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## Opinion on the re-evaluation of ascorbyl palmitate (E 304i) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups

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### Abstract

Ascorbyl palmitate (E 304(i)) was re-evaluated in 2015 by the former EFSA Panel on Food Additives and Nutrient sources added to Food (ANS). As a follow-up to this assessment, the Panel on Food Additives and Flavourings (FAF) was requested to assess the safety of ascorbyl palmitate (E 304(i)) for its uses as food additive in food for infants below 16 weeks of age belonging to food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and as carry over in line with Annex III, Part 5 Section B to Regulation (EC) No 1333/2008. In addition, the FAF Panel was requested to address the issues already identified during the re-evaluation of the food additive when used in food for the general population. The process involved the publication of a call for data to allow the interested business operators to provide the requested information to complete the risk assessment. On the basis of the data submitted by interested business operators and the considerations from the Panel, a revision of the existing EU specifications for ascorbyl palmitate (E 304 (i)) has been recommended. Based on in vitro data, the FAF Panel assumed that ascorbyl palmitate fully hydrolyses pre-systemically to ascorbic acid and palmitate. The Panel concluded that the intake of both metabolites, at the MPLs for ascorbyl palmitate as a food additive in infant formula belonging to FC 13.1.1 or in food for special medical purposes belonging to FC 13.1.5.1, does not raise health concerns.

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## Summary

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

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

As follow-up of the above, this Opinion addresses the data gaps previously identified during the re-evaluation of ascorbyl palmitate (E 304(i)) as food additive and the safety in the special subpopulation of infants below 16 weeks of age.

The process followed involved the publication of a dedicated call for data allowing all interested business operators to provide the requested information for completing the assessment and to confirm that the additive is used as food additive in food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and present as carry over in line with Annex III, Part 5 Section B to Regulation (EC) No 1333/2008. In addition, the interested business operators were requested to provide data to address the issues already identified during the re-evaluation of the food additive when used in food for the general population. The data submitted in response to the call for data on ascorbyl palmitate (E 304(i)) comprised technical information, human data and the outcome of literature searches.

On the basis of the data submitted by interested business operators and the considerations from the Panel, a revision of the existing EU specifications for ascorbyl palmitate (E 304(i)) has been recommended. In particular, the Panel recommended to lower the current maximum limits in the specifications for lead, arsenic and mercury, considering also other sources of exposure, and to include limits for solvents.

Dietary exposure to ascorbyl palmitate (E 304(i)) from its use as a food additive was estimated for infants below 16 weeks of age. The exposure scenario is based on the recommended consumption levels from the relevant Scientific Committee Guidance which recommends values of 200 and 260 mL formula/kg body weight (bw) per day as conservative mean and high-level consumption values for 14–27 days old infants. For infants below 16 weeks of age consuming infant formulae (Food category 13.1.1), the exposure to ascorbyl palmitate (E 304(i)) was estimated to be 2 mg/kg bw per day (mean consumption) and 2.6 mg/kg bw per day (high-level consumption). For infants below 16 weeks of age consuming infant formula for special medical purposes (FSMP) (Food category 13.1.5.1), the exposure to ascorbyl palmitate (E 304(i)) was estimated to be 20 mg/kg bw per day (mean consumption) and 26 mg/kg bw per day (high-level consumption).

No new animal data were provided. Literature searches were performed by the interested business operators. The literature search identified one paper in which toxicological endpoints (cytotoxicity and induction of apoptosis) were studied and which was published after the last evaluation. According to the Panel, this publication does not contribute to the assessment of the safety of ascorbyl palmitate as a food additive.

Several clinical studies were submitted in which formulas were tested which contained ascorbyl palmitate among other food additives. None of the studies was intended to test the safety and tolerability of ascorbyl palmitate specifically. In these studies, no remarkable deviations from the normal growth and development were noted. All the studies have limitations in design and reporting. In a risk of bias assessment, five of the six studies were allocated to tier 3 and one study to tier 2 in which the Panel noted that ascorbyl palmitate was present in a relatively low concentration of [REDACTED]. The absence of appropriate control groups which were not exposed to ascorbyl-palmitate is the most severe limitation. Therefore, the studies provide limited evidence for the safe use of ascorbyl palmitate. From the post-marketing information, it can be deduced that adverse effects were rarely reported.

For the current assessment of ascorbyl palmitate, the FAF Panel assumed based on in vitro data that also infants below the age of 16 weeks are able to hydrolyse ascorbyl palmitate pre-systemically to ascorbic acid and palmitate.

Exposure to ascorbyl palmitate (E 304(i)) from infant formulae (FC 13.1.1) was estimated at 2.6 mg/kg bw per day at the 95 percentile which corresponds to 1.11 mg/kg bw per day ascorbic acid. Taking into account the molecular weight of the moiety of ascorbic acid in the additive E 304(i), ascorbic acid corresponds to 42.5% of the weight. EFSA has proposed an adequate vitamin C intake of 20 mg/day for infants up to 6 months. Hence, considering the default body weight of 4.8 kg for infants from 0 to 3 months of age, the amount of vitamin C resulting from the exposure to ascorbyl palmitate (E 304(i)) for the scenario with infant formulae (FC 13.1.1) results to be 26.6% of the adequate vitamin C intake. For the exposure according to the scenario with special infant formulae (FC 13.1.5.1), the exposure to ascorbyl palmitate (E 304(i)) was estimated to be 10-fold higher than the previous one and thus should be 2.66 fold (i.e. 266%) the adequate vitamin C intake. Compared to the regulatory content of vitamin C in infant formulae of minimum 4 and maximum 30 mg ascorbic acid/100 kcal (Reg (EU) 2016/127), high-level intake (p95) of ascorbic acid from the use of E 304(i) in infant formulae (FC 13.1.1) represents between 2.0 and 17.7%. Compared to the regulatory content of vitamin C in special infant formulae (i.e. food for special medical purposes developed to satisfy the nutritional requirements of infants) of minimum 4 and maximum 30 mg ascorbic acid/100 kcal (Reg (EU) 2016/128), high-level intake (p95) of ascorbic acid from the use of E 304(i) in special infant formulae (FC 13.1.5.1) represents between 20 and 177%. The Panel noted that the SCF and the NDA Panel concluded that the available data did not allow to set an upper limit of intake level for vitamin C at any age. Given the fact that adverse effects in adults are only reported with doses of several grams of vitamin C (e.g. 5 g/adult = 71.4 mg/kg bw per day), the Panel considered that the exceedance of the statutory maximum content is of no safety concern.

No regulatory guidance currently exists for palmitate intake. The Panel noted that palmitate provides approximately 25% of all breast milk fatty acids and that triglycerides represent 98–99% of breast milk fat content, i.e. 35 g/L. Considering a high daily breast milk intake of 1,200 mL for infants from 0 to 6 months (EFSA NDA Panel, 2013) and the default value of 4.8 kg for infants between 0 and 3 months (EFSA Scientific Committee, 2012), the palmitate intake in an exclusively breast fed infant can be estimated to around 2,200 mg/kg bw per day. Exposure to palmitate from the use of 304(i) in infant formula (FC 13.1.1) and in special infant formulae (FC 13.1.5.1) represents therefore less than, respectively, 0.1% and 1% of palmitate intake in exclusively breastfed infants between 0 and 3 months.

Hence, the intake of both, ascorbic acid and palmitate, at the MPL for food according to FC 13.1.1 and food according to FC 13.1.5.1 does not raise health concerns for infants below 16 weeks of age.

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## 1. Introduction

The present opinion deals with:

- the risk assessment of ascorbyl palmitate (E 304(i)) in food for infants below 16 weeks of age in the food categories 13.1.1 (Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC<sup>1</sup>) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) as defined in Annex II and III to Regulation (EC) No 1333/2008<sup>2</sup> on food additives.
- the follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of ascorbyl palmitate (E 304(i)) as a food additive (EFSA ANS Panel, 2015a).

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

#### 1.1.1. Background

The composition of food intended for infants and young children, as defined by Regulation (EU) No 609/2013<sup>3</sup>, is regulated at EU level and such rules include requirements concerning the use of substances as food additives.

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

In accordance with Regulation (EU) No 257/2010<sup>4</sup>, EFSA is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. However, the risk assessment approach followed until now has not covered the use of food additives in food for infants below 12 weeks of age. Consequently, EFSA published several scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 but not addressing their use in food for infants below 12 weeks of age.

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

On 31 May 2017, EFSA published a guidance document (EFSA Scientific Committee, 2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additives used in food for infants below 12 weeks of age.<sup>5</sup> Now EFSA is expected to launch dedicated calls for data to be able to perform such risk assessments.

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

In accordance with the current EC approach for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified, a

<sup>1</sup> Commission Delegated Regulation (EU) 2016/127/EC of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. OJ L 25, 2.2.2016, p. 1–29.

<sup>2</sup> Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance). OJ L 354, 31.12.2008, p. 16–33.

<sup>3</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

<sup>4</sup> Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

<sup>5</sup> See Section 1.1.3.



specific call for data would be published by the EC on DG SANTE's website<sup>6</sup> on food additives and additional (missing) information would then be provided by interested business operators to the EC.

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

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

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

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

### 1.1.2. Terms of reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002<sup>7</sup>, and as part of EFSA's work in completing its risk assessments concerning the use of food additives in food for infants below 12 weeks of age, covered by the re-evaluation programme and its terms of reference, the European Commission requests the European Food Safety Authority to address all the data gaps specified in the recommendations made in its scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 (food for infants and young children) of Annex II to Regulation (EC) No 1333/2008.

### 1.1.3. Interpretation of the Terms of Reference

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

This risk assessment addresses the use of ascorbyl palmitate (E 304 (i)) in its technological function as food additive and not as source of vitamin C. However, it should be considered that ascorbic acid and also palmitic acid are released from E 304(i) adding to the vitamin C and fatty acids content of the formula.

## 1.2. Previous evaluations of ascorbyl palmitate (E 304(i)) for use in foods for infants

Ascorbyl palmitate was previously evaluated by JECFA (1974) and considered by the SCF in 1983, 1989, 1991, 1997 and 1998 (SCF, 1983, 1989a,b, 1991, 1997, 1998). JECFA established an ADI of 1.25 mg/kg bw per day for ascorbyl palmitate or ascorbyl stearate or the sum of both on the basis of data from a chronic toxicity study in rats (Fitzhugh and Nelson, 1946) where 'diet-related' effects were not observed up to the highest tested dose (JECFA, 1974).

In 1983, the Scientific Committee on Food (SCF) evaluated ascorbyl palmitate as source of vitamin C and antioxidant in infant formulae and in 'follow-up milks based on cows' milk proteins' and considered its use in those food categories as acceptable; scientific data and explanations were not given (SCF, 1983). The use in other types of foods for infants and young children was supported in its following evaluations (SCF, 1989a, 1991, 1997, 1998). In particular, in its opinion on additives in nutrient preparations for use in infant formulae, follow-on formulae and complementary foods, the use

<sup>6</sup> [https://ec.europa.eu/food/safety/food\\_improvement\\_agents/additives/re-evaluation\\_en](https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en)

<sup>7</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

of ascorbyl palmitate and tocopherols for infant formulae and follow-on formulae was considered acceptable when the total level of the substances (as nutrients and as antioxidants) is less than 1.5 mg/100 kcal (SCF, 1997). In 1998, SCF considered the use of ascorbyl palmitate as antioxidant in infant formulae and follow-on formulae as acceptable for healthy infants and young children at levels up to 10 mg/L in infant formulae and at corresponding levels in food for special medical purposes (FSMP) for the same age group (SCF, 1998).

In 2015, the EFSA ANS Panel issued a scientific opinion on the safety of ascorbyl palmitate (E 304 (i)) when used as food additive in the food categories specified in Annexes II and III to Regulation (EC) No 1333/2008. This opinion did not cover the uses as food additives in food categories relevant for infants below 12 weeks of age; the risk assessment approach followed in the re-evaluation of food additives did not apply to this age group. The ANS Panel has, therefore, specified in its opinion that the re-evaluation of uses for this age group will be performed separately (EFSA ANS Panel, 2015a).

### 1.3. Summary of the previous EFSA re-evaluation of ascorbyl palmitate (E 304 (i)) for uses in foods for all population groups except for infants below 12 weeks of age

Under the frame of Regulation (EC) No 257/2010, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) has re-evaluated the safety of ascorbyl palmitate (E 304(i)) when used as a food additive (EFSA ANS Panel, 2015a).

In its scientific opinion, the ANS Panel reviewed available technical, biological and toxicological data on ascorbyl palmitate (E 304 (i)). The ANS Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available and the data available following a public call for data.

Regarding the technical data, the ANS Panel recommended that the limits for residual solvents used in the manufacturing process should be included in the EU specifications. The ANS Panel also considered that the maximum limits for heavy metals (lead, mercury and arsenic) in the EU specification for ascorbyl palmitate (E 304(i)) should be revised in order to ensure that ascorbyl palmitate (E 304(i)) as food additive will not be a significant source of exposure to these toxic elements in food.

Ascorbyl palmitate is assumed to be completely hydrolysed in the gastrointestinal tract and not systemically available. This assumption was supported by an *in vitro* study in simulated intestinal fluid (Beck et al., 2014) and some limited information from human studies (DeRitter et al., 1951 and Johnston et al., 1994). Therefore, the ANS Panel considered that the toxicity of ascorbyl palmitate can be extrapolated from the data available for ascorbic acid and palmitic acid (EFSA ANS Panel, 2015a,b).

Acute toxicity studies demonstrating a low acute toxicity of ascorbyl palmitate were available (Bächtold, 1972, 1973a,b). The chronic toxicity study (Fitzhugh and Nelson, 1946) was considered by the ANS Panel as not adequate.

One *in vitro* mutagenicity study (Ames test) was available in which ascorbyl palmitate did not show any mutagenic activity. No studies on reproductive and developmental toxicity were available.

Based on the available information, the ANS Panel concluded that (i) the toxicological data were too limited to establish an ADI and (ii) the toxicity of ascorbyl palmitate can be extrapolated from the data for ascorbic acid and palmitic acid. The ANS opinion referred to a JECFA report (JECFA, 1986) establishing a 'not specified' ADI for various fatty acids including palmitic acid and stating that '*Their safety is based on their occurrence in edible fats and oils that have a long history of use as foods or food components. In addition, the even-chain fatty acids from C4 to C18 have been shown to undergo oxidation to give acetoacetic acid and ketone bodies. The metabolic products are utilized and excreted.*' The ANS Panel noted that from the literature (Nkondjock et al., 2003; Ramirez-Silva et al., 2011; Huang et al., 2012), the intake of palmitic acid ranged from 8,400 to 14,200 mg/person per day with a mean of 12,200 mg/person per day. The ANS Panel noted that intake of palmitic acid from the use of ascorbyl palmitate as food additive represents only a limited fraction (around 3%) of their daily intake from the regular diet. Additionally, the ANS Panel evaluated the safety of ascorbic acid and concluded that '*given the fact that adequate data on exposure and toxicity were available and no adverse effects were reported in animal studies, there is no safety concern*' (EFSA ANS Panel, 2015a,b).

Considering the available data, the ANS Panel concluded that there is no safety concern for the use of ascorbyl palmitate (E 304(i)) as food additive at the reported uses and use levels (EFSA ANS Panel, 2015a). The ANS Panel noted that the use of ascorbyl palmitate (E 304(i)) as a food additive in food for infants below 12 weeks was not covered in the assessment and that these uses would require a specific risk assessment.



## 2. Data and methodologies

### 2.1. Data

EFSA launched a public call for data<sup>8</sup> to collect relevant information from interested business operators.

The Panel based its assessment on information submitted to EFSA following the public call for data, information from previous evaluations and additional available literature up to 30 April 2020.

To verify the use of the food additive ascorbyl palmitate (E 304(i)) in food products, the Mintel's GNPD was used. This database is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3 million food and beverage products of which more than 1,100,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries, and Norway and UK presented in the Mintel GNPD.

### 2.2. Methodologies

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

In order to conclude on the safety of ascorbyl palmitate (E 304(i)) for all population groups and to address the data gaps identified during the re-evaluation, the FAF Panel assessed the information provided:

- for the follow-up on issues that have been raised in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of ascorbyl palmitate (E 304(i)) as a food additive (EFSA ANS Panel, 2015a); and
- for the risk assessment of ascorbyl palmitate (E 304(i)) in food for infants below 16 weeks of age in the food categories 13.1.1 (Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) as in Regulation (EC) No 1333/2008 on food additives.

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

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

Dietary exposure to ascorbyl palmitate (E 304(i)) from its use as a food additive in foods for infants below 16 weeks of age was estimated combining the mean and high-level consumption values reported for the period of 14–27 days of life which corresponds to 200 and 260 mL/kg bw per day (EFSA Scientific Committee, 2017), respectively, with the maximum levels according to Annex II and

<sup>8</sup> Call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Available from: <https://www.efsa.europa.eu/en/consultations/call/181010-3>

Annex III, Part 5 Section B to Regulation (EC) No 1333/2008 and reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

### 3. Assessment

#### 3.1. Technical data

##### 3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012, the food additive E 304(i) is named ascorbyl palmitate. A synonym according to that Regulation also indicates the stereoisomer form and it is L-ascorbyl palmitate. It is an ester made by the reaction of the primary alcohol group of ascorbic acid (vitamin C) with the carboxylic acid group of palmitic acid. Being a simple monoester, the ascorbyl and palmitate moieties are in a 1:1 mole ratio. Allowing for the mass gain by addition of one molecule of water, hydrolysis of 1 g of ascorbyl palmitate will form 0.425 g (42.5 % w/w) of ascorbic acid and 0.618 g (61.8% w/w) of palmitic acid.

##### 3.1.2. Specifications

The specifications for ascorbyl palmitate (E 304(i)) as defined in the Commission Regulation (EU) No 231/2012 are listed in Table 1.

**Table 1:** Specifications for ascorbyl palmitate (E 304(i)) according to Commission Regulation (EU) No 231/2012

<b>Commission Regulation (EU) No 231/2012</b>	
<b>Definition</b>	
Chemical names	Ascorbyl palmitate; L-ascorbyl palmitate; 2,3-didehydro-L-threo-hexono-1,4-lactone-6-palmitate; 6-palmitoyl-3-keto-L-gulofuranolactone
Synonym	L-ascorbyl palmitate
EINECS number	205-305-4
Chemical formula	C <sub>22</sub> H <sub>38</sub> O <sub>7</sub>
Molecular weight	414.55
Assay	Content not less than 98% on the dried basis
Description	White or yellowish-white powder with a citrus-like odour
<b>Identification</b>	
Melting point	107-117°C
Specific rotation	[α] <sub>D</sub> <sup>20</sup> between + 21° and + 24° (5 % w/v in methanol solution)
<b>Purity</b>	
Loss on drying	Not more than 2% (vacuum oven, 56–60°C, 1 h)
Sulfated ash	Not more than 0.1%
Arsenic	Not more than 3 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg

##### 3.1.2.1. Analytical data from commercial samples of the food additive

###### *Analytical data on toxic elements*

Analytical data on the current levels of toxic elements in ascorbyl palmitate (E 304(i)) have been provided by two interested business operators in response to the call for data (Documentation provided to EFSA n. 1 and 3). Upon request, further clarifications on the data provided were submitted (Documentation provided to EFSA n. 4 and 5).

Concerning toxic elements, in the case of the first interested business operator, the analytical procedure used was acid digestion followed by ICP-MS analysis. The limit of quantification (LOQ) values were 0.05 (As), 0.05 (Pb) and 0.005 (Hg) mg/kg. Limits of detection (LODs) were not determined. The interested business operator stated that it is reasonable to assume that the LOD ranges from

one-third to one-quarter of the corresponding LOQ values. Therefore, the Panel considered that the LODs would be ca. 0.015 (As), 0.015 (Pb) and 0.0015 (Hg) mg/kg. The analytical results on five independent batches of the food additive were reported as As < 0.1, Pb < 0.1 and Hg < 0.01 mg/kg, corresponding to two times the LOQ and defined as the internal quality control on the product (Documentation provided to EFSA n. 3, 4).

The second interested business operator also analysed five batches of the food additive using acid digestion followed by ICP-MS analysis (carried out according to DS/EN ISO 1724-2m:2016/ICP\_MS). The LOD was reported to be equal to the LOQ of the analysis and these were 0.1 (As), 0.05 (Pb) and 0.005 (Hg) mg/kg. All five batches analysed were below the LOD/LOQ values for all three elements (Documentation provided to EFSA n. 1, 5). The Panel emphasises that LOD values would normally be expected to be at least three times lower than limit of quantification (LOQ) rather than numerically the same as stated.

On the question of the lowest technologically achievable level for these elements, the first interested business operator stated that this depends mainly on the raw materials used to manufacture the food additive (Documentation provided to EFSA n. 3). Data were not made available as to what extent any individual production/processing/purification step(s) can potentially reduce these impurities levels. It was their view that a reasonable specification that can be met with a high level of confidence must therefore be based on the corresponding limits set or routinely met for the raw materials. On that basis, maximum limits for the specifications of toxic elements in ascorbyl palmitate were proposed as: As = 1, Pb = 1, Hg = 0.5 mg/kg.

According to the second interested business operator (Documentation provided to EFSA n. 1, 5), lowest technological achievable levels are As = 2, Pb = 1, Hg = 1 mg/kg and have been defined using an uncertainty factor to account for sources of uncertainty and taking into account the limit for the toxic elements in the raw materials, ascorbic acid (E 300) and palmitic acid, used in the manufacturing of E 304(i). The Panel noted that the proposed limits are more than 10-fold higher than the LOQ.

An overview of the data provided and proposed maximum limits for As, Pb and Hg is presented in Table 2.

**Table 2:** Overview of the data on toxic elements submitted by the two interested business operators

	Documentation provided to EFSA n. 1,5		Documentation provided to EFSA n. 3,4	
Analytical data (mg/kg)	As < 0.1		As < 0.1	
	Pb < 0.05		Pb < 0.1	
	Hg < 0.005		Hg < 0.01	
LOD and LOQ (mg/kg) (ICP-MS)	LOQ	Declared that LOD = LOQ	LOQ	LOD not determined and stated that it reasonably ranges from 1/3 to 1/4 of LOQ values)
	As = 0.1		As = 0.05	
	Pb = 0.05		Pb = 0.05	
	Hg = 0.005		Hg = 0.005	
Proposed maximum limits (mg/kg) <sup>(a)</sup>	As = 2		As = 1	
	Pb = 1		Pb = 1	
	Hg = 1		Hg = 0.5	

LOD = Limit of detection; LOQ = Limit of quantification.

(a): Based on the lowest technologically achievable levels.

#### Analytical data on residual solvents

Analytical data on current levels of residual solvents in ascorbyl palmitate (E 304(i)) have been provided by two interested business operators in response to the call for data (Documentation provided to EFSA n. 1,3,4 and 5).

The first interested business operator (Documentation provided to EFSA n. 3, 4) indicated that the solvents used during the production process of ascorbyl palmitate are removed to a major extent; however, their residues are technologically unavoidable in the final product. The information provided during the re-evaluation of the food additive (EFSA ANS Panel, 2015a,b) was mentioned and the maximum limits for acetone (300 mg/kg), petrol ether (150 mg/kg, CAS no. 8032-32-4) and mesityl oxide (50 mg/kg) as updated EU specifications were proposed. The Panel noted that CAS no. 8032-32-4 refers to petroleum ether, the 'Ligroin' fraction, that is a family of aliphatic hydrocarbons and

considered that 'petroleum ether' should be used instead of 'petrol ether'. The analytical method used was reported to be HS-GC-FID. The LOQs for these solvents ranged from 1 to 2.5 mg/kg, and the limits of detection (LODs) being about three to four times lower than the LOQ. Analytical data from the analysis of five non-consecutive lots were submitted and the results ranged from 22 to 94 mg/kg for acetone, 8–22 mg/kg for petroleum ether and 10–18 mg/kg for mesityl oxide. It was mentioned that in the past 9 years of production, the results of the samples analysed were not higher than 150 mg/kg for acetone, 50 mg/kg for petroleum ether and 50 mg/kg for mesityl oxide.

The second interested party (Documentation provided to EFSA n. 1, 5) provided information on another manufacturing process for E 304(i) where [REDACTED]

The interested business operator proposed lowest technologically achievable levels of [REDACTED] for each of the three residual solvents, to be used as reference to define the maximum limits for these impurities in the EU specifications for E 304(i).

An overview of the data provided and proposed maximum limits for residual solvents is presented in Table 3.

**Table 3:** Overview of the data on residual solvents submitted by the two interested business operators

	Documentation provided to EFSA n. 3, 4	Documentation provided to EFSA n. 1, 5
Analytical data (mg/kg)	Acetone: 22–94	[REDACTED]
	Petroleum ether: 8–22	[REDACTED]
	Mesityl oxide: 10–18	[REDACTED]
Proposed maximum limits (mg/kg)	Acetone: 300	[REDACTED]
	Petroleum ether: 150	[REDACTED]
	Mesityl oxide: 50	[REDACTED]

The proposed revisions of the EU specification are provided under Section 3.5.

### 3.1.2.2. Information on particular specifications requirements in the additive for use in infant formulae

The data provided along with the proposals for amended specifications were indicated by the interested business operators to apply for all uses and for all population groups, including infants below 16 weeks of age. So, no particular specification requirements are/were foreseen for ascorbyl palmitate (E304(i)) intended for use in infant formulae.

### 3.1.3. Stability of the substance, and reaction and fate in food

No information on reaction products of ascorbyl palmitate in food was provided by the interested business operators. The food additive acts as a primary or a secondary food antioxidant, and therefore, it is intended to prevent (auto)oxidation and the formation of undesirable oxidation/rancid products in the food. As a result of its function as an antioxidant, it can be expected to become oxidised itself, similar to ascorbic acid (Saltmarsh, 2013; EFSA ANS Panel, 2015a,b). The Panel agrees with the suggestion made by interested business operators that ascorbyl palmitate is stable in infant formula.

## 3.2. Authorised uses and use levels

Maximum levels of ascorbyl palmitate (E 304(i)) in foods for infants below 16 weeks of age are defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this opinion, these levels are named maximum permitted levels (MPLs).

Currently, ascorbyl palmitate (E 304(i)) is authorised in dietary foods for infants in the food categories 13.1.1 (Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC<sup>1</sup>) at a maximum level of 10 mg/kg and in food category 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) at a maximum level of 100 mg/kg (Table 4).

According to Annex III, Part 5 section B, to Regulation (EC) No 1333/2008, ascorbyl palmitate (E 304(i)) is authorised for uses in all nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded (Table 5).

**Table 4:** MPLs of ascorbyl palmitate (E 304(i)) in foods for infants below 16 weeks of age according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
13.1.1	Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC	E 304(i)	–	10
13.1.5.1 <sup>(a)</sup>	Dietary foods for infants for special medical purposes and special formulae for infants	E 304(i)	–	100

MPL: maximum permitted level.

(a): This category covers dietary foods for infants for special medical purposes and special formulae such as premature infant formulae, hospital discharge formulae, low and very low birth weight formulae, and human breast milk fortifiers.

**Table 5:** Authorisation of ascorbyl palmitate (E 304(i)) in foods for infants below 16 weeks of age according to the Annex III to Regulation (EC) No 1333/2008

Authorisation according to	E-number	Maximum level	Nutrient to which the food additive may be added	Food category
Annex III, Part 5 Section B	E 304(i)	For uses in nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded	All nutrients	Foods for infants and young children

Infant formulae and infant formulae for special medical purposes shall comply with the compositional requirements as set out in Annex I to Commission Delegated Regulation (EU) No 2016/127 and in Annex 1, Part A, Table 1 of Commission Delegated Regulation (EU) No 2016/128, respectively. According to Regulation (EU) No 609/2013, L-ascorbyl 6-palmitate is a substance authorised to be added as a source of vitamin C, for satisfying these compositional requirements. Commission Delegated Regulations (EU) No 2016/127 and No 2016/128 provides minimum and maximum vitamin C contents in infant formulae and follow on formulae of 4 mg/100 kcal and 30 mg/100 kcal.

### 3.3. Exposure data

Some food additives are authorised in the EU in infants' formulae as defined by Regulation (EC) No 1333/2008 (FC 13.1.1) and in dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual use levels are required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call<sup>8</sup> for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. In response to this public call, updated information on the actual use levels of ascorbyl palmitate (E 304(i)) in foods was made available to EFSA by interested business operators.

#### 3.3.1. Reported use levels in food categories 13.1.1 and 13.1.5.1

Interested business operators did not provide EFSA with use levels but indicated that the current use levels of ascorbyl palmitate (E 304(i)) as food additive correspond to the MPL of 10 mg/kg in infant formulae (FC 13.1.1) and of 100 mg/kg in special formulae for infant (FC 13.1.5.1).

Therefore, the assumption of 10 mg/kg in infant formulae (FC 13.1.1) and of 100 mg/kg in special formulae for infants (FC 13.1.5.1) is also the one taken on for the refined exposure assessment scenario.



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

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3.3 million food and beverage products of which more than 1,200,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries and, Norway and UK presented in the Mintel GNPD.<sup>9</sup>

For the purpose of this Scientific Opinion, Mintel's GNPD<sup>10</sup> was used for checking the labelling of food and beverage products and food supplements for ascorbyl palmitate (E 304(i)) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, ascorbyl palmitate (E 304(i)) was labelled on 66 products of 'Baby formula (0–6 months)' between January 2015 and April 2020, which represent 20% of the total number of food products belonging to this subcategory. It is noted that for the uses authorised according to Annex III (uses in nutrient preparations), the labelling is not mandatory.

### 3.4. Exposure estimates for infants below 16 weeks

Exposure to ascorbyl palmitate (E 304(i)) from its uses as a food additive in formulae for infants below 16 weeks was estimated. This scenario is based on the recommended consumption levels from SC Guidance (EFSA Scientific Committee, 2017). This guidance 'recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age'. These recommended consumption levels correspond to 14–27 days old infants consumption.

#### 3.4.1. Dietary exposure to ascorbyl palmitate (E 304(i)) from infant formulae (FC 13.1.1)

Table 6 summarises the estimated exposure to ascorbyl palmitate (E 304(i)) from its use as a food additive in infant formulae (FC 13.1.1) for infants below 16 weeks of age.

**Table 6:** Dietary exposure to ascorbyl palmitate (E 304(i)) in infant formulae (FC 13.1.1) for infants below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
<b>Regulatory maximum level exposure assessment scenario/refined estimated exposure assessment scenario (10 mg/kg)</b>	
• Mean consumption (200 mL/kg bw per day)	2
• High level consumption (95th percentile, 260 mL/kg bw per day)	2.6

#### 3.4.2. Dietary exposure to ascorbyl palmitate (E 304(i)) from FSMP infant formulae (FC 13.1.5.1)

Table 7 summarises the estimated exposure to ascorbyl palmitate (E 304(i)) from its use as a food additive in special formulae for infant (FC 13.1.5.1) for infants below 16 weeks of age.

**Table 7:** Dietary exposure to ascorbyl palmitate (E 304(i)) in special formula for infants (FC 13.1.5.1) below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
<b>Regulatory maximum level exposure assessment scenario/refined estimated exposure assessment scenario (100 mg/kg)</b>	
• Mean consumption (200 mL/kg bw per day)	20
• High level consumption (95th percentile, 260 mL/kg bw per day)	26

<sup>9</sup> Missing Cyprus, Luxembourg and Malta.

<sup>10</sup> <http://www.gnprd.com/sinatra/home/> accessed on 29/4/2020.



Exposure from the use of ascorbyl palmitate (E 304(i)) according to Annex III, i.e. from carry-over from nutrient preparations, is covered in these exposure estimates from authorisation according to Annex II.

### 3.4.3. Dietary exposure to ascorbyl palmitate using maximum level of vitamin C as set in Regulation (EU) No 2016/127

Level of vitamin C set in the Commission Delegated Regulation (EU) No 2016/127 and No 2016/128, respectively, for infant formula and special infant formula, is maximum 30 mg vitamin C/100 kcal. Ascorbyl palmitate may be used as a nutritional source for vitamin C in infant formulae.

Considering that 100 mL infant formulae lead to an intake of minimum 60 kcal and maximum 70 kcal (Reg (EU) No 2016/127), an amount of 260 mL/kg bw per day corresponds to an intake of minimum 156 kcal/kg bw per day and maximum 182 kcal. The amount of vitamin C from such intakes is minimum 46.8 mg/kg bw per day and maximum 54.6 mg/kg bw per day, which would correspond to 110 mg ascorbyl palmitate/kg bw per day minimum and 128.3 mg ascorbyl palmitate/kg bw per day maximum (see Table 8).

The same calculation for an average consumption of 200 mL/kg bw per day would result in minimum 36 mg vitamin C/kg bw per day and maximum 42 mg vitamin C/kg bw per day, thus minimum 84.7 mg ascorbyl palmitate/kg bw per day and maximum 98.8 mg ascorbyl palmitate/kg bw per day (see Table 8).

For comparison, the data for ascorbic acid and palmitate exposure from infant formulae (FC 13.1.1) and special formula for infants (FC 13.1.5.1) for infants below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 are also given in Table 8.

**Table 8:** Dietary exposure to ascorbyl palmitate using maximum level of vitamin C as set in Regulation (EU) No 2016/127 and dietary exposure to ascorbyl palmitate (E 304(i)) from infant formulae (FC 13.1.1) and special formula for infants (FC 13.1.5.1) for infants below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008, and their corresponding intake of ascorbic acid and palmitate

		<b>Ascorbyl palmitate (mg/kg bw per day)</b>	<b>Ascorbic acid (mg/kg bw per day)</b>	<b>Palmitate (mg/kg bw per day)</b>
<b>From maximum level of vitamin C set in Regulation (EU) No 2016/127</b>	Mean consumption (200 mL/kg bw per day)	84.7–98.8	36–42	48.7–56.8
	High-level consumption (95th percentile, 260 mL/kg bw per day)	110–128.3	46.8–54.6	63.2–73.8
		<b>E 304(i) (mg/kg bw per day)</b>	<b>Ascorbic acid (mg/kg bw per day)</b>	<b>Palmitate (mg/kg bw per day)</b>
<b>FC 13.1.1</b>	Mean consumption (200 mL/kg bw per day)	2	0.9	1.2
	High-level consumption (95th percentile, 260 mL/kg bw per day)	2.6	1.1	1.5
<b>FC 13.1.5.1</b>	Mean consumption (200 mL/kg bw per day)	20	8.5	11.5
	High-level consumption (95th percentile, 260 mL/kg bw per day)	26	11.1	15.0

### 3.4.4. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA Scientific Committee, 2007), the following sources of uncertainties have been considered and summarised in Table 9.

**Table 9:** Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction <sup>(a)</sup>
Consumption data: one reference point only to estimate exposure during the period of up to 16 weeks of age	+
Uncertainty in possible national differences in use levels of food categories	+/-
Regulatory maximum level/refined exposure assessment scenario: – exposure calculations based on the MPL according to Annex II and III to Regulation (EC) No 1333/2008 for FC 13.1.1 and FC 13.1.5.1	+/-

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

Ascorbyl palmitate (E 304(i)) is authorised as a food additive in food categories 13.1.1 and 13.1.5.1 according to Annex II and III to Regulation (EC) N°1333/2008.

Based on the assumption that carers would be brand-loyal to an infant formula (FC 13.1.1), the exposure assessment scenario would in general result in an average reliable estimation of exposure.

Based on the assumption that carers of children with medical disorder would be brand-loyal to an infant formula for special medical purposes (FC 13.1.5.1) that suits the child's medical disorder, the exposure assessment scenario would in general result in an average reliable estimation of exposure.

### 3.5. Proposed revision to existing EU Specifications for ascorbyl palmitate (E 304(i))

#### 3.5.1. Proposal for revision of the EU specifications for E 304 (i) regarding toxic elements

Whereas the interested business operators have proposed new specification limits for arsenic, lead and mercury, the analytical data submitted show that the actual concentrations in the food additive are substantially lower. The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the food additive. Therefore, if the European Commission decides to revise the current limits in the EU specifications to more appropriate values, the estimations of toxic elements intake as below could be considered.

Where analytical results provided are below LOQ, the Panel considered that the LOQ can be taken as a starting point to establish maximum limits for the specifications for E 304(i). The LOQ of the methods of analysis (ICP-MS) used by both interested business operators are the same for Pb and Hg, at 0.05 and 0.005 mg/kg, respectively. In the case of As, the Panel considered that an LOQ of 0.05 mg/kg is achievable. Multiplying these values by an 'uncertainty' factor of 10, e.g. to cover uncertainties, such as representativeness, homogeneity and analytical measurement uncertainty, the maximum limit values for the revision of the EU specifications could be derived as 0.5, 0.5 and 0.05 mg/kg, for Pb, As and Hg, respectively. The Panel emphasises that the choice of the 'uncertainty' factor and other considerations, such as on multiple sources of exposure to conclude on the maximum limits for toxic elements in the specifications is in the remit of risk management.

The potential exposure to these toxic elements from the use of the food additive ascorbyl palmitate (E 304 (i)) can be calculated by assuming that the impurities in the food additive may be up to the specifications limit values and then by calculation pro-rata to the estimates of exposure to the food additive itself. In the previous opinion on ascorbyl palmitate (EFSA ANS Panel, 2015a), the ANS Panel estimated chronic exposure to two fatty acid esters of ascorbic acid (E 304), these being ascorbyl palmitate (E 304(i)) and ascorbyl stearate (E 304(ii)). Making the assumption that the two antioxidants considered were mutually exclusive, the ANS Panel estimated the exposure to fatty acid esters of ascorbic acid (E 304) using the highest concentration reported for ascorbyl palmitate (E 304 (i)) or ascorbyl stearate (E 304(ii)) for each food category. It is noteworthy that both food additives also have the same limit values in the EU specifications for the content of As, Pb and Hg.

For the brand-loyal consumer exposure scenario, the highest P95 exposure to fatty acid esters of ascorbic acid (E 304) was 9.9 mg/kg bw per day for children (EFSA ANS Panel, 2015a). In the current opinion, the dietary exposure to ascorbyl palmitate (E 304(i)) for infants below 16 weeks of age is estimated to be up to 2.6 mg/kg bw per day from infants formula (Table 6) and up to 26 mg/kg bw per day from infants formula for special medical purposes (Table 7).

The above-mentioned potential maximum limits calculated for the toxic elements (0.5 (As), 0.5 (Pb) and 0.05 (Hg) mg/kg), combined with the estimated intakes of ascorbyl palmitate (9.9, 2.6 or 26 mg/kg bw per day) could result in an exposure which can be compared with the following reference points or health-based guidance values for the three toxic elements; a BMDL01 of 0.3–8 µg/kg bw per day for arsenic (EFSA CONTAM Panel, 2009), a BMDL01 of 0.5 µg/kg bw per day for lead (EFSA CONTAM Panel, 2010) and a TWI of 4 µg/kg bw for mercury (EFSA CONTAM Panel, 2012).

The outcome of such an exercise illustrates the health impact that would result if the potential specification values calculated above were to be used: for Pb and As, the MOS/MOE could be as low as 38 and 23, respectively, and for Hg, the exhaustion of the health-based guidance value could be up to 0.2% (Table 10).

**Table 10:** Exposure to toxic elements based on potential specification values calculated by the Panel based on the analytical data provided

Exposure to the additive (mg/kg bw/day)	MOS/MOE for As at 0.5 mg/kg	MOS/MOE for Pb at 0.5 mg/kg	% of the TWI for Hg at 0.05 mg/kg
9.9 <sup>(a)</sup> (EFSA ANS Panel 2015a)	61–1,616	101	0.1%
2.6 <sup>(b)</sup> (Table 6)	231–6,154	385	0.02%
26 <sup>(c)</sup> (Table 7)	23–615	38	0.2%

MOS: Margin of safety; MOE: Margin of exposure; TWI: Tolerable weekly intake.

(a): Children considering brand-loyal consumer scenario.

(b): Infants from infant formulae.

(c): Infants from infants' formula for special medical purposes.

Using the specifications proposed by the interested business operators (i.e. 1, 1, 0.5; As, Pb, Hg) would result: for Hg, the exhaustion of the health-based guidance value could be up to 2.3%, and for Pb and As, the MOS/MOE could be as low as 19 and 12, respectively (Table 11).

**Table 11:** Exposure to toxic elements based on specifications proposed by the interested party (Documentation provided to EFSA n. 1, 3, 4, 5)

Exposure to the additive (mg/kg bw/day)	MOS/MOE for As at 1 mg/kg	MOS/MOE for Pb at 1 mg/kg	% of the TWI for Hg at 0.5 mg/kg
9.9 <sup>(a)</sup> (EFSA ANS Panel 2015a)	30–808	51	0.9%
2.6 <sup>(b)</sup> (Table 6)	115–3,077	192	0.2%
26 <sup>(c)</sup> (Table 7)	12–308	19	2.3%

MOS: Margin of safety; MOE: Margin of exposure; TWI: Tolerable weekly intake.

(a): Children considering brand-loyal consumer scenario.

(b): Infants from infant formulae.

(c): Infants from infants' formula for special medical purposes.

Using the existing specifications (i.e. 3, 2, 1; As, Pb, Hg) would result: for Hg, the exhaustion of the health-based guidance value could be up to 4.6%, and for Pb and As, the MOS/MOE could be as low as 10 and 4, respectively (see Table 12).

**Table 12:** Exposure to toxic elements based on the existing specifications

Exposure to the additive (mg/kg bw/day)	MOS/MOE for As at 3 mg/kg	MOS/MOE for Pb at 2 mg/kg	% of the TWI for Hg at 1 mg/kg
9.9 <sup>(a)</sup> (EFSA ANS Panel 2015a)	10–270	25	1.7%
2.6 <sup>(b)</sup> (Table 6)	39–1,026	96	0.5%
26 <sup>(c)</sup> (Table 7)	3.8–103	9.6	4.6%

MOS: Margin of safety; MOE: Margin of exposure; TWI: Tolerable weekly intake.

(a): Children considering brand-loyal consumer scenario.

(b): Infants from infant formulae.

(c): Infants from infants' formula for special medical purposes.

These calculations support the Panel recommendation to decrease the current maximum limits for arsenic, lead and mercury, considering also other sources of exposure to these toxic elements.

### 3.5.1.1. Proposal for revision of the EU specifications for E 304 (i) regarding residual solvents

Based on the information provided on the manufacturing processes of ascorbyl palmitate (E 304(i)), different residual solvents (acetone, ██████████ mesityl oxide, ██████████ and petroleum ether) can remain in the food additive. For many food categories (and their associated production and processing methods), any solvent residue in the food additive may be partially or even extensively removed by evaporation. A worse-case exposure to these solvents can be estimated by assuming the maximum residual content proposed combined with the estimates of exposure to the food additive itself, this being 9.9 (children in the refined brand-loyal exposure scenario (EFSA ANS Panel, 2015a)) and 26 mg/kg bw per day (for FC 13.1.5.1 for special formulae for infants, see Section 3.4). A ██████████ residual limit for solvent in the additive would give exposure to the solvent of ██████████ µg/kg bw per day, respectively.

Acetone and ██████████ are extraction solvents authorised to be used in the production of foodstuffs and food ingredients (Directive 2009/32/EC<sup>13</sup>). The Panel noted that the proposed limit for acetone by the interested party (300 mg/kg, Table 3) is much higher than the reported analytical results and the results obtained from samples analysed in the last 9 years (not higher than 150 mg/kg); therefore, the Panel considered that a level of 150 mg/kg is realistic. A level of ██████████ for ██████████ and 150 mg/kg for acetone would be acceptable for the EU specifications.

Petroleum ether is listed in the Guideline for residual solvents in pharmaceuticals for human use (ICH, 2019), where it is indicated that no toxicological data was found to establish a permissible daily exposure (PDE) for petroleum ether and manufacturers should supply justification for residual levels of this solvent in pharmaceutical products. The Panel noted that the proposed limit for petroleum ether by the interested party (150 mg/kg, Table 3) is much higher than the reported analytical results and the results obtained from samples analysed in the last 9 years (not higher than 50 mg/kg). Therefore, the Panel considered that a level of 50 mg/kg is realistic and would be acceptable for the EU specifications.

██████████ is classified as a solvent 'to be limited' (Class 2) and a PDE of ██████████ is mentioned in the Guideline for residual solvents in pharmaceuticals for human use (ICH, 2019). Taking this PDE value into consideration and the estimated exposure to ██████████, the level of ██████████ proposed by the business operator for the EU specifications would be acceptable.

The Panel noted that ██████████ can be used in the manufacturing of organic materials coming into contact with drinking water with a maximum allowable concentration at the consumer's tap of 2.5 µg/L ██████████. Taking this value into consideration and the estimated exposure to ██████████ from the use of the food additive E 304(i) ██████████, the level of ██████████ proposed by the business operator for the EU specifications would be acceptable.

Mesityl oxide is included in the Union list of flavouring substance (FL 07.101, Annex I to Regulation (EC) No 1334/2008). It was evaluated by EFSA (EFSA CEF Panel, 2013) and it was considered not to be of concern with respect to genotoxicity and was assigned to Cramer class II which has a toxicological threshold of 540 µg/person/day (equivalent to 9 µg/kg bw per day). Taking this value into consideration and the estimated exposure to mesityl oxide from the use of the food additive E 304(i) up to 1.3 µg/kg bw per day, the level of 50 mg mesityl oxide/kg E 304(i) proposed by the business operator for the EU specifications would be acceptable.

Taking into account the considerations mentioned above, the Panel considered that maximum limits for residual solvents as presented in Table 13 could be included in the EU specifications for E 304(i).

**Table 13:** Maximum limits for residual solvent that could be included in the EU specifications for E 304(i)

Solvent	Max limit for EU specifications (mg/kg)
Acetone	150
Petroleum ether (CAS no. 8032-32-4)	50
Mesityl oxide	50
██████████	██████████
██████████	██████████
██████████	██████████

<sup>13</sup> Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. (Text with EEA relevance) OJ L 141, 6.6.2009, p. 3–11.

### 3.5.1.2. Proposal for revision of the EU specifications for E 304(i)

Based on the considerations provided above (Sections 3.1.2.1), the Panel recommends the revisions listed in Table 14.

**Table 14:** Proposal for revision of the existing EU Specifications for ascorbyl palmitate (E 304 (i))

	<b>Commission Regulation (EU) No 231/2012</b>	<b>Comment/justification for revision</b>
<b>Definition</b>		
Chemical names	Ascorbyl palmitate; L-ascorbyl palmitate; 2,3-didehydro-L-threo-hexono-1,4-lactone-6-palmitate; 6-palmitoyl-3-keto-L-gulofuranolactone	Unchanged
Synonym	L-ascorbyl palmitate	Unchanged
CAS numbers	–	Include CAS number 137-66-6
EINECS number	205-305-4	Unchanged
Chemical formula	C <sub>22</sub> H <sub>38</sub> O <sub>7</sub>	Unchanged
Molecular weight	414.55	Include dimension (g/mol)
Assay	Content not less than 98% on the dried basis	Unchanged
Description	White or yellowish-white powder with a citrus-like odour	Unchanged
<b>Identification</b>		
Melting point	107–117°C	Unchanged
Specific rotation	[α] <sub>D</sub> <sup>20</sup> between + 21° and + 24° (5% w/v in methanol solution)	Unchanged
<b>Purity</b>		
Loss on drying	Not more than 2% (vacuum oven, 56–60°C, 1 h)	Unchanged
Sulfated ash	Not more than 0.1%	Unchanged
Arsenic		Current levels (3 mg/kg) should be lowered on the basis of the analytical data provided taking into account the considerations of the Panel
Lead		Current levels (2 mg/kg) should be lowered on the basis of the analytical data provided taking into account the considerations of the Panel
Mercury		Current levels (1 mg/kg) should be lowered on the basis of the analytical data provided taking into account the considerations of the Panel
Acetone		150 mg/kg should be included
Petroleum ether		50 mg/kg should be included
Mesityl oxide		50 mg/kg should be included

## 3.6. Biological and Toxicological data

### 3.6.1. Summary of the information previously reported and relevant for infants

The following text (in italics) is from the opinion published in 2015 (EFSA ANS Panel, 2015a). New information related to the specific age group below 16 weeks of age is added.



*Absorption, distribution, metabolism and excretion (ADME) data on ascorbyl palmitate (E 304(i)) (...) are sparse and the safety assessment for their use as food additives is based on the assumption that ascorbyl palmitate (...) fully hydrolyses pre-systemically to ascorbic acid and their respective fatty acids. This assumption was supported by an in vitro study reporting near-complete hydrolysis of ascorbyl palmitate in simulated intestinal fluid (Beck et al., 2014) and by human data. (...)*

*The only chronic and carcinogenicity study available was performed with ascorbyl palmitate (Fitzhugh and Nelson, 1946). It was not performed according to current standards. The Panel considered the study not adequate for the re-evaluation of ascorbyl palmitate as a food additive because the group sizes were too small, only one sex was used, histopathological examination was limited and only two doses were tested. There was only one in vitro mutagenicity study on ascorbyl palmitate available, in which ascorbyl palmitate did not show any mutagenic activity. (...)*

*No studies on reproductive and developmental toxicity on ascorbyl palmitate or ascorbyl stearate were available. (...)*

*The Panel concluded that ascorbyl palmitate (...) are hydrolysed and not systemically available and therefore their toxicity can be extrapolated from the data available for ascorbic acid, palmitic acid (...).*

*The Panel noted that it cannot exclude the possibility that some absorption of the parent compounds may take place before hydrolysis in the gut. However, the Panel considered that any absorbed intact ascorbyl palmitate (...) would be completely hydrolysed in the hepatic portal plasma and/or liver and therefore would not be systemically available.*

### 3.6.2. Newly available data

#### 3.6.2.1. Toxicological data

No new animal data were provided. Literature searches were performed by the interested business operators (Documentation provided to EFSA n. 1 and 2). The literature search identified one paper published after the last evaluation (EFSA ANS Panel, 2015a), in which cytotoxicity and induction of apoptosis by ascorbyl palmitate were investigated in vitro in Human Umbilical Vein Endothelial Cells (HUVECs) (Sohrabi et al., 2018). The Panel considered that, due to several methodological shortcomings, including the fact that experimental conditions do not resemble gut physiology, this study is not relevant for the safety assessment of ascorbyl palmitate as a food additive.

#### 3.6.2.2. Clinical studies

Several clinical studies were submitted in which infant formulas were tested which contained ascorbyl palmitate among other food additives (Documentation provided to EFSA n. 3). However, according to those publications (Auestad et al., 1997; Borschel et al., 2013, 2014a,b; Fleddermann et al., 2014; Ahrens et al., 2018) no study was performed with the aim to investigate the safety and tolerability of ascorbyl palmitate. Moreover, the publications do not contain information on the presence of ascorbyl palmitate (E 304(i)) in the composition of studied formulas. The ascorbyl palmitate levels were provided separately on request from EFSA by interested business operators (a clarification letter was received from two Specialised Nutrition Europe (SNE) members in September 2019) and were not accompanied by certificates as a confirmation of the additive levels.

The reviewers gave identical RoB scores for the individual studies (see Figure 1). Only minor inconsistencies on the scoring for some of the questions/elements were noted and clarified. Five studies (Auestad et al., 1997; Borschel et al., 2013, 2014a,b; Ahrens et al., 2018) were allocated to tier 3 (high risk of bias). The study from Fleddermann et al., 2014 was allocated to tier 2 (moderate risk of bias). The Panel considered that the studies allocated to a RoB tier 3 could only be used as supporting evidence. These studies are briefly described here. The elements considered for RoB appraisal by the reviewers are summarised in Figure 1.



	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Incomplete outcome data (attrition bias)	Confidence in exposure to the interventions	Confidence in outcome assessment	Selective reporting (reporting bias)	Other potential treats to internal validity	Tier
Auestad et al. (1997)	+	-	-	+	--	+	+	+	3
Borschel et al. (2013)	++	++	-	--	+	+	-	--	3
Borschel et al. (2014a)	+	-	+	--	--	+	++	-	3
Borschel et al. (2014b)	+	-	-	+	--	+	++	-	3
Fleddermann et al. (2014)	+	+	+	+	-	+	++	+	2
Ahrens et al. (2018)	++	-	-	+	-	-	++	+	3

Definitely low risk of bias (++), Probably low risk of bias (+), Probably high risk of bias (-), Definitely high risk of bias (--).

**Figure 1:** 'Risk of bias summary' (modified from Cochrane Risk of Bias Tool; NTP-OHAT (2015, 2019); see Appendix B for further details)

The study of **Auestad et al. (1997)** was aimed at measuring the effect of adding polyunsaturated fatty acids (docosahexaenoic acid (experimental group 1); combined arachidonic acid and docosahexanoic acid (experimental group 2)) to formulas on red blood cell phospholipid levels of these fatty acids, on visual acuity and on growth during the first year of life compared to control formula without polyunsaturated fatty acids (control formula, not further defined). All the formulas contained ascorbyl-palmitate in a concentration of [REDACTED] (according to a clarification letter dated 6 September 2019 from a Specialised Nutrition Europe-SNE member). Infants aged < 7 days (n = 134) were randomly assigned to one of the three treatment groups and 63 breast-fed infants served as reference group controls. Whereas the authors mentioned that parents recorded the occurrence of spit-off, vomiting and frequency, colour and consistence of stools, the results of these parameters were not reported. Body weight, crown-heel lengths and head circumference were not different between the groups and also compared to the breast-fed infants and within normal limits (as measured by z-scores) at all the study visits. The study has important limitations (e.g. no appropriate control group without ascorbyl-palmitate) and was allocated to tier 3 (high risk of bias).

In the study of **Borschel et al. (2013)**, an extensively hydrolysed casein-based formula (eHF) was compared to an amino acid-based formula. The amino acid-based formula (AAF) contained ascorbyl-palmitate (E 304(i)) in a concentration of [REDACTED] (according to a clarification letter dated 6 September 2019 from a Specialised Nutrition Europe-SNE member). It has to be mentioned that the AAF contains [REDACTED] mg ascorbic acid/L according to the study report (documentation provided to EFSA n. 6). The randomised, double-blind parallel group study was performed in 213 infants entering the study between 0 and 9 days old of age; 107 infants were randomised to AAF whereas 106 infants were randomised to eHF. The study duration was 112 days. The formulas were given ad libitum and were the sole source of nutrition throughout the study. The primary endpoint was weight gain between 14 and 112 days of age. Secondary endpoints included length, head circumference, study formula intake, daily stool number, mean rank stool consistency and serum albumin concentration. Seventy-nine infants (37%) dropped out, with similar demographic characteristics between groups. The numbers of infants who finished the study early because of intolerance symptoms were similar. The group not receiving ascorbyl palmitate (E 304(i)) had a significantly greater number of daily stools and average mean rank stool consistency. This finding may be explained by the differences in protein

composition between the two study formulae. No statistically significant differences between groups in weight, length, head circumference or mean serum albumin concentration were observed. The Panel noted that 40 of the 107 infants randomised to the formula (AAF) containing ascorbyl palmitate (E 304(i)) dropped out from the study, 21 of them because of intolerance which was not further specified. From the 66 infants who completed the study no adverse events were noted. The study has substantial limitations (e.g. no appropriate control group, i.e. AAF without ascorbyl-palmitate, a high number of dropouts which is seen as invalidating study results, see Genaidy et al., 2007) and was allocated to tier 3 (high risk of bias).

In the study of **Borschel et al. (2014a)**, an extensively hydrolysed casein-based formula was studied in the form of ready to feed as compared to a powder form (PWD). The randomised group study was performed in 195 infants entering the study between 0 and 9 days old. The PWD formula contained ascorbyl-palmitate in a concentration of [REDACTED] (according to a clarification letter dated 6 September 2019 from a Specialised Nutrition Europe-SNE member). The study duration was 112 days. The primary endpoint was weight gain between 14 and 112 days of age. Secondary endpoints included length, head circumference, study formula intake, daily stool number, mean rank stool consistency; percentage of feeds with spit up and/or vomits. Fifty-eight infants (29.7%) dropped out, with similar demographic characteristics between groups. The numbers of infants who finished the study early because of intolerance symptoms were similar. No statistically significant differences between groups in weight, length and head circumference were noted. The average percentage of feeds with spit-up and/or vomit was not different as was the average stool consistency, whereas the number of stools per day was higher in the ready to feed compared to the powder formula, the difference being statistically significant at every study visit (28, 56, 84 and 112 days). The Panel considered that this finding is not clinically meaningful. The study has some limitations (e.g. no PWD control group without ascorbyl-palmitate, a high number of dropouts which is seen as invalidating study results, see Genaidy et al., 2007). The study was allocated to tier 3 (high risk of bias) and provides limited evidence for the safe use of ascorbyl-palmitate.

In the study of **Borschel et al. (2014b)**, two partially hydrolysed casein-based formulas (freshly prepared from powder) were studied, a commercially available control formula (CF) and the experimental formula from Abbott laboratories (EF). The study formulas contained identical protein sources but differed in fat and carbohydrates sources and also used different emulsifiers/stabilisers. In the table on composition, ascorbyl palmitate is not mentioned at all. The randomised blinded group study was performed in 209 infants entering the study between 0 and 9 days old. The EF contained ascorbyl-palmitate in a concentration of [REDACTED] according to a letter to EFSA (according to a clarification letter dated 6 September 2019 from a Specialised Nutrition Europe-SNE member). The study duration was 119 days. The primary endpoint was weight gain between 14 and 112 days of age. Secondary endpoints included length, head circumference, study formula intake, daily stool number, mean rank stool consistency; numbers of feeds with spit up and/or vomits. Thirty-two infants dropped out, with similar demographic characteristics between groups. However, the text of the publication mentioned 55 infants which discontinued the study formula prematurely. According to Table 3 of the publication, the number of infants in the CF group was 61 at the end of the study whereas 99 infants entered the study which is a difference of 38 infants lost during the study period. The number of infants in the EF group is 69 at the end of the study whereas 107 infants entered the study which is a difference of 38 infants lost during the study period. No statistically significant differences between groups in weight, length and head circumference were noted. The average percentage of feeds with spit-up and/or vomit was not different as was the average stool consistency, whereas the number of stools per day was somewhat higher in the ready to feed compared to the formula freshly prepared from powder, the difference being statistically significant at every study visit (28, 56, 84 and 112 days), however, considered not to be relevant. The study has limitations (e.g. a high number of dropouts which is seen as invalidating study results, see Genaidy et al., 2007) and was allocated to tier 3 (high risk of bias).

In the study of **Fleddermann et al. (2014)**, two formulas were studied which differed in their content of protein, lipids and carbohydrates (Intervention formula vs. Control formula: protein 1.3 vs. 1.5 g/100 mL, lipids 3.6 vs. 3.3 g/100 mL and carbohydrates 7.5 vs. 7.8 g/100 mL). The randomised blinded group study was performed in 213 infants entering the study up to 28 days old. Both formulas contained ascorbyl palmitate in a concentration of [REDACTED] according to a letter to EFSA (according to a clarification letter dated 18 September 2019 from a Specialised Nutrition Europe-SNE member). The study duration was 120 days. The primary endpoint was weight gain between 30 and 120 days of age. Secondary endpoint included length and head circumference. Recorded were study formula intake,

daily stool number, mean rank stool consistency; flatulence, colic, regurgitation, vomiting in the last 3 days before each visit (baseline, 30, 60, 90, 120 days). Forty-six (21.6%) infants dropped out without comparative information on the demographic characteristic of the infants. No statistically significant differences between groups in weight, length and head circumference were noted. The better tolerability was attributed by the authors to a higher content of  $\alpha$ -lactalbumin, which was said to have immunostimulatory and antibacterial effects. The study did not include an untreated control group; nevertheless, the study was allocated to tier 2 (moderate risk of bias) and provides limited evidence for the safe use of ascorbyl palmitate in a relatively low concentration of [REDACTED].

In a European multi-center randomised trial (Ahrens et al., 2018), 402 formula-fed infants were randomly assigned to four groups: low protein (LP) formulas (1.9 g protein/100 kcal) as partially hydrolysed formula (pHF) with or without synbiotics (unspecified combination of probiotics and prebiotics), LP-Extensively hydrolysed formula (eHF) formula with synbiotics, or regular protein eHF (2.3 g protein/100 kcal). One hundred and one breast-fed infants served as observational reference group. The primary endpoint was daily weight gain during the first 4 months of life when comparing the LP group to a regular protein eHF group. Healthy term new-borns were included into the study where visit 1 (inclusion) was at  $28 \pm 3$  days. The infants were observed until visit 4 at  $112 \pm 4$  days. All formula contained ascorbyl palmitate in a concentration of [REDACTED] (a clarification letter dated 18 September 2019 was received from an SNE member). Weight gain, length gain and head circumference, as well as selected laboratory parameters (albumin, blood urea nitrogen, serum bicarbonate, base excess) were within normal limits throughout the study. Reported adverse events and specifically serious adverse events were of similar frequency compared to a group of breast-fed infants ( $n = 95$ ). The study has major limitations (no sufficient information on blinding, no information on the existence of ascorbyl palmitate in the formulas in the publication) and was allocated to tier 3 (high risk of bias).

#### Post marketing monitoring

[REDACTED]

### 3.7. Discussion

Taking into account the data submitted by interested business operators (Section 3.1.2.1) and the considerations from the Panel (Section 3.1.2.1), a revision of the existing EU specifications for ascorbyl palmitate (E 304 (i)) as listed in Table 14 has been recommended.

In the EFSA ANS opinion on ascorbyl palmitate (E 304 (i)) (EFSA ANS Panel, 2015a), the ANS Panel concluded that the available toxicological data were too limited to establish an ADI for ascorbyl palmitate (E 304(i)) or ascorbyl stearate (E 304(ii)). The ANS Panel further concluded that ascorbyl palmitate fully hydrolyses pre-systemically to ascorbic acid and palmitate. For ascorbic acid, the conclusion was that based on adequate data on the toxicity, there is no safety concern and no need for a numerical ADI (EFSA ANS Panel, 2015b). Concerning palmitic acid (E 570), no adverse effects were observed in subchronic animal studies in which the substance was given in the diet in a concentration of up to 10%. Furthermore, there was no genotoxic concern (EFSA ANS Panel, 2017).

For the current assessment of ascorbyl palmitate, the FAF Panel assumed that also infants below the age of 16 weeks are able to hydrolyse ascorbyl palmitate pre-systemically to ascorbic acid and palmitate. This assumption is based on the existence of mRNA of CES2, the carboxylesterase present in the duodenum in the first days of life (Chen et al., 2015). The Panel was not provided with new toxicological information on ascorbyl palmitate. A literature search did not result in relevant toxicological publications since the date of the publication year of the EFSA ANS opinions on ascorbyl palmitate (2015), palmitate and ascorbic acid. Several clinical studies were submitted in which formulas were tested which contained ascorbyl palmitate among other food additives. None of the studies was intended to test the safety and tolerability of ascorbyl palmitate specifically. In these studies, no remarkable deviations from the normal growth and development were noted. All the studies have limitations in design and reporting. In a risk of bias assessment, five of the six studies were allocated to tier 3 (Ahrens et al., 2018; Auestad et al., 1997; Borschel et al., 2013, Borschel et al., 2014 a, Borschel et al., 2014b) and one study to tier 2 (Fleddermann et al., 2014) in which the Panel noted that ascorbyl palmitate was present in a relatively low concentration of [REDACTED]. The absence of an appropriate control groups which were not exposed to ascorbyl-palmitate is the most

severe limitation. Therefore, the studies provide limited evidence for the safe use of ascorbyl palmitate. From the post-marketing information, it can be deduced that adverse effects were rarely reported.

Exposure to ascorbyl palmitate (E 304(i)) from infant formulae (FC 13.1.1) was estimated at 2.6 mg/kg bw per day at the 95 percentile which corresponds to 1.11 mg/kg bw per day ascorbic acid. Taking into account the molecular weight of the moiety of ascorbic acid in the additive E 304(i), ascorbic acid corresponds to 42.5% of the weight. EFSA has proposed an adequate vitamin C intake of 20 mg/day for infants up to 6 months (EFSA NDA Panel, 2013). Hence, considering the default body weight of 4.8 kg for infants from 0 to 3 months of age (EFSA Scientific Committee, 2012), the amount of vitamin C resulting from the exposure to ascorbyl palmitate (E 304(i)) for the scenario with infant formulae (FC 13.1.1) results to be 26.6% of the adequate vitamin C intake (Table 6). For the exposure according to the scenario with special infant formulae (FC 13.1.5.1) (Table 7), the exposure to ascorbyl palmitate (E 304(i)) was estimated to be 10-fold higher than the previous one and thus should be 2.66-fold (i.e. 266%) the adequate vitamin C intake. Compared to the regulatory content of vitamin C in infant formulae of minimum 4 and maximum 30 mg ascorbic acid/100 kcal (Reg (EU) 2016/127), high-level intake (p95) of ascorbic acid from the use of E 304(i) in infant formulae (FC 13.1.1) represents between 2.0 and 17.7%. Compared to the regulatory content of vitamin C in special infant formulae (i.e. food for special medical purposes developed to satisfy the nutritional requirements of infants) of minimum 4 and maximum 30 mg ascorbic acid/100 kcal (Reg (EU) 2016/128), high-level intake (p95) of ascorbic acid from the use of E 304(i) in special infant formulae (FC 13.1.5.1) represents between 20 and 177%. The Panel noted that the SCF and the NDA Panel concluded that the available data did not allow to set an upper limit of intake level for vitamin C at any age (EFSA, 2004). Given the fact that adverse effects in adults are only reported with doses of several grams of vitamin C (e.g. 5 g/adult = 71.4 mg/kg bw per day; Hanck, 1982), the Panel considered that the exceedance of the statutory maximum content is of no safety concern.

Exposure to palmitate from the use of E304(i) in infant formula (FC 13.1.1) and in special infant formulae (FC 13.1.5.1) corresponds to 1.49 mg/kg bw per day and 14.9 mg/kg bw per day, respectively, at the 95 percentile. These levels correspond, respectively, to 0.66 kcal per day or 6.6 kcal per day using the default value of 4.8 kg bw for infant between 0 and 3 months. No regulatory guidance currently exists for palmitate intake. The Panel notes that palmitate provides approximately 25% of all breast milk fatty acids and that triglycerides represent 98–99% of breast milk fat content, i.e. 35 g/L (Koletzko, 2016). Considering a high daily breast milk intake of 1,200 mL for infants from 0 to 6 months (EFSA NDA Panel, 2013) and the default value of 4.8 kg for infants between 0 and 3 months (EFSA Scientific Committee, 2012), the palmitate intake in an exclusively breast fed infant can be estimated to around 2,200 mg/kg bw per day. Exposure to palmitate from the use of 304(i) in infant formula (FC 13.1.1) and in special infant formulae (FC 13.1.5.1) represents therefore less than, respectively, 0.1% and 1% of palmitate intake in exclusively breastfed infants between 0 and 3 months.

Hence, the intake of both, ascorbic acid and palmitate, at the MPL of ascorbyl palmitate (E 304(i)) as a food additive in infant formula for food according to FC 13.1.1 and food according to FC 13.1.5.1 does not raise safety concerns.

## 4. Conclusions

Taking into account the data submitted by interested business operators and the considerations from the Panel, a revision of the existing EU specifications for ascorbyl palmitate (E 304 (i)) has been recommended.

Based on the presumption that ascorbyl palmitate fully hydrolyses pre-systemically to ascorbic acid and palmitate, the Panel concluded that the intake of both metabolites, at the MPLs for ascorbyl palmitate as a food additive in infant formula belonging to FC 13.1.1 or in food for special medical purposes belonging to FC 13.1.5.1 does not raise safety concerns.

## 5. Recommendation

The Panel recommends the European Commission to revise current specifications for the food additive ascorbyl palmitate (E 304(i)) in line with the proposals made in Table 14.

In addition, the European Commission should consider harmonising the name of the food additive (E 304(i)) between Regulation (EC) No 1333/2008 and Regulation (EU) No 231/2012.



## 6. Documentation as provided to EFSA

- 1) DuPont, 2019. Submission of data in response to the call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on February 2019.
- 2) HiPP, 2019. Submission of data in response to the call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on July 2019.
- 3) Specialised Nutrition Europe (SNE), 2019. Submission of data in response to the call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on July 2019.
- 4) Specialised Nutrition Europe (SNE), 2020. Submission of clarifications on the data submitted in the call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on March 2020.
- 5) DuPont, 2020. Submission of clarifications on the data submitted in the call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on February 2020.
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## Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANSES	French Agency for food, environmental and occupational health and safety
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
As	Arsenic

BMDL	Benchmark Dose Modelling
bw	body weight
CAS	Chemical Abstract Service
CES2	Carboxylesterase 2
CF	Control Formula
CHO	Carbohydrates
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
DG Santé	Directorate General for Health and Food Safety
DNA	Deoxyribonucleic acid
E 304	(i) Ascorbyl palmitate
EF	Experimental Formula
eHF	extensively Hydrolysed Formula
FAF Panel	EFSA Panel on Food Additives and Flavourings
FAO/WHO	Food and Drug Organisation/World Health Organisation
FC	Food category
FSMP	Food for special medical purposes
Hg	Mercury
HS-GC-FID	Headspace Gas Chromatography with Flame Ionisation Detection
HUVECs	Human Umbilical Vein Endothelial Cells
IC50	Half maximal inhibitory concentrations
ICH	International Council for Harmonisation
ICP/MS	Inductively Coupled Plasma / Mass Spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit of detection
LOQ	Limit of quantification
LP	low protein
██████	████████████████████
Mintel	GNPD Mintel's Global New Products Database
MOE	margin of exposure
MOS	margin of safety
MPL	maximum permitted levels
mRNA	messenger ribonucleic acid
NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
Pb	Lead
PDE	permissible daily exposure
pHF	partially Hydrolysed Formula
PND	postnatal day
PWD	Powder
SC	Scientific Committee of EFSA
SCF	Scientific Committee on Food
SNE	Specialised Nutrition Europe
TD	Toxicodynamics
TK	Toxicokinetics
TWI	Total Weekly Intake
UK	United Kingdom

## Appendix A – Data requested in the call for data (Call for technical and toxicological data on ascorbyl palmitate (E 304(i)) for uses as a food additive in foods for all population groups including infants below 16 weeks of age<sup>11</sup>

Kind of data	Data requested in the call for data	Responses from interested business operators	Comment
<b>A. Information regarding the follow-up of the conclusions and the recommendations for ascorbyl palmitate (E 304(i)) for all uses (EFSA ANS Panel, 2015a,b)</b>			
<b>1. Technical data</b>	Analytical data on current levels of arsenic, lead and mercury commercial samples of the food additive	Received	Assessed, no further follow-up
	The lowest technologically achievable level for lead, mercury and arsenic in order to adequately define their maximum limits in the specifications	Received	Assessed, no further follow-up
	The lowest technologically achievable level for residual solvents which can be used when manufacturing ascorbyl palmitate (E 304(i))	Received	Assessed, no further follow-up
<b>2. Literature searches</b>	Literature searches from 31/9/2015 up to the date of the data submission	Received	Assessed, no further follow-up
<b>B. Information required for the risk assessment of ascorbyl palmitate (E 304(i)) for uses in foods for infants below 16 weeks of age</b>			
<b>1. Technical data</b>	Information on the levels of use of ascorbyl palmitate (E 304(i)) as well as analytical data on the concentration of ascorbyl palmitate in these foods	Received	Assessed, no further follow-up
	Information on the fate and the reaction products of ascorbyl palmitate (E 304(i))	No specific data	Assessed, no further follow-up
	Proposals for particular specification requirements for identity and purity of ascorbyl palmitate (E 304(i)) used in these food categories (e.g. toxic elements, solvents)	No specific data	Assessed, no further follow-up
<b>2. Toxicological data</b>	Post-marketing surveillance reports on undesired and adverse reactions, indicating the ages and other relevant data of the exposed infants and young children and the use levels in the marketed products	Received	Assessed, no further follow-up
	Published and unpublished case reports (e.g. available nutriviigilance data) on undesired and adverse effects, associated with the oral administration of ascorbyl palmitate in any form to infants and young children	No data besides the postmarketing surveillance data	Assessed, no further follow-up
	No other studies are required provided that the total amount of vitamin C (nutrient and food additive use) resulting from the use of ascorbyl palmitate (E 304(i)) in food for infants (FC 13.1.1 and FC 13.1.5.1) does not exceed the levels of vitamin C as currently authorised in the Council Directive 2006/141/ EC	Received	Assessed, no further follow-up
<b>3. Literature searches</b>	Literature searches should be conducted relevant for the safety evaluation of ascorbyl palmitate (E 304(i)) when used in foods for infants below 16 weeks of age	Received	Assessed, no further follow-up

<sup>11</sup> Available from: <https://www.efsa.europa.eu/en/consultations/call/181010-3> and responses from interested business operators

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

### Decision rules

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

Tier 1:

- All the key questions are scored + /++  
AND
- No more than one non-key question is scored -  
AND
- No non-key question is scored –

Tier 2:

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

Tier 3:

- Any question is scored –  
OR
- More than one key question is scored –

### Elements considered in the assessment

Question	Rating	Explanation for expert judgement
1. Was the administered dose or exposure level adequately randomised?	++	There is direct evidence that subjects (or clusters) were allocated to any study group including controls using a method with a random component. Acceptable methods of randomisation include referring to a random number table, using a computer random number generator, coin tossing, shuffling cards or envelopes, throwing dice or drawing of lots (Higgins and Green, 2011). Restricted randomisation (e.g. blocked randomisation) to ensure particular allocation ratios will be considered low risk of bias. Similarly, stratified randomisation and minimisation approaches that attempt to minimise imbalance between groups on significant prognostic factors (e.g. body weight) will be considered acceptable
<b>Key question</b>	+	There is indirect evidence that subjects (or clusters) were allocated to study groups using a method with a random component (i.e. authors state that allocation was random, without description of the method used)  <b>OR</b> It is deemed that allocation without a clearly random component during the study would not appreciably bias results. For example, approaches such as biased coin or urn randomisation, replacement randomisation, mixed randomisation and maximal randomisation may require consultation with a statistician to determine risk-of-bias rating (Higgins and Green, 2011)

Question	Rating	Explanation for expert judgement
	NR	There is insufficient information provided about how subjects (or clusters) were allocated to study groups
	–	There is indirect evidence that subjects (or clusters) were allocated to study groups using a method with a non-random component <i>NOTE: Non-random allocation methods may be systematic but have the potential to allow participants or researchers to anticipate the allocation to study groups. Such 'quasi-random' methods include alternation, assignment based on date of birth, case record number, or date of presentation to study</i>
	---	There is direct evidence that subjects (or clusters) were allocated to study groups using a non-random method including judgement of the clinician, preference of the participant, the results of a laboratory test or a series of tests, or availability of the intervention (Higgins and Green, 2011)
2. Was the allocation to study groups adequately concealed?	++	There is direct evidence that at the time of recruitment, the research personnel and subjects did not know what study group subjects were allocated to, and it is unlikely that they could have broken the blinding of allocation until after assignment was complete and irrevocable. Acceptable methods used to ensure allocation concealment include central allocation (including telephone, web-based and pharmacy-controlled randomisation); sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes; or equivalent methods
	+	There is indirect evidence that the research personnel and subjects did not know what study group subjects were allocated to and it is unlikely that they could have broken the blinding of allocation until after recruitment was complete and irrevocable <b>OR</b> It is deemed that lack of adequate allocation concealment would not appreciably bias results (e.g. some crossover designs)
	NR	There is insufficient information provided about allocation to study groups
	–	There is indirect evidence that at the time of recruitment, it was possible for the research personnel and subjects to know what study group subjects were allocated to, or it is likely that they could have broken the blinding of allocation before assignment was complete and irrevocable <i>NOTE: Inadequate methods include using an open random allocation schedule (e.g. a list of random numbers); assignment envelopes used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; or any other explicitly unconcealed procedure. For example, if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed</i>
	---	There is direct evidence that at the time of recruitment, it was possible for the research personnel and subjects to know what study group subjects were allocated to, or it is likely that they could have broken the blinding of allocation before recruitment was complete



Question	Rating	Explanation for expert judgement
3. Were the research personnel and human subjects blinded to the study group during the study?	++	There is direct evidence that the subjects and research personnel were adequately blinded to study group, <b>AND</b> it is unlikely that they could have broken the blinding during the study. Methods used to ensure blinding include central allocation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes; or equivalent methods
	+	<p>There is indirect evidence that the subjects and research personnel were adequately blinded to study group, <b>AND</b> it is unlikely that they could have broken the blinding during the study</p> <p><b>OR</b></p> <p>There is direct evidence for no blinding during the study (including where it was not possible to implement) <b>AND</b> it is deemed that no blinding would appreciably bias results <b>BUT</b> bias minimising measures have been adequately implemented</p> <p><b>OR</b></p> <p>It is deemed that lack of adequate blinding or no blinding during the study would not appreciably bias results (e.g. controls unlikely to behave differently for factors other than sodium intake) (e.g. cross-over)</p>
	NR	There is insufficient information provided about blinding to study group during the study (including possible breaking and minimising measures)
	-	There is indirect evidence that it was possible for research personnel or subjects to infer the study group <b>AND</b> it is deemed that lack of adequate blinding or no blinding during the study would appreciably bias results (e.g. no comparable treatment of controls, including not comparable exposure to factors other than the interventions of interest; differential behaviour) <b>AND</b> no bias minimising measures have been adequately implemented
	---	There is direct evidence for lack of adequate blinding of the study group (including no blinding or incomplete blinding) of research personnel and subjects <b>AND</b> it is deemed that lack of adequate blinding or no blinding during the study would appreciably bias results (e.g. no comparable treatment of controls, including not comparable exposure to factors other than the interventions of interest, differential behaviour) <b>AND</b> no bias minimising measures have been adequately implemented

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

Question	Rating	Explanation for expert judgement
5. Can we be confident in the exposure characterisation? <b>Key question</b>	++	There is direct evidence that the exposure (including compliance with the treatment, if applicable) was independently characterised <b>AND</b> that exposure was consistently administered (i.e. with the same method and time frame) across treatment groups
	+	There is indirect evidence that the exposure (including compliance with the treatment, if applicable) was independently characterised <b>AND</b> there is indirect evidence that exposure was consistently administered (i.e. with the same method and time-frame) across treatment Groups
	NR	There is insufficient information provided to judge the exposure characterisation
	-	There is indirect evidence that the exposure (including compliance with the treatment, if applicable) was assessed using poorly validated methods (e.g. FFQs, spot urine etc.) <b>OR</b> There is indirect evidence that the exposure assessment was probably biased
	---	There is direct evidence that the exposure (including compliance with the treatment, if applicable) was assessed using poorly validated methods (e.g. FFQs, spot urine etc.) <b>OR</b> There is direct evidence that the exposure assessment was biased
6. Can we be confident in the outcome assessment? <b>Key question</b>	++	There is direct evidence that the outcome was assessed using well-established methods (e.g. for office BP: according to a clearly described methodology, including e.g. repeated measurements per visit, trained technician, resting period before each measurement) <b>AND</b> There is direct evidence that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding prior to reporting outcomes
	+	There is indirect evidence that the outcome was assessed using acceptable methods (i.e. deemed valid and reliable but not the gold standard) <b>OR</b> it is deemed that the outcome assessment methods used would not appreciably bias results <b>AND</b> There is indirect evidence that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding before reporting outcomes <b>OR</b> it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results
	NR	There is insufficient information provided about blinding of outcome assessors or the method of measurement
	-	There is indirect evidence that the outcome assessment method is an unacceptable method <b>OR</b> There is indirect evidence that it was possible for outcome assessors to infer the study group before reporting outcomes

Question	Rating	Explanation for expert judgement
	---	<p>There is direct evidence that the outcome assessment method is an unacceptable method</p> <p><b>OR</b></p> <p>There is direct evidence for lack of adequate blinding of outcome assessors (including study subjects if home BP is the outcome), including no blinding or incomplete blinding</p>
7. Were all measured outcomes reported?	++	<p>There is direct evidence that all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract and/or introduction (that are relevant for the evaluation) have been reported</p>
	+	<p>There is indirect evidence that all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract and/or introduction (that are relevant for the evaluation) have been reported</p> <p><b>OR</b></p> <p>Analyses that had not been planned in advance (i.e. retrospective unplanned subgroup analyses) are clearly indicated as such and it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g. appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not)</p>
	NR	<p>There is insufficient information provided about selective outcome reporting</p>
	-	<p>There is indirect evidence that all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract and/or introduction (that are relevant for the evaluation) have not been reported</p> <p><b>OR</b></p> <p>There is indirect evidence that unplanned analyses were included that may appreciably bias result</p>
	---	<p>There is direct evidence that all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results</p>

Question	Rating	Explanation for expert judgement
<p>8. Were there no other potential threats to internal validity?</p> <p><b>NOTE:</b> Baseline characteristics should be appraised <b>only if</b> Q1 (randomisation) was rated with ++/+ and Q2 (allocation concealment) was rated with ++/+ /NR</p>	<p>++</p>	<p>There is evidence that variables, other than the exposure and outcome, did not differ between groups during the course of the intervention in a way that could bias results</p> <p><b>AND</b>, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported':</p> <p>There is no evidence of differences in baseline characteristics</p> <p><b>OR</b></p> <p>There is no information on both BUT no concern</p>
	<p>+</p>	<p>1. There is evidence that variables, other than the exposure and outcome, differed between groups during the course of the intervention</p> <p><b>AND</b> it is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis)</p> <p><b>AND</b>, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported':</p> <p>There is evidence that reported variables differed between groups at baseline</p> <p><b>AND</b></p> <p>It is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis)</p> <p><b>OR</b></p> <p>2. There is evidence that variables, other than the exposure and outcome, did not differ between groups during the course of the intervention in a way that could bias results</p> <p><b>AND</b>, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported':</p> <p>There is evidence that reported variables differed between groups at baseline</p> <p><b>AND</b></p> <p>It is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis)</p> <p><b>OR</b></p> <p>3. There is evidence that variables, other than the exposure and outcome, differed between groups during the course of the intervention.</p> <p><b>AND</b> it is deemed that these differences would not appreciably bias results (no concern or adequately addressed by analysis)</p> <p><b>AND</b>, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported':</p> <p>There is no evidence of differences in baseline characteristics</p> <p><b>OR</b></p> <p>There is no information BUT no concern</p>



Question	Rating	Explanation for expert judgement
	–	<p>There is no information on baseline characteristics <b>AND/OR</b> there is no information about differences between groups during the course of the intervention</p> <p><b>AND</b></p> <p>There is concern</p>
	---	<p>There is evidence that variables, other than the exposure and outcome, differed between groups during the course of the intervention</p> <p><b>AND</b></p> <p>It is deemed that these differences appreciably biased results (there is concern (e.g. not adequately addressed by analysis)) <b>OR</b>, in case randomisation is rated 'probably low'/'definitely low' RoB and allocation concealment is rated 'probably low'/'definitely low' RoB or 'not reported':</p> <p>There is evidence that reported variables differed between groups at baseline</p> <p><b>AND</b></p> <p>It is deemed that these differences appreciably biased results (there is concern (e.g. not adequately addressed by analysis))</p>