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Infant formulae supplemented with prebiotics: Are they better than unsupplemented formulae? An updated systematic review

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Abstract

In 2011, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition systematically reviewed published evidence related to the safety and health effects of the administration of formulae supplemented with pro- and/or prebiotics compared with unsupplemented formulae. We updated evidence on the effects of the administration of prebiotic-supplemented infant formulae (IF) compared with unsupplemented IF. Five databases were searched up to March 2017 for randomised controlled trials. In all, forty-one publications were identified, including twenty-five new publications. The administration of currently evaluated prebiotic-supplemented formulae to healthy infants does not raise safety concerns with regard to growth and adverse effects. Some favourable clinical effects are possible, primarily stool softening, which may be beneficial in some infants. Currently, there is no existing robust evidence to recommend the routine use of prebiotic-supplemented formulae. The latter conclusion may reflect the small amount of data on specific prebiotics and outcomes, rather than a genuine lack of an effect. The efficacy and safety should be considered for each prebiotic(s)-supplemented formula.

Key words: Infants: Nutrition: Microbiota: Feeding

In 2011, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) systematically reviewed published evidence related to the safety and health effects of the administration of formulae supplemented with pro- and/or prebiotics compared with unsupplemented formulae[1].

With regard to probiotics, in line with the 2011 ESPGHAN document[1], our recent updated systematic review[2] concluded that the available scientific data suggest that the administration of currently evaluated probiotic-supplemented formulae to healthy infants does not raise safety concerns with regard to growth and adverse effects. Some beneficial clinical effects are possible; however, there is no existing robust evidence to recommend their routine use. The latter conclusion may reflect the small amount of data on a specific probiotic strain(s) and outcomes, rather than a genuine lack of an effect. The efficacy and safety should be considered for each probiotic(s)-supplemented formula.

With regard to prebiotic-supplemented formulae, in 2011 the ESPGHAN Committee concluded that the administration of currently evaluated prebiotic-supplemented formulae to healthy infants does not raise safety concerns with regard to growth and adverse effects. The Committee did not support the routine use of prebiotic-supplemented formulae in infants[1]. Subsequent to the Committee review, new evidence on the effects of supplementation of infant formulae with prebiotics was published. Here, we aimed to update the 2011 evidence on the effects of the administration of prebiotic-supplemented infant formulae to find out whether there is a need to revise current recommendations.

Methods

Criteria for considering studies for this review

The same methodology that has been already presented in two previous reviews[1,2] was followed. In brief, only randomised controlled trials (RCT) were eligible for inclusion. Participants had to be healthy term infants. Only studies that compared use of infant formula or follow-on formula supplemented with prebiotics (with prebiotic specification) during the manufacturing process compared with unsupplemented formula were included. Studies in which prebiotics were not administered during the manufacturing process, but thereafter, for example in capsules, the contents of which were supplemented to infant formula or feeds, were excluded. Studies in which synthetic human milk oligosaccharides were used were excluded. We also excluded trials evaluating fermented, acidified, and partially or extensively

Abbreviations: AOS, acidic oligosaccharide; ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition; FOS, long-chain fructo-oligosaccharide; GOS, galacto-oligosaccharides; MD, mean difference; PDX, polydextrose; RCT, randomised controlled trial; RR, risk ratio.

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hydrolysed formulae supplemented with prebiotics. We focused on growth and such clinical outcomes as gastrointestinal infections, respiratory infections, tolerance, etc. However, no firm definitions of any of these clinical outcomes were applied. Of note, in some trials, these outcomes were reported as the primary or secondary outcomes; in others, these were reported as adverse events. In this review, we evaluated them as referred to in the original publications.

Search methods for identification of studies

Five databases were searched up to March 2017 to identify potential studies: Cochrane Library, MEDLINE, EMBASE, Web of Science and CINAHL. No restrictions by either date or language were used in the search strategies. The search results were imported into Endnote bibliographic software, providing a total of 3035 records to be screened by the research team. The search strategy for one of the databases (EMBASE) is provided in the online Supplementary Table S1. We also searched reference lists of identified studies, key review articles and previous systematic reviews. Certain publication types (i.e. letters to the editor, abstracts, proceedings from scientific meetings) were excluded, unless a full set of data were obtained from the authors. Three registers for clinical trials (www.clinicaltrials.gov, www.clinicaltrialsregister.eu; www.anzctr.org.au) were screened.

Data collection, analysis, extraction and management

Titles and abstracts of the papers identified by the search strategy were independently screened by three researchers (A. S., M. K., M. P.-L.). Full texts of the potentially relevant publications were obtained and assessed. Any disagreements were discussed within the study team until a consensus was reached. Data extraction was performed using standard data-extraction forms. In addition to data such as methods, participants, interventions and outcomes, we collected information about sample size calculation and the funding of each study.

Assessment of risk of bias in included studies

The Cochrane Collaboration's tool for assessing risk of bias was used to establish the risk of bias. Type of randomisation method (selection bias), allocation concealment (selection bias), blinding of participants, personnel, intervention (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other forms of bias were considered[3] (Fig. 1).

Measures of treatment effect

For reporting the effect, the results for individual studies and pooled statistics (if applicable) are reported as the risk ratio (RR), or mean difference (MD), between the experimental and control groups with 95 % CI. In other circumstances, we report the findings as reported by the authors of the included studies.

Data synthesis (statistical methods)

If appropriate, the data were analysed using Review Manager (RevMan) (Computer program; version 5.3, 2014; The Cochrane Collaboration). As various prebiotics differ in their effects, we did not perform pooling data (meta-analysis) of all prebiotic trials. Instead, we report evidence related to a specific prebiotic or their combinations separately. Compared with the 2011 report, no new meta-analyses were performed.

Results

Description of studies

For a flow diagram documenting the identification process for eligible trials (online Supplementary Fig. S1). Overall, forty-one publications met our inclusion criteria. In addition to the previously identified sixteen publications, twenty-five new publications were identified. In addition, four registered trials were identified: still recruiting at the time of the writing of this manuscript: NCT01143233, NCT03090360 and NCT02948114; active but not recruiting at the time of the writing of this manuscript: ACTRN12616001571460.

Table 1 summarises the characteristics of all included RCT evaluating the effects of prebiotic-supplemented formulae. Among new studies identified, there were three studies that were not included in the previous Committee systematic review despite having been published before the review[10,16,18]. A number of RCT described the same study population but reported different outcome measures[6,7,11,24,26,30,31,34–42]. The online Supplementary Table S2 summarises the characteristics of the excluded trials, with reasons for exclusion. All of the included studies were carried out in healthy term infants. The vast majority of trials were conducted in Westernised countries and the majority were industry-funded trials.

The studies varied in the types of prebiotics used. As previously reported by the ESPGHAN Committee on Nutrition, the most commonly studied prebiotic was a 9:1 mixture of short-chain galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) (GOS/FOS)[16–23,30,36–38,40,42,43].

Other prebiotics studied were as follows:

(1) GOS[5–7,10,11,14,15,33,39];
(2) acidic oligosaccharides (AOS)[4];
(3) FOS[8,9,12,13,29,32];
(4) GOS/FOS/AOS[4,24,31,34,35,44];
(5) oligofructose plus inulin (SYN-1)[23,28]; and
(6) polydextrose (PDX) plus GOS with lactulose (PDX + GOS + LOS)[25,27] or without lactulose (PDX + GOS)[5,25–27,41].

The doses of prebiotics ranged from 0·1 to 0·8 g/100 ml, and the duration of the intervention ranged from 2 weeks to 12 months. All but five RCT[9,10,14,31,34] reported the prebiotic supplementation of an infant formula. In these five trials, prebiotics were used to supplement follow-on formula.

Risk of bias in included trials

The quality of the included RCT varied (Fig. 1). Almost all of the included trials had a number of methodological limitations. The most common problems were a lack of description of randomisation procedures and/or allocation concealment and/or blinding.

Table 1. Characteristics of included randomised controlled trials in healthy term infants fed formulae supplemented with pro- and/or prebiotics compared with unsupplemented formulae (search up to March 2017). Overview of the findings: clinical results
(Mean values with their standard errors; medians amd 25–75 percentiles; risk ratios (RR), mean differences (MD) and 95 % confidence intervals)

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
Growth						
Fanaro *et al.*[4] (Italy)*	Healthy term infants	GOS/FOS + AOS (0·8 g/100 ml) (n 15) AOS (0·2 g/100 ml) (n 16)	IF (*n* 15)	6 weeks	No info	NS
Ashley *et al.* [5] (USA)†	Healthy term infants	GOS (0·4 g/100 ml) (*n* 138)	IF (*n* 142)	120 d	Unclear	NS
Ben *et al.*[6,7] (China)*	Term infants	GOS (0·24 g/100 ml) (*n* 69)	IF (*n* 52) BF (*n* 26)	6 months	No info	NS
Bettler-Euler *et al.*[8] (USA)*	Healthy term infants	FOS (0·3 g/100 ml) (*n* 101) FOS (0·15 g/100 ml) (*n* 98)	IF (*n* 98)	12 weeks	No info	NS
Brunser *et al.*[9] (Chile)*	Healthy term infants, enrolled at 3·5 months	FOS (0·2 g/100 ml) (*n* 32) L johnsonii La1 10^8 CFU/g (*n* 25)	FF (*n* 33) BF (*n* 26)	13 weeks (15 weeks)	No info	NS
Fanaro *et al.*[10] (Italy)†	Healthy infants 4–6 months	GOS (0·5 g/100 ml) (*n* 77 including 15 preterm)	IF (*n* 82 including 13 preterm	18 weeks	Humana GmbH, Herford, Germany	NS
Giovannini *et al.*[11] (Italy)†	<15 d	GOS (0·4 g/100 ml) (*n* 83)	IF (*n* 80) BF (*n* 199)	6 months	No info	NS
Paineau *et al.*[12] (France)†	Healthy, term infants <7 d	FOS 0·4 g/100 ml (*n* 31)	IF (*n* 27)	4 months	No info	NS
Ripoll *et al.*[13] (Spain)†	Healthy infants 4 months ± 2 weeks	FOS (0·5 g/ml) (*n* 38)	IF (*n* 37)	6 months	Syrallberia S.A.U. Saragossa, Spain	NS
Sierra *et al.*[14] (Spain)†	Healthy term infants <2 months	GOS (0·44 g/100 ml in IF and 0·50 g/dl in FF) (*n* 188)	IF (*n* 177)	12 months	Hero Group	NS
Williams *et al.*[15] (USA)†	Healthy term <8 d	GOS (0·4 g/100 ml) (*n* 46) GOS (0·8 g/100 ml) (*n* 43)	IF (*n* 44)	119 d	Abbott Nutrition	NS
Bisceglia *et al.*[16] (Italy)†	Healthy term infants	GOS/FOS (0·8 g/100 ml) (*n* 39)	IF (*n* 37)	28 d	Partially financed by Numico Friedrichsdorf Germany	NS
Costalos *et al.*[17] (Greece)*	Healthy term infants, enrolled ≤14 d	GOS/FOS (0·4 g/100 ml) (*n* 80)	IF (*n* 80)	12 weeks	No info	NS
Bruzzese *et al.*[18] (Italy)*	Healthy infants 15–120 d	GOS/FOS (0·4 g/100 ml) (*n* 169)	IF (*n* 173)	12 months	An unrestricted grant from Numico Research, Friedrichsdor, Germany	Increased mean body weight at 3 and 6 months of follow-up ($P < 0·01$). Increased mean body length at all-time intervals ($P < 0·05$)
Decsi *et al.*[19] (Hungary)*	Healthy term infants	GOS/FOS (0·4 g/100 ml) (*n* 14)	IF (*n* 13) BF (*n* 42)	12 weeks	No info	NS
Ivakhnenko *et al.*[20] (Ukraine)†	Newborns	GOS/FOS (0·8 g/100 ml) (*n* 80)	IF (*n* 80) BF (*n* 80)	2 months (follow-up 18 months)	Pediatrics Department of Lviv National Medical University, state registration number „ 0108U101130	NS
Moro *et al.*[21] (Italy)*	Term infants	GOS/FOS (0·4 g/100 ml) (*n* 30) GOS/FOS (0·8 g/100 ml) (*n* 27)	IF (*n* 33)	4 weeks	No info	NS
Salvini *et al.*[22] (Italy)†	Healthy newborns of hepatitis C virus-infected mothers	GOS/FOS (0·8 g/100 ml) (*n* 10)	IF (*n* 10)	6 months	Danone Research, Centre for Specialised Nutrition	NS
Veereman-Wauters *et al.*[23] (Belgium)†	Healthy term infants <5 d	Oligofructose-enriched inulin (SYN1) (0·4 g/100 ml) (*n* 21) Oligofructose-enriched inulin (SYN-1) (0·8 g/100 ml) (*n* 20) GOS:FOS (0·8 g/100 ml) (*n* 19)	IF (*n* 21) BF (*n* 29)	28 d	Beneo-Orafti	NS

Table 1. *Continued*

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
Piemontese *et al.*[24] (Italy, Switzerland, Germany, Austria, the Netherlands) (The same population as van Stuijvenberg)†	Healthy, term infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 years	No info	NS
Nakamura *et al.*[25] (USA)*	Healthy term infants 13 to 92 d	PDX + GOS (0·4 g/100 ml) (*n* 27) PDX + GOS + LOS 0·4 g/100 ml (*n* 27) PDX + GOS + LOS 0·8 g/100 ml (*n* 25)	IF (*n* 25) BF (*n* 30)	28 d	Mead Johnson & Company, Evansville, IN	NS
Scalabrin *et al.*[26] (USA)†	Healthy term infants 21–30 d	PDX/GOS (0·4 g/100 ml) (*n* 100)	IF (*n* 101) BF (*n* 88)	60 d	Mead Johnson Nutrition	NS
Ziegler *et al.*[27] (USA)*	Healthy term infants <14 d	PDX + GOS (0·4 g/100 ml) (*n* 74) PDX + GOS + LOS (0·8 g/100 ml) (*n* 76)	IF (*n* 76)	120 d	Mead Johnson & Co	NS
Closa-Monasterolo *et al.*[28] (Spain)†	Newborns	Oligofructose-enriched inulin (SYN1) (0·8 g/100 ml) (*n* 128)	IF (*n* 124) BF (*n* 136)	4 months	BENEO Institute (an Initiative of BENEO GmbH).	NS
Tolerance						
Fanaro *et al.*[4] (Italy)*	Healthy term infants	GOS/FOS + AOS (0·8 g/100 ml) (*n* 15) AOS (0·2 g/100 ml) (*n* 16)	IF (*n* 15)	6 weeks	No info	NS (crying, regurgitation, vomiting episodes)
Paineau *et al.*[12] (France)†	Healthy, term infants <7 d	FOS 0·4 g/100 ml (*n* 31)	IF (*n* 27)	4 months	No info	NS (abdominal pain, crying, nausea)
Ripoll *et al.*[13] (Spain)†	Healthy infants 4 months ± 2 weeks	FOS (0·5 g/ml) (*n* 38)	IF (*n* 37)	6 months	Syrallberia S.A.U. Saragossa, Spain	NS (constipation, crying, regurgitation) Number of days with vomiting was lower ($P = 0·05$) in the FOS group.
Xia *et al.*[29] (USA)†	Healthy term infants <7 d	FOS (0·2 g/100 ml) (*n* 25) FOS (0·3 g/100 ml) (*n* 26)	IF (*n* 24) BF (*n* 22)	4 weeks	Abbott Nutrition	NS (spitting up or vomiting)
Ashley *et al.*[5] (USA)†	Healthy term infants	GOS (0·4 g/100 ml) (*n* 138) PDX/GOS (0·4 g/100 ml)	IF (*n* 142)	120 d	Unclear	NS (fussiness, gassiness)
Ben *et al.*[6,7] (China)*	Term infants	GOS (0·24 g/100 ml) (*n* 69)	IF (*n* 52) BF (*n* 26)	6 months	No info	NS (crying, regurgitation, vomiting)
Fanaro *et al.*[10] (Italy)†	Healthy infants 4–6 months	GOS (0·5 g/100 ml) (*n* 77 including 15 preterm)	IF (*n* 82 including 13 preterm	18 weeks	Humana GmbH, Herford, Germany	NS (crying, regurgitation, vomiting, flatulence)
Bruzzese *et al.*[18] (Italy)*	Healthy infants 15–120d	GOS/FOS (0·4 g/100 ml) (*n* 169)	IF (*n* 173)	12 months	An unrestricted grant from Numico Research, Friedrichsdorf, Germany	NS (no data)
Decsi *et al.*[19] (Hungary)*	Healthy term infants	GOS/FOS (0·4 g/100 ml) (*n* 14)	IF (*n* 13) BF (*n* 42)	12 weeks	No info	NS (excessive irritability, vomiting, regurgitation, atopy)
Moro *et al.*[21] (Italy)*	Term infants	GOS/FOS (0·4 g/100 ml) (*n* 30) GOS/FOS (0·8 g/100 ml) (*n* 27)	IF (*n* 33)	4 weeks	No info	NS (crying, regurgitation, vomiting)
Costalos *et al.*[17] (Greece)*	Healthy term infants, enrolled ≤14 d	GOS/FOS (0·4 g/100 ml) (*n* 80)	IF (*n* 80)	12 weeks	No info	NS (tolerance posseting)
Knol *et al.*[30] (Germany) (The same study as Haarman 2005 (– different outcomes)*	Fully formula-fed infants 4–8 weeks	GOS/FOS (0·8 g/100 ml) (*n* 24)	IF (*n* 23) BF (*n* 21)	6 weeks	Numico Research	NS (flatulence, posseting)
Veereman-Wauters *et al.*[23] (Belgium)†	Healthy term infants <5 d	GOS:FOS (0·8 g/100 ml) (*n* 19)	IF (*n* 21) BF (*n* 29)	28 d	Beneo-Orafti	NS (regurgitation and vomiting scores)
Piemontese *et al.*[24] (Italy, Switzerland, Germany, Austria, the Netherlands) (The same population as van Stuijvenberg)†	Healthy, term infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 year	No info	NS (spitting, posseting, vomiting, colic, flatulence, cramps)

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Table 1. *Continued*

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
van Stuijvenberg *et al.*[31] (The Netherlands, Austria, Switzerland, Italy, Germany)†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 year	Danone Research	NS (duration of vomiting, number of vomiting episodes)
Veereman-Wauters *et al.*[23] (Belgium)†	Healthy term infants <5 d	Oligofructose-enriched inulin SYN1 (0·4 or 0·8 g/100 ml)	IF (*n* 21) BF (*n* 29)	28 d	Beneo-Orafti	NS (regurgitation and vomiting scores) no data
Nakamura *et al.*[25] (USA)*	Healthy term infants 13 to 92 d	PDX/GOS (0·4 g/100 ml)	IF (*n* 25) BF (*n* 30)	28 d	Mead Johnson & Company, Evansville, IN.	NS (fussiness, gassiness)
Scalabrin *et al.*[26] (USA)†	Healthy term infants 21–30 d	PDX/GOS (0·4 g/100 ml) (*n* 100)	IF (*n* 101) BF (*n* 88)	60 d	Mead Johnson Nutrition	NS (fussiness, gassiness)
Stool frequency						
Fanaro *et al.*[4] (Italy)*	Healthy term infants	AOS (0·2 g/100 ml) (*n* 16)	IF (*n* 15)	6 weeks	No info	NS
Wernimont *et al.*[32] (USA)†	Healthy term infants 5–15d	FOS (0·3 g/100 ml) (*n* 47)	IF (*n* 48) BF (*n* 50)	8 weeks	Wyeth Nutrition	NS
Xia *et al.*[29] (USA)†	Healthy term infants <7 d	FOS (0·2 g/100 ml) (*n* 25) FOS (0·3 g/100 ml) (*n* 26)	IF (*n* 24) BF (*n* 22)	4 weeks	Abbott Nutrition	NS
Ashley *et al.*[5] (USA)†	Healthy term infants	GOS (0·4 g/100 ml) (*n* 138)	IF (*n* 142)	120 d	Unclear	mean stool frequency higher week 1 (3·9 (SE 0·2) *v.* 2·2 (SE 0·2); $P<0·05$) week 2 (3·7 (SE 0·2) *v.* 2·2 (SE 0·2); $P<0·05$). This pattern continued through 60 d of age; by 90 d of age – NS
Ben *et al.*[6,7] (China)*	Term infants	GOS (0·24 g/100 ml) (*n* 69)	IF (*n* 52) BF (*n* 26)	6 months	No info	Increased stool frequency (no data)
Fanaro *et al.*[10] (Italy)†	Healthy infants 4–6 months	GOS (0·5 g/100 ml) (*n* 77 including 15 preterm)	IF (*n* 82 including 13 preterm	18 weeks	Humana GmbH, Herford, Germany	NS
Matsuki *et al.*[33] (Japan)†	Healthy term infants 31–54 d	GOS (0·3 g/100 ml) (*n* 17)	IF (*n* 18)	2 weeks	Yakult Central Institute	NS
Sierra *et al.*[14] (Spain)†	Healthy term infants <2 months	GOS (0·44 g/100 ml in IF and 0·50 g/dl in FF) (*n* 188)	IF (*n* 177)	12 months	Hero Group	At 3 months: 1·45 (SE 0·97) *v.* 1·26 (SE 0·83); $P<0·05$); At 4 months: (1·5 (SE 0·99) *v.* 1·26 (SE 0·94); $P<0·05$); At 6, 9 and 12 months – NS
Williams *et al.*[15] (USA)†	Healthy term <8 d	GOS (0·4 g/100 ml) (*n* 46) GOS (0·8 g/100 ml) (*n* 43)	IF (*n* 44)	119 d	Abbott Nutrition	NS
Bisceglia *et al.*[16] (Italy)†	Healthy term infants	GOS/FOS (0·8 g/100 ml) (*n* 39)	IF (*n* 7)	28 d	Partially financed by Numico Friedrichsdorf Germany	At 28 d: 3·4 (SE 0·7) *v.* 1·7 (SE 0·9); $P<0·001$
Costalos *et al.*[17] (Greece)*	Healthy term infants, enrolled ≤14 d	GOS/FOS (0·4 g/100 ml) (*n* 80)	IF (*n* 80)	12 weeks	No info	1·9 (range: 1·2–2·1) *v.* 1·6 (1·1–1·9); $P=0·031$
Knol *et al.*[30] (Germany) (The same study as Haarman 2005 (– different outcomes)*	Fully formula-fed infants 4–8 weeks	GOS/FOS (0·8 g/100 ml) (*n* 24)	IF (*n* 23) BF (*n* 21)	6 weeks	Numico Research	NS
Moro *et al.*[21] (Italy)*	Term infants	GOS/FOS (0·4 g/100 ml) (*n* 30) GOS/FOS (0·8 g/100 ml) (*n* 27)	IF (*n* 33)	4 weeks	No info	NS
Veereman-Wauters *et al.*[23] (Belgium)†	Healthy term infants <5 d	GOS/FOS (0·8 g/100 ml) Oligofructose-enriched inulin SYN1 (0·4 or 0·8 g/100 ml)	IF (*n* 21) BF (*n* 29)	28 d	Beneo-Orafti	NS

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Table 1. *Continued*

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
Fanaro *et al.*[4] (Italy)*	Healthy term infants	GOS/FOS + AOS (0·8 g/100 ml) (*n* 15)	IF (*n* 15)	6 weeks	No info	Increased stool frequency (no exact data)
Piemontese *et al.*[24] (Italy, Switzerland, Germany, Austria, the Netherlands) (The same population as van Stuijvenberg)†	Healthy, term infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 year	No info	NS
Closa-Monasterolo *et al.*[28] (Spain)†	Newborns	Oligofructose-enriched inulin (SYN1) (0·8 g/100 ml) (*n* 128)	IF (*n* 124) BF (*n* 136)	4 months	BENEO Institute (an Initiative of BENEO GmbH).	Significantly higher frequency of depositions at any time month 1, $P<0·001$; month 2, $P<0·01$; month 3, $P<0·01$; month 4, $P<0·05$
Ashley *et al.*[5] (USA)†	Healthy term infants	PDX/GOS (0·4 g/100 l)	IF (*n* 142)	120 d	Unclear	Higher frequency of stools in the prebiotic (PDX/GOS)-supplemented group at some time intervals
Scalabrin *et al.*[26] (USA)†	Healthy term infants 21–30 d	PDX/GOS (0·4 g/100 ml) (*n* 100)	IF (*n* 101) BF (*n* 88)	60 d	Mead Johnson Nutrition	No differences between the groups in stool frequency during all study periods
Gastrointestinal infections						
Paineau *et al.*[12] (France)†	Healthy, term infants <7 d	FOS 0·4 g/100 ml (*n* 31)	IF (*n* 27)	4 months	No info	NS (diarrhoea number of days)
Ripoll *et al.*[13] (Spain)†	Healthy infants 4 mo ± 2 weeks	FOS (0·5 g/ml) (*n* 38)	IF (*n* 37)	6 months	Syrallberia S.A.U. Saragossa, Spain	NS (diarrhoea and gastroenteritis)
Sierra *et al.*[14] (Spain) †	Healthy term infants <2 months	GOS (0·44 g/100 ml in IF and 0·50 g/dl in FF) (*n* 188)	IF (n 177)	12 months	Hero Group	NS (episodes of diarrhoea per infant; number of infants with at least one episode of diarrhoea per year)
Bruzzese *et al.*[18] (Italy)*	Healthy infants 15–120d	GOS/FOS (0·4 g/100 ml) (n 169)	IF (*n* 173)	12 months	An unrestricted grant from Numico Research, Friedrichsdorf, Germany	Lower rate of diarrhoeal episodes/child (0·12 (SE 0·04) *v.* 0·29 (SE 0·05), $P=0·015$), lower number of children with at least 1 episode of acute diarrhoea 10/96 (10·4 %) *v.* 26/109; $P=0·01$
Ivakhnenko *et al.*[20] (Ukraine)†	Newborns	GOS/FOS (0·8 g/100 ml) (n 80)	IF (n 80) BF (n 80)	2 month (follow-up 18 months)	Pediatrics Department of Lviv National Medical University, state registration number „ 0108U101130.	Lower incidence of GI infections (0·28 (SE 0·05) *v.* 0·78 (SE 0·12) episodes/child/18 months, MD −0·5 (95 % CI −0·53, −0·47).
van Stuijvenberg *et al.*[34] (The Netherlands, Austria, Switzerland, Italy, Germany†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (n 414)	IF (*n* 416) BF (*n* 300)	1 year	Danone Research	NS (number of episodes of gastroenteritis)
van Stuijvenberg *et al.*[31] (The Netherlands, Austria, Switzerland, Italy, Germany) follow-up as above†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (n 414)	IF (*n* 416) BF (*n* 300)	1 year	Danone Research	NS: Febrile episodes due to diarrhoea: 0·51 (25–75 percentile 0·03–1·07) *v.* 0·53 (25–75 percentile 0·03–1·51), $P=0·22$ Shorter duration of diarrhoea: 1·0 (25–75 percentile 0·0–4·0) *v.* 2·0 (25–75 percentile 0·0–7·0), $P=0·01$
Respiratory tract infections						
Sierra *et al.*[14] 2015 (Spain)†	Healthy term infants <2 months	GOS (0·44 g/100 ml in IF and 0·50 g/dl in FF) (*n* 188)	IF (*n* 177)	12 months	Hero Group	NS: Number of URTI episodes and the number of infants with recurrent URTI
Bruzzese *et al.*[18] (Italy)†	Healthy infants 15–120 d	GOS/FOS (0·4 g/100 ml) (*n* 169)	IF (*n* 173)	12 months	An unrestricted grant from Numico Research, Friedrichsdorf, Germany	Number of children with one episode of URTI – NS Number of children with more than 3 episodes of URTI–NS

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Table 1. *Continued*

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
Ivakhnenko *et al.*[20] (Ukraine)†	Newborns	GOS/FOS (0·8 g/100 ml) (n 80)	IF (n 80) BF (n 80)	2 months (follow-up 18 months)	Pediatrics Department of Lviv National Medical University, state registration number „ 0108U101130.	Incidence of URT infections: 2·81 (SE 0·51) *v.* 5·78 (SE 0·97) episodes/child per 18 months; MD −2·97 (95 % CI −3·26, −2·68).
van Stuijvenberg *et al.*[34] (The Netherlands, Austria, Switzerland, Italy, Germany)†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (n 416) BF (n 300)	1 year	Danone Research	URTI, ear infection, pneumonia/bronchiolitis – NS
van Stuijvenberg *et al.*[31] (The Netherlands, Austria, Switzerland, Italy, Germany) follow-up as above†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (n 414)	IF (*n* 416) BF (*n* 300)	1 year	Danone Research	Episodes and duration of coughing, wheezing, and blocked nose at 3 to 5 years – NS
Allergic diseases						
Sierra *et al.*[14] (Spain)†	Healthy term infants <2 months	GOS (0·44 g/100 ml in IF and 0·50 g/dl in FF) (*n* 188)	IF (*n* 177)	12 months	Hero Group	Allergic manifestations and allergic sensitisation – NS
Ivakhnenko *et al.*[20] (Ukraine)†	Newborns	GOS/FOS (0·8 g/100 ml) (*n* 80)	IF (*n* 80) BF (*n* 80)	2 months (follow-up 18 months)	Pediatrics Department of Lviv National Medical University, state registration number „ 0108U101130.	Allergic reactions to food products: 3/62 *v.* 9/53, RR 0·28 (95 % CI 0·09, 0·9) Allergic reactions to cows' milk protein: 2/62 *v.* 8/53, RR 0·2 (95 % CI 0·05, 0·84) Atopic dermatitis: 3/62 *v.* 9/53, RR 0·28 (95 % CI 0·09, 0·9) Respiratory allergic symptoms (recurrent wheezing): 3/62 *v.* 7/53, RR 0·36 (95 % CI 0·1, 1·2) GI food allergy: 2/62 *v.* 7/63, RR 0·24 (95 % CI 0·06, 0·98).
Grüber *et al.*[35] (the Netherlands, Austria, Switzerland, Italy Germany (The same population as van Stuijvenberg)†	healthy infants <8 weeks	GOS/ FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	12 months	Danone Research, Friedrichsdorf, Germany	Atopic dermatitis: 5·7 *v.* 9·7 %; $P = 0{\cdot}04$.
Antibiotic therapy						
Ripoll *et al.*[13] (Spain)†	Healthy infants 4 months ± 2 weeks	FOS (0·5 g/ml) (*n* 38)	IF (*n* 37)	6 months	Syrallberia S.A.U. Saragossa, Spain	Number of infants with concomitant treatments (including antibiotics) – NS ($P = 0{\cdot}12$)
Sierra *et al.*[14] (Spain)†	Healthy term infants <2 months	GOS (0·44 g/100 ml in IF and 0·50 g/dl in FF) (*n* 188)	IF (*n* 177)	12 months	Hero Group	NS
Bruzzese *et al.*[18] (Italy)*	Healthy infants 15–120 d	GOS/FOS (0·4 g/100 ml) (*n* 169)	IF (*n* 173)	12 months	An unrestricted grant from Numico Research, Friedrichsdorf, Germany	Significantly lower mean rate of antibiotic courses: 1·03 (SE 0·15) *v.* 1·48 (SE 0·16); MD 0·45, 95 % CI −0·49, −0·4; $P = 0{\cdot}038$; Multiple antibiotic courses/year: 24/60 *v.* 43/65; $P = 0{\cdot}004$
van Stuijvenberg *et al.*[34] (The Netherlands, Austria, Switzerland, Italy, Germany)†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (n 416) BF (n 300)	1 year	Danone Research	NS: number of fever episodes requiring systemic antibiotics during the 1st year of life

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Table 1. *Continued*

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
van Stuijvenberg *et al.*[31] (The Netherlands, Austria, Switzerland, Italy, Germany) follow-up as above†	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 year	Danone Research	NS: number of fever episodes requiring systemic antibiotics at 3 and 5 years follow-up
Adverse events						
Bettler-Euler *et al.*[8] (USA)*	Healthy term infants	FOS (0·3 g/100 ml) (n 101) FOS (0·15 g/100 ml) (n 98)	IF (*n* 98)	12 weeks	No info	0·3 g/100 ml *v.* 0·15 g/100 ml: reduced constipation ($P=0·03$), increased vomiting ($P=0·016$). Flatulence, diarrhoea, loose stools, dehydration or allergic reaction – NS
Brunser *et al.*[9] (Chile)*	Healthy term infants, enrolled at 3·5 months	FOS (0·2 g/100 ml) (*n* 32) L johnsonii La1 10^9 CFU/g (*n* 25)	FF (*n* 33) BF (*n* 26)	13 weeks (15 weeks)	No info	The number of adverse events per infant, including upper and lower respiratory infections and diarrhoeal episodes – NS ($P>0·05$; data not shown)
Paineau *et al.*[12] (France)†	Healthy, term infants <7 d	FOS 0·4 g/100 ml (*n* 31)	IF (*n* 27)	4 months	No info	NS (not related to the tested formula (bronchiolitis))
Ripoll *et al.*[13] (Spain)†	Healthy infants 4 months ± 2 weeks	FOS (0·5 g/ml) (*n* 38)	IF (*n* 37)	6 months	Syrallberia S.A.U. Saragossa, Spain	NS (total AEs, diarrhoea, gastroenteritis)
Wernimont *et al.*[32] (USA)†	Healthy term infants 5–15 d	FOS (0·3 g/100 ml) (*n* 47)	IF (*n* 48) BF (*n* 50)	8 weeks	Wyeth Nutrition	Total AE: 15/19 *v.* 13/20 (RR 1·2; 95 % CI 0·8, 1·8, NS); Feeding-related GI AEs: 13/19 *v.* 13/20 (RR 1·0; 95 % CI 0·7, 1·6, NS) Withdrawals (vomiting, spitting up, and abdominal pain) 7/19 *v.* 2/20 (RR 3·7; 95 % CI 0·9, 15·6, NS)
Ashley *et al.*[5] (USA)†	Healthy term infants	GOS (0·4 g/100 ml)	IF (*n* 142)	120 d	Unclear	Incidence of at least one medically confirmed AE, 77 *v.* 78 % – NS. Eyes, ears, nose, and throat system – NS GI reflux, emesis, or diarrhoea – NS Excessive spitting – significantly higher in GOS group ($P<0·05$) SAEs (all unrelated to study formulas) – NS
Ben *et al.*[6,7] (China)*	Term infants	GOS (0·24 g/100 ml) (*n* 69)	IF (*n* 52) BF (*n* 26)	6 months	No info	NS (crying, regurgitation, vomiting)
Bisceglia *et al.*[16] (Italy)†	Healthy term infants	GOS/FOS (0·8 g/100 ml) (*n* 39)	IF (*n* 37)	28 d	Partially financed by Numico Friedrichsdorf Germany	NS (none)
Costalos *et al.*[17] (Greece)	Healthy term infants, enrolled ≤14 d	GOS/FOS (0·4 g/100 ml) (*n* 80)	IF (*n* 80)	12 weeks	No info	NS
Giovannini *et al.*[11] (Italy)†	<15 d	GOS (0·4 g/100 ml) (*n* 83)	IF (*n* 80) BF (*n* 199)	6 months	No info	Diarrhoeal, crying, and vomiting episodes – NS Infantile colic – NS or reduced (inconsistent results) Higher stool frequency $P<0·05$ Regurgitation $P<0·05$
Matsuki *et al.*[33] (Japan)†	Healthy term infants 31–54 d	GOS (0·3 g/100 ml) (*n* 17)	IF (*n* 18)	2 weeks	Yakult Central Institute	Regurgitations, gassiness, or rejections – no reported
Piemontese *et al.*[24] (Italy, Switzerland, Germany, Austria, the Netherlands) (The same population as van Stuijvenberg)†	Healthy, term infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 year	No info	AE (otitis media, bronchitis, gastroenteritis, URTI, varicella, bronchiolitis, pharyngitis, UTI): 31 *v.* 30 %; $P>0·05$) SAEs: 10·6 *v.* 9·4 %; NS

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Table 1. *Continued*

References (country)	Participants (age at enrolment)	Intervention	Comparison	Duration of intervention (follow-up)	Funding	Effect (prebiotic-supplemented formula group *v.* unsupplemented formula group)
Salvini *et al.*[22] (Italy)†	Healthy newborns of hepatitis C virus-infected mothers	GOS/FOS (0·8 g/100 ml) (*n* 10)	IF (*n* 10)	6 months	Danone Research, Centre for Specialised Nutrition	No AE recorded
Scholtens *et al.*[36] (Belgium) (Part of a study by Alliet)*	Healthy term infants	GOS/FOS (0·6 g/100 ml) (*n* 27)	IF (*n* 29) BF (*n* 31/39)	6 months (26 weeks)	Numico Research, Wageningen, The Netherlands	NS
van Stuijvenberg *et al.*[34] (The Netherlands, Austria, Switzerland, Italy, Germany)*	Healthy infants <8 weeks	GOS/FOS + AOS (0·8 g/100 ml) (*n* 414)	IF (*n* 416) BF (*n* 300)	1 year	Danone Research	No serious AE related to the intervention
Veereman-Wauters *et al.*[23] (Belgium)†	Healthy term infants <5 d	GOS/FOS (0·8 g/100 ml) Oligofructose-enriched inulin SYN1 (0·4 or 0·8 g/100 ml)	IF (*n* 21) BF (*n* 29)	28 d	Beneo-Orafti	No SAE related to the intervention
Williams *et al.*[15] (USA)†	Healthy term <8 d	GOS (0·4 g/100 ml) (*n* 46) GOS (0·8 g/100 ml) (*n* 43)	IF (*n* 44)	119 d	Abbott Nutrition	Higher proportion of SAE, primarily respiratory tract infections ($P<0·05$)
Closa-Monasterolo *et al.*[28] (Spain)†	Newborns	Oligofructose-enriched inulin (SYN1) (0·8 g/100 ml) (*n* 128)	IF (*n* 124) BF (*n* 136)	4 months	BENEO Institute (an Initiative of BENEO GmbH).	Regurgitation, digestive discomfort – NS Crying (min/d) – NS (except at 4 months – longer in the prebiotic group; $P<0·05$) Total AE: NS (except less loose stools in the prebiotic group: 2 *v.* 8 %, $P<0·05$)
Ashley *et al.*[5] (USA)†	Healthy term infants	PDX/GOS (0·4 g/100 ml)	IF (*n* 142)	120 d	Unclear	NS
Nakamura *et al.*[25]*	Healthy term infants 13 to 92 d	PDX/GOS (0·4 g/100 ml)	IF (*n* 25) BF (*n* 30)	28 d	Mead Johnson & Company, Evansville, IN	NS (data not shown)
Scalabrin *et al.*[26] (USA)†	Healthy term infants 21–30 d	PDX/GOS (0·4 g/100 ml) (*n* 100)	IF (*n* 101) BF (*n* 88)	60 d	Mead Johnson Nutrition	NS: 1 or more AE or when analysed by type of event
Ziegler *et al.*[27] (USA)*	Healthy term infants <14 d	PDX + GOS (0·4 g/100 ml) (*n* 74) PDX + GOS + LOS (0·8 g/100 ml) (*n* 76)	IF (*n* 76)	120 d	Mead Johnson & Co.	Serious AE (none related to the study products): NS Diarrhoea (control *v.* PG4, 4 *v.* 18 %, $P=0·008$), Eczema (PG4 *v.* control, 18 *v.* 7 %, P.0·046; PG4 *v.* PGL8, 18 *v.* 4 %, $P=0·008$), Irritability (control *v.* PGL8, 4 *v.* 16 %, $P=0·027$)
Nakamura *et al.*[25]*	Healthy term infants 13 to 92 d	PDX/GOS (0·4 g/100 ml)	IF (*n* 25) BF (*n* 30)	28 d	Mead Johnson & Company, Evansville, IN	NS (data not shown)

GOS, short-chain galactooligosaccharides; FOS, long-chain fructo-oligosaccharides; AOS, acidic oligosaccharides; IF, infant formula; BF, breast-feeding; BAE, adverse event; FF, follow-on formula; SYN1, 50:50 mixture of long chain inulin (Orafti HP) and oligofructose (b(2-1)-linked fructo-oligosaccharides with less than nine fructose moieties and partially containing a terminal glucose unit; PDX, polydextrose; LOS, lactulose; CFU, colony-forming units; GI, gastrointestinal; SAE, serious adverse event; URTI, upper respiratory tract infection; UTI, urinary tract infection.

* The effects as reported in a 2011 review (for details, see 1).

† Newly identified evidence (for details, see text).

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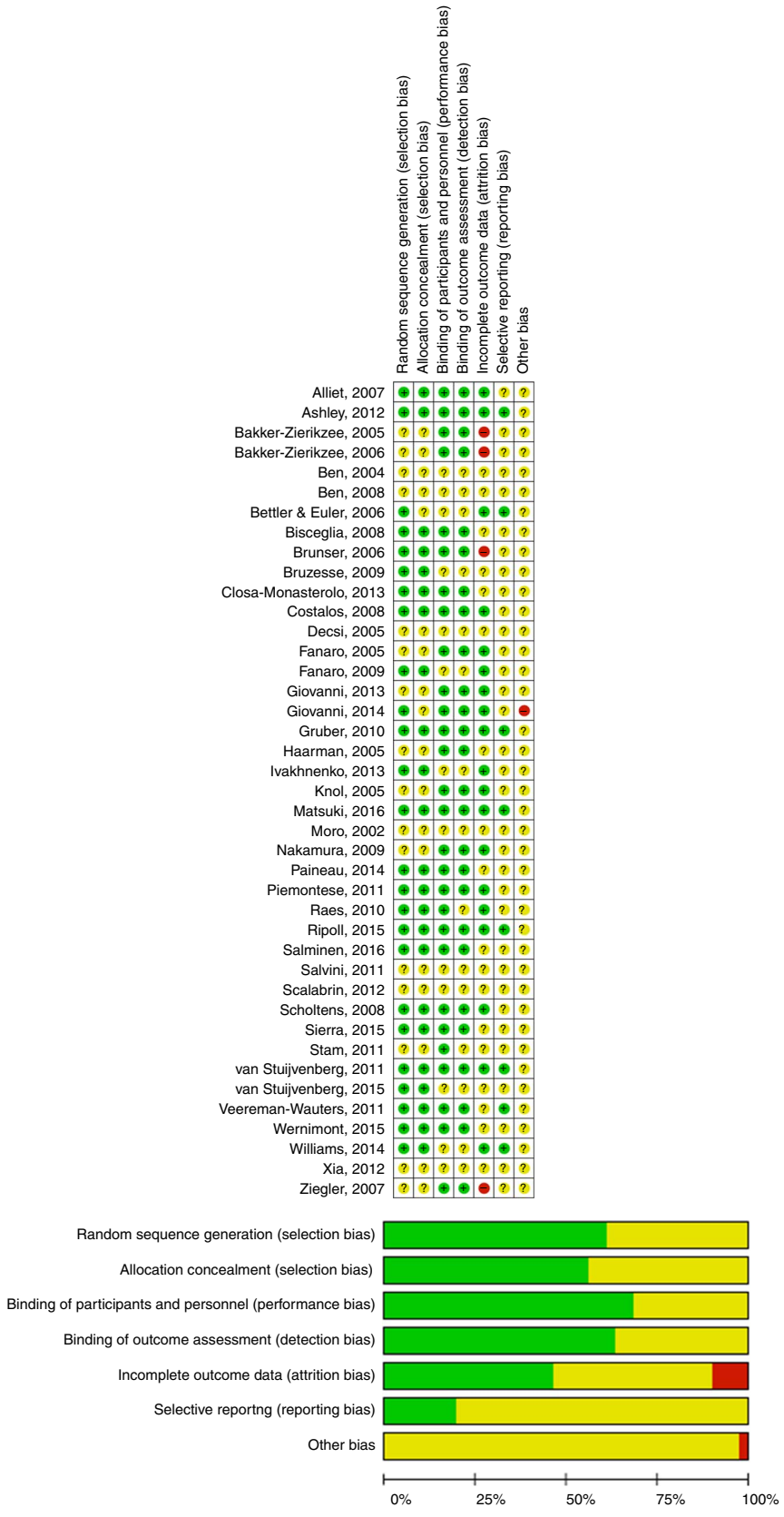

Fig. 1. Assessment of the risk of bias in included trials and the review authors' judgements about each risk of bias item presented as percentages across all included studies. ■, Low risk of bias; ■, unclear risk of bias; ■, high risk of bias. For a colour figure, see the online version of the paper.

Clinical effects

Below, we summarise evidence from trials reporting clinical outcomes that have not been included in the earlier report by the ESPGHAN Committee on Nutrition[1].

Growth. A number of trials assessed the effects of prebiotic supplementation on growth, including FOS supplementation of infant formula, either 0·4 g/100 ml[12] or 0·5 g/100 ml,[13] and GOS supplementation (with various GOS contents)[5,10,11,14,15]. In both cases, there were no significant differences between the prebiotic-supplemented and the unsupplemented formula groups. In 4 studies investigating the effects of the administration of formula supplemented with 0·8 g/100 ml of GOS/FOS, no significant differences between the prebiotic and control groups in anthropometric parameters (weight, length and head circumference) were found at any time[16,20,22,23]. However, in a study by Bruzzese *et al.* the authors observed a transient increase in body weight. In the GOS/FOS-supplemented formula group compared with the control formula group, there was a significant increase in the mean body weight at 3 and 6 months of follow-up ($P<0·01$), whereas it was similar in the two groups at 9 and 12 months of follow-up. Mean body length was significantly greater in the GOS/FOS-supplemented formula group at all-time intervals ($P<0·05$), whereas mean head circumference was similar in the two groups at 3, 6, 9 and 12 months of follow-up (data not shown)[18]. The supplementation with FOS/GOS + AOS[24] and the supplementation with two different doses of oligofructose-enriched inulin (0·4 g/100 ml and 0·8 g/100 ml supplementation)[23,28] had no significant effects on growth. Similarly, the supplementation with PDX/GOS[5,26] resulted in no differences in anthropometric measures between infants receiving a control formula or a formula supplemented with 0·4 g/100 ml of a prebiotic blend of PDX and GOS from 14 to 60 d of age[26] or from 14 to 120 d of age[5].

Tolerance. Several new studies evaluated the influence of prebiotic-supplemented formulae on tolerance. Although there were different definitions of tolerance, there were no significant differences in tolerance between infants who received unsupplemented and prebiotic-supplemented formulae in the majority of studies. There were no differences in the number of days of abdominal pain (with crying) (0·22 (SE 0·28) *v.* 0·23 (SE 0·23), respectively; *P* value not shown) or nausea (0·05 (SE 0·12) *v.* 0·03 (SE 0·07), respectively, *P* value not shown) between infants who received unsupplemented or FOS-supplemented (0·4 g/100 ml) formula[12], no significant differences between infants fed FOS-supplemented formulae (0·2 g/100 ml or 0·3 g/100 ml) or unsupplemented formula in the frequency of spitting up or vomiting (no exact data shown)[29], and no significant differences between infants fed FOS-supplemented formula (0·5 g/100 ml) or unsupplemented formula in the mean number of days with regurgitation (30·0 (SE 5·8) *v.* 27·7 (SE 5·0), respectively; $P=0·79$), crying (20·7 (SE 6·0) *v.* 18·67 (SE 5·2), respectively; $P=0·85$), and constipation (18·2 (SE 4·7) *v.* 9·8 (SE 2·4), respectively; $P=0·23$). However, there was a significantly reduced number of days with vomiting (4·1 (SE 0·7) *v.* 7·7 (SE 1·3); $P=0·05$) in the FOS-supplemented formula group[13]. No significant differences were found in tolerance symptoms, such as fussiness and gassiness, between an unsupplemented formula group and either a GOS-supplemented formula (0·4 g/100 ml) group[5] or a PDX/GOS-supplemented formula group[5,26]. Regurgitation and vomiting scores were evaluated in infants receiving GOS/FOS- or oligofructose-enriched inulin-supplemented formulae[23], with no significant differences found between the supplemented and unsupplemented formula groups. Tolerance was also defined as the incidence of episodes of crying, regurgitation, vomiting and flatulence. No differences between groups were found in a studies comparing GOS-[10] (no data shown), GOS/FOS- and AOS- (0·8 g/100 ml) supplemented formulae with unsupplemented formula ($P>0·05$)[24]. There were also no differences in either the duration of vomiting (median 2·0 (25–75 centile 0·0–4·0) *v.* 2·0 (25–75 centile 0·0–5·0), respectively; $P=0·13$) or the number of episodes of vomiting (median 0·87 (25–75 centile 0·4–1·56) *v.* 0·91 (25–75 centile 0·9–1·55), respectively; $P=0·46$) in the supplemented formula group compared with the unsupplemented formula group[31].

Stool frequency. Stool frequency was evaluated in twelve new trials. Although different prebiotics were used in the majority of studies, there were no significant effects on stool frequency between prebiotic-supplemented and unsupplemented formula groups, including FOS supplementation (two RCT)[29,32], GOS supplementation (five RCT)[10,15,33], FOS/GOS supplementation[23], FOS/GOS/AOS supplementation[24], oligofructose-enriched inulin supplementation[23] and PDX/GOS supplementation[26]. Only four studies reported a higher frequency of stools in the prebiotic-supplemented formula group, either at all study time points (FOS/GOS)[16] and (oligofructose-enriched inulin)[28] or at some time intervals (GOS at 7, 14 and 60 d),[5] (GOS at 3 and 4 months)[14] and (PDX/GOS at 7, 14 and 60 d)[5].

Stool consistency. A number of new trials assessed the effects of prebiotic supplementation on stool consistency, including FOS supplementation (three RCT)[13,29,32], GOS supplementation (four RCT)[5,10,14,15], FOS/GOS supplementation (two RCT)[18,23], FOS/GOS/AOS supplementation (one RCT)[24], PDX/GOS supplementation (two RCT)[5,26] and oligofructose-enriched inulin supplementation (two RCT)[23,28]. These trials documented a softening effect of prebiotic supplementation of formula on stool consistency (Table 1).

Gastrointestinal infections/diarrhoea. Six new studies evaluated the effects of prebiotic supplementation on the incidence of episodes of diarrhoea/gastrointestinal infections. Neither addition of FOS, GOS, nor FOS/GOS/AOS had an influence on the frequency of these episodes, with no differences between the supplemented and unsupplemented formula groups. Paineau *et al.*[12] found no difference between control and FOS-supplemented groups in the number of days with diarrhoea, defined as the number of days with liquid stools (0·10 (SE 0·16) *v.* 0·18 (SE 0·24), respectively; *P* value not shown). There were no significant differences between unsupplemented and GOS-supplemented formula groups in either the number of episodes of diarrhoea (defined as semiliquid or liquid faeces in

three or more depositions per day for at least 3 d) per infant (0·20 (SE 0·52) *v.* 0·27 (SE 0·67); respectively; MD 0·07, 95 % CI −0·05, 0·19; $P=0·36$) or the number of infants with at least 1 episode of diarrhoea/year (15·9 *v.* 18·2 %, respectively; $P=0·8$)[14]. There were also no significant differences in the number of episodes of gastroenteritis (diagnosed as a fever episode accompanied by vomiting and diarrhoea, or only diarrhoea) between infants receiving either standard or follow-up formula supplemented with 0·8 g/10 ml of GOS/FOS/AOS or control formula during the first 12 months of the intervention[34], as well as in the adjusted frequency of febrile episodes due to diarrhoea analysed prospectively at 3–5 years (0·51 (25–75 centile 0·03–1·07) *v.* 0·53 (25–75 centile 0·03–1·51), respectively; $P=0·22$); however, the authors reported a shorter duration (d) of diarrhoea in the experimental group ((1·0 (25–75 centile 0·0–4·0) *v.* 2·0 (25–75 centile 0·0–7·0), respectively; $P=0·01$)[31]. Only studies investigating formulae supplemented with GOS/FOS reported a beneficial effect of prebiotic supplementation. Ivakhnenko & Nyankovskyy[20] found a significant reduction in the incidence of gastrointestinal tract infections at the age of 18 months in the GOS/FOS-supplemented (0·8 g/100 ml) formula group compared with the unsupplemented formula group (0·28 (SE 0·05) *v.* 0·78 (SE 0·12) episodes/child/18 months, respectively; MD −0·5; 95 % CI −0·53, −0·47; $P<0·001$). Bruzzese *et al.*[18] reported, in the GOS/FOS-supplemented formula group compared with the unsupplemented formula group, a reduced rate of diarrhoeal episodes (defined as 3 or more loose or watery stools/d lasting for at least 3 d) (0·12 (SE 0·04) *v.* 0·29 (SE 0·05) episodes/child per 12 months, respectively; MD −0·17; 95 % CI −0·18, −0·16) and a reduced number of children with at least one episode of acute diarrhoea (10/96 *v.* 26/109, respectively; RR 0·37; 95 % CI 0·17, 0·82).

Respiratory tract infections. Only five studies reported the effects of prebiotic supplementation on the incidence of respiratory tract infections. No significant differences between groups in the number of episodes of upper respiratory tract infections per infant (1·65 (SE 1·8) *v.* 1·84 (SE 2·0), respectively; $P=0·44$) or in the number of infants with at least three episodes of upper respiratory tract infections per year (15·9 *v.* 16·7 %, respectively; $P=0·9$)[14] were reported when GOS-supplemented formula and unsupplemented formula were compared. No significant differences between groups were reported in the median adjusted number of upper respiratory tract infections as a suspected cause of fever as well as the number of episodes or duration in days of coughing (episodes 2·55 (25–75 centile 1·09–4·07) *v.* 2·63 (25–75 centile 1·09–4·56), respectively, $P=038$; duration: 22·5 (25–75 centile 8·0–42·5) *v.* 24·0 (25–75 centile 9·0–50·0), respectively, $P=0·27$) or runny or blocked nose (episodes: 2·59 (25–75 centile 1·02–5·26) *v.* 2·91 (25–75 centile 0·99–4·95), respectively, $P=0·93$; duration: 21·0 (25–75 centile 5·0–61·0) *v.* 24·0 (25–75 centile 6·0–60·0), respectively, $P=0·66$) between the prebiotics (FOS/GOS) and control groups during 12 months and at 3–5 years[31,34]. Similar results were reported by Bruzzese *et al.*[18]. These authors found no significant differences between the FOS/GOS-supplemented formula group and the unsupplemented formula group in the number of patients with at least 1 episode of upper respiratory tract infections (60/94 *v.* 65/109, respectively; RR 1·07; 95 % CI 0·86, 1·33) and >3 episodes of upper respiratory tract infections (17/60 *v.* 29·65, respectively; RR 0·64; 95 % CI 0·39, 1·03). Also, Closa-Monasterolo *et al.*[28] reported no significant differences in the mean number of infections between control and oligofructose-enriched inulin formula-fed groups. However, a significant reduction in the incidence of upper respiratory tract infections at the age of 18 months in the FOS/GOS-supplemented (0·8 g/100 ml) formula group compared with the control formula group (2·81 (SE 0·51) *v.* 5·78 (SE 0·97), respectively; MD −2·97; 95 % CI −3·26, −2·68) was reported by Ivakhnenko & Nyankovskyy[20].

Allergic manifestations. Only two studies evaluated the effects of prebiotic supplementation on allergic manifestation. Sierra *et al.*[14] reported no significant difference between the unsupplemented and GOS-supplemented formula groups in the number of allergic manifestations (atopic dermatitis, wheezing, food allergy) (28/132 *v.* 39/172, respectively; RR 1·39; 95 % CI 0·91, 2·12; $P=0·12$)[14]. Whereas Ivakhnenko & Nyankovskyy[20] found, in the GOS/FOS-supplemented formula group compared with the unsupplemented formula group, a significant reduction in the number of infants with allergic reactions to food (3/62 *v.* 9/53, respectively; RR 0·28; 95 % CI 0·09, 0·9), allergic reactions to cows' milk protein (2/62 *v.* 8/53, respectively; RR 0·2; 95 % CI 0·05, 0·84), atopic dermatitis (3/62 *v.* 9/53, respectively; RR 0·28; 95 % CI 0·09, 0·9), and gastrointestinal symptoms of food allergy (2/62 *v.* 7/53, respectively; RR 0·24; 95 % CI 0·06, 0·98); however, there was no effect of formula supplementation on respiratory system allergic symptoms (3/62 *v.* 7/53, respectively; RR 0·36; 95 % CI 0·1, 1·2).

Antibiotic treatment. Five studies reported on the effects of prebiotic supplementation on frequency of antibiotic treatment. Four of them reported no significant difference between the unsupplemented and supplemented formula groups. Ripoll *et al.*[13] evaluated FOS-supplemented formula and reported no significant difference ($P=0·12$) in the number of infants with concomitant treatments (including antibiotics) between study groups. Sierra *et al.*[14] reported no significant difference between the unsupplemented and GOS-supplemented formula groups in the percentage of patients with antibiotic treatment (19·8 *v.* 17·8 %, respectively; RR 0·88; 95 % CI 0·5, 1·45; $P=0·48$). In the studies by van Stuijvenberg *et al.*[34], there were no significant differences between the experimental (FOS/GOS-supplemented formula) and control groups in the median adjusted numbers of fever episodes for which systemic antibiotics were used during the 1st year of life as well as during follow-up of that group at 3–5 years[31]. However, Bruzzese *et al.*[18] found, in the FOS/GOS-supplemented formula group compared with unsupplemented formula group, a significantly reduced rate of antibiotic courses prescribed for children (1·03 (SE 0·15) *v.* 1·48 (SE 0·16), respectively; MD 0·45; 95 % CI −0·49, −0·4, $P=0·038$) and a significantly reduced number of children receiving more than two antibiotic courses/year (24/60 *v.* 43/65, respectively; RR 0·6; 95 % CI 0·42, 0·86); however, there was only a borderline significant difference between groups in the number of children receiving at least 1 antibiotic course (46/60 *v.* 58/65, respectively; RR 0·86; 95 % CI 0·73, 1·01).

Adverse events. A number of studies reported data on adverse events (Table 1), but none of them reported serious adverse events

related to the tested formulae. Some authors defined adverse events as concomitant infections (nasopharyngitis, bronchitis, gastroenteritis and diarrhoea), whereas others defined adverse events as feeding-related gastrointestinal symptoms or symptoms of intolerance. No significant differences in the number of adverse events between supplemented and unsupplemented formula groups were reported by Ripoll *et al.*[13] and Wernimont *et al.*[32] evaluating FOS supplementation, by Piemontese *et al.*[24] evaluating the FOS/GOS/AOS supplementation, and by Scalabrin *et al.*[26] evaluating PDX/GOS supplementation. Ashley *et al.*[5] evaluated GOS supplementation and PDX/GOS supplementation and found no differences in the overall incidence of at least one medically confirmed adverse event between supplemented and unsupplemented formula groups. However, these authors reported that the frequency of excessive spitting was significantly higher in the GOS-supplemented formula group (7·5 *v.* 0 %, $P<0·05$) and gas was significantly less frequent in the PDX/GOS-supplemented group ($P<0·05$) compared with the unsupplemented group. Also, Williams *et al.*[15] reported no significant differences among feeding groups in the proportions of subjects with specific adverse events, with one exception; there was a significantly higher proportion of serious adverse events, primarily respiratory tract infections, in the group that received a higher concentration of GOS (0·8 g/100 ml *v.* 0·4 g/100 ml; $P<0·05$). In two studies by Giovannini *et al.*[11,39], there were no differences in crying episodes, vomiting, or diarrhoeal episodes between the groups. However, the authors reported significantly lower regurgitation rates and episodes of infantile colic in the GOS-supplemented formula group ($P<0·05$), and lower stool frequency in the unsupplemented formula group ($P<0·05$). In a study evaluating oligofructose-enriched inulin supplementation, the frequencies of the majority of adverse events were generally equal in both groups; exceptions were the amount of time spent crying (min/d), which was significantly longer in the prebiotic group compared with the control group ($P<0·05$), as well as the number of episodes of loose stools, which was slightly fewer in the prebiotic group compared with the control group (2 *v.* 8 %; $P<0·05$)[28]. In some studies, no adverse events were observed throughout the study period in the control and experimental groups (GOS[33], FOS/GOS)[16,18,22,23].

Non-clinical outcomes

The online Supplementary Table S3 provides an overview of the non-clinical findings in all RCT that evaluated the effects of prebiotic-supplemented formulae. The findings were not consistent. However, differences differences in microbiota composition and immune parameters in infants fed prebiotic-supplemented formula *v.* unsupplemented formula (e.g. higher stool colony counts of bifidobacteria; increased SCFA concentrations; reduce faecal pH) were found.

Discussion

Summary of findings

We updated the 2011 evidence on the effects of the administration of prebiotic-supplemented infant formulae compared with unsupplemented formulae. Forty-one eligible trials were identified, described in twenty-five new publications.

Considering all of the evidence available (i.e. included in the earlier report by the Committee on Nutrition and the current up-date), supplementation of infant formula with FOS alone had no effect on growth, tolerance (assessed as some of following: abdominal pain, crying, nausea, vomiting, regurgitation), gastrointestinal infections, and stool frequency. However, it was associated with a softer stool consistency. Supplementation of infant formula with GOS alone had no effect on growth, tolerance (assessed as some of following: crying, regurgitation, fussiness, gassiness, vomiting, flatulence), gastrointestinal infections, respiratory tract infections, and allergic manifestations. However, it was associated with an increased stool frequency with a softer stool consistency in some trials. Supplementation of infant formula with FOS/GOS had no effect on growth or tolerance (assessed as some of following: crying, regurgitation, fussiness, gassiness, vomiting, flatulence, cramps). The effects on gastrointestinal and respiratory tract infections remain unclear, as this was documented in only some trials. Furthermore, the methodological quality of some them was poor. There was no effect of supplementation of infant formula with FOS/GOS on stool frequency. However, it was associated with a softer stool consistency. Supplementation of infant formula with AOS alone had no effect on growth, tolerance (assessed as crying, regurgitation, or vomiting), and stool frequency. However, it was associated with a softer stool consistency. Supplementation of infant formula with FOS/GOS/AOS had no effect on growth or tolerance (assessed as crying, regurgitation, vomiting). The effects on gastrointestinal and respiratory tract infections remain unclear, as this was documented in only some trials. Furthermore, the methodological quality of some them was poor. Supplementation of infant formula with FOS/GOS/AOS was associated with an increased stool frequency, with a softer/lower stool consistency. Supplementation of infant formula with PDX/GOS had no effect on growth or tolerance (as assessed by fussiness, gassiness). However, it was associated with a softer stool consistency. Supplementation of infant formula with oligofructose-enriched inulin had no effect on growth or tolerance (assessed as fussiness, regurgitation, or vomiting). However, it was associated with a softer stool consistency. Overall, adverse events were frequently reported; however, with minor exceptions, no differences were found between the prebiotic-supplemented and unsupplemented formula groups. None of the trials reported serious adverse events related to use of the study products.

Based on clinical outcomes, a number of studies analysed the effects of different prebiotics on growth, an essential outcome measure for evaluating the safety of infant formulae. All of these trials concluded that prebiotic supplementation of infant formulae did not have any significant effects on growth. All trials consistently showed that prebiotic supplementation of infant formulae has the potential to soften stools. However, the clinical significance of these findings remains unclear, although it may be beneficial in small infants with hard stools. Many, albeit not all, studies showed that prebiotic supplementation of infant formulae significantly increased stool frequency. Again, the clinical significance of these findings remains unclear. There is some inconsistent evidence suggesting that supplementation of infant formula with GOS/FOS may be associated with a reduced risk of

gastrointestinal infections. However, the effect size was small and the confidence intervals were wide, so these results should be interpreted with caution. The supplementation of infant formula with GOS/FOS/AOS may be associated with a reduction in the duration of diarrhoea. With one exception, the available data showed no effect of prebiotic supplementation of infant formulae on respiratory tract infections. The only trial suggesting that supplementation of infant formula with GOS/FOS may be associated with a reduced risk of upper respiratory tract infections needs to be interpreted with caution, as the confidence intervals were wide. The effects of prebiotic supplementation of infant formulae on allergic diseases were inconclusive. One study with methodological limitations demonstrated that supplementation of infant formula with GOS/FOS resulted in a significant decrease in some allergic reactions. Supplementation with GOS/FOS/AOS reduced the risk of eczema. Supplementation with GOS had no effect on the rates of allergic manifestations and sensitisation. Thus, there is still too much uncertainty to draw reliable conclusions from the available data. The effects of prebiotic supplementation of infant formulae on antibiotic use were inconclusive. One study demonstrated that supplementation of infant formula with GOS/FOS resulted in a significant decrease in the number of fever episodes for which systemic antibiotics were used; the authors of other studies reported no significant decrease in antibiotic use with prebiotic supplementation. The reporting of symptoms such as crying, fussiness, regurgitation, and vomiting was not consistent. In trials in which these symptoms were evaluated as tolerance, no significant differences were found between the prebiotic-supplemented and unsupplemented formula groups. In trials in which these symptoms were evaluated as adverse events, differences were observed in some of the trials, but the effects were not consistent.

Strengths and limitations

An important strength of this systematic review is the use of rigorous methodology developed by the Cochrane Collaboration. The review addressed a clear question that was defined in terms of the study design, participants and interventions; however, the primary outcomes were not determined in advance.

We employed several methods to reduce bias (i.e. comprehensive literature search, pre-specified criteria for methodological assessment and analysis, no restrictions by language or year of publication). However, we cannot exclude the risk of publication bias, which was not formally addressed due to the limited number of included studies. The methodological quality of the included studies was generally moderate to low, which increases the risk of bias. Some of the prebiotics were evaluated in single trials only. The included studies were likely to be underpowered for addressing some outcomes (also adverse events).

The majority of included studies were industry-supported trials. There is bias associated with study funding sources. Compared with non–industry-sponsored studies, industry-sponsored studies tended to have more favourable effectiveness and harm findings and more favourable conclusions[45]. Funding of research by manufacturers of infant formulae may be considered even more controversial because of the need for protection and promotion of breast-feeding. However, in the case of studies involving infant formulae, industry involvement is unavoidable, as investigators lack the means to manufacture quality infant products.

Comparison with other studies

Overall, even as new data have become available, the conclusions made previously did not change. With regard to growth, the 2011 systematic review by the ESPGHAN concluded that prebiotic-supplemented formulae do not raise safety concerns, that is, they do not have adverse effects on growth[1]. Our review does not confirm the findings of a 2012 systematic review by Mugambi *et al.*, which concluded that prebiotic-supplemented formula increased weight gain; however, currently, more data are available. Similar to our review, Mugambi *et al.*[46] concluded that use of prebiotic-supplemented formula increased stool frequency but, in contrast to our findings, it had no impact on stool consistency.

Our review found that all of the prebiotic-supplemented formulae were well tolerated; no serious adverse effects related to use of the study products were observed in any of the studies, regardless of the dose of prebiotics used. The dose is important. As recently concluded by Gibson *et al.*[47], 'an appropriate dose must be sufficient to generate a prebiotic effect, but not too high to induce unwanted or adverse effects such as excessive gas formation or non-selective utilisation.'

Previously, the ESPGHAN Committee on Nutrition concluded that prebiotic supplementation of infant formulae has the potential to affect a number of non-clinical outcomes[1]. Overall, one of the most consistent findings was that the use of prebiotic-supplemented formulae resulted in significantly higher stool colony counts of bifidobacteria. It is generally accepted that the establishment of gut microbiota is of great importance to gastrointestinal physiology and appears to modulate the health and well-being of the host organism. The lower incidence of gastrointestinal and other infections found in breast-fed infants may, in part, be related to a predominance of *Bifidobacterium* and *Lactobacillus* in their gut microbiota. Therefore, the establishment of a gut microbiota closer to that of breast-fed infants in formula-fed infants after supplementation with prebiotics might be considered in the context of the current hypothesis on the role of the gut microbiota in health and disease. The development of not only infectious diseases but also non-communicable diseases (e.g. allergy, diabetes, obesity) may be related to aberrant gut microbiota early in life. At least in some studies, it has been documented that prebiotic supplementation stimulates the production of SCFA, primarily acetic acid. SCFAs are measurable products of bacterial fermentation and play a role in normal colonic functions. However, whether the increase in SCFA concentrations *per se* is of benefit is currently not well established.

The same applies to other stool parameters, such as the reduced faecal pH values in infants who have received prebiotic-supplemented formulae. The effects of prebiotic-supplemented formulae on some immunologic parameters were not consistent. However, some effects may be potentially important. For example, prebiotic supplementation increased

faecal IgA secretion. Considering that IgA is an antibody that plays a critical role in mucosal immunity, prebiotic supplementation may have an impact on the development of the immune system in infants. However, taken together, the interpretation of the non-clinical findings was difficult. Whether a change in any of these parameters *per se* is of benefit to the infants is currently not established.

Conclusions

In line with the 2011 ESPGHAN document, the available scientific data suggest that the administration of currently evaluated prebiotic-supplemented formulae to healthy infants does not raise safety concerns with regard to growth and adverse effects. Some favourable clinical effects are possible, primarily stool softening, which may be beneficial in some infants. Currently, there is no existing robust evidence to recommend the routine use of prebiotic-supplemented formulae. The latter conclusion may reflect the small amount of data on specific prebiotics and outcomes, rather than a genuine lack of an effect. The efficacy and safety should be considered for each prebiotic(s)-supplemented formula.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518000120

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