates naturally present in food and derived from additives in diets nutritionally adequate in respect of calcium.

EFSA

EFSA published a re-evaluation of the safety of phosphates as food additives in 2019 (EFSA 2019). EFSA considered phosphates to be of low acute oral toxicity and expressed no concern for genotoxicity and carcinogenicity, as well as developmental and reproductive toxicity. In repeated dose toxicity studies the only significant adverse effect of phosphates was calcification of the kidney and tubular nephropathy. Renal impairment was also reported in some human studies. EFSA derived a group ADI for phosphates expressed as phosphorus of 40 mg/kg bw/day, based on a no observed adverse effect level (NOAEL) of 167 mg/kg bw/day phosphate in rats and applying a chemical-specific adjustment factor of 4.

With respect to infants under the age of 16 weeks, EFSA noted that plasma levels of phosphate are two-fold higher in the first 6 months of life compared with adult plasma levels. Clinical studies in infants consuming either standard formula or food for special medical purposes with added phosphates demonstrated that growth is similar to WHO growth

standards. In addition, a clinical study with infant formula of high phosphorus content did not find any significant increase of serum inorganic phosphate, which was still within the normal range. EFSA concluded that the available data do not give rise to safety concerns in infants below 16 weeks of age consuming formula and food for medical purposes.

2.3.4 Discussion and conclusions

JECFA established a group MTDI for phosphorus from all sources of 70 mg/kg bw/day, expressed as phosphorus, at its 26th meeting. The MTDI was established on the basis of findings of nephrocalcinosis in studies in rats. EFSA re-evaluated the use of phosphates as food additives in 2019, and concluded that based on the available data their use did not give rise to safety concerns in infants below 16 weeks of age consuming formula and food for special medical purposes.

Sodium phosphates, potassium phosphates and calcium phosphates are currently permitted forms of electrolytes for addition to infant formula products, food for infants and food for special medical purposes in Schedule 29—7 of the Code. Phosphoric acid is not currently permitted but it is included in the JECFA MTDI that applies to phosphoric acid and phosphate salts. Therefore the of use phosphoric acid, sodium phosphates, potassium phosphates and calcium phosphates as food additives does not raise additional toxicological concerns provided:

- the levels of calcium content in infant formula are consistent with the required minimum and recommended maximum set out in S29—9 and S29—10 of the Code
- that limits on sodium, potassium and phosphorus content in infant formula, prescribed in S29—9 are maintained, and
- that the ratio of calcium to phosphorus in infant formula must be no less than 1.2:1 and no more than 2:1

The maximum limit for phosphorus or calcium is not expected to be exceeded, but at the proposed MPL for sodium phosphates and potassium phosphates of 450 mg/kg as phosphorus, the maximum levels of sodium or potassium set out in S29—9 could be exceeded. For example, based on the proposed maximum energy content of 295 kJ/100 mL in formula ready for consumption, use of trisodium phosphate at the MPL would be expected to result in a sodium concentration of approximately 100 mg/100 mL, compared with a permitted maximum of approximately 44 mg/100 mL. Use of tripotassium phosphate at the MPL may result in a potassium concentration of approximately 171 mg/100 mL, compared with a permitted maximum of approximately 147.5 mg/100 mL.

3 Citric and fatty acid esters of glycerol (CITREM; INS 472c)

Citric acid and fatty acid esters of glycerol (CITREM) is currently listed in Schedule 15 of the Code for use in infant formula products for specific dietary use based on a protein substitute, at an MPL of 9000 mg/L. These food additives are also permitted at GMP in section S16-2 of the Code.

3.1 Codex permissions

Codex recently listed CITREM for use as an emulsifier in all types of powdered and liquid infant formula with MPLs of 7500 mg/L and 9000 mg/L, respectively (Codex 2016). Listing in CODEX STAN 72-1981 followed a safety evaluation of these food additives by JECFA at its 79th meeting (WHO 2014, 2015). CITREM is also listed in the Codex General Standard for

Food Additives for use in complementary foods for infants and young children at a maximum level of 5000 mg/kg (Codex 2018).

3.2 JECFA evaluation of CITREM

Prior to the 79th meeting, CITREM had been reviewed by JECFA at its 17th, 35th and 61st meetings. An ADI 'not specified' was established in 1973, based on biochemical and metabolic studies showing that CITREM is completely hydrolysed in the gastrointestinal tract into components that are normal constituents of the diet, together with information on the metabolism and lack of toxicity of citric acid, glycerol and fatty acid esters of glycerol (WHO 1974).

At its 79th meeting, JECFA evaluated the safety of CITREM for use as an emulsifier in infant formula and formula for special medical purposes intended for infants. CITREM was intended to replace the combined use of three emulsifiers, lecithin (INS 322), monoglycerides and diglycerides of fatty acids (INS 471) and diacetyl tartaric acid ester of monoglycerides and diglycerides (INS 472e).

Proposed use levels considered by JECFA were up to 7500 mg/L as consumed in reconstituted infant formula powder and up to 9000 mg/L in ready-to-feed liquid infant formula.

3.2.1 Chemical and technical considerations

CITREM is a white to ivory coloured, oily to waxy material obtained by esterification of glycerol with citric acid and food-grade fatty acids, or by reacting a mixture of monoglycerides and diglycerides of food-grade fatty acid with citric acid. It is primarily composed of glycerol (8-33%), fatty acids (37-81%) and citric acid (13-50%). JECFA observed that it could contain up to 4% free glycerol and minor amounts of free fatty acids, free citric acid and monoglycerides and diglycerides.

JECFA noted that the fatty acid moieties present in CITREM have chain lengths that most commonly range from C12 to C22. CITREM can be manufactured from any edible oil, and the profile distribution of the fatty acids will vary depending on the fat/oil source.

At the 79th meeting, JECFA observed that if lead was present in CITREM at the maximum limit permitted in its specification (2 mg/kg) this could result in exceedance of the maximum limit for lead in infant formula (as consumed) of 0.01 mg/kg established by the Codex Committee on Contaminants in Foods (Codex 2014). The Committee considered that introduction of a lead limit of 0.5 mg/kg would ensure that use of CITREM at the maximum use level would not result in the maximum level of lead in the final infant formula being exceeded.

Data on the levels of lead present in CITREM were reviewed at the 82nd JECFA meeting. The current limit for lead in CITREM was maintained at 2 mg/kg for general use, and a limit of 0.5 mg/kg was introduced for use in infant formula (WHO 2016).

The specification for CITREM was further reviewed at the 86th and 87th JECFA meetings.

3.2.2 Toxicokinetics and metabolism

JECFA reviewed an *in vitro* study on the digestibility of CITREM, and a CITREM-containing infant formula, using a two-stage model with different pH values and bile salt concentrations as a means of simulating the preterm and term human infant stomach and duodenum. Extensive (but not complete) hydrolysis of CITREM by gastric and pancreatic lipases was

seen under the conditions tested. When CITREM in infant formula was tested, however, hydrolysis of CITREM into its components was lower than expected, in the range of 14-28%.

Further investigations found that hydrolysis of pure citric and fatty acid esters of glycerol (i.e. the main components of CITREM with negligible glycerol, free citric acid, free fatty acids or free glycerides) was about two-fold higher (47-58%) than for CITREM in infant formula.

JECFA considered that this finding indicates a negative effect of free glycerides on the action of lipases and that, *in vivo*, hydrolysis of CITREM is likely to continue lower down the small intestine as the glycerides and free fatty acids from breakdown of the fats in infant formula and CITREM form micelles and are absorbed by enterocytes. This absorption of micelle contents occurs mainly in the proximal jejunum, which was not simulated in the experimental model. The model also did not include lingual lipase which would also contribute to hydrolysis.

JECFA concluded that CITREM was likely to be substantially hydrolysed in the gastrointestinal tract *in vivo*, and that any partially hydrolysed products such as glycerol citric acid esters would not be of safety concern.

3.2.3 Toxicological data

Previous JECFA evaluations of CITREM identified very few toxicological studies, and the only new information available at the 79th meeting was an *in vitro* bacterial mutagenicity study which was negative, and a short-term study on the effects of CITREM on fat absorption in the rat, which was not considered useful to the evaluation because a very high amount of CITREM was tested.

JECFA considered a hypothesis that food emulsifiers may decrease the integrity of the intestinal epithelial barrier. The Committee concluded that the evidence for this hypothesis is very limited, as it is primarily based on *in vitro* studies in which surfactants and emulsifiers have been applied directly to cells at concentrations (e.g. 1 mg/mL) that are likely to exceed those occurring in the gut lumen after consuming emulsifier-containing foods.

In addition, JECFA noted that the monolayers of intestinal cells used in these studies (Caco2 cells) do not fully replicate the complex architecture of the intestine or physiological conditions: for example they do not have the protective layer of intestinal mucus that would be present *in vivo*. Further, none of the *in vitro* studies used CITREM, and they were performed using emulsifiers with considerably higher hydrophilic-lipophilic balance (HLB) values than CITREM (i.e. more hydrophilic and more effective in modulating tight junctions between intestinal mucosal cells than surfactants with lower HLB values). The Committee concluded that based on the available data it is not possible to conclude that CITREM will affect the intestinal barrier under *in vivo* conditions.

3.2.4 Human studies

Eleven paediatric clinical studies have been conducted with infant formulas containing CITREM at concentrations ranging from 950 to 1620 mg/L. No adverse effects, including on growth or haematological or biochemical parameters were reported, but these studies were not primarily aimed at investigating safety. Infant formula containing sodium and potassium citric acid salts added at a concentration of 2500 mg/L was also well tolerated by infants.

A summary of five case reports on infants aged 2-11 months given a liquid peptide based formula containing CITREM at 8560 mg/L for two or four weeks was also submitted to JECFA. All infants had increases in weight, length and head circumference while on the formula, but no conclusions could be drawn on tolerance from these reports as all the infants

had pre-existing gastrointestinal disorders or diseases, some also had other health problems and some had loose or soft stools prior to being given the formula.

JECFA also considered limited available evidence on whether free citric acid in the gut might cause diarrhoea. One study found no effects in 13 infants given formula containing citrate salts with an exposure of 500 mg/kg bw/day, while in another study diarrhoea (severity not reported) was observed in four of eight infants given free citric acid by gavage (vehicle unspecified) in divided doses over 24 hours equivalent to approximately 400-700 mg/kg bw/day free citrate. The Committee noted that the diarrhoea may have been a result of osmolality and the gavage mode of administration, whereas enzymatic release of free citrate from CITREM-containing infant formula would be more gradual. JECFA also noted that citrate salts have been used in oral rehydration solutions for the treatment of diarrhoea in infants (2940 mg/L trisodium citrate or 3240 mg/L tripotassium citrate). Oral sodium or potassium citrate is also prescribed to alkalinise the urine for treatment of urinary tract infections, hypocitraturia and kidney stones, including at doses of 1-4 mEq/kg bw/day (108-430 mg/kg bw/day) in infants and children. Diarrhoea is listed as an occasional side effect of sodium citrate or potassium citrate treatment, due to the irritant effect in the gut.

Based on these considerations, JECFA concluded that it is unlikely that consumption of formulas containing typical levels of CITREM in powdered formulas (up to 2700 mg/L as reconstituted), which is equivalent to an estimated exposure to citrate of 440 mg/kg bw/day for the very young infant at the 95th percentile energy intake, would cause diarrhoea. At the high end of potential uses (up to 9000 mg/L), equivalent to estimated citrate exposure of 1140 mg/kg bw/day for the very young infant at the 95th percentile energy intake, diarrhoea might occur in some infants. These exposure estimates are conservative as they were based on an assumption of complete hydrolysis of CITREM and the maximum citric acid content of 50%, and basal levels of citric acid measured in prepared infant formula (640 mg/L) were also included.

3.2.5 Assessment of dietary exposure

JECFA considered that citric acid was the only component of CITREM for which a dietary exposure estimate was needed. Exposures were estimated based on the upper end of the reported range of citric acid in CITREM (13 – 50%) and basal levels of citric acid in ‘typical’ prepared infant formulas (640 mg/L).

At typical concentrations of CITREM used in formula (up to 2700 mg/L as reconstituted), median estimated exposures to citric acid for infants aged 0 – 6 months were 120 – 360 mg/kg bw/day. At the high end of intended use (9000 mg/L) median citric acid exposure estimates were 230 – 930 mg/kg bw/day. For 95th percentile consumers estimated citric acid exposures were up to 440 mg/kg bw/day at the 2700 mg/L use level and up to 1140 mg/kg bw/day at the 9000 mg/L use level.

3.2.6 Conclusion of JECFA evaluation

JECFA concluded that the use of CITREM in infant formula and formula for special medical purposes at concentrations up to 9000 mg/L does not raise any toxicological concerns.

It was considered that it is unlikely that consuming formulas containing typical levels of CITREM in powdered formulas (up to 2700 mg/L as reconstituted) would cause diarrhoea. However, there is a possibility of diarrhoea from free citric acid released from CITREM-containing formula at the high end of the requested range. The Committee noted that it was difficult to assess the risk of diarrhoea given the paucity of clinical data available and that citric acid exposure assumptions were maximised, but the risk was considered to be low.

3.3 Assessments by other regulatory agencies

3.3.1 EFSA

The EFSA Panel on Food Additives and Flavourings (FAF) re-evaluated the use of CITREM as a food additive in 2020, as part of an assessment of a range of esters of mono- and diglycerides of fatty acids (EFSA 2020b). The EFSA opinion specifically excluded an evaluation of the use of CITREM as a food additive in infant formula for infants under the age of 12 weeks, for which a separate risk assessment is due to be performed.

EFSA concluded that CITREM is expected to be extensively hydrolysed either in the gastrointestinal tract or (pre)-systemically after absorption and are unlikely to be present intact systematically. The hydrolysis products (citric acid, free fatty acids and glycerol) are all normal dietary constituents that are metabolised or excreted intact. No adverse effects relevant for humans were identified from the toxicological database for the group of substances. Given these considerations it was considered that there was no need to establish an ADI for CITREM.

3.4 Discussion and conclusions

A literature search using PubMed did not identify any new information that would indicate a need to amend the conclusions of the 2014 JECFA evaluation of CITREM as an additive in infant formula. The most recent assessment of CITREM by EFSA did not include infants under the age of 12 weeks, but concluded there is no need for a numerical ADI in the absence of any identifiable hazard.

The use of CITREM in all infant formula products at the maximum level assessed by JECFA of 9000 mg/L does not raise any toxicological concerns. CITREM is expected to be substantially hydrolysed in the gut and any partially hydrolysed products, such as glycerol citric acid esters would not be of safety concern.

Free citric acid released from CITREM containing-formula is unlikely to cause diarrhoea at lower use levels (e.g. up to 2700 mg/L). At the higher use levels (up to 9000 mg/L) there is a possibility of diarrhoea from free citric acid. It is not possible to quantify the risk based on the very limited data available, but it is likely to be low.

4 Starch sodium octenyl succinate (INS 1450)

Starch sodium octenyl succinate, also referred to as octenyl succinic acid (OSA)-modified starch, is not currently listed for use in infant formula in the Code.

4.1 Current Codex permissions

Starch sodium octenyl succinate is listed for use as a thickener in the Codex Standard for infant formula and formulas for special medical purposes intended for infants. The Codex permission is for use in hydrolysed protein and/or amino acid based infant formula only. The maximum level permitted is 2 g/100 mL (20,000 mg/L) of the product ready for consumption (Codex 2016). Starch sodium octenyl succinate is also listed in the Codex General Standard for Food Additives for use in complementary foods for infants and young children, at a maximum level of 50,000 mg/kg (Codex 2018).

The safety of starch sodium octenyl succinate has been evaluated by JECFA on several occasions, and most recently at its 79th meeting (WHO 2014, 2015).

4.2 JECFA evaluation of starch sodium octenyl succinate

An ADI 'not specified' was allocated to starch sodium octenyl succinate by JECFA at its 26th meeting.

The safety of starch sodium octenyl succinate for use as an emulsifier in infant formula and in formula for special medical purposes intended for infants was assessed by JECFA at its 79th meeting. Because OSA is produced from starch sodium octenyl succinate in the intestine by hydrolysis of ester bonds, a number of studies on the toxicology and metabolism of OSA were also evaluated.

The proposed use levels evaluated by JECFA were 9000 mg/100 g in infant formula powder (equivalent to ~12,000 mg/L as consumed) and 2000 mg/100 mL (20,000 mg/L) in ready-to-feed formula.

4.2.1 Chemical and technical considerations

Starch sodium octenyl succinate is derived by modifying food starch with OSA, in a process involving controlled esterification by the introduction of lipophilic octenyl succinic groups from n-octenyl succinic anhydride to waxy starch pre-treated with acid. The resulting n-octenyl succinic anhydride ester slurry undergoes several processing steps before being cooked under controlled temperature and pressure and spray-dried. JECFA noted that the final starch sodium octenyl succinate product should not contain more than 3% octenyl succinyl groups and not more than 0.3% free OSA.

At its 79th meeting, JECFA observed that if lead was present in starch sodium octenyl succinate at the maximum limit permitted in its specification (2 mg/kg) the maximum limit for lead in infant formula (as consumed) of 0.01 mg/kg established by the Codex Committee on Contaminants in Foods (Codex 2014) could be exceeded. The Committee considered that introduction of a lead limit of 0.1 mg/kg for starch sodium octenyl succinate would ensure that the maximum level of lead in the final infant formula was not exceeded, at the maximum use level reviewed by JECFA.

The limit for lead in modified starches was reduced from 2 to 0.2 mg/kg at the 86th meeting.. The limit for lead in starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg (WHO 2019).

4.2.2 Toxicokinetics and metabolism

In vitro and *in vivo* studies performed in experimental animals and humans have shown that starch sodium octenyl succinate is at least partially hydrolysed in the gastrointestinal tract by digestive enzymes to form OSA and native starch. Digestibility of starch sodium octenyl succinate *in vitro* ranged from 83% to 98% of its corresponding native starch, and was comparable to that reported for other modified food starches.

The starch component undergoes typical carbohydrate digestion and absorption, while OSA is absorbed and either excreted unchanged or metabolised via a combination of ω-, ω-1 and β-oxidation steps, similar to the metabolism of other branched-chain fatty acids, and then excreted.

These processes are similar in rats, dogs and human infants, although the amount of OSA excreted unchanged in the urine differs (dogs > rats; variable in human infants). JECFA concluded that while the degree of metabolism may vary between species, in general the same metabolites are produced.

4.2.3 Toxicological data

When starch sodium octenyl succinate was reviewed by JECFA at its 26th meeting, the only significant finding in a 90-day feeding study of starch sodium octenyl succinate at dietary levels up to 30% in the rat was corticomedullary calcium deposition in the kidney. The effect was more severe in females than males, and occurred in animals fed both the control starch and starch sodium octenyl succinate. The meeting noted that urinalysis showed higher concentrations of urinary calcium and magnesium in females, but not males, compared with controls, and that nephrocalcinosis in the rat may be related to a marginal magnesium deficiency when carbohydrate comprises a major proportion of the diet.

At its 79th meeting, JECFA noted that such effects are commonly encountered with poorly absorbed osmotically active materials, resulting in caecal enlargement which in turn leads to an increase in renal calcium deposition. JECFA further observed that there have been a large number of reports of nephrocalcinosis in response to exposures to carbohydrates with no changes in renal function, demonstrating that nephrocalcinosis in rats given large amounts of carbohydrates is not relevant to humans.

Further studies reviewed by JECFA at the 79th meeting included a 90-day oral toxicity study in which rats were fed diets containing 30% starch sodium octenyl succinate, equal to approximately 37,000 mg/kg bw/day, and a long-term dietary study in which diet containing 30% starch sodium octenyl succinate (equal to 17,000 and 20,000 mg/kg bw /day for males and females, respectively) was fed to rats for up to 120 weeks. No treatment-related adverse effects were reported in these studies.

Starch sodium octenyl succinate was not genotoxic *in vitro*, and no evidence of carcinogenicity was observed in the long-term rat study. No data on reproductive or developmental toxicity were available.

4.2.4 Special studies in young animals

Two studies conducted with neonatal animals were also evaluated by JECFA, one in Beagle pups and one in Yorkshire crossbred piglets. A study in three-month-old Beagle dogs was also reviewed.

In the study in neonatal Beagles, pups aged 5-9 days old were dosed twice daily by oral gavage for 6 weeks with starch sodium octenyl succinate at doses of 0 (water control), 5000 or 10,000 mg/kg bw/day, or with a control starch at doses of 5000 or 10,000 mg/kg bw/day. Body weight gains were lower than expected in all groups during the first 3 weeks of the study; this was attributed by the study authors to a limited milk supply due to the large litter size (each treatment group of eight puppies (4/sex/group) was nursed by a single dam resulting in a limited milk supply). Pups in the high-dose group were less active after 15 days and had decreased body weight gain compared with pups in the other groups. This effect could not be clearly attributed to treatment due to the limited milk supply as noted above. No other toxicologically relevant findings were reported.

A follow-up study in 3-month old Beagle pups to investigate the reduced body weight gain in the high dose group found no clinical or histopathological changes following administration of starch sodium octenyl succinate at dietary concentrations of 0%, 5.5%, 11.0% or 22.0% (equivalent to 0, 3000, 6000 and 12,000 mg/kg bw/day) for 42 days. Body weight gain was decreased in the high dose group compared with starch-fed controls. The Committee considered that the differences in body weight gain reported in these two dog studies were likely to be due to incomplete digestion of OSA-modified starch, resulting in a lower calorie intake.

A GLP-compliant toxicity study conducted in neonatal piglets was considered to be the more relevant of the two neonatal animal studies. In this study, starch sodium octenyl succinate was administered at doses of 0, 1000, 2000 or 10,000 mg/kg bw/day via a feeding device 6 times per day for 3 weeks. The test substance was administered from 2 days after birth for 3 weeks, to model the 0-12 week period of development in human infants where infant formula may be provided as the sole source of nutrition. Starch sodium octenyl succinate was well tolerated by piglets and no adverse effects were observed on growth, clinical pathology parameters, macroscopic or histopathological evaluation. Males in the high dose group had a reduced body weight and body weight gain compared with control males, but these differences were not statistically significant and were not considered to be toxicologically relevant by JECFA. Similar changes were not observed in high dose females. The NOAEL was 10,000 mg/kg bw/day, the highest dose tested.

4.2.5 Human studies

A single dose of 25 g of starch sodium octenyl succinate was well tolerated by fasting healthy non-diabetic adults, and attenuated the post-prandial glycaemic response compared to glucose.

Two randomised, multicentre, double-blind clinical studies with infants investigated the effects of formula supplemented with starch sodium octenyl succinate. In one study, infants received control formula or formula containing 1.33-1.47 g/100 mL starch sodium octenyl succinate from 2-16 days of age for 120 days. Formula intake at 90 days of age was higher in the group given starch sodium octenyl succinate compared with controls (1114 mL/day versus 947 mL/day, respectively), although the study authors noted that formula intake was not accurately determined for some subjects. No effects on growth were observed and there was no difference in illnesses or symptoms of concern as reported by parents between the two groups. In a second study, two similar casein hydrosylate formulas, both containing less than 2% starch sodium octenyl succinate (concentration not further specified), were compared with a control formula from days 0-8 of life to day 28. No issues with tolerability were reported.

Post-marketing surveillance of an infant formula containing 2% starch sodium octenyl succinate to be used for special medical purposes also indicated that it was well tolerated by infants, although the Committee acknowledged that patient exposure at the time of evaluation was fairly limited. Only 167,424 patient treatment days (defined as 0.8 L of prepared formula per treatment day) had been distributed as of 30 October 2013. JECFA noted that adverse events reported were primarily related to gastrointestinal symptoms that are within the expected safety profile of the product.

4.2.6 Assessment of dietary exposure

The proposed maximum use level of starch sodium octenyl succinate (20 g/L formula) was estimated to result in median exposures of up to 3700 mg/kg bw/day in infants aged 0-6 months. Estimated exposures for infants with high (95^{th} percentile) energy intakes were up to 4400 mg/kg bw/day.

4.2.7 Conclusions of JECFA evaluation

JECFA considered that all of the new data available to the Committee confirmed the very low toxicity of starch sodium octenyl succinate, and the previously established ADI ‘not specified’ was confirmed.

Several of the new studies were relevant to assessing the safety of starch sodium octenyl succinate in infant formula and formula for special medical purposes intended for infants. The

study in neonatal piglets was considered the more relevant of the two neonatal animal studies evaluated because of the similarity of the digestive systems of neonatal pigs and human infants. In this study the NOAEL of starch sodium octenyl succinate was 10,000 mg/kg bw/day, the highest dose tested. Several studies in human infants have demonstrated that starch sodium octenyl succinate at concentrations up to 2% in infant formula is well tolerated. In one of these studies exposure was estimated to be 2500 mg/kg bw/day. Post-marketing surveillance of an infant formula containing 2% starch sodium octenyl succinate also indicated that it is well tolerated.

Margins of exposure (MOEs) compared with the NOAEL of 10,000 mg/kg bw/day in the neonatal piglet study were 2.3 for infants with a 95th percentile energy intake (4400 mg/kg bw/day starch sodium octenyl succinate) and 2.7 at the median energy intake (3700 mg/kg bw/day starch sodium octenyl succinate).

JECFA noted that if an additive is proposed for use in infant formula at relatively high levels (e.g. 0.1% or greater), conducting toxicological studies in neonatal animals at doses two or more orders of magnitude greater than the anticipated human exposure, which is the approach commonly taken for food additives, may not be feasible. The Committee concluded that MOEs in the region of 1-10 can be interpreted as low risk for the health of infants aged 0-12 weeks consuming a food additive in infant formula, when appropriate toxicological and dietary exposure considerations have been taken into account (WHO 2014).

Taking into account the overall low toxicity of starch sodium octenyl succinate, the conservatism in the NOAEL which was the highest dose tested in neonatal animals, the conservatism in the dietary exposure estimates and the supporting evidence from clinical trials and post-marketing surveillance, JECFA concluded that the consumption of starch sodium octenyl succinate in infant formula or formula for special medical purposes intended for infants is not of concern at use levels up to 20 g/L.

4.3 Additional studies

One study relating to digestibility of starch sodium octenyl succinate that was not reviewed by JECFA was identified in a literature review. This study is summarised below.

4.3.1 Toxicokinetics and metabolism

In vitro and in vivo digestion of octenyl succinic starch (Ai et al. 2013) Regulatory status: Non-GLP

Normal corn starch (NCS) and high-amylose corn starch (HA7), a form of resistant starch containing high concentrations of amylose, were modified with 3% and 10% (w/w) OSA. The study did not report whether the modified starch products complied with JECFA specifications for starch sodium octenyl succinate. The *in vitro* digestibility of raw and cooked modified and unmodified starches was assessed by incubation with porcine pancreatin extract and amyloglucosidase. In the *in vivo* part of the study, male Fisher-344 rats aged 7 weeks (10 per diet) were fed diets containing 55% of boiled NCS, HA7 or OSA (10%)-modified HA7 starch (equivalent to 55,000 mg/kg bw/day) for 9 weeks. Food consumption and body weights were recorded and faecal samples were collected weekly. At the end of the study rats were killed and caecum tissue weight, content weight and pH were measured for each rat.

The *in vitro* digestion study showed that approximately 87-93% of boiled OSA-treated NCS was digestible, compared with approximately 99% in boiled untreated NCS. The digestibility of OSA-treated boiled HA7 starch was around 76-79%, and around 76% in boiled untreated HA7 starch.

In the *in vivo* study, body weights of rats in the three groups were similar throughout the course of the study and all groups gained weight as expected. Faecal weights of rats fed OSA (10%) - modified HA7 starch were significantly higher than those of control and HA7-fed rats from the end of the first week of the study, and faeces from OSA (10%)-HA7 rats also had higher starch contents. It was calculated that 20.2-31.1% of starch in the OSA (10%)-HA7 diet was not being utilised *in vivo*, compared with ≤4.9% in the HA7 diet and ≤0.2% in the control diet. Caecum tissue and content weights were significantly larger in OSA (10%)-HA7 and HA7-treated rats compared with controls. Caecal pH was significantly different between all three groups (controls: pH 7.53; HA7: pH 5.33; OS (10%)-HA7: 5.79), and the lower pH values in the resistant starch groups were considered to indicate increased fermentation by gut microflora in these groups.

4.4 Assessments by other regulatory agencies

4.4.1 EFSA

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) reviewed the safety of a range of modified starches, including starch sodium octenyl succinate, as food additives in 2017. Use of starch sodium octenyl succinate in dietary foods for special medical purposes and special formulas for infants was considered as part of this review (EFSA 2017b).

The studies on starch sodium octenyl succinate reviewed by EFSA were the same as those reviewed by JECFA. EFSA concluded that there was no safety concern for use of modified starches, including starch sodium octenyl succinate, as food additives for the general population at the reported uses and use levels, and there was no need for a numerical ADI.

With respect to use of starch sodium octenyl succinate in dietary foods for special medical purposes and special formulas for infants, EFSA noted that infants and young children consuming foods belonging to these food categories may show higher susceptibility to gastrointestinal effects of modified starches than their healthy counterparts, due to their underlying medical condition. While no adverse effects on body weight and food intake were observed in neonatal pigs at exposures up to 10,000 mg/kg bw/day starch sodium octenyl succinate for 21 days, gastrointestinal symptoms were reported in post-marketing surveillance of infants consuming hypoallergenic infant formula containing 2% of starch sodium octenyl succinate. EFSA also noted that the clinical studies in infants are based on formulas containing less than 2% starch sodium octenyl succinate, and the available information on these studies is limited.

The EFSA ANS Panel therefore concluded that the available data are not sufficient for an adequate assessment of the safety of starch sodium octenyl succinate in dietary foods for special medical purposes and special formulas for infants at the presently authorised maximum use level in Europe of 20,000 mg/kg.

In a follow-up evaluation, the EFSA FAF Panel assessed the safety of starch sodium octenyl succinate as a food additive in foods for infants below 16 weeks of age (EFSA 2020a). Additional data submitted for the assessment included the full report of the 3-week dietary toxicity study in piglets considered in the previous evaluation (also reviewed by JECFA), six clinical studies in infants below 16 weeks of age, and post-marketing surveillance reports.

The FAF Panel was unable to identify a reference point for risk assessment from the post-natal piglet study, due to a lack of a dose-response in the effect on body weights observed in male piglets and the absence of effects in female animals.

Five of the six clinical trials submitted were considered to have low internal validity with a high risk of bias, based on use of a risk of bias assessment tool. The one clinical study with a moderate risk of bias had a very low starch sodium octenyl succinate content (not reported). Given these limitations the Panel concluded that a reference point for risk assessment could not be derived from the clinical studies.

Although it was not possible to derive a reference point from the available piglet and clinical studies, the FAF Panel noted that both data sources did not clearly indicate an adverse effect of starch sodium octenyl succinate. The Panel concluded that there is no indication of a safety concern at use levels of starch sodium octenyl succinate in food for infants below 16 weeks within the range reported in the clinical studies (up to 2725 mg/kg bw/day). The conclusion of the ANS Panel that there was no need for a numerical ADI was reiterated.

4.5 Discussion and conclusions

The 2014 evaluation by JECFA indicates that starch sodium octenyl succinate is of very low toxicity. No adverse effects were seen in a 3-week toxicity study in neonatal pigs at doses up to 10,000 mg/kg bw/day, the highest dose tested. In addition, clinical studies in human infants have shown that starch sodium octenyl succinate is well tolerated in infant formula at concentrations approaching 2%.

While EFSA considered it was not possible to derive a reference point for risk assessment for starch sodium octenyl succinate from the available toxicity and clinical studies, both data sources did not clearly indicate an adverse effect. EFSA concluded that for infants below 16 weeks of age, there is no indication of a safety concern at use levels within the range reported in the clinical studies (up to 2725 mg/kg bw/day).

A literature search identified a recent *in vitro* digestion study that was not reviewed by JECFA. The digestibility of starch sodium octenyl succinate in this study was within the same range as that reported by JECFA.

Using JECFA's dietary exposure assessment based on a maximum level of 20,000 mg/L and the NOAEL of 10,000 mg/kg bw/day in the neonatal piglet study, MOEs are 2.3 for infants with a 95th percentile energy intake (4400 mg/kg bw/day) and 2.7 at the median energy intake (3700 mg/kg bw/day). MOEs in the region of 1-10 can be interpreted as low risk for the health of infants aged 0-12 weeks consuming a food additive in infant formula, when appropriate toxicological and dietary exposure considerations have been taken into account (WHO 2014).

Based on the available data, the consumption of starch sodium octenyl succinate in IFPSDU at the maximum level assessed by JECFA of 20,000 mg/L does not raise health concerns.

5 Carob (locust) bean gum (INS 410)

Carob (locust) bean gum, also referred to as locust bean gum, is used as a thickener, stabiliser, emulsifier and gelling agent.

Carob bean gum is listed in Schedule 15 of the Code for use as a thickener in infant formula products at an MPL of 1000 mg/L.

5.1 Current Codex permissions

Carob bean gum is listed for use as a thickener in the Codex Standard for infant formula and formulas for special medical purposes intended for infants, with a maximum level of 0.1

g/100 mL (i.e. 1000 mg/L) in all types of infant formula (Codex 2016). Carob bean gum is also listed in the Codex General Standard for Food Additives for use in complementary foods for infants and young children at a maximum level of 2000 mg/kg (Codex 2018).

The safety of carob bean gum has been evaluated by JECFA on several occasions, most recently at its 82nd meeting in 2016 (WHO 2016, 2017).

5.2 JECFA evaluation of carob bean gum

JECFA has reviewed the safety of carob bean gum at its 13th, 18th, 19th, 24th, 25th and 82nd meetings. A temporary ADI 'not specified' was assigned at the 19th meeting, and a full ADI 'not specified' was established at the 25th meeting following the provision of additional toxicity studies (WHO 1981).

At its 82nd meeting, JECFA evaluated the safety of carob bean gum for use as a thickener in infant formula and formula for special medical purposes intended for infants in the context of the (therapeutic) dietary management of gastro-oesophageal reflux.

The proposed use level evaluated by JECFA was up to 10,000 mg/L for infant formula, although the sponsor suggested a typical use level of 5000 mg/L.

5.2.1 Chemical and technical considerations

Carob bean gum is obtained from the endosperm of the carob (locust) tree, *Ceratonia siliqua*.

The seeds are dehusked and the endosperm is milled. The gum may be washed with ethanol or isopropanol to control the microbiological load (washed carob bean gum). Native carob bean gum may be further clarified by dispersal in hot water followed by recovery with isopropanol or ethanol, filtering, drying and milling; this form is referred to as clarified carob bean gum. JECFA has established separate specifications for carob bean gum and for carob bean gum (clarified). The maximum protein content in these specifications is 7.0% and 1.0%, respectively.

Carob bean gum and carob bean gum (clarified) mainly consist of high molecular weight (in the range of 50 – 3000 kDa) galactomannans. The mannose to galactose ratio of carob bean gum is approximately 4:1, with mannose and galactose concentrations of 73 – 86% and 14 – 27%, respectively.

Carob bean gum has the capacity to form very viscous solutions at relatively low concentrations, and its thickening properties have been used in infant formulas for the dietary management of infant regurgitation in EU countries for more than 20 years.

Specifications for carob bean gum and carob bean gum (clarified) were updated in 2016 (WHO 2016). A limit of 2 mg/kg for lead was maintained for general use and a limit of 0.5 mg/kg was introduced for use in infant formula. The limit for lead when used in infant formula was established following the introduction of a maximum limit of 0.01 mg/kg for lead in infant formula (as consumed) by the Codex Committee on Contaminants in Foods (Codex 2014), in order to ensure that this would not be exceeded.

5.2.2 Toxicokinetics and metabolism

In vitro tests with human enzyme preparations and *in vivo* studies in rats indicate that carob bean gum is largely non-digestible, although some reductions in galactomannan chain length may occur through fermentation by gut microflora.

Increased microbial activity with higher caecum and caecum weights have been observed in rats. JECFA noted that this finding supports the conclusion that fermentation of carob bean gum occurs in the gastrointestinal tract. JECFA further commented that fermentation of carob bean gum by gut microflora produces oligosaccharides or monosaccharides, which will be converted to short-chain fatty acids (SCFA). These SCFAs can be absorbed and metabolised in normal biochemical pathways.

5.2.3 Toxicological data

JECFA concluded at its 25th meeting that carob bean gum is of low acute oral toxicity and is not mutagenic in bacterial test systems. No adverse effects were observed in short-term toxicity or long-term toxicity and carcinogenicity studies in rats or mice, in reproductive toxicity studies in rats, or in developmental studies in rats, mice, hamsters and rabbits. In dogs, diets containing 10% carob bean gum resulted in gastrointestinal hypermotility, soft bulky stools and reduced digestibility.

Several additional short-term toxicity studies were evaluated by JECFA at its 82nd meeting. Caecal enlargement in several rat studies was considered an adaptive response in rodents administered diets containing high levels of indigestible carbohydrates. In 90-day studies, no adverse effects were observed in mice or rats fed carob bean gum at concentrations of up to 100,000 mg/kg feed, equivalent to 15,000 and 10,000 mg/kg bw/day, respectively.

The 82nd meeting also reviewed a bacterial reverse mutation assay, with and without metabolic activation, with *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 and TA1537. Negative results were observed and JECFA concluded that carob bean gum is not mutagenic.

5.2.4 Special studies

Several *in vitro* studies using a continuous flow dialysis system to simulate the upper gastrointestinal tract of infants less than 6 months of age were reviewed by JECFA at the 82nd meeting. These studies indicated that infant formulas containing carob bean gum at concentrations higher than 4000 mg/L may reduce the levels of calcium, zinc and iron available for absorption compared to non-thickened formulas and a human milk standard.

In newly weaned 5-week-old piglets fed a diet containing 1% carob bean gum (equal to 240 mg/kg bw/day) for 11-12 days, slight changes in the mitotic index of intestinal crypts compared with controls were not considered adverse in the absence of accompanying morphological or histopathological changes. Addition of a 10% carob tree meal to the diet affected the bacteriological and morphological characteristics of the small intestine. However, JECFA noted the diet contained a significant portion of unidentified components, including polyphenols with antibacterial properties, making it difficult to determine whether carob bean gum was the cause of these effects.

Effects on immunological parameters of intestinal function following oral *Escherichia coli* challenge infection were assessed in newly weaned piglets (aged 4 weeks) fed a diet containing 0.5% carob bean gum for 14 days. There were no significant differences in immunoglobulin (Ig) A levels or expression of toll-like receptors 2 and 4 messenger RNA (mRNA) in the ileum and/or mesenteric lymph node compared with controls. A significant repression of C-reactive protein expression after *E. coli* challenge was seen in piglets fed locust bean gum-containing diet, which JECFA considered may indicate a reduction in the acute inflammatory response caused by the challenge.

JECFA considered the relevance of the studies in newly weaned piglets to infants aged 0-12 weeks. The Committee noted that it had previously concluded that the neonatal period from 0

– 28 days in humans corresponds to days 0 – 15 days in the pig, and the period 1 – 23 months in human infants corresponds to weeks 2 – 4 in the pig. The newly weaned piglet aged over 4 weeks does not mimic the infant gut over the 12-week developmental period and is therefore not a suitable neonatal animal model.

5.2.5 Human studies

No adverse gastrointestinal effects in infants or adults were reported in the feeding studies reviewed by JECFA at its 25th meeting.

Results of 13 clinical studies in healthy term infants were evaluated by JECFA at the 82nd meeting. In total, around 400 term infants were included in these trials, which ranged from 1 week to 3 months at carob bean gum concentrations ranging from 3500 – 6000 mg/L. The formulas were generally well tolerated and there were no effects on growth or serious adverse effects reported. Some studies reported increased bowel movements, but none of the studies reported statistically significant levels of severe adverse gastrointestinal effects, including severe diarrhoea. Reduced frequency of regurgitation was often observed.

The potential concerns around reduced mineral and nutrient bioavailability suggested by *in vitro* studies were evaluated in one of the clinical studies. Twenty healthy infants were given a control whey-predominant formula or a casein-based formula containing 4000 mg/L carob bean gum for 13 weeks. All infants showed normal growth and there were no differences in blood levels of iron, calcium, phosphorus, zinc and iron-binding capacity. Slight differences in urea and albumin levels were observed between groups, but these were attributed to the differences between the whey- and casein-based formulas.

A single case of allergenicity was reported for one 5-month-old infant following exposure to a carob bean gum-thickened formula, and a single case report of an adult with carob bean gum hypersensitivity were identified by the Committee. A study in 12 peanut-allergic children found that some participants produced an IgE-specific response or were positive in skin prick tests (SPTs), but none showed clinical reactivity in double-blind, placebo-controlled food challenges with either raw or cooked carob. JECFA considered that the available data indicated that the prevalence of hypersensitivity to carob bean gum after ingestion is expected to be very low in the overall population and not of concern.

The Committee evaluated two case reports of isolated adverse events in extremely low birth weight infants fed formula containing carob bean gum. In one report, an association between feeding formula containing 2000 – 5000 mg/L carob bean gum to preterm infants and a higher frequency of defecation, metabolic acidosis and hypokalaemia was hypothesised to be due to a shortened gastrointestinal passage time or binding of potassium to the carob bean gum. The other report was of two cases of fatal necrotising enterocolitis (NEC) in extremely low birth weight, premature infants. Histopathology was not investigated in these two cases. JECFA concluded that the effects in either case report could not be attributed to carob bean gum.

5.2.6 Assessment of dietary exposure

Based on the maximum proposed use level for carob bean gum in infant formula of 10,000 mg/L, average dietary exposure was estimated to range from 600-1800 mg/kg bw/day in infants aged 0-12 weeks, while exposures of infants with 90th percentile energy intakes were estimated to be up to 2100 mg/kg bw/day.

5.2.7 Conclusions of JECFA evaluation

JECFA concluded that the available data were not sufficient for the evaluation of carob bean

gum for use in infant formula at the proposed use level (10,000 mg/L), due to the absence of toxicological studies in neonatal animals. While human infant feeding studies do not report any serious adverse effects and support tolerability up to 6000 mg/L, these studies were not designed to evaluate effects on infant gut morphology of health.

The Committee noted that the current use level of carob bean gum for infant formula or formula for special medical purposes intended for infants allowed in the relevant Codex Standard (1000 mg/L) is much lower than the proposed use level, but did not comment on the safety of this lower use level.

Toxicological data from studies in neonatal animals, adequate to evaluate safety for use in infant formula, were requested in order for JECFA to complete its evaluation of the use of carob bean gum in infant formula at concentrations up to 10,000 mg/L.

5.3 Additional studies

A number of studies and case reports published since the 82nd JECFA meeting containing information relevant to the safety assessment of carob bean gum were found in a literature search. These studies are summarised below.

5.3.1 Toxicokinetics and metabolism

In vitro study of gut fermentability (González-Bermúdez et al. 2018) Regulatory status: Non-GLP

Several thickening agents including carob bean gum were evaluated for their *in vitro* fermentability and associated effects on infant gut microbiota. Fresh faecal samples from three healthy infants ranging from 2 to 3 months old (sex not specified) were combined with a minimal basal medium and inoculated with 1% (w/v) carob bean gum, maize hydroxypropylated distarch phosphate (MHDP) or pre-gelatinised rice starch (gRS) at 37ºC for 48 hours. Inulin and d-glucose were also used as prebiotic positive and negative controls, respectively. Samples were collected at 0, 5, 8, 10, 24 and 48 hours after inoculation and pH, gas pressure, SCFAs and bacterial populations were evaluated.

Carob bean gum resulted in significantly lower gas production than MHDP, gRS or d-glucose, and the time taken for pH to decrease from ~7.5 to ~5 was similar to that of inulin and longer than MHDP, gRS or d-glucose (24 hours versus 5 hours). Incubation with carob bean gum also resulted in greater SCFA production, including a higher production of propionate. These properties were considered to have enhanced the development of a more varied faecal microbiota compared with MHDP or gRS. The authors concluded that locust bean gum can be defined as a slowly fermented ingredient.

5.3.2 Observations in humans

Hypersensitivities

Food protein-induced enterocolitis syndrome induced by locust bean gum in an infant (Jędrzejczyk et al. 2020) Regulatory status: Non-GLP

A case report describes an 11-week old boy with chromosome 21 trisomy fed with cow's milk formula admitted to the emergency department due to persistent vomiting, watery diarrhoea, non-responsiveness and drowsiness. The child had been hospitalised five times previously with similar symptoms, and infectious factors, endocrine disorders, immunodeficiency and IgE-dependent food allergy had been excluded.

Based on the clinical picture it was suspected that the child had food protein-induced enterocolitis syndrome (FPIES) due to cow's milk protein. Cow's milk formula was replaced by a casein-based extensively hydrolysed formula (EHF), resulting in a rapid improvement in the child's condition. Over the next three months the child was readmitted three times with similar symptoms. An oral food challenge with cow's milk formula confirmed the diagnosis of cow's milk protein FPIES. Detailed questioning of the child's caregivers indicated full adherence to the use of EHF, however carob bean gum had been added to the EHF as a thickener. An oral food challenge was then performed with EHF containing carob bean gum (0.5 g, increased to 1.0 g after 60 minutes), and the patient presented with vomiting and lethargy after 100 minutes and diarrhoea after 6 hours. Laboratory tests showed increased C-reactive protein, white blood cells and faecal calprotectin, similar to test results after oral food challenge with cow's milk formula. EHF was then maintained and the patient's diet was gradually expanded from age 10 months. Rice cereals, carrot, potato and turkey were introduced with no pathological reaction.

The authors noted that this case appeared to be the first description of FPIES triggered by carob bean gum in an infant.

Occupational rhinitis due to inhaled carob bean gum (La Vázquez de Torre et al. 2020); Regulatory status: Non-GLP

A 44-year old woman with a history of rhinoconjunctivitis to pollen who had worked in an ice cream factory for nine years presented with a two-year history of ocular and nasal itching, rhinorrhoea, nasal congestion and sneezing when handling Cremodan SL29, a powder used as a stabiliser. Cremodan SL29 contains carob bean gum, dextrose, milk proteins, gelatin, pectin and carrageenan. The patient was able to ingest ice cream prepared with Cremodan SL29. One year after her initial symptoms, the patient developed pharyngeal itching, eyelid angioedema and dyspnoea when ingesting chickpea, and cough after ingesting almonds, pistachio and sunflower seeds. The patient subsequently avoided tree nuts and legumes except peas and green beans, which she tolerated.

Prick-prick testing with Cemodan SL29 was positive as were SPTs to carob tree seed extract and carob bean gum. SPTs to dextrose, milk protein, gelatin, pectin and carrageenan were negative. SPTs to legume extracts were positive for chickpea, soybean and lentil and negative for peanut, pea and white, red and green bean. An SPT with tree nut extract was negative whereas prick-prick tests with walnut, almond, cashew, pistachio, pine nut and sunflower seeds were negative. Prick-prick testing with hazelnut was negative. A single-blind oral challenge with cooked white beans resulted in oral itching and cough 30 minutes after the second dose.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) showed a similar IgE-binding protein band profile when the patient's serum was incubated with Cremodan SL29, carob bean gum and carob tree seed extract. Cross-reactivity studies showed complete inhibition of immunoglobulin (Ig) E binding to carob bean gum and carob tree seed extract by pre-incubation with Cremodan SL29, chickpea and almond. Pre-incubation with Cremodan SL29 completely inhibited IgE binding to chickpea and partially inhibited binding to almond.

The authors concluded that to their knowledge this is the first report of occupational IgE-mediated sensitisation to inhaled carob bean gum proteins with clinical consequences for ingestion of legumes.

Allergic sinusitis and asthma caused by occupational exposure to carob bean gum (Hawley

et al. 2017) Regulatory status: Non-GLP

A case of an adult male who began to experience wheezing and other respiratory symptoms including sinusitis a year after beginning work as a cheesemaker has been reported. His symptoms progressively worsened over the following 16 years, developing into severe asthma. The cause of his asthma was identified as occupational exposure to finely powdered carob bean gum in the course of preparing ingredients for cheese mixtures.

Immune reactivity to carob bean gum (Vojdani and Vojdani 2015) Regulatory status: Non-GLP

Serum samples from 288 healthy individuals (144 male and 144 female) of different ethnicities, aged 18 to 65 years, were obtained from a commercial source and screened for IgG and IgE antibodies against extracts of a range of gums, including carob bean gum. The percentage of serum samples showing significant elevations in IgG and IgE responses to the various gums, defined as two standard deviations above the mean, ranged from 1.4 – 27.1% and 12.8 – 29.1%, respectively. A significant increase in IgG and IgE antibody response against carob bean gum was observed in 11.5% and 15.6% of serum samples, respectively. Enzyme-linked immunosorbent assay (ELISA) inhibition studies assessing potential cross-reactivity found less than 45% inhibition of the carob bean gum antigen-antibody reaction following pre-incubation with other gums, sesame albumin, lentil, corn, rice, pineapple, peanut, pea protein, shrimp or kidney bean.

Paediatric tolerance studies

Efficacy and safety of infant anti-regurgitation formula thickened with carob bean gum, pectin and other starches (Dupont and Vandenplas 2020)

A retrospective analysis of four open-label, interventional, single-group, multi-centre clinical trials of anti-regurgitation formula thickened with four different combinations of carob bean gum (source and specification not reported), pectin and tapioca or corn starch was reported. The trials shared an identical study design and were conducted in France and Belgium between 2013 and 2017. Exclusively formula fed infants aged < 5 months presenting with at least five regurgitations per day were enrolled and administered study formula for 14 months. Parents collected 3-day diaries on the number, volume and severity of regurgitation episodes, stool consistency and frequency and volume of formula consumed. Diaries were recorded at the start of the intervention (baseline) and again on days 11 – 13, before an investigator visit on day 14. The investigators performed a physical examination including measurement of anthropometric parameters and recorded information on adverse events. Parents were given the opportunity to continue with the study formula for an additional period of 3 months, with diaries completed over the final 3 days and a further visit at around day 90. While each of the four studies used a different test formula, the carob bean gum concentration in all four was 650 mg/L.

The primary outcome was the daily number of regurgitations after 2 weeks of feeding the test formula. Secondary outcomes included the number and severity score of regurgitations at day 3, stool consistency and frequency and growth at day 14 and all parameters at day 90 if parents agreed to continue with the test formula. Adverse events were assessed in all infants who consumed the test formula at least once, and the efficacy analysis was performed on all infants with information on the number of regurgitations at day 14.

In total 392 infants were included across the four trials. The mean age at study inclusion was 65.2 ± 37.9 days. Baseline characteristics were similar in all trials. Although the inclusion criteria specified infants < 5 months old, six patients were between 5 months and 5 months and 21 days old. All but one of the infants received the test formula at least once, and

information regarding the primary outcome was obtained in 346 infants. The mean number of daily regurgitation episodes and regurgitation severity scores were significantly reduced at days 3, 14 and 90 compared with baseline. Stool frequency was unchanged by the interventions. A trend towards a reduction in the number of infants with abnormal stool consistency at day 90 versus baseline was seen in three of the four studies, although it was only significant in one study. Weight-for-age and length-for-age z-scores were below the reference means in all studies at baseline, but increased towards the 50th percentile at day 90, indicating normalisation. A total of 141 adverse events were reported. Seven adverse events were classed as serious but none of these were considered related to the study formula by the investigators. Twenty-nine non-serious adverse events were considered related to the study formulas, the majority of which were related to the gastrointestinal tract: constipation (n = 8), diarrhoea (n = 5), colic/abdominal pain (n = 7) and worsening of regurgitations (n = 5).

The study authors concluded that infant formula containing a combination of carob bean gum, pectin and starch is safe and effective for the management of regurgitations in young infants.

Tolerance study with carob bean gum thickened infant formula (Georgieva et al. 2016)
Regulatory status: Non-GLP

In a randomised clinical trial, 56 full-term infants, aged 1-6 months, with gastroesophageal reflux (GER) were randomised to receive infant formula containing 3300 mg/L of cold-soluble carob bean gum galactomannans (Formula A), 4500 mg/L cold-soluble carob bean gum galactomannans (Formula B), or 4500 mg/L hot-soluble carob bean gum galactomannans (Formula C) for two weeks. A comparison of the specifications of these substances with the JECFA specification for carob bean gum was not reported. The cold-soluble form of carob bean galactomannans is heated during production to be pre-gelatinised and becomes gelatinised when dissolved in lukewarm water. The hot-soluble form is minimally heated during production and needs to be dissolved in hot water in order to be gelatinised. Due to the differences in water temperature needed for formula preparation, the intervention was double-blind for the two groups given cold soluble carob bean galactomannans, but not the group given the hot soluble form. A control group receiving standard infant formula was not included.

All infants were fed a standard infant formula for seven consecutive days prior to the intervention. At the end of the run-in period, baseline anthropometric and 24 hour oesophageal pH impedance measurements were conducted, with further measurements performed at the end of the two-week intervention. Mothers were given three-day diaries at the start and end of the intervention period in order to record tolerance indices, consisting of frequency of colics and the type and frequency of defecations. Intention to treat analysis was conducted on 16 infants given Formula A, 15 infants given Formula B, and 16 infants given Formula C.

Significant reductions from baseline to follow up were observed for the Boix Ochoa Score (an index of oesophageal acid exposure) and number of refluxes per day for infants given Formula A. Non-significant reductions in these indices were observed for infants given Formulas B or C. Body weight was significantly increased in infants given Formulas A and C at the end of the intervention period compared with baseline. Body weight was not significantly increased in infants given Formula B, although changes in body weight from baseline to follow-up were not significantly different between the three intervention groups. Recumbent body length increased significantly in all three groups, with no significant between-group differences. The total number of defecations and of diarrhoeic defecations was significantly increased in infants given Formula B, and the number of diarrhoeic defecations was significantly decreased in infants given Formula C. Formula A had no

significant effects on the number of defecations or diarrhoeic defecations. The number of colics per day was significantly reduced at the end of the intervention in all three groups. The study authors hypothesised that the increased number of total and diarrhoeic defecations among infants fed Formula B may explain the lack of a significant increase in body weight in this group. The study authors concluded that Formula A, containing 3300 mg/L of cold-soluble carob bean gum galactomannans, was more effective in reducing certain pH-monitoring indices of GER than the other two formulas, supported body weight gain and was well-tolerated by infants.

5.4 Assessments by other regulatory agencies

5.4.1 EFSA

The EFSA ANS Panel re-evaluated the use of carob bean gum as a food additive in 2016. The opinion specifically excluded an evaluation of use as a food additive in infant formula for infants under the age of 12 weeks, for which a separate risk assessment is due to be performed (EFSA 2017a).

The ANS Panel considered that sufficient toxicity data in animals were available demonstrating no adverse effects at highest doses tested up to 7500 mg/kg bw/day. It was concluded that a numerical ADI for carob bean gum was not needed.

EFSA noted the two case reports of adverse effects or fatality in premature or low birth weight infants that were also reviewed by JECFA. EFSA observed that the origin of such effects could be related to the very immature state of their gastrointestinal tract. In considering the two cases of fatal NEC in two extremely low birth weight premature infants, EFSA commented that the authors had hypothesised thickened milk may have led to a bowel obstruction leading to NEC, but pathophysiology of these cases was not investigated.

EFSA concluded that there was no safety concern for the general population from carob bean gum's reported uses as a food additive. For infants over 12 weeks of age, EFSA noted that infants (over the age of 12 weeks) and young children consuming foods for special medical purposes may show a higher susceptibility to gastrointestinal effects of carob bean gum because of their underlying medical condition. EFSA concluded there was a lack of specific clinical data addressing the safety of carob bean gum in the food categories 'dietary foods for special medical purposes and special formulas for infants' and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' at the defined maximum use levels (10,000 mg/L and 10,000 – 20,000 mg/L respectively).

EFSA concluded that the available data did not allow an adequate safety assessment of carob bean gum in these foods for infants and young children.

5.5 Discussion and conclusions

JECFA has assessed the toxicity of carob bean gum on a number of occasions. It is a non-digestible galactomannan that is not bioavailable or hydrolysable, but may be fermented by microflora in the gastrointestinal tract.

Carob bean gum is of low toxicity following oral exposure, with no adverse effects observed in short-term toxicity or long-term toxicity studies in rats or mice, reproductive toxicity studies in rats and developmental toxicity studies in several mammalian species. Carob bean gum is not mutagenic.

Studies involving direct oral administration to neonatal animals are required for evaluation of food additives in infant formula. Such studies are not available for carob bean gum. Although

human infant feeding studies reviewed by JECFA did not report any serious adverse effects and suggest tolerability up to 6000 mg/L, these studies were not designed to evaluate effects on infant gut morphology or health.

EFSA has not yet completed a re-evaluation of carob bean gum in foods for infants under 12 weeks of age, however it noted that infants and young children consuming foods for special medical purposes may show a higher susceptibility to gastrointestinal effects due to their underlying medical condition. EFSA concluded that the available data did not allow an adequate safety assessment of carob bean gum in these foods for infants and young children.

There are two case reports of isolated adverse events in extremely low birth weight infants fed formula containing carob bean gum, including fatal NEC. Based on the available information it is not possible to determine if there is a causal association with carob bean gum based on the available information. JECFA reached the same conclusion in considering these case reports, while EFSA also noted the lack of pathophysiology for the cases of NEC.

New studies published subsequent to the JECFA evaluations include an *in vitro* study showing fermentation of carob bean gum by infant gut microbiota, adding further support to previous evidence of fermentation from *in vitro* and animal studies.

Results of a paediatric clinical trial suggest that infant formula containing 3300 mg/L cold-soluble carob bean gum galactomannans or 4500 mg/L hot-soluble galactomannans appears to be well-tolerated and supports body weight gain. Formula containing 4500 mg/L cold-soluble carob bean gum galactomannans was less well-tolerated, with increases in total and diarrhoeic defecations, which may have contributed to the lack of a significant body weight gain observed in this group.

The combined results of four clinical trials in infants with regurgitation indicated that thickened infant formulas containing 650 mg/L carob bean gum together with pectin and tapioca or corn starch were well tolerated.

JECFA identified a single case of allergenicity in one 5-month old infant following exposure to carob bean gum, and a single adult case report of carob bean gum hypersensitivity. Several new studies related to immune responses were identified by FSANZ.

A single case was reported of an infant with FPIES related to carob bean gum used as a thickener in infant formula. FPIES is a non-IgE-mediated gastrointestinal food allergy that occurs predominantly in infants and young children. FPIES may be caused by almost any food with common triggers being cow's milk, rice, soy and oats, while other triggers include poultry meats, egg yolk, cereal grains, fruits and vegetables (Caubet et al. 2019). A survey in Australia reported that the incidence of FPIES in infants < 24 months was 15.4/100,000 per year. The most common food trigger in Australian infants was rice (45%), followed by cow's milk (33%), egg (12%), oats (9%) and chicken (8%) (Mehr et al. 2017). FPIES has also been reported in exclusively breastfed infants, although this appears to be very rare (Baldo et al. 2020; Mehr et al. 2017).

A case report of an occupationally exposed woman with inhalation allergy to carob bean gum who developed food allergies to some legumes and tree nuts was also identified. This study indicated that the food allergies may have been due to cross-reactivity with carob bean gum, although the woman was able to eat ice cream containing carob bean gum.

Other new data were a case report of asthma resulting from occupational exposure to carob bean gum and a study of serum antibody reactivity to carob bean gum.

Overall, the currently available evidence suggests that food allergy to carob bean gum is likely to be rare.

No toxicological studies with neonatal animals were found that would change the conclusions of the JECFA evaluation at the 82nd meeting that the available data are not sufficient for the evaluation of carob bean gum for use in infant formula at a maximum use level of 10,000 mg/L.

JECFA did not specifically comment on the safety of carob bean gum at the MPL of 1000 mg/L currently permitted in the Codex Standard and the Code, although it was noted that this level is much lower than the proposed use level. Studies in infants at concentrations up to 6000 mg/L carob bean gum, 3300 mg/L cold soluble carob bean gum galactomannans or 4500 mg/L hot soluble carob bean gum galactomannans did not report any serious adverse events, indicating that use in infant formula at the current MPL is unlikely to be of toxicological concern.

6 Xanthan gum (INS 415)

Xanthan gum has technical functions as an emulsifier, foaming agent, stabiliser or thickener. Xanthan gum is not currently listed for use in infant formula products in Schedule 15 of the Code.

6.1 Current Codex permissions

Xanthan gum is currently listed in the Codex General Standard for Food Additives for use in complementary foods for infants and young children at an MPL of 10,000 mg/kg (Codex 2018). It is not currently permitted by Codex for use in infant formula.

The safety of xanthan gum has been evaluated by JECFA on several occasions, most recently at its 82nd meeting where its use as a thickener in infant formula was considered (WHO 2016, 2017).

6.2 JECFA evaluation of xanthan gum

JECFA reviewed the safety of xanthan gum at its 18th, 29th and 30th meetings. An ADI 'not specified' was established at the 30th meeting based on the absence of adverse effects in toxicological studies in rats and dogs, and supported by a lack of adverse effects in studies involving humans (WHO 1987).

In its 82nd meeting, JECFA evaluated the safety of xanthan gum for use as a thickener in protein hydrosylate infant formula, follow-on formula and formula for special medical purposes intended for infants.

The maximum proposed use level evaluated by JECFA was 1000 mg/L (WHO 2017).

6.2.1 Chemical and technical considerations

Xanthan gum has a high molecular weight (around 1000 kDa) and is a water soluble polysaccharide containing D-glucose and D-mannose as the dominant hexose units, together with D-glucuronic acid and pyruvic acid. It is produced by fermentation of a carbohydrate source in a pure culture of *Xanthomonas campestris*, a naturally occurring bacterium. The fermentation medium contains sources of carbohydrate and nitrogen as well as mineral salts.

Xanthan gum is recovered from the broth by ethanol or isopropanol precipitation following

fermentation, in the form of a sodium, calcium or potassium salt. The resulting coagulum is further processed, resulting in a final product that is a cream-coloured powder.

The specification for xanthan gum was reviewed by JECFA at the 82nd meeting. The Committee maintained the previous limit for lead for general use (2 mg/kg) and introduced a lead limit of 0.5 mg/kg for use in infant formula and formula for special medical purposes intended for infants. The limit for lead when used in infant formula was established following introduction of a maximum limit of 0.01 mg/kg for lead in infant formula as consumed by the Codex Committee on Contaminants in Foods (Codex 2014), in order to ensure that this would not be exceeded.

6.2.2 Toxicokinetics and metabolism

In vitro studies and studies involving oral administration to rats have demonstrated that xanthan gum is largely not digested by enzymes in the upper gastrointestinal tract and is poorly absorbed. The majority of ingested xanthan gum is excreted unchanged in the faeces, however *in vitro* studies indicate it is also susceptible to microbial degradation in the lower parts of the gut. Xanthan gum ingestion by rats, dogs and human subjects was associated with variable changes in faecal and/or caecal SCFA concentrations.

6.2.3 Toxicological studies

JECFA noted that xanthan gum shows low acute oral toxicity in mice, rats and dogs, with LD_{50} values ranging from > 1000 mg/kg bw to > 45,000 mg/kg bw.

In the majority of short-term toxicity studies, effects in animals occurred mainly in the intestine at doses greater than 700 mg/kg bw/day. These included faecal bulking and water binding accompanied by some increases in intestinal tissue mass. Reduced nutrient absorption was reported at doses greater than 700 mg/kg bw/day and was associated with reduced weight gain and lower liver weights. No changes in the weights of other organs were observed, and no gross morphological or histopathological changes were reported. Dogs fed a diet containing xanthan gum for 12 weeks showed stool softening at 500 mg/kg bw/day. Occasional diarrhoea was seen in a 2-week dog study at 1000 mg/kg bw/day, and persistent diarrhoea occurred at 2000 mg/kg bw/day.

New short-term dietary toxicity studies reviewed by JECFA in 2016 included a report of increased caecal tissue weight, increased faecal output and increased faecal SCFAs following dietary administration of xanthan gum at 5% (equivalent to 2500 mg/kg bw/day) to male rats for 4 weeks. Heavier intestinal tissue weights and small but significant increases in the length of the small and large intestines were observed in a 2-week study in rats fed 5% xanthan gum. Increased concentrations of total bile acid and bile volume and enhanced digestive enzyme activity were also observed. The study authors suggested that the enlargement of the digestive organs and increased biliary secretions may result from xanthan gum decreasing absorption from the intestinal tract and digestive processes. No effects on body weight gain were reported.

A NOAEL of 3301 mg/kg bw/day, the highest dose tested, was reported in a 90-day study of a new xanthan gum product in rats. In two dog studies, dietary administration of xanthan gum (1.2% or 14%, equivalent to 300 and 4200 mg/kg bw/day, respectively) for up to 10 days was associated with increased stool moisture and softened stools.

Xanthan gum was reported to be well tolerated in rats and dogs at doses up to 1000 mg/kg bw/day in the diet for 2 years, with no increase in tumour incidence. No adverse effects on reproduction or *in utero* or postnatal development were reported in a three-generation reproductive toxicity study in rats, at dietary doses up to 500 mg/kg bw/day.

JECFA noted that in special studies, xanthan gum reduced the postprandial glucose and insulin response, increased bile acid secretion and reduced plasma cholesterol and triglyceride levels, and reduced serum uric acid concentrations in rats with adenine-induced renal dysfunction.

In immune studies, xanthan gum induced DNA synthesis in mouse splenic B cells and thymocytes *in vitro*, and stimulated polyclonal IgM and IgG antibody responses in B cells. Incubation of two mouse macrophage cell lines with xanthan gum resulted in production of interleukin 12 and tumour necrosis factor alpha. Splenocytes from xanthan gum-treated mice had greater natural killer cell activity than those from mice treated with a vehicle control, and oral administration of xanthan gum inhibited the growth of transplanted tumour cells. JECFA concluded that these observations indicate biological activity of xanthan gum on exposed cells, but the human relevance of these findings is not known.

A study in rats assessing the potential effects of several fluid thickeners on water absorption found no significant differences in the amount of water absorbed following treatment with xanthan gum-containing fluid, compared with pure water.

6.2.4 Special studies in neonatal animals

Two toxicological studies with xanthan gum in neonatal pigs were reviewed by JECFA. The studies used the same protocol and were conducted by the same laboratory within a 2-month period, and were considered together as a single study by the Committee in its evaluation. In the first study, neonatal pigs (6/sex/group) were administered xanthan gum in a milk replacer at doses of 0, 375 or 3750 mg/kg bw/day (dosing concentrations of 0, 750 and 7500 mg/L, respectively) from lactation day 2 for 20 days. In the second, follow-up study, neonatal pigs (6/sex/group) received xanthan gum at doses of 0 or 750 mg/kg bw/day (concentration of 1500 mg/L), again from lactation day 2 for 20 days.

All animals survived to scheduled necropsy on postnatal day 22. Green discoloured faeces, soft faeces, watery faeces and increased defecation were observed at 3750 mg/kg bw/day. At the two lower doses, soft and/or watery faeces were seen, but were considered an expected effect of xanthan gum. Body weight gains at 375 and 750 mg/kg bw/day were similar to controls, whereas terminal body weights in pigs at the highest dose were about 40% lower than those of controls. Marked increases in absolute and relative weights of the caecum and colon of both sexes were seen at 3750 mg/kg bw/day, while changes in intestinal weights at the two lower doses were smaller and not statistically significant. Treatment-related histological changes (primarily goblet cell hypertrophy/hyperplasia) were seen in the large and small intestine at the highest dose. These changes were rated as minimal to moderate severity. Similar histological changes were seen in fewer animals at the two lower doses and when present, were minimal in severity. JECFA considered the changes at the two lower doses to be adaptive and non-adverse. The Committee concluded that the NOAEL in neonatal pigs was 750 mg/kg bw/day, based on intolerability and histological changes in the intestines at 3750 mg/kg bw/day.

6.2.5 Observations in humans

Observations in humans reviewed by JECFA included a series of studies assessing growth outcomes and tolerability as well as mineral absorption in full-term infants fed protein hydrosylate formula containing xanthan gum. In addition, post-marketing surveillance data collected by one manufacturer were available to the Committee.

In a 1-week study, infants (aged ≤ 28 days) fed formula containing up to 1500 mg/L xanthan gum (doses up to 232 mg/kg bw/day) showed better tolerability than infants given the same

formula without xanthan gum. Infants fed the xanthan gum had a decreased percentage of watery stools and number of stools per day compared with controls.

Infants (aged ≤ 8 days) fed xanthan gum-containing formula at a concentration of 750 mg/L (dose of 120-126 mg/kg bw/day) with or without starch sodium octenyl succinate for 20-28 days had similar growth outcomes (body weight and body weight gain) and mean rank stool consistencies as infants fed formula without xanthan gum. No clinically relevant differences in serious adverse events between groups were reported, and the presence of xanthan gum in the formula decreased vomiting. The highest rate of dropouts due to intolerance of formula was in the group consuming xanthan gum-free formula.

Feeding of xanthan gum-containing reconstituted protein hydrosylate formula (750 mg/L, equivalent to 102 mg/kg bw/day) for up to 112 days to infants aged ≤ 9 days did not adversely affect growth or development. Infants receiving the control, ready-to-feed formula had greater intakes of formula and passed more stools per day than infants receiving the xanthan gum-containing formula.

Two special studies examining potential effects of xanthan gum-containing formula on mineral absorption (750 mg/L; infants aged less than 6 months or 1500 mg/L; infants aged 60 – 105 days) found slight decreases in mineral absorption. Fractional calcium absorption[1] was lower in infants fed formula with xanthan gum compared with controls (39% versus 62%, respectively), but net calcium absorption[2] was similar to that of the infants fed control formula (50.2 and 45.3 mg/kg bw/day, respectively). Total and net zinc absorption[3] were lower in infants given the xanthan gum-containing formula compared with controls, although the difference was not statistically significant. Despite these differences in mineral absorption, no effects on the growth of infants fed xanthan gum-containing formula were reported in these studies or in the 112-day infant growth study.

JECFA noted that cases of late-onset NEC in (mostly premature) newborns consuming formula to which a xanthan gum thickener was added have been reported. In a summary of 22 case reports that had been submitted to the US Food and Drug Administration (FDA), 21 of the affected infants were born premature. Concentrations of xanthan gum were not reported, but based on the descriptions of the preparations having a honey/nectar consistency the concentration was presumed to be higher than that in marketed infant formula (up to 1000 mg/L). The authors noted that a well-designed analytical study would be needed to establish a true association between use of viscous xanthan gum-containing formula and late-onset NEC. JECFA also considered it was not possible to conclude whether there is any causal association based on the available information.

JECFA also considered post-marketing surveillance data collected by one manufacturer from June 2010 to May 2015, during which time distribution of hydrolysed powder containing xanthan gum provided exposure of a total of 96 million treatment days (where a treatment day is assumed to be equivalent to the consumption of 0.8 L of product by an infant in a 24-hour period). The formula was to be reconstituted resulting in xanthan gum concentrations up to 1000 mg/L. Formula containing xanthan gum was not associated with an increased rate of adverse events. The Committee noted that these data provide additional support for the safe use of xanthan gum in infant formula.

In addition, JECFA reviewed a number of studies in which xanthan gum was given to adult humans with no significant adverse effects reported.

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[1] Fractional absorption refers to the proportion of an ingested substance that is absorbed.
[2] Net absorption refers to the amount of a substance that is absorbed, minus the amount that is excreted.
[3] Total absorption refers to the total amount that is absorbed.

6.2.6 Assessment of dietary exposure

At the maximum proposed use level for xanthan gum in infant formula of 1000 mg/L, estimated dietary exposure ranged from 60 – 180 mg/kg bw/day in infants aged 0 – 12 weeks, based on recommended average daily formula intake or estimated median energy requirements for formula-fed infants. For infants with high (95th percentile) energy requirements, intakes were estimated to reach 220 mg/kg bw/day.

6.2.7 Conclusions of JECFA evaluation

JECFA considered that the new short-term toxicity and special studies in rats and dogs that have become available since the previous evaluation of xanthan gum confirm the absence of any adverse effects due to consumption of xanthan gum. It was noted that clinical studies with infants indicate that formulas containing xanthan gum at concentrations up to 1500 mg/L (232 mg/kg bw/day) were well tolerated.

The Committee noted that a NOAEL of 750 mg/kg bw/day (given as a 1500 mg/L formulation) was established in neonatal pigs. The neonatal pig is an appropriate animal model for assessing the safety of food additives for infants, as exposure of neonatal pigs for the first 3 weeks of life models the 0-12-week period of development in humans during which infant formula may be the sole source of nutrition.

Comparison of the conservative upper estimate of dietary intake for (220 mg/kg bw/day) with the NOAEL from the neonatal pig study provides a margin of exposure of 3.4. MOEs in the range of 1-10 may be interpreted as indicating a low risk for the health of infants aged 0-12 weeks consuming a food additive in infant formula, subject to a number of considerations related to the toxicological point of departure and exposure assessment. JECFA took the following considerations into account for xanthan gum:

- The toxicity of xanthan gum is low.
- The NOAEL is derived from two studies in neonatal pigs, which are considered a relevant animal model for human infants.
- Clinical studies in infants support the tolerability of formula containing concentrations of xanthan gum up to 1500 mg/L.
- No adverse effects were reported in post-marketing surveillance conducted by one manufacturer over a 5-year period on formulas containing xanthan gum at concentrations up to 1000 mg/L.

Based on these considerations JECFA concluded that the consumption of xanthan gum in infant formula or formula for special medical purposes intended for infants is of no safety concern at the proposed use level of 1000 mg/L. The Committee recognised that infants requiring formula for special medical purposes may have a variety of medical conditions and that these infants would normally be under medical supervision.

6.3 Additional studies

A small number of studies published since the 82nd JECFA meeting containing information relevant to the safety assessment of xanthan gum were identified in a literature search. These studies are summarised below.

6.3.1 Special studies

Effects of dietary fibres on gastrointestinal functions, food intake and body weight in male

rats (Tan et al. 2017); Regulatory status: Non-GLP

Male Sprague Dawley rats aged 10 weeks (8 per group) were assigned to receive a control diet containing 12% wheat bran as a fibre source, control diet with 2% Konjac flour replacing 2% of wheat bran, or control diet containing 2% pre-gelatinised waxy maize starch (PWMS) plus guar gum (85.7% PWMS + 14.3 % guar gum) or PWMS plus xanthan gum (95% PWMS + 5% xanthan gum; equivalent to approximately 70 – 90 mg/kg bw/day xanthan gum) replacing 2% of wheat bran. All animals were fed a control diet for 7 days prior to the start of the treatment period, which lasted 21 days. Food intake was recorded daily and body weights were measured at the beginning and end of the experiment. Digestive tracts were removed at study termination, tissue and content weights were recorded and physicochemical analysis of the fibre materials and digesta was performed.

All three experimental diets had higher viscosity, swelling capacity and water binding capacity compared with the control diet, with the highest values found in the diet containing PWMS and guar gum. Animals fed Konjac flour or PWMS plus guar gum had reduced daily food intakes compared with controls, but there was no significant difference in food intake between the control and PWMS plus xanthan gum groups. No differences in final body weights and body weight gains were found in any group. There were no between group differences in tissue and fresh digesta weights of the stomach, small intestine and colon. Empty caecum weights were similar in all four groups, however fresh caecum digesta weight was increased in animals that consumed Konjac flour or PWMS plus guar gum compared with controls, as was caecum moisture content. These effects were not observed in the PWMS plus xanthan gum group. Compared with controls, the stomach digesta from animals in the Konjac flour and PWMS plus guar gum groups had higher water binding capacity and swelling capacity, but this was not seen in the PWMS plus xanthan gum group. The study authors hypothesised that PWMS plus xanthan gum may not have performed as well as the other interventions due to lower water binding capacity and swelling capacity.

6.3.2 Observations in humans

Effects of xanthan gum on postprandial glycaemia (Tanaka et al. 2018); Regulatory status: Non-GLP

Five healthy volunteers (2 males and 3 females) aged 21-22 years were fasted for 12 hours prior to assessment of fasting blood glucose levels on two experimental days, held seven days apart. In the first experiment, participants ingested 150 mL of an enteral nutrient (Meibalance®, Meiji, Japan, 17.5 g/100 mL total carbohydrate) and blood glucose levels were measured 20, 40, 60 and 120 minutes after ingestion. In the second experiment, participants ingested 150 mL of Meibalance® supplemented with 1.0% (w/v) xanthan gum and blood glucose levels were assessed at the same times as in the previous experiment.

Fasting blood glucose levels were similar on both experiment days. Blood glucose levels 20 minutes after ingestion were significantly lower following consumption of xanthan gum than after consuming the nutrient alone (84.0 ± 5.5 mg/dL versus 107.4 ± 7.8 mg/dL, respectively, $P < 0.05$). Blood glucose levels in the xanthan gum group were slightly lower than in the nutrient alone group at 40 and 60 minutes following consumption, while levels were similar in both groups 120 minutes after ingestion. Total glucose exposure over the 120 minute period, measured as the area under the blood concentration-time curve (AUC), was significantly lower in the xanthan gum group than in the nutrient alone group (14.1 ± 5.1 mg/dL·h versus 29.5 ± 7.8 mg/dL·h, respectively, $P < 0.05$). The study authors suggested that xanthan gum may inhibit glucose absorption.

Effects of xanthan gum on blood sugar following rice consumption (Fuwa et al. 2016);

Regulatory status: Non-GLP

Fasting and post-prandial blood glucose levels were assessed in 11 healthy females, aged 19-39 years, who had never been diagnosed with diabetes. Participants consumed a portion of either standard rice or rice mixed with xanthan gum powder (XGP; 0.5, 1.0 or 1.5%) or xanthan gum sol (XGS; 0.5, 1.0 and 2.5%) containing 50 g of carbohydrate. Blood glucose levels were measured before and 15, 30, 45, 60, 90 and 120 minutes after consumption of the test sample. XGS was prepared by dispersing xanthan gum in water and heating at 98ºC for 60 minutes with stirring. Glucose release from rice *in vitro* was also assessed using simulated mastication and incubation with pancreatin and invertase.

Addition of ≥1.0% XGP to rice significantly reduced postprandial blood sugar levels at 15 and 30 minutes compared with standard rice, and levels at 45 minutes remained significantly lower in participants consuming 1.5% XGP. Glycaemic index and the amount of glucose released *in vitro* were significantly reduced at all concentrations of XGP compared with standard rice.

Post-prandial blood glucose levels were significantly lower at 15 – 60 minutes following consumption of rice mixed with XGS (all concentrations) compared with standard rice. Glycaemic index and *in vitro* glucose release in comparison with standard rice was significantly lower at all concentrations of XGS. Suppression of blood glucose levels by XGS was more effective when consumed together with rice rather than before or after rice consumption. The authors concluded that coating rice with XGS was the most effective method of suppressing blood glucose levels after rice consumption.

6.4 Assessments by other regulatory agencies

6.4.1 EFSA

EFSA's ANS Panel re-evaluated the use of xanthan gum as a food additive in 2017. The opinion specifically excluded an evaluation of use as a food additive in infant formula for infants under the age of 12 weeks, for which a separate risk assessment is due to be performed (EFSA 2017d).

The ANS Panel noted that xanthan gum is unlikely to be absorbed intact and is likely to be fermented by intestinal microbiota. No adverse effects were reported in chronic and carcinogenicity studies at the highest doses tested, and there is no concern for genotoxicity. The Panel also noted that repeated oral intake by adults of up to 214 mg/kg bw/day xanthan gum for ten days was well tolerated; some individuals experienced abdominal discomfort, which was considered undesirable but not an adverse effect.

On the basis of these considerations together with refined exposure assessments, the ANS Panel concluded there was no need for a numerical ADI and there was no safety concern for the general population from use of xanthan gum as a food additive.

For infants and children over 12 weeks of age, the Panel noted that consumption of xanthan gum in infant formula or formula for special medical purposes was well tolerated by infants in clinical studies at concentrations up to 1500 mg/L (232 mg/kg bw/day). In addition, no cases of adverse effects were reported from post-marketing surveillance of formula containing approximately 750 mg/L xanthan gum, supporting the results of the clinical studies. The Panel also noted that the cases of NEC described in the literature are not related to the use of xanthan gum as a food additive in manufacturing of infant formula but relate to its addition to formula or human milk as a thickener prior to consumption. The doses of xanthan gum associated with these cases, while unknown, were expected to be in gram amounts.

The ANS Panel therefore concluded there were no safety concerns from the use of xanthan gum in foods for special medical purposes consumed by infants and young children at concentrations reported by the food industry.

6.5 Discussion and conclusions

The 2016 evaluation by JECFA indicates that xanthan gum is of low toxicity and is well tolerated by infants. Using JECFA's dietary exposure assessment for infant formula, based on a maximum level of 1000 mg/L (220 mg/kg bw/day for fully formula-fed infants with high energy requirements) and the NOAEL of 750 mg/kg bw/day from a toxicity study in neonatal pigs, the MOE was 3.4. JECFA has previously advised that MOEs in the region of 1-10 could be interpreted as low risk for the health of infants aged 0-12 weeks consuming a food additive in infant formula, when appropriate toxicological and dietary exposure considerations have been taken into account (WHO 2014).

Cases of late-onset NEC in (mostly premature) newborns consuming formula to which a xanthan gum thickener was added have been reported. Based on the available information it is not possible to determine if there is a causal association with xanthan gum. JECFA reached the same conclusion in considering these case reports and noted that the xanthan gum concentrations in these case reports was likely to be higher than that the maximum level of 1000 mg/L. EFSA noted that the described cases are not related to the food additive use of xanthan gum in infant formula but relate to its addition to formula or human milk as a thickener prior to consumption. The doses of xanthan gum associated with these cases, while unknown, were expected to be in gram amounts, i.e. higher than the proposed MPL. The US FDA has also stated that further study is needed to determine if there is an actual link between consumption of xanthan gum-based thickener and development of NEC.

New studies with xanthan gum published since the JECFA evaluation do not indicate a need to revise the conclusions of JECFA's risk assessment.

A study of the effect of dietary fibres on food intake, body weight and gastrointestinal parameters in rats found no significant adverse effects from a diet containing xanthan gum together with pre-gelatinised waxy maize starch.

Two studies in humans reported reductions in postprandial blood glucose levels following consumption of rice or a semi-solid enteral nutrient source. No adverse effects were reported in these studies. These findings are consistent with reductions in postprandial glucose reported in animal and human studies reviewed by JECFA.

Based on the available data, the consumption of xanthan gum in all infant formula products at concentrations up to 1000 mg/L, the maximum level assessed by JECFA, does not raise safety concerns.

7 Pectins (INS 440)

Pectins are used as gelling, thickening and stabilising agents. Pectins are not currently listed for use in infant formula products in the Code.

7.1 Current Codex permissions

Pectins are not currently permitted for use in infant formula in Codex standards, but are listed in the Codex General Standard for Food Additives for use in complementary foods for infants and young children and for use in follow-up formula, both at an MPL of 10,000 mg/kg (Codex 2018).

The safety of pectins has been evaluated by JECFA on several occasions, most recently at its 82nd meeting when its use as a thickener in infant formula was assessed (WHO 2016, 2017).

7.2 JECFA evaluation of pectins

The safety of pectins was evaluated by JECFA at its 13th, 18th, 19th, 25th, 29th, 79th and 82nd meetings. An ADI 'not specified' was established for pectin and amidated pectin at its 25th meeting in 1981. The Committee noted that non-amidated pectins and their salts (as specified) are normal constituents of the human diet and have also been administered to humans intravenously at high levels without acute toxic effects (WHO 1981).

JECFA evaluated the safety of pectins for use in infant formula and formula for special medical purposes intended for infants at its 79th and 82nd meetings. At the 82nd meeting, JECFA considered additional safety data requested following the 79th meeting, and the maximum proposed use level was reduced from 5000 mg/L to 2000 mg/L (WHO 2016, 2017).

7.2.1 Chemical and technical considerations

Pectin is a complex heteropolysaccharide consisting mainly of partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts. Pectin is obtained by aqueous extraction of edible plant material, usually citrus fruits or apples. The molecular weight of pectin used in food is variable depending on its source and processing, but is expected to range from 100 – 200 kDa.

Pectin is used in infant formula as a thickener to increase the viscosity and as a stabiliser to maintain the homogeneity of the formula during its shelf-life.

JECFA was made aware that a further pectin product is available on the market. Pectin-derived acidic oligosaccharides (pAOS) is produced by enzymatic hydrolysis of pectin. pAOS has not been evaluated by JECFA and is not covered by the existing specification for pectins.

At the 82nd JECFA meeting, the specification for pectins was modified to reduce the limit for lead from 5 to 2 mg/kg for general use, and to introduce a limit for lead of 0.5 mg/kg for use in infant formula.

7.2.2 Toxicokinetics and metabolism

JECFA concluded that pectin is a non-digestible carbohydrate that undergoes extensive fermentation by gut microflora to oligogalacturonic acids. The oligogalacturonic acids are then further metabolised to SCFAs such as acetate, propionate and butyrate.

pAOS is a digestion product of food-grade pectin consisting of small polymers that predominantly have a molecular weight ≤ 3800 Da. JECFA noted that manufactured pAOS is similar to products formed from pectin in the gastrointestinal tract. JECFA therefore concluded that studies on pAOS can support conclusions reached based on data from studies with pectin.

7.2.3 Toxicological studies

JECFA concluded that the NOAEL of pAOS in short-term toxicity studies was around 7000 mg/kg bw/day, the highest dose tested. It was concluded that pAOS is not genotoxic. Diffuse

hyperplasia of the bladder epithelium was observed in rats at the high dose, however this was caused by a concurrent increase in urinary sodium levels and pH, a condition known to predispose rats to hyperplasia of the bladder epithelium. Diffuse hyperplasia of the bladder epithelium due to increased urinary sodium ion concentration and increased urinary pH is a well-known phenomenon in rats and not frequently observed in other animal species. JECFA concluded that this effect is not of relevance to humans.

7.2.4 Special studies in neonatal animals

In a 3-week study with neonatal pigs fed a pectin-containing milk replacer, decreased feed intake and body weight gain were reported at 1.0% (reported to be equal to 3013 mg/kg bw/day). At its 79th meeting, JECFA considered that while no overt toxicological effects were observed in the study, decreased food intake and body weight gain would be undesirable in human infants. The NOAEL in this study was 0.3%, reported as equal to 847 mg/kg bw/day.

At the 82nd JECFA meeting, an updated statistical analysis of the neonatal pig study was reviewed, as well as an additional 3-week study with neonatal pigs. Reanalysis of the previously assessed neonatal pig study proposed a combined analysis of growth data from both sexes, and confirmed there were no effects on growth at 0.05% or 0.3% pectin compared with controls. At the highest dose, pectin did not significantly affect consumption of milk replacer, but did significantly decrease body weight and feed conversion efficiency, irrespective of sex. The dose levels were recalculated using measured pectin concentrations rather than the target concentrations, resulting in exposures of 131, 1049 and 4015 mg/kg bw/day for males and 130, 1064 and 4062 mg/kg bw/day for females. JECFA concluded the NOAEL in this study was 1049 mg/kg bw/day.

In the new study, neonatal pigs (6/sex/dose) were given pectin in milk replacer as the sole source of nutrition for 3 weeks at target concentrations of 0.2% or 1% (equal to 704 and 4461 mg/kg bw/day, respectively, for males and females combined). The focus of the new study was growth and nutrient digestibility. There were no differences between the control and 0.2% groups in any aspect of growth, including average daily milk replacer consumption, daily body weight, average body weight gain, feed conversion efficiency and final body weight. At the high dose, growth was significantly reduced compared with controls, and associated with significantly lower milk replacer consumption and reduced nutrient digestibility. JECFA concluded that the reduced milk replacer consumption was likely due to delayed gastric emptying and/or prolonged gut transit due to the highly viscous 1% pectin diet. The NOAEL in this study was 0.2% pectin, equal to 704 mg/kg bw/day for males and females combined.

7.2.5 Human studies

One study investigating effects of pectin in preterm human infants was reviewed by JECFA. Infants fed fortified human milk containing 0.085% pectin had a significantly higher linear growth rate and greater increases in weight, length and head circumference compared with control infants. No treatment-related adverse events were reported.

In four studies with term infants fed infant formula containing pAOS at concentrations up to 0.2%, pAOS was well tolerated. Two of these studies reported no adverse effects on growth, while no effects on faecal counts of *Bifidobacterium* sp., *Clostridium* sp., *E. coli* or *Enterobacter* sp. were observed in the other two studies.

7.2.6 Assessment of dietary exposure

At the maximum proposed use level of 2000 mg/L evaluated in 2016, JECFA concluded that estimated median dietary exposure to pectin from infant formula ranged from 120 to 360

mg/kg bw/day for infants aged 0-12 weeks. Infants with high (95th percentile) daily energy intakes may reach an exposure level of 440 mg/kg bw/day.

7.2.7 Conclusions of JECFA evaluation

At its 79th meeting, JECFA concluded that estimated exposure to pectin in infant formula at the proposed use level of 5000 mg/L was in the region of the NOAEL in the neonatal pig study and close to the LOAEL, which was based on decreased feed intake and body weight gain. This was considered to be of concern.

In 2016, JECFA noted that the newly submitted data confirm these effects in neonatal pigs and indicate they are due to delayed gastric emptying and/or prolonged gut transit resulting from the viscosity of the milk replacer at 1% pectin. Re-evaluated dose levels using measured concentrations of pectin in milk replacer indicate a NOAEL of 1049 mg/kg bw/day. The NOAEL in the second neonatal pig study (704 mg/kg bw/day) was lower than that of the previous study, but this was due to differences in dose spacing. The critical NOAEL was 1049 mg/kg bw/day.

At the new maximum proposed use level of 2000 mg/L, estimated exposures of infants aged 0-12 weeks would be up to 360 and 440 mg/kg bw/day at mean and high consumption, respectively. The MOEs are 2.9 and 2.4 for average and high consumers, respectively.

MOEs in the range of 1-10 may be interpreted as indicating a low risk for the health of infants aged 0-12 weeks consuming a food additive in infant formula, subject to a number of considerations related to the toxicological point of departure and exposure assessment. JECFA took the following considerations into account for pectin:

- The toxicity of pectin is low.
- The NOAEL is derived from studies in neonatal pigs, a relevant animal model.
- The adverse effects in neonatal pigs are likely to be related to the viscosity of pectin at 1%.
- Clinical studies provide support for the tolerance of infants to pectin at concentrations up to 0.2%.
- The exposure estimates are conservative.

Based on these considerations, JECFA concluded that the MOEs for the use of pectin at 2000 mg/L in infant formula indicate a low risk for the health of infants and therefore are not of concern. The Committee noted that there is variability in medical conditions among infants requiring formula for special medical purposes, and that these infants would normally be under medical supervision.

7.3 Additional studies

A small number of studies published since the 2016 JECFA evaluation containing information relevant to the safety assessment of pectins were identified in a literature search. These studies are summarised below.

7.3.1 Toxicokinetics and metabolism

Effects of pectin supplementation on carbohydrate fermentation patterns in rats (Tian et al. 2016) Regulatory status: Non-GLP

Male Wistar rats (8/group, age not specified) were fed a control diet or diets containing 3% (w/w) of low-methyl esterified citrus pectin (LMP), high-methyl esterified citrus pectin (HMP), sugar beet pectin (SBP) or soy pectin (SSPS) for 7 weeks. Rats were killed at the end of the

study period and caecal and colonic digesta were collected and analysed for digestibility of starch, protein and carbohydrates, concentrations of lactate, SCFAs and microbiota composition.

Samples were pooled for the digestibility analysis so no statistical analysis could be performed. Caecal digestibility of added pectin was calculated to be 91%, 82%, 96% and 101% in LMP-, HMP-, SBP and SSPS-fed rats, respectively. Digestibility in the colon was 93%, 92%, 112% and 98%, respectively. The main fermentation products found in the digesta were SCFAs including acetate, propionate and butyrate. In the caecum, LMP- and SSPS-fed rats had significantly higher total SCFA, propionate and butyrate concentrations than controls or the other pectin-fed groups. No significant differences in SCFA concentrations between groups were observed in the colon. Lactate concentrations were similar in both groups in the caecum and the colon. Effects on microbiota were generally more pronounced in the caecum than in the colon. Members of the *Lactobacillus* genus were increased in the caecum in LMP- and SBP-fed rats, and levels of Firmicutes in caecal and colonic digesta were higher in pectin-fed rats than controls.

Effects of pectin on fermentation characteristics, carbohydrate utilisation and microbial composition in the gastrointestinal tract of weaning pigs (Tian et al. 2017)
Regulatory status: Non-GLP

Dutch Landrace X Large White piglets were randomly assigned at weaning (age 3 weeks) to be fed a control diet or diets containing a LMP, a HMP or a hydrothermal-treated soybean meal for 28 days. LMP and HMP formed 3% (w/w) of the diet while autoclaved soybean meal formed 7% (w/w) of the diet. Each group comprised four piglets, sex unspecified. Faecal samples were collected on study days 14 and 28, and on day 28 two piglets in each group were killed and digesta were collected from the distal ileum, proximal colon, mid colon and distal colon. Digesta and faeces were analysed for digestibility of nutrients, concentrations of lactate, succinate and SCFAs and for microbiota composition.

All piglets were reported to be healthy for the duration of the study. The LMP diet was associated with reduced digestion of starch in the ileum compared with the other diets, resulting in higher starch fermentation in the colon. LMP was fermented more efficiently than HMP in the ileum. HMP was efficiently fermented in the proximal colon resulting in an absence of HMP in the mid and distal colon. LMP was of limited digestibility in the proximal colon, where a high amount of starch was utilised. In the mid colon and distal colon 10% and 4% of LMP were fermented, respectively. Lower concentrations of SCFAs were observed in the ileum of LMP- and HMP-fed pigs compared with controls. Concentrations of SCFAs in LMP-fed pigs were higher in all parts of the colon compared with controls. HMP-fed pigs had lower levels of SCFAs than controls in the proximal colon but greater levels in the mid colon. The LMP and HMP diets decreased the relative abundance of the genus *Lactobacillus* and increased that of *Prevotella* in the colon compared with controls.

7.3.2 Toxicological data

Subchronic oral toxicity study of cocoa pod husk pectin in rats (Adi-Dako et al. 2018b)
Regulatory status: Non-GLP; Protocol based on OECD TG 408

Groups of six week-old Sprague Dawley rats (6/sex/group) were administered 0, 0.714, 7.14 or 71.4 mg/kg bw/day pectin derived from cocoa pod husks by oral gavage for 90 days. The cocoa pod husk pectin was extracted from minced fresh cocoa pod husks by hot aqueous extraction, and was reported to have an ash value of 1.0%, a moisture content of 0.19 ± 0.06% and a low degree of esterification (26.8 ± 2.5%). The molecular weight was reported as 43-82 kDa. Water was used as the vehicle and negative control. Animals were observed daily for clinical signs, and feed and water consumption were measured at an unspecified

frequency. Urine and blood samples were taken on days 30, 60 and 90 for urinalysis and serum biochemistry assessment. At the end of the study period rats were killed and gross necropsy was conducted. Wet organ weights relative to body weight were determined for the heart, liver, lungs, spleen and kidneys. Histopathology was performed on tissues from the heart, liver, lung and kidney. Sleeping time following intraperitoneal injection of pentobarbital was also assessed.

All animals survived the duration of the study and no clinical signs of toxicity were reported. Feed and water intake was similar in all groups in study weeks 0, 4, 8 and 13. Body weights and body weight gains were not reported. Relative wet organ weights were similar in all groups, apart from a significant decrease in kidney and spleen weights in females given 71.4 mg/kg bw/day. Serum biochemistry parameters and urinalysis were not affected by treatment with cocoa pod husk pectin. No adverse effects were observed on gross necropsy or histopathology of the kidney and other organs evaluated.

Pentobarbital-induced sleeping time was similar in all groups at the end of the study.

Effects on haematological parameters in a 90-day oral toxicity study of cocoa pod husk pectin in rats (Adi-Dako et al. 2018a) Regulatory status: Non-GLP

Sprague Dawley rats (6/sex/group), aged 6 weeks, were administered 0, 0.714, 7.14 or 71.4 mg/kg bw/day cocoa pod husk pectin by oral gavage for 90 days. Cocoa pod husk pectin was extracted with hot water and hot aqueous citric acid, and had a degree of esterification of 26.8%. Clinical signs were monitored daily and body weights were measured weekly. Food and water consumption was monitored for the 90-day period at unspecified time intervals. Blood samples were collected on study days 30, 60 and 90 and analysed for haematology parameters and bilirubin levels. Rats were killed at the end of the study period and the spleen was collected for histopathological examination. It is not clear if this is the same study as that discussed above, where effects of cocoa pod husk pectin at the same doses on other parameters were reported, where decreased spleen weights (relative to body weight) were observed in females administered the highest dose (Adi-Dako et al. 2018b).

No mortality occurred during the study and no significant differences in food and water consumption was reported. Animals gained body weight over the 90 day period and no significant differences between groups were reported. There were no treatment-related effects on red blood cell indices or on the levels of white blood cells, lymphocytes and neutrophils. Mean corpuscular volume was significantly reduced compared with controls in high dose males on study day 30, but similar changes were not seen on days 60 or 90. Platelet indices and bilirubin concentrations were also unaffected by treatment with cocoa pod husk pectin. No histological changes in the spleen were reported.

7.3.3 Special studies

In vitro study of cytotoxicity and oxidative DNA damage induced by citrus pectin and apple pectin in breast cancer cells (Salehi et al. 2018) Regulatory status: Non-GLP

Citrus pectin and apple pectin were evaluated for their ability to cause apoptosis and oxidative DNA damage in breast cancer cells *in vitro*. Both citrus pectin and apple pectin inhibited cell proliferation and induced apoptosis in cultures of human breast cancer cells (MDA-MB-231, T47D and MCF-7 cell lines). In contrast, cell proliferation was not inhibited in non-tumourigenic L929 mouse fibroblast cells. Citrus pectin and apple pectin increased the production of intracellular reactive oxygen species (ROS) in MDA-MB-231 cells and reduced the mitochondrial membrane potential. Increased formation of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative DNA damage was observed following treatment with either form of pectin. Citrus pectin and apple pectin induced DNA strand breaks as measured using a

comet assay at concentrations associated with significant induction of ROS (800 and 200 µg/mL). These concentrations were twice the respective IC_{50} of citrus and apple pectin for cell growth inhibition in 72 hour cell cultures. Further analysis suggested that citrus pectin and apple pectin may interact *in vitro* with double stranded DNA by intercalation and groove binding plus partial intercalation, respectively.

Immunomodulation of cytokine levels in the spleen of BALB/c mice by natural and modified citrus pectin (Merheb et al. 2019) Regulatory status: Non-GLP

Groups of five female BALB/c mice (aged 8 weeks) were assigned to receive 0, 1.5%, 3% or 5% citrus pectin, or 1.5%, 3% or 5% modified citrus pectin via drinking water for 21 days. The form of modified citrus pectin used was PectaSol-C, which is prepared by enzymatic treatment and has a molecular weight between 5 and 10 kDa. The molecular weight of citrus pectin was reported to be higher than that of modified citrus pectin, but was not specified. At the end of the treatment period mice were killed, spleens were collected and the levels of a range of cytokines in the spleen were evaluated using ELISA kits. Some increases in levels of pro-inflammatory cytokines (TNF-α, IFN-ɣ, IL-17) were found following treatment with citrus pectin and/or modified citrus pectin, however no consistent dose-related changes were observed.

Effects of supplementation of mice with oligosaccharides during pregnancy or lactation on sensitisation and allergy of offspring (Hogenkamp et al. 2015) Regulatory status: Non-GLP

Female pathogen-free C3H/HeOuJ mice (aged 3 weeks) were assigned to one of three groups (n=9). One group was sensitised intragastrically to hen's egg ovalbumin using cholera toxin as an adjuvant, a second, non-sensitised group received cholera toxin only, while a third sham group received vehicle (phosphate buffered saline only). Mice were mated two weeks after the last sensitisation and fed either a control diet or diet supplemented at 2% with a 9:1:2 mixture of short-chain galacto-oligosaccharides (scGOS), long-chain fructo-oligosaccharides (lcFOS) and pAOS (scGOS/lcFOS/pAOS) during pregnancy or lactation. After birth, pups were randomly allocated to be nursed by sensitised or non-sensitised dams that were supplemented with scGOS/lcFOS/pAOS during pregnancy, supplemented during lactation or not supplemented at any point. After weaning, all offspring were fed a control diet and sensitised with ovalbumin plus cholera toxin. Acute allergic skin responses, shock symptoms, body temperature and ovalbumin-specific plasma immunoglobulins (IgE, IgG1 and IgG2a) were measured upon intradermal ovalbumin challenge.

Offspring of sensitised dams fed scGOS/lcFOS/pAOS during pregnancy (S-Preg) or during lactation (S-Lact) had significantly smaller allergic skin responses to ovalbumin than offspring of sensitised dams that were fed control diet during both pregnancy and lactation (S-Cont). S-Lact offspring also had an absence of shock symptoms, as well as lower ovalbumin-specific IgE and IgG1 and higher ovalbumin-specific IgG2a concentrations compared to S-Cont and S-Preg offspring.

Offspring of non-sensitised dams fed scGOS/lcFOS/pAOS during lactation (NS-Lact) had significantly lower anaphylactic shock scores and less change in body temperature than NS-Cont or NS-Preg offspring. Allergic skin responses among NS-Lact offspring were non-significantly lower than NS-Cont or NS-Preg offspring, and similar to sham-treated mice, whereas responses in the NS-Cont and NS-Preg offspring were significantly higher than those of sham-treated mice. NS-Lact offspring had significantly lower ovalbumin-specific IgE and IgG1 compared with NS-Cont and NS-Preg offspring.

The study authors concluded that scGOS/lcFOS/pAOS supplementation during pregnancy led to fewer allergic symptoms of offspring sensitised before mating, while supplementation

during lactation led to reduced allergic symptoms in offspring regardless of whether the dam had been sensitised before mating. Given the mixture of substances given to the animals it is not possible to attribute effects to a particular component.

Protective effects of a mixture of oligosaccharides against rotavirus gastroenteritis in suckling rats (Rigo-Adrover et al. 2017) Regulatory status: Non-GLP

Suckling Lewis rats were distributed into four groups and administered either vehicle control, vehicle control plus rotavirus inoculation, heat-treated fermented milk components or a mixture of scGOS, lcFOS and pAOS by oral gavage from day 3 of life until termination on day 14 (n=9) or day 21 (n=12). Each group included 3 litters containing 7 pups. The scGOS/lcFOS/pAOS group received a dose of 8000 mg/kg bw/day, 15% of which was pAOS (i.e. 1200 mg/kg bw/day pAOS). All animals apart from controls were inoculated with rotavirus on day 7 of life, and the incidence of diarrhoea was monitored and faecal samples were collected. Body weights were recorded between days 2 and 14 of life. At termination on day 14 or 21 samples of blood and the small intestine were collected for analysis of anti-rotavirus antibodies.

All animals gained body weight and no weight loss was associated with the infection process. The group given scGOS/lcFOS/pAOS had a significantly lower body weight slope than the other groups from day 6 to day 13, but at day 14 there were no significant differences in body weight or body weight gain. Administration of scGOS/lcFOS/pAOS resulted in softer faeces but when this was adjusted for scGOS/lcFOS/pAOS was found to reduce the incidence and severity of diarrhoea induced by rotavirus. Intestinal anti-rotavirus IgA concentrations were increased at day 14 in the scGOS/lcFOS/pAOS groups compared with rats given rotavirus alone. Data on viral shedding and from an *in vitro* blocking assay suggested that scGOS/lcFOS/pAOS may be able to bind the rotavirus viral particles. The study authors concluded that scGOS/lcFOS/pAOS may have protective properties against rotavirus infection. Given the mixture of substances given to the animals it is not possible to attribute effects to a particular component.

Effects of dietary pectin on intestinal bile acid profile and transport in young pigs (Fang et al. 2018) Regulatory status: Non-GLP

Male Duroc X Large White crossbred piglets (age unspecified) were randomly divided into two groups of six animals and allowed to adapt for a week before being fed diets containing 5% apple pectin or cornstarch for 72 days. At the end of the study, blood samples were collected as well as samples of liver, intestinal digesta and intestinal mucosa. Faeces were collected the day before study termination. Serum was analysed for bile acids, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Bile acids were quantified in luminal contents and faeces, and the abundance of mRNA of a range of bile acid-related genes (related to bile acid transport, receptors, signalling and biosynthesis) was measured in mucosa of the duodenum, jejunum, ileum, caecum and proximal colon using real-time PCR.

Average daily food intake and body weights on study days 0 and 72 were similar in the pectin and control groups. Serum TC and LDL-C were significantly lower in pigs fed pectin compared with controls, and plasma HDL-C was significantly higher than controls. The LDL-C:HDL-C ratio was significantly lower in the pectin-fed pigs compared with controls. Serum TG levels were lower in pigs given pectin, but the difference was not significantly different. No between-group differences in total serum bile acids were observed. No statistically significant differences in concentrations of individual bile acids were observed in the ileum, caecum, colon and faeces, although there was a non-significant reduction in caecal ursodeoxycholic acid and hyocholic acid in pectin-fed pigs. Expression of the bile acid receptor (FXR) mRNA was significantly higher in the ileum of pectin-treated animals

compared with controls. Expression of the apical bile acid transporters ASB2 and MRP2 were also increased in the ileum, but expression of transporters on the basolateral membrane were unaffected, suggesting increased transport of bile acid in and out of enterocytes across the apical membrane. In the caecum, FXR expression was significantly increased in pigs fed pectin compared with controls, as was expression of efflux receptors on the apical (MRP2) and basolateral (MRP3) membrane. No significant differences in expression of bile acid-related genes were observed in the colon. In the liver, expression of enzymes involved in biosynthesis of bile acids were not affected by pectin consumption.

Effects of pectin on obesity development and duodenal alkaline phosphatase activity in Sprague Dawley rats (Šefčíková and Raček 2016) Regulatory status: Non-GLP

Groups of 8 male Sprague Dawley rats (aged 30 days) were given either a standard diet (9.5% fat) or a high-fat diet (30% fat) for 10 days. Additional groups received the same diets supplemented with 15% pectin from citrus peel (60% degree of esterification) or diets where food was restricted to achieve the average amount of energy consumed each day from the respective pectin-containing diets. Diets supplemented with pectin resulted in significantly lower energy intake, body weight and body weight gain compared with controls. Among the groups given standard diet, reductions in body weight and weight gain in pectin-fed animals were similar to those of the food-restricted animals. Among those given the high-fat diet, reductions in body weight and body weight gain were greater in the pectin group than in the food-restricted group. Significant reductions in epididymal and perirenal fat pad weights were seen in both pectin-fed groups compared with controls. A significant decrease in duodenal alkaline phosphatase activity was also observed in pectin-fed rats. Food-restricted rats had similar fat pad weights and duodenal alkaline phosphatase activity as the controls, despite their lower energy intake and body weight gain.

Effects of pectin on satiety, body weight, adiposity and caecal fermentation in high fat diet-induced obese rats (Adam et al. 2016) Regulatory status: Non-GLP

Male Sprague Dawley rats (8/group; age 13 weeks) with high fat diet-induced obesity were administered high fat diets alone or supplemented with 10% w/w highly esterified apple pectin, casein protein or pea protein (40% food energy content) for 28 days. Additional groups were fed high fat diets containing casein or pea protein plus 10% pectin. Pectin decreased food intake by 23% and induced a 23% fat loss compared to controls. Body weight gain was significantly lower in rats on the three pectin-containing diets compared with rats on the high-fat diets alone or in combination with casein or pea protein. Plasma concentrations of the satiety-associated hormones peptide tyrosine tyrosine (PYY) and glucagon-like peptide 1 (GLP-1) were increased by dietary pectin compared with controls, and concentrations of leptin and insulin were decreased. Caecal weight and caecal SCFA concentrations were significantly increased in the three pectin-containing diets compared with controls.

7.3.4 Human studies

Hypersensitivities

Case report of anaphylaxis associated with pectin in albedo of citrus fruit (Uno et al. 2017) Regulatory status: Non-GLP

An abstract of a case report written in Japanese describes an incident where a 7-year old girl developed a barking cough and pruritis approximately 2 hours after eating a frozen *Citrus unshiu* (satsuma). The patient had a history of anaphylaxis induced by consuming cashew nuts. Skin testing and basophil activation tests with a commercially available pectin product were positive. An oral food challenge test was conducted in which the patient only felt

abdominal pain and nausea after eating *Citrus unshui* fruit together with the albedo. The authors concluded that the case was induced by pectin present in the albedo of *Citrus unshui*, but not by the fruit itself. The authors suggested that patients with cashew nut allergies may have a possibility of pectin allergies.

Studies in infants and children

Efficacy and safety of infant anti-regurgitation formula thickened with pectin, carob bean gum and other starches (Dupont and Vandenplas 2020)

A retrospective analysis of four open-label, interventional, single-group, multi-centre clinical trials of anti-regurgitation formula thickened with four different combinations of pectin (source and specification not reported), carob bean gum and tapioca or corn starch was reported. This paper is also discussed in the section on carob bean gum (Section 5). The trials shared an identical study design and were conducted in France and Belgium between 2013 and 2017. Exclusively formula fed infants aged < 5 months presenting with at least five regurgitations per day were enrolled and administered study formula for 14 months. Parents collected 3-day diaries on the number, volume and severity of regurgitation episodes, stool consistency and frequency and volume of formula consumed. Diaries were recorded at the start of the intervention (baseline) and again on days 11 – 13, before an investigator visit on day 14. The investigators performed a physical examination including measurement of anthropometric parameters and recorded information on adverse events. Parents were given the opportunity to continue with the study formula for an additional period of 3 months, with diaries completed over the final 3 days and a further visit at around day 90. Each of the four studies used a different test formula, with the pectin concentration ranging from 2000 – 4200 mg/L.

The primary outcome was the daily number of regurgitations after 2 weeks of feeding the test formula. Secondary outcomes included the number and severity score of regurgitations at day 3, stool consistency and frequency and growth at day 14 and all parameters at day 90 if parents agreed to continue with the test formula. Adverse events were assessed in all infants who consumed the test formula at least once, and the efficacy analysis was performed on all infants with information on the number of regurgitations at day 14.

In total 392 infants were included across the four trials. The mean age at study inclusion was 65.2 ± 37.9 days. Baseline characteristics were similar in all trials. Although the inclusion criteria specified infants < 5 months old, six patients were between 5 months and 5 months and 21 days old. All but one of the infants received the test formula at least once, and information regarding the primary outcome was obtained in 346 infants. The mean number of daily regurgitation episodes and regurgitation severity scores were significantly reduced at days 3, 14 and 90 compared with baseline. Stool frequency was unchanged by the interventions. A trend towards a reduction in the number of infants with abnormal stool consistency at day 90 versus baseline was seen in three of the four studies, although it was only significant in one study. Weight-for-age and length-for-age z-scores were below the reference means in all studies at baseline, but increased towards the 50th percentile at day 90, indicating normalisation. A total of 141 adverse events were reported. Seven adverse events were classed as serious but none of these were considered related to the study formula by the investigators. Twenty-nine non-serious adverse events were considered related to the study formulas, the majority of which were related to the gastrointestinal tract: constipation (n = 8), diarrhoea (n = 5), colic/abdominal pain (n = 7) and worsening of regurgitations (n = 5).

The study authors concluded that infant formula containing a combination of pectin, carob bean gum and starch is safe and effective for the management of regurgitations in young infants.

Randomised controlled trial of a thickened amino acid formula in infant with cow's milk allergy – interim analysis (Dupont et al. 2014) Regulatory status: Non-GLP

In a multicentre, double-blind, randomised controlled trial, infants aged < 18 months (mean age 6.2 ± 4.3 months) with cow's milk protein allergy (CMPA) failing to respond to extensively hydrolysed formulas were assigned to receive either a pectin-based thickened amino acid formula (TAAF) or a commercially available reference amino acid formula (RAAF) for 3 months. The TAAF included the presence of a patented thickening mixture including fibres (5000 mg/L) composed mainly of pectin (source unspecified), which thickens at gastric pH. Visits were carried out at 1, 3, and 6 months following randomisation and anthropometric data and CMPA symptoms were evaluated. Parents were asked to report their child's feeding, behaviour, sleep and digestive symptoms in a diary covering the 3 days prior to the visit. The intention to treat population included 46 infants receiving TAAF and 40 receiving RAAF, 42 and 33 of whom, respectively, had proven CMPA and intolerance to extensively hydrolysed formulas. Tolerance/hypoallergenicity was assessed among the infants with proven CMPA plus intolerance to extensively hydrolysed formulas.

Results of an interim analysis after 1 month of feeding were reported. All infants tolerated the formulas and the major allergic symptoms disappeared within 1 month in 62% and 52% of infants receiving TAAF and RAAF, respectively. Regurgitations disappeared in 67% and 42% of infants, respectively. Two serious adverse events occurred in the TAAF group, one case of oesophagitis and one case of gastroenteritis. One case of oesophagitis occurred in the RAAF group. None of these serious adverse events resulted in study discontinuation, and the study authors did not consider they were related to the study formula. Infants fed the TAAF had significant reductions in mean crying time and increased sleep duration, while those receiving RAAF had significant improvements in mean crying time only. Infants consuming TAAF had a significantly higher proportion of normal stools that those fed RAAF after one month (91% versus 67%, respectively, P = 0.011). Changes in weight, weight-for-length, body mass index and head circumference z-scores were similar between groups. The only significant difference between groups was the length-for-age z-score (0.0 ± 0.5 for TAAF versus 0.3 ± 0.6 for RAAF, P = 0.029), but the authors did not consider this to be of clinical significance. The authors concluded that both formulas were well tolerated by infants with CMPA plus extensively hydrolysed formula intolerance and supported appropriate growth following 1 month of feeding, with the pectin-containing TAAF providing additional comfort.

Randomised controlled trial of a thickened amino acid formula in infants with cow's milk allergy (Dupont et al. 2015) Regulatory status: Non-GLP

In a follow-up paper, results from the study described above (Dupont et al. 2014) at 3 and 6 months from the start of the intervention were reported. Additional experimental details were also provided. At the end of the 3 month intervention period during which infants received TAAF or RAAF, infants from both groups were fed the pectin-based TAAF for 3 additional months, and anthropometric data were collected. Blood samples were obtained at study entry and at 3 months from a subset of infants (32 in the TAAF group and 29 in the RAAF group). Biochemical parameters (not specified), IgG, IgA, IgM, serum ferritin and complete blood count were analysed. Faecal samples were collected at baseline, and at 3 months, and analysed for bacterial populations and faecal eosinophil-derived neurotoxin (EDN), a marker of intestinal immune stimulation related to allergic inflammatory responses, especially eosinophilic infiltration.

No infants with CMPA plus extensively hydrolysed formula intolerance dropped out for reasons of intolerance during the study. At 3 months, the dominant allergic symptom had disappeared in 76% of infants fed TAAF and 52% of those fed RAAF. Greater improvements in the Scoring Atopic Dermatitis Index were observed with TAAF than with RAAF, and regurgitations disappeared in 66.7% of infants fed TAAF and 44.0% fed RAAF. Normal stools

(soft or formed consistency) were seen in 93% and 76% of infants fed TAAF and RAAF, respectively. All seven infants fed RAAF for 3 months who had hard or liquid stools at the end of the 3 month intervention showed soft or formed stools at the 6 month observation, following 3 months of TAAF consumption.

Infants' growth was similar between the two groups at 3 months and at 6 months. Significant improvement of weight-for-age z scores were observed at 6 months for infants fed TAAF for the entire study, and mean weight-for-age z scores significantly increased between 3 and 6 months in the infants initially fed RAAF. The most common adverse events were gastrointestinal tract affections and infections and were not considered to be related to the study product by the study authors. In total, four serious adverse events were recorded between 1 and 3 months (3 in the TAAF group and 1 in the RAAF group) and three were recorded between 3 and 6 months. None led to study dropout and none were considered to be related to the test formula by the study authors.

A significant reduction in plasma eosinophil concentrations was observed in both groups from inclusion to 3 months, with no significant differences between interventions. All other blood parameters measured were reported to be within normal ranges at 3 months. Amino acids were measured in a subset of infants at 3 months (25 and 22 in the TAAF and RAAF groups, respectively). Values were similar to those reported for breast-fed infants and there were no differences between the two study groups apart from significantly higher valine levels in the RAAF group. No significant differences in faecal microbiota composition were observed between groups. Infants from both groups showed a decrease of faecal EDN levels at 3 months. The authors concluded that the pectin-based TAAF was tolerated by all infants with CMPA plus extensively hydrolysed formula intolerance, and that the anthropometric and clinical data demonstrated the safety of the formula.

Neurodevelopment of pre-term infants given neonatal supplementation of oligosaccharides (van den Berg et al. 2016) Regulatory status: Non-GLP

In a randomised controlled trial, 114 very pre-term infants (<32 weeks and/or birth weight <1500 g) were given human milk or pre-term formula supplemented with placebo (maltodextrin) or a mixture of scGOS, lcFOS and pAOS (scGOS/lcFOS/pAOS). Supplementation with the mixture was administered in increasing doses from day 3 of life to day 30, to 1500 mg/kg per day. The paper is unclear as to whether these doses relate to concentrations in formula/milk or doses on a body weight basis, and the timing of dose escalation is also not described. The proportion of pAOS in the mixture was previously reported to be 20% (Westerbeek et al. 2008). At 24 months corrected age, cognitive and motor development were assessed using the Mental Development Index (MDI) and Psychomotor Development Index (PDI) of the Bayley Scales of Infant and Toddler Development.

Twelve infants died before the age of 2 years, 4/55 in the scGOS/lcFOS/pAOS group and 8/59 in the placebo group. After loss of some other infants to follow-up, 38 and 39 infants in the scGOS/lcFOS/pAOS and placebo groups, respectively, were evaluated for neurodevelopment. No significant differences in MDI and PDI were found between the two groups. Supplementation of scGOS/lcFOS/pAOS during the neonatal period did not influence the incidence of cerebral palsy or neurodevelopmental impairment.

This study is considered of limited utility for regulatory purposes because the doses of pAOS were not clearly described.

Pneumococcal vaccine responses in pre-term infants after neonatal oligosaccharide

supplementation (van den Berg et al. 2015) Regulatory status: Non-GLP

In a further assessment of the cohort of pre-term infants described above (van den Berg et al. 2016), antibody responses to vaccination with a heptavalent conjugate pneumococcal vaccine were analysed. Infants received four doses of the vaccine at 2, 3, 4 and 11 months of age. Serum samples were collected at birth and at 5 and 12 months of age and analysed for IgG antibodies to the seven pneumococcal vaccine (PCV7) serotypes. Blood samples taken from a group of term infants at birth and at age 5 months were also analysed for the PCV7 serotypes. At 5 months of age, mean IgG responses to the 7 pneumococcal vaccine serotypes were significantly lower in the scGOS/lcFOS/pAOS group compared with the placebo group, but were similar to those found in term infants. Serotypes for 5 of the 7 vaccine serotypes were significantly higher in the placebo group compared with term infants. At 12 months, following booster vaccination at 11 months, antibody levels were not different in the scGOS/lcFOS/pAOS and placebo groups. The proportion of pre-term infants with a protective antibody concentration (>0.35 µg/mL) was not significantly different for 6 of the 7 serotypes in both groups, while a significantly lower proportion of infants in the scGOS/lcFOS/pAOS were protected from serotype 6B compared with placebo. After the booster vaccination at 12 months, no significant differences in protective antibody concentrations were observed between the two groups.

Occurrence of febrile episodes in children previously fed pAOS-supplemented infant formula (van Stuijvenberg et al. 2015) Regulatory status: Non-GLP

In a follow-up study of children enrolled in a randomised controlled trial that was reviewed by JECFA, term-born children who had been fed an infant formula containing pAOS at 1.2 g/L plus a 9:1 mixture of scGOS and lcFOS (6.8 g/L) were assessed for the incidence of febrile episodes and other illnesses between the children's third to fifth birthday, using parent diaries. In the follow-up study 672 (60% of the original study population) participated: 232 (56%) from the pAOS/scGOS/lcFOS group, 343 (58%) from the control group and 197 (66%) from a non-randomised breast-fed group. No significant differences were observed between the pAOS/scGOS/lcFOS group and controls in the occurrence of fever, coughing, wheezing, runny/blocked nose, vomiting and diarrhoea. The duration of illness episodes was similar between groups, apart from a significantly shorter duration of diarrhoea in the pAOS/scGOS/lcFOS group compared with the controls. No significant differences in use of antibiotics or antipyretics were found between the two study formula groups.

Studies in adults

Effect of phytic acid, tannic acid and pectin on fasting iron bioavailability (Jaramillo et al. 2015) Regulatory status: Non-GLP

In a study of 28 fasted, apparently healthy adult females, a single dose of 250 mg pectin from citrus fruit (55-70% esterified) had no significant effect on the absorption of 5 mg non-heme iron ($FeSO_4$) in both the presence (n=13) and absence (n=15) of calcium (800 mg $CaCl_2$).

Influence of pectin esterification on fasting iron bioavailability (Jaramillo et al. 2018) Regulatory status: Non-GLP

Effects of the degree of esterification of pectins on non-heme iron absorption was assessed in 13 fasted, apparently healthy adult women. Each participant consumed 5 mg iron ($FeSO_4$) either alone or accompanied by 5 g citrus pectin. Three forms of pectin were used, one with 27% esterification and 20% amidation, one with 36% esterification and 14% amidation and one with 67-73% esterification. Each intervention was performed on different days. No statistically significant differences in iron absorption were observed with any form of pectin. The study authors concluded that the degree of pectin esterification does not influence the

bioavailability of non-heme iron in women.

Impact of pectin on intestinal barrier function in healthy young adults and elderly (Wilms et al. 2019) Regulatory status: Non-GLP

The effect of dietary supplementation with sugar beet derived pectin on gastrointestinal barrier function was assessed in a randomised, double-blind, placebo-controlled parallel study. In total 52 health young adults (18 – 40 years) and 48 healthy elderly (65 – 75 years) were assigned to receive 15 g/day pectin or placebo (maltodextrin) for four weeks. Segment-specific gastrointestinal permeability was assessed pre- and post-intervention using a multi-sugar test. Saliva, blood and faeces samples were collected at baseline and at the end of the intervention for analysis of secretory IgA (sIgA) antibodies (saliva and faeces) and IgA antibodies (serum). Gastrointestinal tolerance was assessed by weekly completion of a gastrointestinal symptom rating scale (GSRS). Sigmoid biopsies were collected post-intervention from a sub-set of participants and used for assessment of permeability (transepithelial electrical resistance and fluorescein flux) and transcription of junctional complex-related genes and defence- and immune-related genes.

In all groups, no significant differences in intestinal permeability, (s)IgA levels and expression of junctional complex, defence and immune related genes were observed following the intervention compared with baseline. Pectin did not alter any GSRS scores in elderly participants. The diarrhoea score was increased in young adults given pectin on week 2 of the study, but no differences were seen in weeks 1, 3 and 4. The study authors concluded that intestinal barrier function was not affected by four weeks pectin supplementation in healthy young adults and healthy elderly individuals.

7.4 Assessments by other regulatory agencies

7.4.1 EFSA

The EFSA ANS Panel re-evaluated the use of pectin and amidated pectin as food additives in 2017 (EFSA 2017c). EFSA concluded that there is no safety concern for the use of pectin and amidated pectin as a food additive for the general population, and that there is no need for a numerical ADI. A risk assessment for infants under the age of 12 weeks was not included in this assessment.

In a follow-up evaluation, the EFSA FAF Panel assessed the safety of pectin and amidated pectin as food additives in foods for infants below 16 weeks of age (EFSA 2021). Pectin and amidated pectin are currently approved in the EU for use as food additives in ‘dietary foods for special medical purposes and special formulas for infants’ or in ‘dietary foods for babies and young children for special medical purposes’ at an MPL of 10,000 mg/kg.

EFSA concluded that a reference point for safety assessment could be identified from the two neonatal piglet studies that were also reviewed by JECFA. A NOAEL of 1069 mg/kg bw/day for both sexes combined was identified. Based on estimated dietary exposures for infants under 16 weeks at the current MPL of 10,000 mg/kg, the MOE compared with the NOAEL was below 1 for both mean and high level consumption (exposures of 2000 and 2600 mg/kg bw/day, respectively. Based on the maximum use level reported by industry (4170 mg/kg), the MOE was above 1 for mean consumption (834 mg/kg bw/day) but below 1 for high level consumption (1084 mg/kg bw/day). An MOE below 1 was considered to be too low.

EFSA also considered the potential for exposure to pectin to increase internal exposure to methanol. Consumption of 75% methylated pectin has been reported to induce a significant increase in the breath, and by inference, in the blood of human volunteers. At a dose of 10 g

methylated pectin, the lowest amount of methanol released was 400 mg per adult person. Based on a reported maximum degree of pectin methylation of 90%, EFSA calculated that dietary methanol exposures at the MPL would be 80 or 104 mg/kg bw/day for mean and high level consumption, respectively. EFSA considered that this exposure could lead to adverse health effects in infants below 16 weeks of age. It was noted that the European Chemicals Agency (ECHA) has established an acute derived no effect level (DNEL) for methanol of 88 mg/kg bw based on ocular toxicity (i.e. blindness in humans). EFSA also noted that metabolic acidosis may become relevant at doses between 100 and 150 mg/kg bw/day.

EFSA recommended a reduction in the MPL for pectin and amidated pectin in the relevant food categories in order to reduce the exposure to both the additives themselves and to methanol.

EFSA also recommended revising the specifications for pectin and amidated pectin in order to reduce exposures to arsenic, lead, cadmium, mercury, aluminium and sulphur dioxide, and the introduction of microbiological criteria.

7.5 Discussion and conclusions

The 2016 evaluation by JECFA indicates that pectins are of low toxicity and well tolerated by infants at concentrations up to 2000 mg/L. JECFA's initial evaluation of pectin in infant formula in 2014 considered a proposed maximum use level of 5000 mg/L, however. The estimated exposure at this proposed use level was in the region of the NOAEL in a neonatal pig study and close to the LOAEL which was based on decreased feed intake and body weight gain. Estimated exposures at 5000 mg/L were therefore considered to be of concern.

Using JECFA's dietary exposure assessment for pectin in infant formula based on a maximum use level of 2000 mg/L (estimated exposures for average and high consumers of 360 and 440 mg/kg bw/day, respectively) and the NOAEL from toxicity studies in neonatal pigs (1049 mg/kg bw/day), MOEs were 2.9 and 2.4 for average and high consumers, respectively. JECFA has previously advised that MOEs in the region of 1-10 could be interpreted as low risk for the health of infants aged 0-12 weeks consuming a food additive in infant formula, when appropriate toxicological and dietary exposure considerations have been taken into account (WHO 2014). JECFA acknowledged the variability in medical conditions among infants requiring formula for special medical purposes, but noted that these infants would normally be under medical supervision.

EFSA published a re-evaluation of the use of pectin and amidated pectin in foods for infants below 16 weeks of age in 2021. EFSA found that estimated exposures at the current EU MPL of 10,000 mg/kg resulted in MOEs lower than 1 compared with the NOAEL in neonatal pigs. At the maximum use level reported by industry of 4170 mg/kg, the MOE was above 1 for mean consumption but below 1 for high level consumption. EFSA also noted that at the current EU MPL internal methanol exposure from methylated pectin could lead to adverse health effects in infants below 16 weeks of age. EFSA has recommended that the current MPL be lowered to address these health concerns.

FSANZ calculated the estimated internal methanol exposure from use of 90% methylated pectin at a maximum use level of 2000 mg/L, using JECFA's dietary exposure estimates and following the same methodology as EFSA (based on release of 400 mg methanol per person from a dose of 10 g 75% methylated pectin). For high consumers, the estimated exposure was 20 mg/kg bw/day, below the acute DNEL of 88 mg/kg bw established by ECHA for methanol ocular toxicity. The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment has previously advised that while there are few studies of chronic exposure to methanol in humans or animals, no adverse health effects have been reported from long-term occupational exposures below an occupational exposure limit (OEL)

of 200 ppm for 8 hours exposure, equivalent to 32 mg/kg bw for a 60 kg individual (UK COT 2011). On this basis, exposure to methanol from use of pectin in infant formula at a maximum use level of 2000 mg/L is not expected to result in adverse health effects.

New studies with pectins identified by FSANZ in a literature search do not indicate a need to revise the conclusions of the JECFA assessment. Studies in rats and pigs demonstrated bacterial fermentation of pectin in the large intestine to form SCFAs including acetate, propionate and butyrate. A subchronic study in rats with pectin derived from cocoa pod husks showed no adverse effects at doses up to 71.4 mg/kg bw/day.

An *in vitro* study with citrus pectin and apple pectin showed induction of oxidative DNA damage in a comet assay in breast cancer cells. However these effects were found at concentrations associated with cytotoxicity and ROS production in longer term cultures. The findings of this study are consistent with information in the JECFA review of pectins, which noted that positive findings in *in vitro* genotoxicity studies were only found at cytotoxic concentrations. Both JECFA and EFSA concluded that pectin and pAOS are not genotoxic.

A clinical trial showed that formula containing fibres including pectin at 5000 mg/L was well tolerated in infants with cow's milk protein allergy, although the pectin concentration was not specified. Another trial found that anti-regurgitation formulas containing 2000 – 4200 mg/L pectin as well as carob bean gum and starch were well tolerated. Follow-up studies of pre-term infants previously fed infant formula containing pAOS in combination with scGOS and lcFOS found no evidence of adverse effects on neurodevelopment or pneumococcal vaccine responses. Finally, a follow-up study of term infants given formula containing 1200 mg/L pAOS found no adverse effects on the incidence of febrile episodes or use of antibiotics and antipyretics. One limitation of these studies is that the source and specifications of the pectins tested were not reported.

A study in healthy young adults and healthy elderly individuals found that daily supplementation with 15 g/day pectin had no effect on gastrointestinal barrier function.

A single case report of a child with cashew nut allergy having anaphylaxis associated with pectin in citrus fruits was identified. EFSA's 2017 re-evaluation of pectins discussed an additional case of a child with a hypersensitivity reaction after ingesting pectin, reported in 2006, which was also considered possibly due to cross-reactivity with cashew and pistachio allergens. EFSA also noted three case reports of occupational asthma caused by pectin inhalation, and one case report of a woman with occupational rhinitis and urticaria from pectin dust who developed anaphylaxis after eating cashew nuts. Pectin is not listed as a food allergen on the WHO/IUIS allergen nomenclature database or Allergen Online database. EFSA did not consider pectins as having allergenic potential, and the limited evidence currently available does not indicate a need to revise that conclusion.

Based on the available data, the consumption of pectins in all infant formula products at concentrations up to 2000 mg/L does not raise safety concerns. No new information was identified that would alter the conclusion of JECFA that consumption of infant formula containing pectin at concentrations ≥ 5000 mg/L is of concern.

8 Gum-based thickeners and gastrointestinal disorders in infants

A submission in 2017 highlighted that there are case reports in the literature suggesting an association between the use of gum-based thickeners and gastrointestinal disorders in infants, including NEC. The majority of case reports involve carob bean gum or xanthan gum.

FSANZ has reviewed the case reports for carob bean gum and xanthan gum as part of the evaluations of these additives in Sections 3 and 4, respectively. Further consideration of the available literature is discussed below.

8.1 Reports of gum-based thickeners associated with adverse outcomes in infants

An abstract of an article published in French states that pectin and silicium have been added to milk in order to thicken feedings for infants with gastro-oesophageal reflux, but they may lead to an obstructive medication bezoar[4]. The authors recommend limiting their use to 3-5% of food (Mercier et al. 1984).

A case report provided details on six premature infants with gastro-oesophageal reflux fed infant formula thickened with carob bean gum (2000-5000 mg/L) who developed an increased frequency of defecation, metabolic acidosis and hypokalaemia (Sievers and Schaub 2003). The study authors hypothesised that increased loss of bicarbonate and potassium may have occurred as a result of the higher frequency of defecation, or that potassium may have been adsorbed to the carob bean gum. The concentration of carob bean gum in these cases was higher than the MPL currently listed in the Code (1000 mg/L).

Two cases of extremely low birth weight premature infants who developed fatal NEC while consuming breast milk thickened with a carob bean gum-based thickener (concentration not specified) have also been reported (Clarke and Robinson 2004). The pathophysiology was not investigated, but the study authors expressed concerns that carob-thickened milk may have played a role.

A report of three cases of NEC in premature infants given formula thickened with xanthan gum-based products noted that unlike classic NEC, all three patients presented with late-onset NEC predominantly in the colon (Woods et al. 2012). The authors speculated that the illness may have been due to stimulation of the immature gut by xanthan gum via increasing levels of water, sugars, SCFA and bile acids in the distal small intestine and colon. In addition, it was suggested that xanthan gum may activate gut lymphocytes and macrophages, leading to inflammation.

A report of 22 adverse event reports submitted to the US FDA involving NEC in infants given a xanthan gum thickening agent (concentrations not reported) has been published (Beal et al. 2012). All but one of the infants were premature, 14 cases required surgery and seven died. The median age at onset was later than that seen in a case series of 202 cases (66 versus 45 days of life, respectively). This led the authors to propose that use of xanthan gum-based thickener may be a risk factor for NEC, although they noted that a well-designed analytical study is needed to establish whether there is a true causal association. The authors also noted that the only study they were aware of looking at thickened feeds as a NEC risk factor did not detect a significant association (Drenckpohl et al. 2010). While the concentrations of xanthan gum were not reported, JECFA noted that based on the descriptions of the preparations having a honey/nectar consistency the concentration was likely to be higher than that in marketed infant formula (up to 1000 mg/L) (WHO 2017).

In May 2011, following the reports of NEC cases after being fed a xanthan gum-based thickener, the US FDA advised against feeding the thickener (commercial name SimplyThick) to infants born before 37 weeks gestation because it could cause NEC[5]. The

[4] A bezoar is a tightly packed collection of partially digested or undigested material that most commonly occurs in the stomach.

[5] https://wayback.archive-it.org/7993/20170722060115/https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm256250.htm

US FDA issued a further advisory in September 2012, indicating that everyone involved in the care of a baby should be aware of the potential risk before deciding whether to feed SimplyThick to infants of any age. The FDA noted that further study is needed to determine if there is an actual link between consumption of xanthan gum-based thickener and development of NEC.

Joint recommendations on clinical practice guidelines for paediatric gastro-oesophageal reflux from the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) state that for babies with significant reflux such that thickening is being considered, breastmilk can be thickened with xanthan gum or carob bean gum based thickeners. However, it is noted that some cautions exist. The recommendations state that carob bean thickeners are approved for use in infants after 42 weeks gestation, while xanthan gum thickeners are approved for infants greater than 1 year old because of concerns of NEC, citing the reports by Woods et al. (2012), Beal et al. (2012) and Rosen et al. (2018).

A literature search did not identify further reports of associations between use of gum-based thickeners in infant formula and gastrointestinal disorders. A recent systematic review of prognostic studies for risk factors for NEC in neonates noted a lack of prognostic evidence for formula feeding (Samuels et al. 2017).

8.2 Discussion and conclusions

Based on the available data it is not currently possible to determine if there is a causal association with NEC and xanthan gum, carob bean gum or other thickeners. JECFA has reached the same conclusion in considering these case reports. EFSA noted the lack of pathophysiology in the case reports for carob bean gum, and that the described cases associated with xanthan gum are not related to the food additive use of this substance in the manufacture of infant formula, but relate to its addition to formula or human milk as a thickener prior to consumption. The US FDA has also noted that further study is needed to determine if there is an actual link between consumption of xanthan gum-based thickener and development of NEC.

JECFA noted that for xanthan gum, no adverse events were reported in post-marketing surveillance conducted by one manufacturer on formulas containing xanthan gum at concentrations up to 1000 mg/L over a 5-year period, and that the concentrations in the case reports were likely to have been higher than food additive use in marketed infant formula. This concentration is the same as the MPL proposed to be listed in the Code.

FSANZ considers that best clinical practice (NHMRC 2012; MoH 2008) should be followed in feeding premature infants and/or infants with gastro-oesophageal reflux disease or other medical conditions, and that such infants should be under medical supervision.

accessed 10/09/19

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