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SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives [1]

EFSA Panel on Food Additives and Nutrient Sources added to food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives. The use of these food additives was evaluated by the Scientific Committee on Food (SCF) that established an acceptable daily intake (ADI) of 6 mg/kg body weight (bw)/day. Intestinal absorption of erythorbate was reported from a mice study and near complete excretion within 24 h from a guinea pig study. The Panel noted that the acute toxicity of erythorbic acid or sodium erythorbate is low, there was no indication of adverse effects from the available subchronic toxicity studies, there is no concern with respect to their genotoxicity neither to respect to carcinogenicity. The Panel identified a no observed adverse effect level (NOAEL) of 650 mg/kg bw/day based on a decrease in body weight from a carcinogenicity study. No maternal and developmental effects were observed from a prenatal developmental toxicity study with sodium erythorbate. The Panel recognised the limitation of the overall toxicological database (no reproductive and chronic toxicity studies), but did not consider necessary to increase the usual uncertainty factor of 100 in deriving an ADI. Therefore, the Panel concluded that there is no reason to revise the current ADI of 6 mg/kg bw/day. Combined dietary exposure to erythorbic acid and sodium erythorbate from their use as food additives was calculated. Considering that the ADI is not exceeded by any population group, the Panel also concluded that the use of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives at the permitted or reported use and use levels would not be of safety concern.

KEY WORDS

food additive, erythorbic acid, E 315, CAS No 89-65-6, sodium erythorbate, E 316, CAS No 6381-77-7

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[1] On request from European Commission, Question No EFSA-Q-2011-00482 and EFSA-Q-2011-00483, adopted on 9 December 2015.

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SUMMARY

Following a request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives.

Erythorbic acid (E 315) and sodium erythorbate (E 316) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated erythorbic acid and sodium erythorbate in 1962, 1974 and 1990, and in its latest evaluation allocated an acceptable daily intake (ADI) 'not specified'. The Scientific Committee on Food (SCF) evaluated erythorbic acid and sodium erythorbate in 1987, 1990 and 1997, and an ADI of 6 mg/kg bw/day was confirmed in the latest evaluation.

The absorption, distribution, metabolism and excretion (ADME) of erythorbates was considered to be similar to that of ascorbic acid. The sodium ion of sodium erythorbate is expected to enter the sodium pool of the body. Although rodents seemed to have less efficient erythorbate absorption than humans, the available study in mouse indicated that gastrointestinal absorption occurs (Tsao and Salimi, 1983). Guinea pig, a species more analogous to human due to its active-carrier mediated transport, has near complete excretion within 24 h.

The Panel noted that erythorbic acid can increase iron bioavailability, which may represent a concern for individuals with iron deposition disorders.

The Panel noted that the acute toxicity of erythorbic acid or sodium erythorbate is low. The Panel also noted that in the available subchronic toxicity studies there were some limitations mainly concerning reporting, however, none of them reported any adverse effects and there was no histopathological indication of any adverse effects even after 36 weeks of exposure up to 900 mg/kg body weight (bw)/day.

The Panel considered that based on the available genotoxicity studies there was no concern with respect to genotoxicity of erythorbic acid or sodium erythorbate.

The Panel noted that there is no chronic toxicity study available, but considered from the available carcinogenicity studies that erythorbic acid or sodium erythorbate did not raise a concern with respect to carcinogenicity. The only reported adverse effect was a decrease in body weight at 1300 mg/kg bw/day in one study in male rats and the Panel identified a no observed adverse effect level (NOAEL) of 650 mg/kg bw/day from this study.

No reproductive toxicity studies with erythorbic acid or sodium erythorbate were available. However, no histopathological effects were observed on male reproductive organs in a 36-week study. In prenatal developmental studies, no maternal and developmental effects were observed when sodium erythorbate was administered during organogenesis.

The Panel recognised the limitation of the overall toxicological database (no reproductive and chronic toxicity studies). However, taking into account that erythorbic acid or sodium erythorbate gave negative results in a subchronic toxicity study up to 36 weeks, in genotoxicity studies, in carcinogenicity studies and in developmental toxicity studies, the Panel did not consider necessary to increase the usual uncertainty factor of 100 in deriving an ADI. Therefore, the Panel concluded that there is no reason to revise the current ADI of 6 mg/kg bw/day based on the decreased body weight reported in one carcinogenicity study.

The combined dietary exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additives was calculated based on (1) maximum levels set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage or analytical data (defined as the *refined exposure assessment scenario*). In both exposure scenarios, all combined exposure estimates were below the ADI of 6 mg/kg bw/day. Therefore, considering that the ADI is not exceeded by any population group, the Panel also concluded that the use of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives at the permitted or reported use and use levels would not be of safety concern.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[4]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[5] of 2001. The report 'Food additives in Europe 2000[6]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008[7] of the European Parliament and of the Council on food additives.

[4] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme 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.

[5] COM(2001) 542 final.

[6] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

[7] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of erythorbic acid (E 315) and sodium erythorbate (E 316) when used as food additives.

According to Annex II of Regulation 1333/2008[8], erythorbic acid (E 315) and sodium erythorbate (E 316) are authorised food additives in the EU. The safety of erythorbic acid and its sodium salt as food additives has been previously reviewed by the Scientific Committee for Food (SCF) (SCF, 1987, 1990, 1997) and by Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1962, 1974, 1990). The SCF established an acceptable daily intake (ADI) of 0–6 mg/kg bw/day (SCF 1990, SCF, 1997). JECFA in its latest evaluation established an ADI 'not specified' for erythorbic acid and sodium erythorbate (JECFA, 1990).

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following an EFSA public call for data.[9,10,11] The Panel noted that not all original studies on which previous evaluations were based were available to the Panel. To assist in identifying any emerging issue, EFSA has outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure, and occurrence levels of erythorbic acid and sodium erythorbate (E 315–316), which covered the period from January 2011 up to the end of 2014. The Panel has performed further update and no additional relevant publications were identified.

2. Technical data

2.1. Identity of substances

2.1.1. Erythorbic acid (E 315)

Erythorbic acid (E 315) has the molecular formula $C_6H_8O_6$. The molecular weight is 176.13 g/mol. The Chemical Abstracts Service (CAS) Registry Number is 89-65-6, the European Inventory of Existing Commercial chemical Substances (EINECS) number is 201-928-0 and the EC name 2,3-didehydro-D-erythro-hexono-1,4-lactone (EC Inventory, online[12]). The IUPAC name is (5*R*)-5-[(1*R*)-1,2-dihydroxyethyl]-3,4-dihydroxy-5-methylfuran-2(5*H*)-one.

The structural formula is shown in Figure 1.

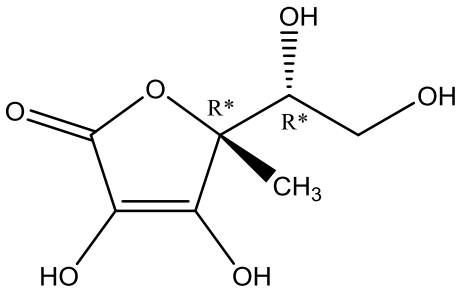
[8] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L295/1 12.00.2011

[9] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.htm

[10] Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324

[11] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 9 March 2014. Available online: http://www.efsa.europa.eu/en/dataclosed/call/datex140310

[12] EC inventory, Available online: http://echa.europa.eu/information-on-chemicals/ec-inventory



R* indicates a chiral C atom

Figure 1: Structural formula of erythorbic acid

Synonyms include: D-*erythro*-hexenonic acid, 3-*keto*, γ-lactone; D-isoascorbic acid; D-araboascorbic acid; erycorbin; isovitamin C (SciFinder[13], online).

Erythorbic acid is described in Commission Regulation (EU) No 231/2012[14], as a white to slightly yellow crystalline solid, which darkens gradually on exposure to light. The substance has a melting point of 164–172°C with decomposition. It is freely soluble in water and soluble in ethanol (JECFA, 2006). The measured Log Po/w value is -1.85[15]. Erythorbic acid is a diprotic acid having pKa's 11.34 and 4.04 (Naval Research Laboratory, 2000).

2.1.2. Sodium erythorbate (E 316)

The Panel noted that in the scientific literature different structural formulae of sodium erythorbate are available. In some of the structural formulae, the exact position of the sodium cation is indicated; in other formulae only the absolute configuration of the erythorbic acid moiety is given without an indication of the exact position of the sodium ion in the molecule.

Sodium erythorbate (E 316) anhydrous has the molecular formula $C_6H_7O_6Na$. The molecular weight is 198.11 g/mol. The CAS Registry Number is 6381-77-7, the EINECS number is 228-973-9 and the EC name is 2,3-didehydro-3-*O*-sodio-D-erythro-hexono-1,4-lactone (EC Inventory, online). The systematic name is D-*erythro*-hex-2-enonic acid, γ-lactone, sodium salt (1:1) (SciFinder, software). Other names include: D-*erythro*-hex-2-enonic acid, γ-lactone, monosodium salt; araboascorbic acid, monosodium salt; erythorbic acid sodium salt; sodium D-isoascorbate; sodium erythorbate (SciFinder, software).

An isomeric form of sodium erythorbate anhydrous ($C_6H_7O_6 \cdot xNa$) has the CAS registry No 7378-23-6, the EC number 230-938-8 and the EC name isoascorbic acid, sodium salt. The systematic name is D-*erythro*-hex-2-enonic acid, γ-lactone, sodium salt (1:X) (SciFinder, software). In this substance the number of sodium ions is undefined.

The molecular formula for sodium erythorbate monohydrate is $C_6H_7O_6Na \cdot H_2O$ and the molecular weight 216.13 g/mol. The chemical name is D-*erythro*-hex-2-enonic acid, γ-lactone, sodium salt hydrate (1:1:1) (SciFinder, software). Other names include: D-*erythro*-hex-2-enonic acid, γ-lactone, monosodium salt, monohydrate; monosodium D-isoascorbate monohydrate (SciFinder, software).

The Panel noted that in Commission Regulation (EU) No 231/2012, the food additive E 316 listed as '*sodium erythorbate monohydrate (E 316)*' is authorised as monohydrate in order to comply with specifications (molecular weight, chemical formula). However, the EINECS number assigned, 228-

[13] SciFinder® the choice for chemistry researchTM.
[14] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1.
[15] Available online: http://www.chemspider.com/Chemical-Structure.16736142.html?rid=aaecda67-f340-4e2c-9afc-8c9d54add5d4

973-9 and three out of four chemical names listed in 'definition' of the EC specifications correspond to the anhydrous form. The CAS Registry Number for sodium erythorbate monohydrate is 63524-04-9 (SciFinder, software); however, no EINECS number assigned to this CAS Registry number (EC Inventory, online).

Sodium erythorbate is described in Commission Regulation (EU) No 231/2012 as a white crystalline solid, freely soluble in water and very slightly soluble in ethanol.

2.2. Specifications

Specifications for erythorbic acid (E 315) and sodium erythorbate (E 316) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (JECFA, 2006).

Table 1: Specifications for erythorbic acid (E 315) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Assay	Content not less than 98% on the anhydrous basis	Not less than 99% on the dried basis
Description	White to slightly yellow crystalline solid which darkens gradually on exposure to light	White to slightly yellow crystalline solid which darkens gradually on exposure to light
Identification		
Melting range	About 164–172°C with decomposition	About 164–172°C with decomposition
Test for ascorbic acid/colour reaction	Passes test	Passes test
Specific rotation	$[\alpha]_D^{25}$ 10% (w/v) aqueous solution between –16.5 ° and –18.0 °	$[\alpha]_D^{25}$: Between –16.5 and –8 °
Solubility	-	Freely soluble in water, soluble in ethanol
Reducing reaction	-	A solution of the sample in water immediately reduces potassium permanganate TS without heating, producing a brown precipitate. A solution of the sample in ethanol will decolourise a solution of 2,6-dichlorophenol-indophenol TS.
Purity		
Loss on drying	Not more than 0.4% after drying under reduced pressure on silica gel for 3 h	Not more than 0.4% (reduced pressure, silica gel, 3 h)
Sulphated ash	Not more than 0.3%	Not more than 0.3%
Oxalate	To a solution of 1 g in 10 mL of water, add 2 drops of glacial acetic acid and 5 mL of 10% calcium acetate solution. The solution should remain clear.	-
Lead	No more than 2 mg/kg	Not more than 2 mg/kg

Table 2: Specifications for sodium erythorbate (E 316) according to Commission Regulation (EU) No 231/2012 and according to JECFA (JECFA, 2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Assay	Content not less than 98% after drying in a vacuum desiccator over sulphuric acid for 24 h expressed on the monohydrate basis	Not less than 98% after drying
Description	White crystalline solid	White to slightly yellow crystalline solid which darkens gradually on exposure to light
Identification		
Solubility	Freely soluble in water, very slightly soluble in ethanol	Freely soluble in water, very slightly soluble in ethanol
Test for ascorbic acid/colour reaction	Passes test	Passes test
Test for sodium	Passes test	Passes test
pH	5.5–8.0 (10% aqueous solution)	5.5–8.0
Specific rotation	$[\alpha]_D^{25}$ 10% (w/v) aqueous solution between +95 ° and +98 °	$[\alpha]_D^{25}$: Between +95.5° and +98.0 ° (10% (w/v) solution)
Reducing activity	-	A solution of the sample will decolourise a solution of 2,6-dichlorophenolindophenol TS.
Purity		
Loss on drying	Not more than 0.25% after drying (in a vacuum desiccator over sulphuric acid for 24 h)	Not more than 0.25% (in vacuum over sulphuric acid, 24 h)
Oxalate	To a solution of 1 g in 10 mL of water, add 2 drops of glacial acetic acid and 5 mL of 10% calcium acetate solution. The solution should remain clear.	To a solution of 1 g in 10 mL of water, add 2 drops of glacial acetic acid and 5 mL of 10% calcium acetate solution. The solution should remain clear.
Arsenic	Not more than 3 mg/kg	-
Lead	No more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	-

The Panel noted that, according to the EC specifications for sodium erythorbate (E 316), impurities of the toxic elements lead, mercury and arsenic are accepted up to a concentration of 2, 1 and 3 mg/kg, respectively, and for erythorbic acid for lead up to 2 mg/kg. Contamination at those levels could have a significant impact on the exposure to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009, 2010, 2012).

The Panel noted that if any solvent (e.g. methanol, acetone and dioxane as mentioned in the manufacturing process, see Section 2.3) is used in the manufacturing process of erythorbic acid (E 315) or sodium erythorbate (E 316), a corresponding maximum limit should be included in the respective EC specifications.

2.3. Manufacturing process

According to information provided by industry to the SCF (Doc. provided to EFSA n. 6), erythorbic acid and sodium erythorbate are manufactured following a multistep process.

2.3.1. Erythorbic acid

Erythorbic acid is manufactured starting from calcium 2-keto-D-gluconate obtained by fermentation of a food-grade starch hydrolysate, together with calcium carbonate by *Pseudomonas fluorescens* (strain not disclosed). The resulting fermentation broth is acidified to produce 2-keto-D-gluconic acid. The acidified broth is then filtered and decalcified (over cation exchange resin). This purified 2-keto-D-gluconic acid solution is concentrated and esterified with methanol under acid conditions to yield methyl 2-keto-D-gluconate. The ester is crystallised by cooling, separated, washed with methanol and

subsequently converted to sodium erythorbate, by heating the suspension after addition of sodium bicarbonate or sodium carbonate. The sodium salt is crystallised by cooling, separated and methanol washed, then it is suspended in a water/methanol mixture and converted to erythorbic acid by acidification with sulphuric acid; sodium sulphate being removed by filtration. Finally, the erythorbic acid solution is concentrated, deionised (over ion exchange resins) and decolourised with activated carbon. The solution is concentrated and crystallised and the crystalline erythorbic acid is separated, washed, dried, sifted and packaged (Doc. provided to EFSA n. 6).

2.3.2. Sodium erythorbate

Sodium erythorbate is synthesised using the same procedure as for the production of erythorbic acid. However, for purification, the isolated sodium erythorbate (obtained as described above) is dissolved in water, and the solution is pH adjusted and filtered. The filtrate is also passed over ion exchange resins and decolourised with activated carbon. The resulting solution is concentrated, and the sodium erythorbate is crystallised by cooling. Crystalline sodium erythorbate is separated, washed with water and methanol, dried, sifted or milled, and packaged (Doc. provided to EFSA n. 6).

The Panel noted that according to Madhavi et al (1995), erythorbic acid could also be produced chemically by reacting 2-keto-D-gluconate, obtained by oxidising potassium diacetone-3-ketogluconate with sodium methoxide. The ester is then converted to sodium erythorbate by treatment with metallic sodium in methanol. Erythorbic acid is obtained by treating the sodium salt with sulphuric acid in the presence of methanol or acetone. Erythorbic acid is purified by crystallisation from dioxane.

2.4. Method of analysis

A number of the papers describing methods for the determination of ascorbic acid include also the analysis of erythorbic acid by using high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection.

Aboul-Enein et al (1990) described an isocratic high-performance liquid chromatographic (HPLC/UV detection) method for the separation and quantitative analysis of L-ascorbic acid and erythorbic acid. Matrices tested were fruits and fruit drinks. No detection limit was provided in this study. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories.

Hidiroglou et al. (1998) used HPLC equipped with PLRP-S column and amperometric detection, in the analysis of ascorbic acid and erythorbic acid in ground meats, dairy products, luncheon meat, meal replacements, diet products, vegetable and fruit drinks, and beverages. The limit of detection was 2 µg/g. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories with the exception of luncheon meat.

Due to the occurrence of drifts when conventional RP-18 is used, Kall and Andersen (1999) proposed a PLRP-S column and demonstrated better performances. In addition to this, by using a post-column derivatization step with o-phenyldiamine they could also determine the dehydro forms of the two acids (ascorbic and erythorbic acid) with a fluorescence detector. Matrices tested were fruits and vegetables, and the quantification range was 1–50 µg/mL for dehydroascorbic acid. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories.

Bognár and Daood (2000) described HPLC method similar to the one described by Kall and Andersen, (1999) but they added an in-line oxidation step in order to measure simultaneously the two epimers (ascorbic and erythorbic acid) and their dehydro forms. Matrices tested were fruit and vegetable extracts, sausage and dairy products, where the limit of detection was estimated to be 1 µg/g for ascorbic acid, but there is no reference on erythorbic acid. The range of analysis covered concentrations up to 100 µg/g. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories.

Fontannaz et al. (2006) reported a HPLC/UV method for the quantification of total ascorbic acid and erythorbic acid in fortified infant food and fruit drinks by applying acidic extraction in the presence of tris(2-carboxyethyl)-phosphine. The limit of detection was estimated to be 1 µg/g for ascorbic acid but there is no reference to erythorbic acid. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories.

Tai and Gohda (2007) tested hydrophilic interaction liquid chromatography (HILIC) with UV detection in tea drinks and dried fruits. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in this foods categories. In this case a diol column was used, with a mobile phase of acetonitrile: 66.7 nM ammonium acetate solution (85:15 v/v) (limit of detection (LOD) 0.3 µg/g). Drivelos et al. (2010) developed a method for the simultaneous determination of ascorbic acid and erythorbic acid also by using HILIC with UV detection in red fish tissue. After having tested a number of combinations of stationary phases with mobile phases, the authors proposed an aminopropyl column with acetonitrile:ammonium acetate solution (100 nM) (90:10 v/v) as a mobile phase (LOD 2.3 µg/g fish). Barros et al. (2010) used also HILIC for chestnuts, ham and orange juice, but this time the stationary phase was TSKgel Amide-80 (LOD 1.23 µg/g). The extraction solvent used was a m-phosphoric acid solution containing EDTA and tris(2-carboxyethyl) phosphine and samples were treated at 40°C. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories.

A number of authors have used capillary zone electrophoresis to separate ascorbic and erythorbic acid. Ling et al. (1992) used a 0.1 M phosphate buffer at a pH of 5.0 to achieve the separation in the analysis fruit juices, demonstrating a detection limit of 0.5 µg/mL. Davey et al. (1996) used a fused silica capillary, with a 200 mM borate buffer at pH 9 as the carrier electrolyte and UV detection. The matrices tested were parsley and mushrooms and the assigned detection limit was 1 µg/mL. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories. Chen et al. (1999) used non-aqueous capillary electrophoresis for the analysis of ascorbic and erythorbic acids in lemon juice. They used indirect laser-induced fluorescence for detection with merocyanine 540 as a fluorophore. The detection limit for erythorbic acid was 0.17 µM (equal to 29.9 µg/mL). The Panel noted that erythorbic acid or sodium erythorbate is not authorised in this food category. Liao et al. (2000) reported similar conditions and equipment to the ones described by Davey et al. (1996) by proposing a new electrolyte of improved performance for separating the two acids (ascorbic and erythorbic acid). No food samples were tested.

Sádecká and Polonský (2001) determined the levels of ascorbic acid and erythorbic acid in beer, juices and in mixtures of additives intended to meat products by using capillary isotachophoresis with conductivity detection. The leading electrolyte contained hydrochloric acid, β-alanine and methyl hydroxyethyl cellulose, and the terminating electrolyte was caproic acid. The detection limit for beer was 7.5 µg/mL. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in the first two food categories.

Finally an official International Organisation of Vine and Wine (OIV)-2008 method intended for the analysis of ascorbic and erythorbic acid in wine involves HPLC/UV for the determination of those acids and includes an additional identification step by using ascorbate oxidase followed by a second injection of the treated sample (OIV, 2008). The LOD for erythorbic acid is 3 mg/l. The Panel noted that erythorbic acid or sodium erythorbate is not authorised in this food category.

2.5. Reaction and fate in food

In aqueous solutions, the first degradation product of erythorbic acid is assumed to be dehydroisoascorbic acid (Hvoslef and Petersen, 1981).

Comparing the oxidative properties of ascorbic acid and erythorbic acid, it is generally accepted that erythorbic acid oxidises more rapidly than L-ascorbic acid in buffered solutions in pH 7.5 (Schulte and Schillinger, 1952), in pH 4 and heating in 60 C (Yourga et al., 1943) and in food products like frozen freestone peaches (Reyes and Luh, 1962), tomato juice (Esselen et al., 1945), peaches, pasteurised

beer and cooked-cured meat (Borenstein, 1965). The Panel noted that erythorbic acid or sodium erythorbate is not authorised in these food categories except from cooked-cured meat.

2.6. Case of need and proposed uses

Maximum levels of erythorbic acid (E 315) and sodium erythorbate (E 316) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives. These levels are referred by the Panel as maximum permitted levels (MPLs) in this document.

Table 3 summarises foods that are permitted to contain erythorbic acid (E 315) and sodium erythorbate (E 316) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 3: Maximum levels of erythorbic acid and sodium erythorbate (E 315-316) in foods according to the Annex II to Regulation (EC) No 1333/2008.

FCS category number	FCS food category description	E-number	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate)
08.3.1	Non-heat-treated meat products	E 315	Only cured meat products and preserved meat products	500[(a)]
		E 316	Only cured meat products and preserved meat products	500[(a)]
08.3.2	Heat-treated meat products	E 315	Only cured meat products and preserved meat products	500[(a)]
		E 316	Only cured meat products and preserved meat products	500[(a)]
09.1.1	Unprocessed fish	E 315	Only frozen and deep-frozen fish with red skin	1,500[(a)]
		E 316	Only frozen and deep-frozen fish with red skin	1,500[(a)]
09.2	Processed fish and fishery products including molluscs and crustaceans	E 315	Only preserved and semi-preserved fish products	1,500[(a)]
		E 316	Only preserved and semi-preserved fish products	1,500[(a)]
09.3	Fish roe	E 315	Only preserved and semi-preserved fish products	1,500[(a)]
		E 316	Only preserved and semi-preserved fish products	1,500[(a)]

FCS, Food Categorisation System (food nomenclature) presented in Annex II to Regulation (EC) No 1333/2008
(a) E 315 and E 316 are authorised individually or in combination, MPL is expressed as erythorbic acid.

2.7. Reported use levels or data on analytical levels of erythorbic acid (E 315) and sodium erythorbate (E 316) in food

Most food additives in the EU are authorised at a specific MPL. However, a food additive is often used at a lower level than the MPL. Therefore, information on actual use levels is 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[16] regarding the re-evaluation of approved food additives, EFSA issued a public call[17] for ocurrence data (usage level and/or concentration data) on erythorbic acid (E 315) and sodium

[16] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme 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.
[17] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 9 March 2014. Available online: http://www.efsa.europa.eu/en/dataclosed/call/datex140310

erythorbate (E 316). In response to this call, both types of data on erythorbic acid (E 315) and sodium erythorbate (E 316) were submitted to EFSA by industry and Member States, respectively.

2.7.1. Data on reported use levels in foods provided by industry

Updated information on the actual usage levels of erythorbic acid (E 315) and sodium erythorbate (E 316) in foods was made available to EFSA by Embutidos del centro, SA (EMCESA) and FoodDrinkEurope (FDE). Industry provided EFSA with usage data on 57 products in 4 food categories (Appendix A). Among those food categories, erythorbic acid (E 315) and sodium erythorbate (E 316) are authorised in non-heat-treated (FCS 08.3.1, 34 products) and heat-treated meat products (08.3.2, 18 products).

In addition, usage levels were also made available for one product of 'fillings of stuffed pasta (ravioli and similar)' (FCS 6.4.5), and 4 products belonging to 'processed foods not covered by categories 1 to 17, excluding foods for infants and young children' (FCS 18)[18].

For the other food categories in which the use of erythorbic acid (E 315) and sodium erythorbate (E 316) is authorised, namely 'unprocessed fish and fishery products' (FCS 9.1.1), 'processed fish and fishery products including molluscs and crustaceans' (FCS 09.2), and 'fish roe' (FCS 09.3), no data on usage levels were submitted to EFSA.

See Appendix A for an overview of the data provided by industry.

2.7.2. Summarised data on analytical results in food submitted by Member States

In total, 5091 analytical results sampled were reported to EFSA between 2000 and 2014: 5047 results by Germany and 44 by Slovakia. All samples were derived from accredited laboratories. Of these samples, 78% was quantified with HPLC, whereas for the remaining 22% the analytical method was not reported.

The 4505 products (including 2337 sausages, 1494 pork meat, 205 beef meat, and 469 mixed meat) submitted to EFSA as FCS 8.3 were considered as misclassified data and grouped in the analysis together with FCS 8.3.1 (i.e. 'heat-treated meat products') and FCS 8.3.2 (i.e. 'non-heat-treated meat products'). The remaining samples were 'fresh meat' (FCS 8.1), 'processed fish and fishery products' (FCS 9.2), 'fats and oils and oil emulsions' (FCS 2), 'fruit and vegetable preparations' (FCS 4.2.4), 'salads and savoury-based sandwich spreads' (FCS 12.7), and other miscellaneous products. Left-censored analytical results were 99% for FCS 8.1, 90% for FCS 12.7, nearly 89% for FCS 8.3.1 and FCS 8.3.2 and 100% for the other FCS.

Data (n=35) above MPL set for authorised uses of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives were reported in meat preparations (FCS 8.3.1 or 8.3.2) (results ranging between 503 and 18,256 mg/kg). For the exposure assessment, EFSA considers analytical data resulting from only authorised uses at levels not exceeding the MPLs; exposure resulting from the presence of food additives in food at levels above the MPL are part of risk management measures, e.g. non-compliance controls. For this reason, such analytical results are not considered in the exposure assessment.

There were also analytical results reported in food categories in which erythorbic acid (E 315) and sodium erythorbate (E 316) are not authorised for direct addition in accordance with Annex II of Regulation (EC) No 1333/2008, including 'flavoured-fermented milk products including heat-treated products' (FCS 1.4), 'fats and oils, and fat and oil emulsions' (FCS 2), 'fruit and vegetable preparations, excluding products covered by 5.4' (FCS 4.2.4), 'other confectionery including breath-refreshing microsweets' (FCS 5.2), 'fresh meat excluding meat preparations as defined by Regulation (EC) No 853/2004 (M42)' (FCS 8.1), 'seasonings and condiments' (FCS 12.2.2), 'sauces' (FCS 12.6), 'salads and savoury-based sandwich spreads' (FCS 12.7), 'fruit juices as defined by Directive 2001/112/EC and vegetable juices' (FCS 14.1.2), 'flavoured drinks' (FCS 14.1.4), 'food supplements

[18] Pizza with chorizo/ham/bacon/other meats, pancakes with meat, croissants and pies with ham, bacon or another kind of meat, rice with ham or other meat.

as defined in Directive 2002/46/EC excluding food supplements for infants and young children' (FCS 17), and other 'processed foods not covered by categories 1 to 17, excluding foods for infants and young children' (FCS 18). However, the Panel noted that all analytical results were below the limit of quantification (LOQ) and in most cases also below the LOD. The Panel also noted that analytical results may be provided for food categories where a given additive is not authorised. Such results might be due to the use of multiscreening methods covering a large range of compounds from food control laboratories analysing the food samples.

Erythorbic acid (E 315) and sodium erythorbate (E 316) were quantified in the following food categories for which they are not authorised: 4 samples of 'fresh meat' (FCS 8.1) (namely beef, pork and poultry) (134–237 mg/kg) and 2 samples of 'salads and savoury-based sandwich spreads' (FCS 12.7), i.e. prepared meat salads. The analytical results of FCS 8.1 were likely to be due to misclassification and were excluded from the analysis. The results on 12.7 were likely due to carry-over via meat products in prepared meat salads. However, 12.7 was not included in the exposure assessment because for this composite food it was not possible to estimate in a reliable way the proportion of meat.

Appendix B shows the analytical results of erythorbic acid in foods as reported by Member States.

2.7.3. Mintel GNDP Database

As an additional source of information on the use of erythorbic acid (E 315) and sodium erythorbate (E 316) in products, the Mintel GNDP[19] database was consulted. In total, nearly 5,000 products identified in Europe, out of the nearly 1 million products sold in Europe listed in the Mintel database, reported on the label to contain erythorbic acid (E 315) or sodium erythorbate (E 316). All the listed products reported erythorbic acid (E 315) or sodium erythorbate (E 316) as an additive of meat products or products contained meat as an ingredient (e.g. pizza containing meat, ready-to-eat meat meals, meat-based spread and filled pasta).

2.8. Information on existing authorisations and evaluations

Erythorbic acid (E 315) and sodium erythorbate (E 316) are authorised as food additives in the EU in accordance with Annex II to Regulation 1333/2008[20] on food additives. Specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

An ADI of 0–5 mg/kg bw/day was derived from the no observed adverse effect level (NOAEL) of 500 mg/kg bw/day from a long-term study in rats (SCF, 1987).

The SCF concluded that the available data were inadequate and did not meet the requirements for a full toxicological evaluation of the substance. The cause for concern arose from the potential of the additive to interfere with absorption and distribution of ascorbic acid (an essential vitamin which is required by the body). The biological competition was deemed potentially detrimental to people with marginal intake of ascorbic acid, which may result in deficiency state. The SCF concluded that the use of erythorbic acid in food and drink was not acceptable and no ADI was established.

A SCF opinion dating to 1990 referred to new data submitted to the Committee, which reconfirmed that erythorbic acid will not interfere with the absorption or biological activity of ascorbic acid. The Committee established an ADI of 0–6 mg/kg bw/day for erythorbic acid *based on a long-term study in rats and satisfactory agreement of those findings with reported human nutritional experience*, however no actual review of any relevant studies was included.

The SCF evaluation of 1997 itself gave a thorough review of available studies, confirming the previously set ADI of 0–6 mg/kg bw/day.

[19] Mintel Global New Products Database (http://www.mintel.com/global-new-products-database). Accessed on 17/07/2015.
[20] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L295/1 12.00.2011

The JECFA evaluation of 1962 on erythorbic acid proposed an ADI of 0–2.5 mg/kg bw/day based on a long-term study in rats. In the JECFA evaluation of 1974, an ADI of 0–5 mg/kg bw/day was established based on the same long-term study in rat (Lehman et al., 1951).

The ADI was thereafter reviewed in a further JECFA evaluation (1990), which reproduced the previously published monograph with additional data. The evaluation concluded that compared to ascorbic acid, erythorbic acid is poorly absorbed and retained in tissues, with rapid excretion and limited reabsorption in the kidney. On this basis, it was concluded that it would interfere with ascorbic acid homoeostasis only if present at concentrations 'an order of magnitude higher than ascorbic acid', and a new ADI of 'not specified' was established for erythorbic acid and sodium erythorbate.

Erythorbic acid and sodium erythorbate are permitted as antioxidants in cosmetic products (European Commission database-CosIng[21]).

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data from national information on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with food consumption data at the level of the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys added in 2015 in the Comprehensive Database[22] were also taken into account in this assessment.[23]

Food consumption data included in the Comprehensive Database were collected through different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used in the exposure calculations, uncertainties can be introduced because of possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

The Panel estimated the chronic exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. For these population groups, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 4).

Table 4: Population groups considered for the exposure estimates of to erythorbic acid (E 315) and sodium erythorbate (E 316).

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 4 up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, United Kingdom
Toddlers	From 12 up to and including 35 months of age	Belgium, Bulgaria, Denmark, Germany, Spain, Finland, United Kingdom, Italy, Netherlands
Children [(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Germany, Denmark, Spain, Finland, France, United Kingdom, Greece, Italy, Latvia, Netherlands, Sweden
Adolescents	From 10 up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Germany, Denmark, Spain, Finland, France, United Kingdom, Italy, Latvia,

[21] Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple
[22] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm
[23] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Population	Age range	Countries with food consumption surveys covering more than one day
		Netherlands, Sweden
Adults	From 18 up to and including 64 years of age	Austria, Belgium, Czech Republic, Germany, Denmark, Spain, Finland, France, United Kingdom, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Sweden
The elderly [(a)]	From 65 years of age and older	Austria, Belgium, Germany, Denmark, Finland, France, United Kingdom, Hungary, Ireland, Italy, Netherlands, Romania, Sweden

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

2.9.1.2. Food categories selected for the exposure assessment to erythorbic acid (E 315) and sodium erythorbate (E 316)

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the FCS as presented in the Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure estimates. The food categories were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

FCS 09.1.1 (i.e. unprocessed fish, only frozen and deep-frozen fish with red skin), in which the use of erythorbic acid (E 315) and sodium erythorbate (E 316) is authorised, was not taken into account in the present estimate because its restriction to fish with red skin is not referenced in the EFSA Comprehensive Database. This might result in an underestimation of the exposure. However, frozen fish with red skin can be considered as a niche product in the EU and it is likely to have a limited impact on exposure estimate.

For the following food categories, the restrictions which apply to the use of erythorbic acid (E 315) and sodium erythorbate (E 316) could not be taken into account, and therefore the whole food category was considered in the exposure assessment. This might result in an overestimation of the exposure:

- 08.3.1 'Non-heat-treated meat products', only cured meat products and preserved meat products
- 08.3.2 'Heat-treated meat products', only cured meat products and preserved meat products
- 09.2 'Processed fish', only preserved and semi-preserved fish products
- 09.3 'Fish roe', only preserved and semi-preserved fish products

FCS 06.4.5 (i.e. fillings of stuffed pasta (ravioli and similar)) was included in the analysis assuming that foods belonging to this category would always contain meat products as an ingredient constituting, on average, 50% of the final product[24]. The other categories provided by manufacturers under FCS 18 include composite products for which no specific codes are available in the EFSA Comprehensive Database. Thus, they were not included in the analysis.

As no concentration levels (either usage or analytical) were available for FCS 09.3 fish roe, exposure via this food category was not considered in the refined exposure assessment scenario.

Overall, 5 food categories were included in the present combined exposure assessment to erythorbic acid (E 315) and sodium erythorbate (E 316) for the MPL scenario and 4 food categories in the refined scenarios (Appendix C).

[24] Clelia D'Onofrio, Il Cucchiaio d'Argento, Editoriale Domus, 1997, ISBN 88-7212-223-6.

2.9.2. Combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additives

The Panel estimated the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) using the highest concentration reported from any of them for each food category.

Dietary exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) was calculated by multiplying the concentration levels (Appendix C) per food category with their respective consumption amount per kg body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period.

These calculations were carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 3). Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large (> 60 subjects) to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Concentration data used to assess the exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) were: (1) MPLs as set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*); and (2) usage and analytical data obtained from manufacturers and Members States (defined as the *refined exposure assessment scenario*).

These two scenarios are discussed in detail below.

2.9.2.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 3 and Appendix C.

The exposure estimates derived from this scenario can be considered as conservative as it is assumed that the consumer will be continuously (over a lifetime) exposed to erythorbic acid (E 315) and sodium erythorbate (E 316) present in food at MPL.

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based mainly on analytical results reported by MSs as they were considerably higher than the usage levels provided by the industry. For filling of stuffed pasta (FCS 6.4.5), we used usage levels provided by the industry because analytical results from MSs were not available. As no concentration levels (either usage or analytical) were available for FCS 9.3 fish roe, exposure via this food category was not considered in the refined exposure assessment scenario.

Appendix C summarises the concentration levels of erythorbic acid (E 315) and sodium erythorbate (E 316) used in refined exposure assessment scenarios.

Based on the available dataset, the Panel calculated two estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to the food additive present at the maximum reported use/analytical level for one food category. This exposure estimate is calculated as follows:

- Combining food consumption with the maximum of the reported use levels or the maximum of the analytical results for the main contributing food category at the individual level.
- Using the mean of the typical reported use levels or the mean of analytical results for the remaining food categories.

- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to the food additive present at the mean reported use/analytical levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results for all food categories.

In the two refined exposure assessment scenarios, the concentration levels considered by the Panel were extracted from the whole dataset (i.e. reported use levels and analytical results). To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ), the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 2009) and the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010) was used. In the present opinion, analytical data below LOD or LOQ were assigned half of LOD or LOQ, respectively (middle-bound). Subsequently, per food category the mean MB concentration was calculated. Non-authorised foods were not considered in the exposure assessment, unless they contain meat and the amount of meat could be estimated (i.e. 6.4.5 'fillings of stuffed pasta (ravioli and similar)').

2.9.2.3. Anticipated combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316)

Table 5 summarises the estimated exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additive in six population groups. Detailed results per population group and survey are presented in Appendix D.

Table 5: Summary of the estimated combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additives in the regulatory maximum level exposure assessment scenario and in the two refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw/day).

	Infants (4–11 months)		**Toddlers (12–35 months)**		**Children (3–9 years)**		**Adolescents (10–17 years)**		**Adults (18–64 years)**		**The elderly (≥ 65 years)**	
Regulatory maximum level exposure assessment scenario												
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Mean	0.05	0.70	0.38	1.52	0.41	1.35	0.18	0.83	0.24	0.54	0.17	0.51
95th percentile	0.15	2.68	1.56	4.40	1.65	3.85	0.54	2.67	0.66	1.65	0.53	1.81
Refined estimated exposure scenario using reported use levels and analytical data												
Brand-loyal scenario												
Mean	0.01	0.12	0.10	0.29	0.08	0.26	0.04	0.17	0.06	0.14	0.04	0.10
95th percentile	0.04	0.48	0.41	0.70	0.39	0.66	0.13	0.52	0.16	0.42	0.12	0.32
Non-brand-loyal scenario												
Mean	0.00	0.02	0.02	0.06	0.02	0.05	0.01	0.03	0.01	0.03	0.01	0.02
95th percentile	0.01	0.09	0.08	0.13	0.08	0.13	0.03	0.10	0.03	0.08	0.02	0.06

The main food categories contributing to the exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) are presented in Appendix E (Tables E1 to E3).

In the *regulatory maximum level exposure assessment* scenario (Appendix E, Table E1), the main food categories contributing to the exposure were heat- and non-heat-treated meat products with a contribution above 42% for all population groups. Processed fish contribution was 5% or more (Table E1).

In both refined exposure scenarios (Appendix E, Tables E2 to E3), meat products were the main contributors (96% or more) to the total mean exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) for all population groups in all population groups. Processed fish and fishery products including molluscs and crustaceans provided minor contributions in all population groups (Table E2 and E3).

2.9.3. Uncertainty analysis

Uncertainties in the exposure assessment of erythorbic acid (E 315) and sodium erythorbate (E 316) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and summarised in Table 6.

Table 6: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction [(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Database: uncertainties to which types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of food categories because of missing FoodEx linkage (n = 1)[25]	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception	
• Regulatory maximum level exposure assessment (n = 4)[26]	+
• Refined estimated exposure assessment (n=3)[27]	+
Concentration data: levels considered applicable to all food items within the entire food category	+/–
Regulatory maximum level exposure scenario: calculations based on the maximum permitted level	+
Refined estimated exposure calculations based on the maximum or mean levels (reported use from industries or analytical data)	+/–
Concentration data: data not available for certain food categories which were excluded from the exposure estimates (n=1 only for the refined scenarios)[28]	–
Uncertainty in possible national differences in use levels of food categories, concentration data not fully representative of foods on the EU market	+/–

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

Overall, the Panel considered that the uncertainties identified would rather lead to an overestimation than an underestimation of the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives in European because the food categories excluded from the refined exposure assessment are only a small proportion of the diet.

2.9.4. Exposure via other sources

Erythorbic acid (E 315) and sodium erythorbate (E 316) are permitted as antioxidants in cosmetic products. The exposure via these routes is unknown, and could therefore not be taken into account in this opinion.

[25] FCS 09.1.1.
[26] FCS 08.3.1, 08.3.2, 09.2, 9.3.
[27] FCS 08.3.1, 08.3.2, 09.2.
[28] FCS 9.3

3. Biological and toxicological data

The Panel considered that sodium erythorbate fully dissociates into sodium ion and erythorbate in the gastrointestinal tract and the sodium ion is not expected to impact on the toxicity of the salt.

3.1. Absorption, distribution, metabolism and excretion

Several experimental studies on the toxicokinetic behaviour of the erythorbic acid in animals have been carried out and previously discussed in the evaluation by JECFA (1990) and the SCF (1997).

3.1.1. Animal studies

3.1.1.1. Absorption

The Panel considered that owing to the ionisation properties of erythorbic acid (pKa 4.03), the unionised form of erythorbic acid and erythorbate should be absorbed by a diffusion process in the stomach. Moreover, absorption of erythorbate and erythorbic acid from the gastrointestinal tract proceeds readily though less efficiently by the same active transport mechanism as for ascorbic acid (Gou1d, 1948; FASEB, 1979 (Doc. provided to EFSA n. 2); Hughes and Jones, 1970). The transport mechanism is located in the brush border membrane vesicles (Toggenburger et al., 1979). But FASEB (1979) indicated that erythorbic acid is well absorbed, but not as rapidly as L-ascorbic acid.

Transport of L-ascorbic acid and D-erythorbic acid is Na-dependent, electro-neutral and saturable. It is competitive and therefore potentially able to reduce ascorbic acid uptake from the intestine, although erythorbic acid is a poorer substrate (Siliprandi et al., 1979). Intact gut segments showed a 16% decrease in ascorbic acid influx if erythorbic acid was present at 10 times the ascorbic acid concentration (Mellors et al., 1977). However, simultaneous oral administration of [1-^{14}C]-erythorbic acid and [6-^{3}H]-ascorbic acid to guinea pigs resulted in similar specific activities in the portal blood for 3.5 h (Hornig, 1977)

3.1.1.2. Distribution

In the Pelletier (1969) study, the authors stated that:'To test the theory that isoascorbic acid (IAA) was retained by organs of animals, guinea pigs were fed a synthetic diet containing ascorbic acid (AA) plus IAA. It was found that organs of the guinea pig retained a significant quantity of IAA which was replaced a corresponding quantity of AA. The incorporated IAA could, in turn, be replaced by AA when only AA was subsequently given in the diet.'

Oral administration of 1.8 mg [6-^{3}H]-ascorbic acid (215.9 µC; specific activity 21.07 mC/mmole) simultaneously with 1.8 mg [1-^{14}C]-erythorbic acid (93.1 µC; specific gravity 9.1 mC/mmole) in 425 µl 0.15 M sodium phosphate buffer at pH 4.2 to fasted (20 hours) guinea pigs (9 animals; age, sex and strain not specified), resulted in the detection of radiolabelled erythorbic acid in the liver, lungs and kidneys after 3.5 h, each accounting for less than 1% of the administered dose (Hornig, 1975). In addition, the authors commented that erythorbic acid was transported into tissues less effectively than ascorbic acid, an effect that was described to occur on membrane level of uptake rather than as a result of absorption through the gastrointestinal tract. There was almost complete excretion of erythorbic acid after oral administration within 24 hours (no further detail available) (Hornig, 1975). The majority of excretion was reported to have occurred via exhaled air (54%) and urine (30%), with some excretion in faeces (4%). Trace amounts (less than 1%) were found in organs, mainly the liver, lungs and kidneys.

Tsao and Salimi (1983) investigated the fate of ascorbic acid and erythorbic acid until steady state was reached in Swiss Webster mice. The results indicated that each isomer has established equilibrium among uptake, catabolism and elimination with no interference from another.

As reported by the SCF (1997) 'tissues reached 60–79% of the ascorbic acid level (Hughes and Hurley, 1969; Hughes and Jones, 1970). Oral doses of 20 or 100 mg erythorbic acid/day per animal

given to guinea pigs, over 16 days, produced detectable tissue levels of erythorbic acid (Suzuki et al., 1987).'

3.1.1.3. Excretion

Pelletier and Godin (1969) reported that 'Guinea pigs given 40 mg erythorbic acid/day per animal for 2 months had excreted 1.9% of the ingested dose in their urine at that time'.

The SCF (1997) reported that 'Rats excreted in their urine 10 times more ^{14}C-labelled erythorbic acid compared to ascorbic acid (Baker et al., 1973 as referred to by SCF, 1997).'

Male F344 rats (five per group, 6-week-old) were given 5% sodium erythorbate in feed for 22 weeks. The rats eliminated totals of 203.3 ~ 33.2 mg erythorbic acid/100 mL and 9.0 ~ 5.1 mg dehydro-erythorbic acid/100 mL during the study. Ascorbic acid and dehydroascorbic acid were not detected. Crystals were detected in urine of rats given basal diet and sodium erythorbate or basal diet alone (Fukushima et al., 1984).

Dogs injected a single i.v. dose of ascorbic acid or erythorbic acid showed nearly identical plasma half-lives, indicating that elimination and reabsorption had the same rate rather than being higher for ascorbic acid (Robinson and Umbreit, 1956, as referred to by SCF, 1997). They excreted 19% of a 5 g dose within 24 h.

3.1.2. Human studies

Loading tests on six volunteers with 165 or 300 mg erythorbic acid showed similar blood levels over 3 h to those obtained with ascorbic acid. Balance studies showed that 50–70% of the erythorbic acid test load compared to 15% of ascorbic acid was excreted within 24 h. Excretion of erythorbic acid was more rapid and more complete suggesting little renal tubular reabsorption (Wang et al., 1962).

Four males, partially depleted of ascorbic acid were given 50 mg erythorbic acid daily for 2 weeks, followed by 100 mg daily for 2 weeks. Although all ascorbic acid concentrations continued to fall throughout the 4 weeks, the urinary excretion of erythorbic acid increased considerably. A loading dose of 300 mg erythorbic acid did not raise the white cell ascorbic acid level, 50–60% of the load appearing in the urine. The decline in white cell ascorbic acid concentration with erythorbic acid supplementation showed that the uptake or tissue fixation of L-ascorbic acid by white cells is structurally specific for the L-configuration about carbon 5 (Rivers et al., 1963).

The absorption of erythorbic acid through the human buccal mucosa was studied in healthy adult subjects. Absorption of a solution of 10 mM erythorbic acid, buffered to pH 6, was 13.0 ± 0.74 µmol/5 minutes compared to 13.0 ± 1.4 µmol/5 minutes for ascorbic acid (Sadoogh-Abasian and Evered, 1979).

Overall, the absorption, distribution, metabolism and excretion (ADME) of erythorbates was considered to be similar to that of ascorbic acid. The sodium ion of sodium erythorbate is expected to enter the sodium pool of the body. Although rodents seemed to have less efficient erythorbate absorption than humans, the available study in mouse indicated that gastrointestinal absorption occurs (Tsao and Salimi, 1983). Guinea pig, a species more analogous to human due to its active-carrier mediated transport, has near complete excretion within 24 h (Hornig, 1975).

3.1.2.1. Interaction of erythorbic acid with ascorbic acid

Although studies by Pelletier (1969), Horrnig (1975) and Hornig and Weiser (1976) seemed to indicate a reduction in the ascorbic acid body pool by 30% by a mixture of erythorbic acid with ascorbic acid (Hormig, 1976) indicating a possible interaction. Neither the study of Turnbull et al. (1979) in cynomolgus monkeys nor a study by Sauberlich (1989) in non-pregnant women confirmed the findings.

In the study by Turnbull et al. (1979), eight male cynomolgus monkeys were kept for 8 weeks on an ascorbic acid depleted diet and then fed for 4 weeks a diet supplemented with either 10 mg/kg bw/day ascorbic acid (4 animals) or 10 mg ascorbic acid with 200 mg erythorbic acid/kg bw/day (4 animals). There was no difference in the blood ascorbate levels of the treated groups suggesting an absence of antagonistic action of erythorbic acid.

In the study by Sauberlich (1989), eleven adult women were maintained for 54 days on an ascorbic acid-free formula diet. Blood ascorbate was reduced markedly during depletion. They then received increasing ascorbic acid supplements with or without 600 mg/day erythorbic acid. Addition of 90 mg ascorbic acid/day for 10 days was needed to restore blood ascorbic acid levels Addition of 600 mg erythorbic acid/day did not cause any adverse effects.

The Panel noted that there is no indication in the literature of an interaction of erythorbate with the kinetic of ascorbic acid.

3.1.2.2. Oxalate formation

Human volunteers given erythorbic acid showed little degradation to oxalate. Ingestion of 3.41 mmol erythorbic acid/day resulted in an increased excretion of only 67–133 µmol oxalate/day (Sauberlich et al., 1989).

3.1.2.3. Effect on metal absorption

Adult male volunteers given daily for 51 days a diet containing 200 g processed meat (uncured, nitrite cured, nitrite +500 µg/g erythorbic acid-cured sausage) showed no significant effects on the bioavailability and absorption of Fe, Zn, Cu, on serum Zn and serum Cu levels, plasma ferritin, transferrin or ceruloplasmin levels (Greger et al., 1984).

In the Fidler et al. (2004) study, iron absorption was monitored in healthy volunteers for 14 to 15 days following dietary intake, by measuring stable-isotope-labelled iron incorporation to erythrocytes. Each woman acted as her own control, which was made possible by a crossover study design. The molar ratios of erythorbic acid to iron (added as ferrous sulphate) were 2:1 and 4:1, respectively. Addition of erythorbic acid increased iron absorption 2.6- and 4.6-fold at 2:1 and 4:1 molar ratio relative to iron respectively, $p < 0.0001$). There was a significant increase in iron absorption as a consequence of increasing molar ratio of erythorbic acid from 2:1 to 4:1 ($p= 0.001$). The authors concluded that erythorbic acid may play a major role in enhancing iron bioavailability.

The Panel noted that the potential increase in iron bioavailability may represent a concern for the population of patients with alteration of their iron metabolism.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The SCF (1997) and JECFA (1990) have previously referenced an unpublished study that reported oral LD_{50} values of 8.3 and 18 g/kg bw in the mouse and the rat, respectively

In male rats, the lowest effect level (LEL) of erythorbic acid was > 2,500 mg/kg bw. In addition, the LEL in dogs was greater than 7,500 mg/kg bw (Doc. provided to EFSA n. 5).

The acute oral LD_{50} of sodium erythorbate in 10 fasted albino rats was > 5,000 mg/kg bw. The treated rats had soft, pasty stools within 3 h of dosing, followed in 2 h by marked diarrhoea that persisted for 24 h (Clairol, 1996, as referred to by Andersen, 1999).

3.2.2. Short-term and subchronic toxicity

3.2.2.1. Mice

B6C3F1 mice (10 animals/sex; 8 weeks old) were administered 0, 0.625%, 1.25%, 2.5%, 5% and 10% sodium erythorbate (equivalent[29] to 0, 1,250, 2,500, 5,000, 10,000 and 20,000 mg/kg bw/day) in drinking water for the 10 weeks (Inai et al., 1989). Controls (20 animals/sex) were given distilled water. Six males and one female died in the 10% dose group by the end of the first week of treatment. There was a reduction in weight gain in males receiving the 5% dose compared to controls, whereas females at the same dose exceeded weight gains of the controls. Histological examination revealed marked atrophy (males at 5 and 10% doses, females at 10% dose only) of the liver cells and lymphoid follicles of spleen, as well as hydropic degeneration of renal tubular epithelium. No further details were provided. Based on available information, the dose level of 5,000 mg/kg bw/day was considered by the authors to be the NOAEL for this study. The Panel noted that the protocol of this study was limited as few of the usual end points were considered.

3.2.2.2. Rats

In a study by Abe et al. (1984), groups of (10 animals/sex; 6 weeks old) F344 rats were given 0, 0.625%, 1.25%, 2.5%, 5% or 10% sodium erythorbate (equivalent[29] to 0, 563, 1,125, 2,250, 4,500 and 9,000 mg/kg bw/day) in drinking water for 13 weeks. No clinical chemistry, haematology and histopathology examinations were performed. All animals at the 10% dose level refused to drink and died within 2 to 5 weeks. In the 5% dose-level group, 3 males and 1 female out of the 10 animals died during the first 4 days. All the other animals survived until the end of the study. A reduction in body weight gains of 12% and 6% in males and females, respectively, was observed at the 2.5% dose level, compared to the non-treated controls. The authors identified the 1,125 mg/kg bw/day dose as the NOAEL. This study was the preliminary range finding study for a 2-year carcinogenicity study, which is discussed in Section 3.2.4.2, and details of its protocol are lacking.

F344 male rats, 6-week-old, were given a diet containing 5% erythorbic acid, sodium erythorbate or basal diet (control) for 24 weeks (Shibata et al. 1985). Parameters of urinary excretion were investigated and the urinary bladder epithelium was examined using light and scanning electron microscopy at weeks 8, 16 and 24. The urine of rats fed erythorbic acid and sodium erythorbate had increased pH, elevated content of crystals and sodium and decreased osmolality at each time point. Histologically, the bladders of rat fed with sodium erythorbate revealed simple hyperplasia at 8 weeks, although this decreased by 16 weeks and was no longer evident at 24 weeks. Scanning electron microscopy study indicated morphological alterations, such as formation of uniform or pleomorphic microvilli and ropy or leafy microridges, on the surface of bladder cells of rat fed with sodium erythorbate. The Panel noted that this study was limited, because it was briefly reported, the number of animal/group was not indicated and only one high-dose (equivalent to 4,500 mg/kg bw/day) was studied.

Ten weanling Osborne–Mendel male rats were fed 0 or 1% erythorbic acid in their diet equivalent[29] to 0 or 900 mg/kg bw/day for 36 weeks (Fitzhugh and Nelson, 1946). There were no significant differences from controls regarding growth, weight gain and mortality. Gross pathology and histopathology of the lung, heart, liver, spleen, pancreas, stomach, small intestine, kidney, adrenal, testis and only occasionally the colon, lymph node, bone, bone marrow, thyroid, parathyroid showed no lesions attributable to erythorbic acid. No clinical chemistry and haematology results were reported.

Overall, the Panel considered that owing to poor reporting and to the absence of clinical chemistry and haematology data, the reliability of the NOAELs in these studies was limited. However, the Panel noted that there was no indication of adverse effects in these studies.

[29] Calculated by the Panel according to EFSA Scientific Committee (2012).

3.2.3. Genotoxicity

A summary of the available *in vitro* and *in vivo* genotoxicity studies is presented in Appendix F.

3.2.3.1. In vitro

Bacterial gene mutation

A bacterial gene mutation tests was performed with and without metabolic activation in a suspension tests using homogenates of the liver, lungs and testes from adult male ICR mice, Sprague–Dawley rats and *Macaca mulatta* (Litton Bionetics, 1974 (Doc. provided to EFSA n. 7)). The indicator organisms were *Salmonella* Typhimurium strains TA1535, TA1537 and TA1538 and the doses tested, determined in a preliminary assay were 0.25 and 0.5% in the culture medium. In parallel, positive control with and without metabolic activation were tested to demonstrate the sensitivity of the tests. An assay using the plate incorporation assay using only a 0.5% solution added in the soft agar. In all strains, erythorbic acid was non-mutagenic. The Panel noted that this study was limited, because it uses an outdated method with a limited number of strains and a limited number of doses.

Sodium erythorbate was not mutagenic in five strains (TA1530, TA1535, TA1536, TA1537 and TA1538) of *Salmonella* Typhimurium in the Ames test, with and without metabolic activation at the dose of 100 mg/plate. The Panel noted that this test was limited concerning the choice of the *S.* Typhimurium strains, the use of one dose-level only and the absence of confirmation of negative results. (Newell, et al. 1974 (Doc. provided to EFSA n. 8))

A reverse mutation assay was conducted with erythorbic acid (99.6% purity) and sodium erythorbate (99.8% purity) at maximum concentrations of 50 mg/plate and 5 mg/plate, respectively, with *Salmonella* Typhimurium tester strains TA92, TA94, TA100, TA1535 and TA1537 with and without rat liver microsome fraction (S9) using the preincubation method (Ishidate et al., 1984). Duplicate plates were used for each concentration. Weak increases of revertant frequencies were observed with the *Salmonella* Typhimurium TA100 tester strain both in the absence and presence of S9 metabolic activation. However, the Panel noted that this result was obtained at a dose-level of 50 mg/plate, clearly exceeding the maximum dose-level of 5 mg/plate recommended by the relevant OECD guideline no. 471. Negative results were obtained for sodium erythorbate up to 5 mg/plate in all tester strains used.

Hayashi et al. (1988) reported positive results in the Ames test for erythorbic acid (99.6% purity). However, the Panel noted that details of the protocol used and results have been already published in the paper by Ishidate et al. (1984) as also mentioned by the present authors.

Zeiger (1993) reported that erythorbate was weakly mutagenic in *Salmonella* Typhimurium. The Panel noted that no information on the strains used and the protocol employed were available in this publication.

A reverse mutation test using *Salmonella* Typhimurium tester strains TA98 and TA100 provided negative results for sodium erythorbate both in the absence and presence of S9 metabolic activation. However, the Panel noted that the study was poorly documented and no information on the range of concentrations used was available (Peters et al., 1983, as referred to by SCF, 1995 (Doc. provided to EFSA n. 10)).

Yeasts gene mutation

Erythorbic acid induced no gene conversion in *Saccharomyces cerevisiae* strain D4 at concentrations of 2% and 4%, as determined in a preliminary assay, using a suspension method with and without metabolic activation (Litton Bionetics, 1974 (Doc. provided to EFSA n. 7)). The Panel noted that this study is limited in the protocol and the assay did not receive further validation and is presently considered obsolete.

At a concentration of 5% (the only dose tested), sodium erythorbate did not increase the mitotic recombination frequency of *Saccharomyces cerevisiae* D3 *in vitro* without metabolic activation (Newell et al., 1974 (Doc. provided to EFSA n. 8)). The Panel noted that this study is very limited in the protocol, and the assay did not receive further validation and is presently considered obsolete.

Chromosomal aberrations

Sodium erythorbate was not clastogenic in a chromosomal aberration test using Chinese hamster cells, both with and without metabolic activation when tested up to 2 mg/mL (Matsuoka, et al. 1979). The Panel noted that the study is poorly documented. Three concentrations were reported to be tested but results were shown only for the high concentration.

No chromosomal aberrations or sister-chromatid exchanges (SCEs) were induced in human fibroblasts (HE2144 cells) at doses of 0.02 mg erythorbic acid /mL for 40–48 h (Kawachi et al., 1980; Sasaki et al., 1980, as referred to by SCF, 1997). The Panel noted that the information available were limited.

A chromosomal aberration assay was carried out using Chinese hamster fibroblast cell Line (CHL) (Ishidate et al., 1984). The cells were exposed to erythorbic acid (99.6% purity) and sodium erythorbate (99.8% purity) at concentrations up to 0.25 mg/l, for 24 and 48 h. No metabolic activation was applied. The number of cells with chromosomal aberrations was recorded on 100 metaphases at 24 and 48 h. A preliminary test to determine maximum dose was carried out by defining a dose with 50% inhibition of cell-growth. Untreated and solvent-treated cells were used as the negative control. The results of the test were negative for polyploidy and structural chromosomal aberrations for both erythorbic acid and erythorbate. The Panel noted that the study is limited as treatments were only performed in the absence of S9 metabolic activation.

Primary DNA Damage test

Erythorbic acid had DNA-damaging potential in the *Bacillus subtilis* Rec assay using strains H17 and M45 (Nonaka, 1989). The Panel noted that information on this test is limited and it is generally not used for genotoxicity risk assessment.

3.2.3.2. In vivo

Host-mediated assay in mice

In mice, sodium erythorbate was not mutagenic in the host-mediated assay using *Salmonella* Typhimurium strain TA1530 and it did not increase the mitotic recombination frequency in the host-mediated *Saccharomyces cereviae* D3 assay at 0.2, 1 or 5 g/kg bw/day *per os* one time or for 5 consecutive days (Newell et al. 1974 (Doc. provided to EFSA n. 8)). The Panel noted that this assay does not belong to the assays currently recommended for the assessment of genotoxicity (EFSA Scientific Committee, 2011).

Micronucleus test

A micronucleus test was carried out in mice bone marrow following intraperitoneal administration of erythorbic acid (Hayashi et al., 1988). Preliminary assays were conducted to determine the maximum dose-levels of test compound at the different sampling times. Erythorbic acid was administered once to ddY mice at 0, 187.5, 375, 750 and 1,500 mg/kg bw. In addition to single dose administration, a multidose study with 750 mg erythorbic acid/kg bw administered 4 times at 24-hour intervals was carried out. Mitomycin C (2 mg/kg bw) served as a positive control. Following exposure to the test compound, the animals were terminated and femoral marrow sampled at 24 h from beginning of treatment. A total of 1,000 polychromatic erythrocytes were scored per animal. The number of micronucleated polychromatic erythrocytes (MNPCE) was recorded, and the proportion of polychromatic erythrocytes (PCEs) evaluated from a total of 1,000 erythrocytes per slide. There were no mortalities in response to the treatment. A clear decrease in the percentage of polychromatic erythrocytes was noted at the top dose. There was no statistically significant induction of micronuclei

in the bone marrow of mice in the single or multidose study. The Panel noted that the number of PCE examined was low but consistent with the internationally recognised protocol at the time for this assay.

Chromosome aberration test in rat bone marrow

In a chromosome aberration test in rat bone marrow cells *in vivo*, a positive response was reported for sodium erythorbate (Kawachi et al., 1980). However, the Panel noted that the results obtained are difficult to interpret because the study is not described in detail.

Dominant lethal assay

Sodium erythorbate was administered orally at doses of 0, 0.2, 1.0 and 5.0 g/kg bw either once or on 5 successive days to proven male rat breeders. A positive control group received a single dose of triethylmelamine (0.2 mg/kg bw i.p.). Following dosing, each male was mated within 2–3 h with two adult female rats for 7 days. The females were then removed, and new females again were added for another week of breeding. This sequence continued for 8 weeks. Effects were evaluated by examining all females for early fetal deaths, late fetal deaths, living fetuses (all of which provide a total implant score), corpora lutea and pre-implantation loss (determined by subtracting the total implant score from the total corpora lutea score). The results of the study show that none of the examined parameters exhibits consistent changes that could be attributed to treatment with sodium erythorbate. Occasional statistically significant differences did not suggest a time or dose-dependent effect (Newell et al., 1974 (Doc. provided to EFSA n. 8)).

Groups of 10 proven breeder male rats were treated (unspecified doses) with sodium erythorbate by gavage at a single dose and with 5 consecutive daily doses; 3 dosage levels were used for each regimen (Jorgenson et al., 1978). Untreated reference controls and positive controls receiving a single i.p. injection of triethylenemelamine were used. Following treatment, each single-dose male was mated to two adult females weekly for 8 weeks; each multiple-dosed male was mated to two adult females weekly for 7 weeks. No consistent responses occurred to suggest that sodium erythorbate was mutagenic to the rat in the dominant lethal assay. The Panel noted that the study was briefly reported and that no information on dose-levels tested were provided.

Heritable translocation test in mice

Male mice received sodium erythorbate in their diets for 7 weeks at the dose levels of 1 and 5 g/kg diet (equivalent[30] to 200 and 1,000 mg/kg bw/ day, respectively) in the diet. Untreated reference controls were included, as well as a positive mutagen control group which received triethylenemelamine (TEM) in the drinking water for 4 weeks. After treatment, the males were mated to virgin females to produce a F_1 generation, the males of which were raised to maturity. One hundred F_1 males per treatment group were selected and bred to three virgin females each. Fetuses of pregnant females were evaluated by predetermined selection criteria to identify suspect F_1 males. These males were rebred to three additional virgin females each. Cytogenetic examinations were made on meiotic cells from males considered as presumptive positives following two successive breedings. All breeding data were evaluated and correlated with the cytogenetic examinations. No increase in reciprocal translocations was observed in the control and sodium erythorbate groups; the TEM group produced, as expected, significant increases in chromosome translocations. The authors concluded that sodium erythorbate administered in the diet over a 7-week period does not induce translocation heterozygosity in male mice (Newell et al., 1974 (Doc. provided to EFSA n. 8)).

Comet assay

Groups of four male ddY mice received by oral route erythorbic acid or its sodium salt at the limit dose of 2,000 mg/kg (Sasaki et al., 2002). They were sacrificed 3 or 24 hours after treatment and eight organs (glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow) were

[30] Calculated by the Panel according to EFSA Scientific Committee (2012).

removed. The test was performed on isolated nuclei. The length of the whole comet and the diameter of the head were measured for 50 nuclei per organ per animal. Mean migration of 50 nuclei from each organ was calculated for each individual animal. The differences between the averages of four treated animals and the untreated control animals were compared with the Dunnett test after one-way ANOVA. A small portion of each organ was fixed in 10% formaldehyde, dehydrated and embedded in paraffin. When positive results were obtained in the comet assay, tissue sections stained by the haematoxylin–eosin and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) methods were observed histopathologically. Erythorbic acid and its sodium salt did not increase DNA damage in any of the organs studied ($p>0.05$).

In summary, data on genotoxicity are available for erythorbic acid and its sodium salt. Erratic positive findings on bacterial gene mutation in studies of limited reliability or at very high dose-levels were observed (Ishidate et al., 1984; Hayashi et al., 1988; Zeiger, 1993). In mammalian cells *in vitro* no chromosomal aberrations were observed both in the absence and presence of S9 metabolic activation (Matsuoka et al., 1979; Ishidate et al., 1984). *In vivo*, a positive finding was reported in a very limited and poorly described rat bone marrow chromosome aberration assay (Kawachi et al., 1980). However, concerning the genotoxic effects at chromosomal level *in vivo*, negative results were observed in a mouse bone marrow micronucleus test for erythorbic acid which followed the internationally recognised protocol at the time when this study was performed (Hayashi et al., 1988), in two dominant lethal assays in rats with sodium erythorbate (Newell et al., 1974 (Doc. provided to EFSA n. 8); Jorgenson et al., 1978) and in a heritable translocation test in mouse for sodium erythorbate (Newell et al., 1974). Furthermore, negative findings were also observed in a 'limit *in vivo* comet assay' (2,000 mg/kg) in eight organs in mice (Sasaki et al., 2002). This last study further corroborates the absence of clastogenic effects *in vivo* by erythorbic acid and adequately clears the limited positive outcomes for gene mutation in bacteria.

The Panel noted that the reliability of most genotoxicity studies was limited or insufficient and that, accordingly, the relevance of their results was limited or low. However, there were also studies of higher reliability and relevance, i.e. a dominant lethal assay, a heritable translocation test and a Comet assay, and these *in vivo* studies were negative. Overall, the Panel concluded that based on the available data there is no concern with respect to genotoxicity of erythorbic acid or sodium erythorbate.

3.2.4. Chronic toxicity and carcinogenicity

3.2.4.1. Mice

In the study by Inai et al. (1989), B6C3F mice (8 weeks of age) were divided into 3 groups (50 animals/sex per group), two were given sodium erythorbate in their drinking water for 96 weeks and the third group was the control group was given distilled water. Males were given 0, 1.25 or 2.5% sodium erythorbate (equivalent[31] to 0, 1,875 and 3,750 mg/kg bw/day), and female were given 0, 2.5 or 5% sodium erythorbate (equivalent to 0, 3,750 and 7,500 mg/kg bw/day). At the end of the 96-week treatment, the mice were kept on a basal diet and distilled water for 14 weeks. The average body weights of the treated mice were generally similar to those of the controls but the final body weights of both surviving male and female mice were higher in the treated groups than in the controls. Also, a better survival of the mice given sodium erythorbate was observed. All mice were necropsied, tumour incidence and time of death recorded. The tumour incidence and the time to death with tumours did not differ significantly from those in the controls. The authors concluded that sodium erythorbate was not carcinogenic to mice on oral administration. The Panel agreed with this conclusion.

3.2.4.2. Rats

Lehman et al. (1951) studied the effect of erythorbic acid (given 1% in the diet; equivalent to 500 mg erythorbic acid/kg bw/day for 2 years) in rats (strain and age unspecified, 10 males/group). Growth rate, mortality and histopathology were not affected by the treatment but no information on the organs

[31] Calculated by the Panel according to EFSA Scientific Committee (2012).

was available. The Panel noted that the study was limited because only 10 animals/dose (only 4/10 survived at the end of the study), only one sex and one dose were studied.

The carcinogenicity of sodium erythorbate was investigated in a 2-year study in F344/DuCrj rats (52 males and 50 females/group; 8 weeks of age) by administering 1.25 or 2.5% (equivalent[31] to 650 and 1,300 mg/kg bw/day for males, and to 712.5 and 1,425 mg/kg bw/day for females) in drinking water. Control rats were given tap water for 120 weeks (Abe et al., 1984). The surviving animals were autopsied after a 16-week recovery period, when the animals received tap water. The rats were observed daily and body weights recorded weekly until termination at 120 weeks. Overall, the authors concluded that sodium erythorbate was not carcinogenic. Reduced body weight gain was evident at the 2.5% dose. The Panel, therefore, considered the NOAEL to be the 1.25% dose, equivalent to 650 mg sodium erythorbate/kg bw/day in males and 712.5 mg/kg bw/day in females for 2 years in the rat.

Fukushima et al. (1984) studied the promoting effects of ascorbic acid, sodium erythorbate on two-stage urinary bladder carcinogenesis in F344 rats initiated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine at a dose of 0.05% in the drinking water. Administration of 5% sodium erythorbate in the diet significantly increased the incidences of preneoplastic lesions, papillomas and malignant tumours of the urinary bladder, whereas administration of 5% ascorbic acid in the diet did not. Administrations of 5% sodium L-ascorbate and 5% sodium erythorbate caused increases in the pH, the sodium content and crystals of $MgNH_4PO_4$ in the urine, whereas ascorbic acid did not induce an increase in $MgNH_4$ crystals. According to the authors, these results showed that sodium erythorbate could promote urinary bladder carcinogenesis, contrary to ascorbic acid. The authors considered that there is a close relationship between the formation of these crystals due to the very high dose of exposure and promotion of urinary bladder carcinogenesis. The Panel agreed with this assumption and considered that this study was not relevant for risk assessment.

The Panel noted that there is no chronic toxicity study available.

The Panel considered that erythorbic acid or sodium erythorbate does not raise a concern with respect to carcinogenicity.

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Reproductive toxicity studies

No reproductive toxicity studies with erythorbic acid or sodium erythorbate were available.

3.2.5.2. Developmental toxicity studies

Sodium erythorbate was administered by gavage to mated female albinos CD-1 outbred mice on gestation days (GD) 6–15 (FDRL, 1974 (Doc. provided to EFSA n. 4)). The test volume was 10 mL/kg bw in water. Body weights were recorded at GD 0, 6, 11, 15 and 17. The mice were observed for appearance and behaviour, as well as feed consumption. All control and test mice survived to term. Of the control mice, 21 of 30 became pregnant. Of the mice given sodium erythorbate, the number of pregnant females per group was 22 of 25 (10.3 mg/kg), 20 of 25 (47.8 and 1,030 mg/kg) and 21 of 28 (221.9 mg/kg), respectively. All dams were subjected to caesarean section on day 17, and the numbers of implantation sites, resorption sites, and the number of live and dead fetuses were recorded. Fetal body weights were determined. The fetuses were examined for the presence of external (gross) congenital abnormalities, and one-third of the fetuses underwent detailed visceral examination. The remaining fetuses were examined for skeletal defects. A cleft palate was observed in a fetus of the 1,030 mg/kg treatment group. The authors concluded that the administration of up to 1,030 mg sodium erythorbate/kg bw/day to pregnant mice for 10 consecutive days had no treatment-related effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the controls. The Panel agreed with this conclusion.

Sodium erythorbate was administered by gavage to mated Wistar rats on GD 6-15 (FDRL, 1974 (Doc. provided to EFSA n. 4)). The test volume was 4 mL water/kg bw/day for the control group and highest dose group and 1 mL /kg bw/day for the other dose groups. Body weights were recorded on GD 0, 6, 15 and 17. The rats were observed for appearance and behaviour, as well as feed consumption. All rats of the control and test groups survived to term. Of the control rats, 20 of 24 became pregnant. Of the rats given sodium erythorbate, the number of pregnant females per group was 20 of 20 (9 and 41.8 mg/kg), 20 of 21 (194 mg/kg) and 20 of 24 (900 mg/kg), respectively. All dams were subjected to caesarean section on day 20, and the numbers of implantation sites, resorption sites, and the number of live and dead fetuses were recorded. Fetal body weights were determined. The fetuses were examined for the presence of external (gross) congenital abnormalities and one-third of the fetuses underwent detailed visceral examination. The remaining fetuses were examined for skeletal defects. The authors concluded that the administration of up to 900 mg sodium erythorbate/kg bw/day to pregnant rats for 10 consecutive days had no treatment-related effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the controls. The Panel agreed with this conclusion.

The potential teratogenicity of sodium erythorbate was investigated in mated Wistar rats (5–7 females/group; 12 weeks of age) (Ema et al., 1985). In addition, 5 pregnant females were allowed to litter and raise their pups until weaning. Pregnant rats were administered 0, 0.05, 0.5 or 5% erythorbate in the diet from GD 7–14. The average total amount of sodium erythorbate intake was calculated by the authors to be 280, 2,650 and 24,800 mg/kg bw/day for the 0.05, 0.5 and 5% dose level, respectively. The Panel recalculated the dose levels and considered to be equivalent[32] to 25, 250 and 2,500 mg/kg bw/day. On GD 20, 5–7 pregnant rats were selected and terminated, and live and dead fetuses recorded. The fetuses were removed and inspected for abnormalities. The placental weight was recorded and half of all fetuses were fixed and examined for skeletal anomalies; the other half were fixed and examined for internal anomalies. The remaining pregnant rats, including control animals, were allowed to deliver spontaneously. The day of delivery was designated day 0 after birth. Dead and alive new-borns were recorded, weighed, sexed and examined on the day of birth and allowed to suckle. The offspring were weaned on day 21 after birth. On the day of weaning, dams were terminated and the number of implantation remnants recorded. The offspring were weighed weekly. No negative effects were recorded for body weight gains and there were no clinical signs of toxicity in the dams. There was no significant difference between the treated group and the control group, in the incidence of intrauterine fetal death, live fetuses per dam, sex ratio of fetuses, fetal body weight and the placental weight. No abnormalities were observed as a result of external, internal and skeletal examinations of the fetuses. No dead new-borns were observed in any group. The live birth index (number of live new-borns at birth divided by number of implants) was similar in all groups. Normal growth and high survival rate were evident in the postnatal development of all offspring from the dams administered sodium erythorbate. The authors concluded that sodium erythorbate did not have developmental effects in rats under the conditions of the study. The Panel agreed with this conclusion and considered that 2,500 mg/kg bw/day, the highest dose tested, was the NOAEL in this study.

Overall, the Panel noted that there was no reproductive toxicity study available for erythorbic acid or sodium erythorbate. In prenatal developmental studies, no maternal and developmental effects were observed when sodium erythorbate was administered during organogenesis, up to a dose of 2,500 mg/kg bw/day.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available as then and the data available following a public call for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for re-evaluation by the Panel.

[32] Calculated by the Panel according to EFSA Scientific Committee (2012).

Erythorbic acid (E 315) and sodium erythorbate (E 316) are authorised as food additives in the EU in accordance with Annex II to Regulation 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

JECFA evaluated erythorbic acid and sodium erythorbate in 1962, 1974 and 1990 and in its latest evaluation allocated an ADI 'not specified'. The SCF evaluated erythorbic acid and sodium erythorbate in 1987, 1990 and 1997, and an ADI of 6 mg/kg bw/day was confirmed in the latest evaluation.

The ADME of erythorbates was considered to be similar to that of ascorbic acid. The sodium ion of sodium erythorbate is expected to enter the sodium pool of the body. Although rodents seemed to have less efficient erythorbate absorption than humans, the available study in mouse indicated that gastrointestinal absorption occurs (Tsao and Salimi, 1983). Guinea pig, a species more analogous to human due to its active-carrier mediated transport, has near complete excretion within 24 h (Hornig, 1975).

The Panel noted that erythorbic acid can increase iron bioavailability which may represent a concern for individuals with iron deposition disorders.

The Panel noted that the acute toxicity of erythorbic acid or sodium erythorbate is low.

The Panel noted that in the three available subchronic toxicity studies there were some limitations mainly concerning reporting. However, none of them reported any adverse effects and there was no histopathological indication of any adverse effects even after 36-week of exposure up to 900 mg/kg bw/day.

Data on genotoxicity were available for erythorbic acid and its sodium salt. The Panel noted that the reliability of most genotoxicity studies was limited or insufficient and that, accordingly, the relevance of their results was limited or low. However, there were also studies of higher reliability and relevance, i.e. a dominant lethal assay, a heritable translocation test and a Comet assay, and these *in vivo* studies were negative. Overall, the Panel concluded that based on the available data there is no concern with respect to genotoxicity of erythorbic acid or sodium erythorbate.

The Panel noted that there is no chronic toxicity study available, but considered from the available carcinogenicity studies that erythorbic acid or sodium erythorbate did not raise a concern with respect to carcinogenicity. The only reported adverse effect was a decrease in body weight at 1,300 mg/kg bw/day in one study in male rats and the Panel identified a NOAEL of 650 mg/kg bw/day from this study.

No reproductive toxicity studies with erythorbic acid or sodium erythorbate were available. However, no histopathological effects were observed on male reproductive organs in a 36-week study. In prenatal developmental studies no maternal and developmental effects were observed when sodium erythorbate was administered during organogenesis.

The Panel recognised the limitation of the overall toxicological database (no reproductive and chronic toxicity studies). However, taking into account that erythorbic acid or sodium erythorbate gave negative results in a subchronic toxicity study up to 36 weeks, in genotoxicity studies, in carcinogenicity studies and in developmental toxicity studies, the Panel did not consider necessary to increase the usual uncertainty factor of 100 in deriving an ADI. Therefore, the Panel considered that there is no reason to revise the current ADI of 6 mg/kg bw/day based on the decreased body weight reported in one carcinogenicity study,

To assess the combined dietary exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additives, the exposure was calculated based on (1) MPLs set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage or analytical data (defined as the *refined exposure assessment scenario*).

Using the *regulatory maximum level exposure assessment scenario*, mean combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additives ranged from 0.05 to 1.52 mg/kg bw/day in six population groups. The high combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) using this scenario ranged from 0.15 to 4.40 mg/kg bw/day.

The refined combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) was up to 0.29 mg/kg bw/day in toddlers for the mean and 0.70 mg/kg bw/day in toddlers for the 95th percentile using the *brand-loyal* exposure scenario. For the non-*brand-loyal* exposure scenario, the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) was up to 0.06 mg/kg bw/day in toddlers for the mean and 0.13 mg/kg bw/day in toddlers and children for the 95th percentile

The Panel noted that in both exposure scenarios, all combined exposure estimates were below the ADI of 6 mg/kg bw/day. The Panel considered that the uncertainties identified in the exposure assessment would rather lead to an overestimation than an underestimation.

CONCLUSIONS

The Panel concluded that there is no reason to revise the ADI of 6 mg/kg bw/day.

Considering that the ADI is not exceeded in any population group, the Panel also concluded that the use of erythorbic acid (E 315) and sodium erythorbate (E 316) as food additives at the permitted or reported use and use levels would not be of safety concern.

RECOMMENDATIONS

The Panel recommended that the maximum limits for the impurities of toxic elements (arsenic, lead and mercury) in the EC specification for erythorbic acid (E 315) or sodium erythorbate (E 316) should be revised in order to ensure that erythorbic acid (E 315) or sodium erythorbate (E 316) as food additives will not be a significant source of exposure to those toxic elements in food.

DOCUMENTATION AS PROVIDED TO EFSA

1. EMCESA (Embutidos del centro, SA), 2014. Data on usage levels of erythorbic acid (E 315) and sodium erythorbate (E 316) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 29 August 2014.

2. FASEB (Federation of American Societies for Experimental Biology) Report (1979). Evaluation of the health aspects of ascorbic acid, sodium ascorbate, calcium ascorbate, erythorbic acid, sodium erythorbate and ascorbyl palmitate as food ingredients. Report prepared for the Bureau of Foods, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D.C., Contract No. FDA 223-75-2004. Submitted by the FDA, April 2015.

3. FDE (FoodDrinkEurope), 2014. Data on usage levels of erythorbic acid (E 315) and sodium erythorbate (E 316) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 September 2014.

4. FDRL (Food and Drug Research Laboratories), 1974. Teratologic evaluation of FDA 71-68 (sodium erythorbate) in mice and rats. Final report, prepared under DHEW contract no. FDA 223-74-2176. Submitted by the FDA, January, 2015.

5. Food Additive Safety Profile, May 2, 1995. Submitted by FDA in response to an FOI request, 4/17/95. Submitted by the FDA, April 2015.

6. Isoacorbic acid (Submitted by Pfizer, 23 March 1990). Scientific Committee for Food. CS/ANT/21. April 1990. Submitted to EFSA from the SCF's archive.

7. Litton Bionetics (1974). Mutagenic evaluation of compound FDA 71-66, erythorbic acid. Report prepared under DHEW contract no FDA 223-74-2104. Submitted by the FDA, April 2015.

8. Newell GW, Jorgenson TA and Simmon VF (1974). Study of the mutagenic effects of sodium erythorbate (FDA No. 71-68). Compound Report No. 4 prepared for U.S. Food and Drug Administration, HEW, Rockville, Maryland. Submitted by the FDA, April 2015.

9. Pre-evaluation document prepared by the Peter Fisk Associates Ltd, United Kingdom. February, 2013.

10. SCF (Scientific Committee for Food), 1995. Isoascorbic acid – monograph– Monograph (Submitted by Prof, P.S. Elias, 8 January 1995 CS/ANT/23-Rev. 2. Submitted to EFSA from the SCF's archive.

REFERENCES

Abe I, Saito S, Hori K, Suzuki M and Sato H, 1984. Sodium erythorbate is not carcinogenic in F344 rats. Experimental and Molecular Pathology, 41, 35-43

Aboul-Enein HY, Al-Duraibi IA, Raluca-Ioan S, Radoi C and Avramescu A, 1990. Analysis of L- and D-ascorbic acid in fruits and "fruitdrinks by HPLC. Seminars in Food Analysis, 4, 31-37.

Andersen FA, 1999. Final report on the safety assessment of ascorbyl palmitate, ascorbyl dipalmitate, ascorbyl stearate, erythorbic acid and sodium erythorbate. International Journal of Toxicology, 18, 1-26.

Baker EM, Tillotspm JA, Kennedy JE and Halver JE, 1973. Comparative metabolism of C14 labeled ascorbic and isoascorbic acid. Federation Proceedings, Abstract, 4009, 665 (as referred to by SCF, 1997).

Barros AI, Silva AP, Gonçalves B and Nunes FM, 2010. A fast, simple, and reliable hydrophilic interaction liquid chromatography method for the determination of ascorbic and isoascorbic acids. Anal Bioanal Chem, 396, 1863-1875.

Bognár A and Daood HG, 2000. Simple in-line postcolumn oxidation and derivatization for the simultaneous analysis of ascorbic and dehydroascorbic acids in foods. Journal of Chromatographic Science, 38, 162-168.

Borenstein B, 1965. The comparative properties of isoascorbic acid and erythorbic acid. Food. Technology, 19, 1719-1722.

Chen MJ, Chen HS, Lin CY and Chang HT, 1999. Indirect detection of organic acids in non-aqueous capillary electrophoresis. Journal of Chromatography A, 853, 171-180.

Clairol, 1996. Mutagenicity of erythorbic acid. Correspondence from JF Corbett, dated June 14, 1996 (1 page) (as referred to by Andersen, 1999).

Davey MW, Bauw G and Montagu MV, 1996. Analysis of ascorbate in plant tissues by high-performance capillary zone electrophoresis. Analytical Biochemistry, 239, 8-19.

Drivelos S, Dasenaki ME and Thomaidis NS, 2010. Determination of isoascorbic acid in fish tissue by hydrophilic interaction liquid chromatography-ultraviolet detection. Analytical and Bioanalytical Chemistry, 397, 2199-2210.

EFSA (European Food Safety Authority), 2006. Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;438, 54 pp. doi:10.2903/j.efsa.2006.438

EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. doi:10.2903/j.efsa.2010.1557

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. doi:10.2903/j.efsa.2011.1970

EFSA Scientific Committee, 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. doi:10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. doi:10.2903/j.efsa.2009.1351

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. doi:10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. doi:10.2903/j.efsa.2012.2985

Ema M, Itami T and Kanoh S, 1985. Effect of sodium erythorbate on pregnant rats and their offpring. Journal of Food Hygienic Society Japan, 26, 448-453.

Esselen WB, Powers JJ and Woodward R, 1945. D-Isoascorbic acid as an antioxidant. Industrial Engineering and Chemistry, 37 (3), 295.

Fidler CF, Davidson L, Zeder C and Hurrell R, 2004. Erythorbic acid is a potent enhancer of nonheme-iron absorption. The American Journal of Clinical Nutrition, 79, 99-102.

Fitzhugh OG and Nelson AA, 1946. Subacute and chronic toxicities of ascorbyl palmitates. Proceedings of the Society for Experimental Biology and Medicine, 61, 195-198.

Fontannaz P, Kilinç T and Heudi O, 2006. HPLC-UV determination of total vitamin C in a wide range of fortified food products. Food Chemistry, 94, 626-631.

Fukushima S, Kurata Y, Shibata MA, Ikawa E and Ito N, 1984. Promotion by ascorbyl acid, sodium erythorbate and ethoxyquin of neoplastic lesions in rats initiated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Cancer letters 23, 29-37.

Greger JL, Lee K, Graham KL, Chinn BL and Liebert JC, 1984. Iron, zinc and copper metabolism of human subjects fed nitrite and erythorbate cured meats. Journal of Agricultural Food Chemistry, 32, 861-865.

Hayashi M, Kishi M, Sofuni T and Ishidate MJ, 1988. Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology, 26, 487-500.

Hidiroglou N, Madere R and Behrens W, 1998. Electrochemical determination of ascorbic acid and isoascorbic acid in ground meat and processed foods by high pressure liquid chromatography. Journal of Food Composition and Analysis, 11, 89-96.

Hornig D, 1975. Distribution of ascorbic acid metabolites and analogues in man and animals. Annals of the New York Academy of Sciences, 258, 103-118.

Hornig D and Weisser H, 1976. Interaction of erythorbic acid with ascorbic acid catabolism. International Journal for Vitamin and Nutrition Research, 46, 40-47.

Hornig D, 1977. Interaction of erythorbic acid with ascorbic acid catabolism, 1977. Acta vitaminologica et enzymologica, 31, 9-14.

Hvoslef J and Petersen B, 1981. Structure of dehydroisoascorbic acid isomers in solution. Carbohydrate Research, 92, 9-20.

Hughes RE and Hurley JE, 1969. The uptake of D-araboascorbic acid(D-isoascorbic acid) by guinea-pig tissues. British Journal of Nutrition, 23, 211-216.

Hughes RE and Jones PR, 1970. D-Araboascorbic acid and guine-pig scurvy. Nutrition report international, 1, 275-279.

Inai K, Akamizu H, Eto R, Nishida T, Ohe K, Kobuke T, Nambu S, Matsuki K and Tokuoka S, 1989. Tumorigenicity study of sodium erythrobate administered orally to mice. Hiroshima journal of medical sciences, 38, 135-140.

Ishidate M Jr., Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chemistry and Toxicology, 22, 623-636.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1962. World health Organization. Technical Report Series No. 228. Evaluation of the toxicity of a number of antimicrobials and antioxidants. Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland. Available online: http://apps.who.int/iris/bitstream/10665/40518/1/WHO_TRS_228.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO Food Additive Series 5. World Health Organization. Geneva, Switzerland. Available online: http://www.inchem.org/documents/jecfa/jecmono/v05je28.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1991. Erythorbic acid and its sodium salt. First draft prepared by Dr R Walker. Available online: http://www.inchem.org/documents/jecfa/jecmono/v28je03.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Monograph 1. Combined compendium of food additive specifications. Available online: http://www.fao.org/ag/agn/jecfa-additives/search.html

Jorgenson TA, Rushbrook CJ, Newell GW and Green S, 1978. In vivo mutagenesis investigations of four GRAS chemicals. Mutation Research 53, 205.

Kall MA and Andersen C, 1999. Improved method for simultaneous determination of ascorbic acid and dehydroascorbic acid, isoascorbic acid and dehydroisoascorbic acid in food and biological samples. Journal of Chromatography B, 730, 101-111.

Kawachi T, Komatsu T, Kada T, Ishidate M, Sasaki M, Sugiyama T and Tazima Y, 1980. Results of recent studies on the relevance of various short-term screening tests in Japan. Applied Methods in Oncology, 3, 253-267.

Klimisch HJ, Andreae M, Tillmann U, 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, 25, 1-5.

Lehman AJ, Fitzhugh OG, Nelson AA and Woodard G, 1951. The pharmacological evaluation of antioxidants. Advances in Food Research, 3, 197-208.

Liao T, Wu JS, Wu MC and Chang HM, 2000. Epimeric separation of L-ascorbic acid and D-isoascorbic acid by capillary zone electrophoresis. Journal of Agricultural and Food Chemistry, 48, 37-41.

Ling LB, Baeyens WR, Van Acker P and Dewaele C, 1992. Determination of ascorbic acid and isoascorbic acid by capillary zone electrophoresis: application to fruit juices and to a pharmaceutical formulation. Journal of Pharmaceutical and Biomedical Analysis, 10, 717-721.

Madhavi DL, Singhal RS and Kulkarni PR, 1995. Food Antioxidants. Technological, toxicological and health perspectives. Eds Madhavi DL, Despande SS and Salunkhe DK. Marcel Dekker Inc. New York, 209–210.

Matsuoka A, Hayashi A and Ishidate JrM, 1979. Chromosomal aberration tests on 29 chemical combined with S9 mix in vitro. Mutation Rearch, 66, 277-290.

Mellors AJ, Nahrwold DL and Rose RC, 1977. Ascorbic acid flux across mucosal border of guinea pig and human ileum. American Journal of Physiology, 223, E374-E379.

Naval Research Laboratory, 2000. Cleaning method of VLF cooling water system development and history of application with step-by-step procedure. Lamontagne RA. NRL/MR/6110-00-8471. 30 June, 2000.

Nonaka M, 1989. DNA repair tests on food additives. Environmental and Molecular Mutagenesis, 14 (Suppl. 15), 143.

OIV (International Organisation of Vine and Wine), 2008. Simultaneous determination of L-ascorbic acid and D-iso-ascorbic acid (erythorbic acid) in wine by HPLC and UV-detection (Resolution Oeno 11/2008) Method OIV-MA-AS313-22. Available online: http://www.oiv.int/oiv/files/6%20-%20Domaines%20scientifiques/6%20-%204%20Methodes%20d%20analyses/6-4-1/EN/OIV-MA-AS313-22.pdf

Pelletier O, 1969, Differential determination of D-isoascorbic acid and L-ascorbic acid in guinea pig organs. Canadian Journal of Biochemistry, 47, 449-453.

Pelletier O, Godin C. 1969, Vitamin C activity of D-isoascorbic acid for the guinea pig. Canadian Journal of Physiology and Pharmacology, 47, 985-991.

Peters JH et al., 1983. Effects of ascorbic and erythorbic acids on the Ames/Salmonella assay of promutagens, mutagens and human urine concentrates. Modulation and mediation of cancer by vitamins pp 104-133. Kager, Basel (as referred to by SCF, 1997).

Phillips AL, Mancini R, Sun Q, Lynch MP and Faustman C, 2001. Effect of erythorbic acid on cooked color in ground beef. Meat Science, 57, 31-34.

Reyes P and Luh BS, 1962. Ascorbic and isoascorbic acids as antioxidants for frozen Freestone peaches. Food Technology, 16, 116-118.

Rivers JM, Huang ED and Dodds ML, 1963. Human metabolism of L-ascorbic acid and erythorbic Acid. Journal of Nutrition, 81, 163-168.

Robinson and Umbreit, 1956. Excretion after oral administration to fasting dogs. Unpublished report. Submitted to WHO by Pfizer Inc (as referred to by SCF, 1997).

Sádecká J and Polonský J, 2001. Determination of acsorbic and isoascsorbic acid in beverages and additives to meat products by capillary isotachophoresis. European Food Research and Technology, 212, 511-517.

Sadoogh-Abasian F and evered DF, 1979. Absorption of vitamin C from human buccal cavity. British Journal of Nutrition, 42, 15-20.

Sasaki S et al., 1980. Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. La Kromosomo II, 20, 574 (as referred to by SCF, 1997).

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K and Tsuda S, 2002. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research, 519, 103-119.

Sauberlich HE, Kretsch MJ, Taylor PC, Johnson ML and Skala JH, 1989. Ascorbic acid and erythorbic acid metabolism in nonpregnant women. American Journal of Clinical Nutrition, 50, 1039-1049.

Siliprandi L, Vanni I, Kessler M, and Semenza G, 1979. Na-dependent, electroneutral L-ascorbate transport across brush border membrane vesicles from guinea pig small intestine. Biochimica et Biophysica Acta, 552, 129-142.

Suzuki E, Kurata T, Koda, M and Arakawa N, 1987. Erythorbic acid content in tissues of guinea pigs administered erythorbic acid. Journal of Nutritional Science and Vitaminology, 33, 169-175.

SCF (Scientific Committee for Food), 1987. Reports of the Scientific Committee for Food (22nd series). Reports of the Scientific Committee for Food on antioxidant. (Opinion on antioxidants expressed on 11 December 1987) report dated 1987.

SCF (Scientific Committee for Food), 1990. Reports of the Scientific Committee for Food (26th series). Opinions of the Scientific Committee for Food on: Second series of food additives of various technological functions. (Opinion expressed on 19 October 1990).

SCF (Scientific Committee for Food), 1997. Reports of the Scientific Committee for Food (Thirty-sixth series). Opinion on iso-ascorbic acid. Expressed on 3 March 1995, pp 43-62.

Schulte KE and Schillinger A, 1952. Comparison of the kinetics of non-fermentative oxidation of D-isoascorbic and L-ascorbic acids. Z. Lebensmittel-Untersuchung, 94(2), 77-85.

Shibata MA, Kurata Y, Ogiso T, Masuo T and Fukushima S, 1985. Response of urinary constituents and bladder epithelium to administration of chemicals and their sodium salts possessing promoting potential sodium salts. Journal of Toxicological Sciences, 10, 264.

Tai A and Gohda E, 2007. Determination of ascorbic acid and its related compounds in foods and beverages by hydrophilic interaction liquid chromatography. Journal of Chromatography B, 853, 214-220.

TemaNord, 2002. Food Additives in Europe, 2000. Status of safety assessment of food additives presently permitted in the EU. TemaNord, 2002, 272–275.

Toggenburger G, Landoldt M and Semenza G, 1979. Na^+-dependent, electroneutral L-ascorbate transport across brush border membrane vesicles from human small intestine. FEBS letters, 108, 473-476.

Tsao CS and Salimi SL, 1983. Influence of erythorbic acid on ascorbic acid retention and elimination in the mouse. International Journal of Nutrition Research, 53, 258-264.

Turnbull JD, Sauberlich HE and Omaye ST, 1979. Effects of ascorbic acid deficiency and of erythorbic acid on blood components in the cynomolgus monkey. International Journal for Vitamin and Nutrition Research, 49, 92-102.

Wang MM, Fisher KH and Dodds ML, 1962. Comparative metabolic response to erythorbic acid and ascorbic acid by the human. Journal of nutrition, 77, 443-447.

WHO (World Health Organization), 2009. Principles and methods for the risk assessment of chemicals in food, International Programme on Chemical Safety, Environmental Health Criteria 240. Chapter 2: risk assessment and its role in risk analysis.

Zeiger E, 1993. Mutagenicity of chemicals added to foods. Mutation Research 209, 53-61.

APPENDICES

Appendix A. Data provided by industry on erythorbic acid (E 315) and sodium erythorbate (E 316) per food category.

FCS category number	FCS food category description	Provided by	N samples	Usage levels (mg/kg) (mean)		
				Min	Typical	Max
6.4.5	Fillings of stuffed pasta (ravioli and similar)	FDE	1	80.0	80.0	80.0
7.2[33]	Fine bakery wares	FDE	1	36	48	60
8.3.1	Non-heat-treated meat products	EMCESA	34	0.9	0.9	0.9
8.3.2	Heat-treated meat products	EMCESA	13	48.4	66.8	92.4
8.3.2	Heat-treated meat products	FDE	5	170.6	236.8	329
18[34]	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	FDE	3	23.7	58.7	164

[33] FCS 7.2 Pancakes (i.e. pancakes and crepes with cheese and ham)

[34] Includes FCS 6.2.2 starches (i.e. 'rice with ham or other meat'), FCS 16 'Desserts excluding products covered in category 1, 3 and 4' (i.e. Croissants and pies with ham, bacon or another kind of meat), FCS 18 'Pizza and pizza-like pies, cheese, meat, mushrooms, and vegetables'.

Appendix B. Summary of analytical results (middle bound mg/kg or mg/L as appropriate) of erythorbic acid as provided by Member States.

FCS category number	FCS food category description	MPL	n	%LC	Range				All data[a]				
					LOD		LOQ		Min	Median	Mean	P95[b]	Max
1.4	Flavoured, fermented milk products including heat-treated products	-	1	100	20	20	20	20	10	10	10	10	10
2	Fats and oils and fat and oil emulsions	-	18	100	0.01	0.07	0.03	0.17	0.02	0.09	0.05	0.09	0.09
4.2.4	Fruit and vegetable preparations, excluding products covered by 5.4	-	6	100	20	20	20	20	10	10	10	10	10
5.2	Other confectionery including breath-refreshing microsweets	-	1	100	17	17	50	50	8.50	8.50	8.50	8.50	8.50
8.1	Fresh meat, excluding meat preparations as defined by Regulation (EC) No 853/2004 (M42) [e]	-	280	99	6.67	50	20	100	4	10	13.9	25	236.5
8.3.1 and 8.3.2	Heat- and non-heat-treated meat products	500	4558	88.8	6.67	50	20	101	4	10	25.3	132	496
9.1.1	Unprocessed fish	1500	1	100	20	20	20	20	10	10	10	10	10
9.2	Processed fish and fishery products including molluscs and crustaceans	1500	132	100	0.01	50	0.03	100	0.02	10	8.08	10	25
12.2.2	Seasonings and condiments	-	2	100	20	20	20	20	10	10	10	10	10
12.6	Sauces	-	2	100	20	20	20	20	10	10	10	10	10
12.7	Salads and savoury-based sandwich spreads [c]	-	20	90	8	50	20	100	4	10	21.6	114	176
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	-	6	100	10	200	25	500	12.5	125	129.2	250	250
14.1.4	Flavoured drinks	-	1	100	40	40	100	100	50	50	50	50	50
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	-	3	100	17	17	50	50	25	25	25	25	25
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	-	24	100	15	50	20	100	7.5	10	10.7	25	25

(a): Under the middle bound assumption.
(b): The 95th percentile based on occurrence data with fewer than 60 analytical results are not reported in the table (EFSA, 2011a).
(c): Prepared meat salads.
%LC: Percentage of left-censored data; Max, maximum; Min, minimum; n, number of data; P95, 95th percentile.

Appendix C. Concentration levels[a] used in the regulatory maximum level and refined exposure scenarios (mg/kg)

FCS category number	FCS food category description	MPL [b]	Concentration levels used in the refined exposure assessment		Data source/comments
			Mean	P95	
6.4.5 [c]	Fillings of stuffed pasta (ravioli and similar)	250	80	80	Usage levels
8.3.1 and 8.3.2	Heat-treated and non-heat-treated meat products, only cured meat products and preserved meat products	500	25.3	132	Analytical data
9.1.1[d]	Unprocessed fish, only frozen and deep-frozen fish with red skin	-	-	-	No data available
9.2	Processed fish and fishery products, only preserved and semi-preserved fish products	1500	8.1	10.0	Analytical data
9.3	Fish roe	1500	-	-	No data available

(a): The additives may be added individually or in combination.
(b): MPL = maximum permitted level
(c): Assuming to consist for 50% of meat.
(d): No food consumption data available.

Appendix D. Summary of total estimated combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from their use as food additives for the regulatory maximum level scenario and the two refined exposure scenarios per population group and survey: mean and high level (mg/kg bw/day)

		Number of subjects	Regulatory maximum level scenario		Refined exposure scenarios			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	P95	Mean	P95	Mean	P95
Infants								
Bulgaria	NUTRICHILD	659	0.05	0.15	0.01	0.04	0.00	0.01
Germany	VELS	159	0.20	1.27	0.05	0.29	0.01	0.06
Denmark	IAT 2006_07	826	0.70	2.68	0.12	0.48	0.02	0.09
Finland	DIPP_2001_2009	500	0.20	0.81	0.05	0.21	0.01	0.04
United Kingdom	DNSIYC_2011	1369	0.34	2.06	0.04	0.25	0.01	0.05
Italy	INRAN_SCAI_2005_06	12	0.06	-	0.02	-	0.00	-
Toddlers								
Belgium	Regional_Flanders	36	1.10	-	0.25	-	0.05	-
Bulgaria	NUTRICHILD	428	0.38	1.56	0.10	0.41	0.02	0.08
Germany	VELS	348	0.96	2.67	0.21	0.58	0.04	0.11
Denmark	IAT 2006_07	917	1.52	3.45	0.29	0.69	0.06	0.13
Spain	enKid	17	0.97	-	0.26	-	0.05	-
Finland	DIPP_2001_2009	500	0.43	1.66	0.11	0.43	0.02	0.08
United Kingdom	NDNS-RollingProgrammeYears1-3	185	1.49	4.27	0.19	0.61	0.04	0.12
United Kingdom	DNSIYC_2011	1314	1.16	4.40	0.14	0.53	0.03	0.11
Italy	INRAN_SCAI_2005_06	36	0.74	-	0.13	-	0.03	-
Netherlands	VCP_kids	322	1.11	3.39	0.24	0.70	0.05	0.13
Children								
Austria	ASNS_Children	128	1.00	3.59	0.19	0.45	0.04	0.09
Belgium	Regional_Flanders	625	1.15	3.27	0.25	0.64	0.05	0.12
Bulgaria	NUTRICHILD	433	0.58	2.29	0.15	0.60	0.03	0.12
Czech Republic	SISP04	389	0.71	2.27	0.17	0.54	0.03	0.10
Germany	EsKiMo	835	0.93	2.65	0.19	0.53	0.04	0.10
Germany	VELS	293	1.03	2.88	0.20	0.45	0.04	0.09
Denmark	DANSDA 2005-08	298	0.83	1.65	0.19	0.40	0.04	0.08
Spain	enKid	156	1.11	3.30	0.26	0.66	0.05	0.13
Spain	NUT_INK05	399	1.01	2.96	0.23	0.55	0.04	0.11
Finland	DIPP_2001_2009	750	0.81	2.31	0.20	0.55	0.04	0.11
France	INCA2	482	1.14	2.94	0.16	0.39	0.03	0.08

		Number of subjects	Regulatory maximum level scenario		Refined exposure scenarios			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	P95	Mean	P95	Mean	P95
United Kingdom	NDNS-RollingProgrammeYears1-3	651	1.20	3.34	0.19	0.51	0.04	0.10
Greece	Regional_Crete	838	0.41	1.89	0.08	0.45	0.02	0.09
Italy	INRAN_SCAI_2005_06	193	0.95	3.80	0.13	0.39	0.03	0.08
Latvia	EFSA_TEST	187	0.58	2.05	0.14	0.50	0.03	0.09
Netherlands	VCP_kids	957	0.99	2.83	0.20	0.56	0.04	0.11
Netherlands	VCPBasis_AVL2007_2010	447	0.99	2.76	0.22	0.60	0.04	0.12
Sweden	NFA	1473	1.35	3.85	0.21	0.51	0.04	0.10
Adolescents								
Austria	ASNS_Children	237	0.51	1.53	0.11	0.32	0.02	0.06
Belgium	Diet_National_2004	576	0.33	1.16	0.07	0.23	0.01	0.04
Cyprus	Childhealth	303	0.18	0.54	0.04	0.13	0.01	0.03
Czech Republic	SISP04	298	0.74	2.21	0.17	0.52	0.03	0.10
Germany	National_Nutrition_Survey_II	1011	0.41	1.44	0.10	0.31	0.02	0.06
Germany	EsKiMo	393	0.71	2.11	0.15	0.43	0.03	0.08
Denmark	DANSDA 2005-08	377	0.33	0.90	0.08	0.21	0.01	0.04
Spain	AESAN_FIAB	86	0.50	1.22	0.12	0.29	0.02	0.06
Spain	enKid	209	0.81	2.67	0.17	0.52	0.03	0.10
Spain	NUT_INK05	651	0.61	1.72	0.14	0.39	0.03	0.07
Finland	NWSSP07_08	306	0.32	0.94	0.08	0.22	0.01	0.04
France	INCA2	973	0.53	1.34	0.09	0.21	0.02	0.04
United Kingdom	NDNS-RollingProgrammeYears1-3	666	0.55	1.71	0.11	0.34	0.02	0.06
Italy	INRAN_SCAI_2005_06	247	0.45	1.35	0.09	0.25	0.02	0.05
Latvia	EFSA_TEST	453	0.49	1.55	0.12	0.35	0.02	0.07
Netherlands	VCPBasis_AVL2007_2010	1142	0.65	1.97	0.14	0.42	0.03	0.08
Sweden	NFA	1018	0.83	2.50	0.13	0.33	0.03	0.06
Adults								
Austria	ASNS_Adults	308	0.35	1.29	0.09	0.33	0.02	0.06
Belgium	Diet_National_2004	1292	0.32	1.08	0.07	0.22	0.01	0.04
Czech Republic	SISP04	1666	0.54	1.65	0.14	0.42	0.03	0.08
Germany	National_Nutrition_Survey_II	10419	0.37	1.20	0.09	0.26	0.02	0.05
Denmark	DANSDA 2005-08	1739	0.27	0.66	0.06	0.16	0.01	0.03
Spain	AESAN	410	0.41	1.22	0.09	0.29	0.02	0.06
Spain	AESAN_FIAB	981	0.40	1.05	0.10	0.24	0.02	0.05
Finland	FINDIET2012	1295	0.35	1.26	0.08	0.27	0.02	0.05

		Number of subjects	Regulatory maximum level scenario		Refined exposure scenarios			
					Brand-loyal scenario		Non-brand-loyal scenario	
			Mean	P95	Mean	P95	Mean	P95
France	INCA2	2276	0.37	0.93	0.07	0.17	0.01	0.03
United Kingdom	NDNS-RollingProgrammeYears1-3	1266	0.38	1.18	0.07	0.22	0.01	0.04
Hungary	National_Repr_Surv	1074	0.49	1.25	0.13	0.33	0.03	0.06
Ireland	NANS_2012	1274	0.30	0.86	0.08	0.22	0.01	0.04
Italy	INRAN_SCAI_2005_06	2313	0.24	0.74	0.06	0.17	0.01	0.03
Latvia	EFSA_TEST	1271	0.35	1.15	0.08	0.27	0.02	0.05
Netherlands	VCPBasis_AVL2007_2010	2057	0.46	1.59	0.09	0.28	0.02	0.05
Romania	Dieta_Pilot_Adults	1254	0.32	0.90	0.08	0.23	0.02	0.04
Sweden	Riksmaten 2010	1430	0.50	1.63	0.07	0.22	0.01	0.04
Elderly and very elderly								
Austria	ASNS_Adults	92	0.32	1.20	0.08	0.32	0.02	0.06
Belgium	Diet_National_2004	1215	0.26	0.80	0.06	0.20	0.01	0.04
Germany	National_Nutrition_Survey_II	2496	0.34	0.98	0.08	0.22	0.02	0.04
Denmark	DANSDA 2005-08	286	0.25	0.53	0.05	0.12	0.01	0.02
Finland	FINDIET2012	413	0.27	0.90	0.06	0.22	0.01	0.04
France	INCA2	348	0.30	0.78	0.06	0.16	0.01	0.03
United Kingdom	NDNS-RollingProgrammeYears1-3	305	0.33	1.15	0.06	0.17	0.01	0.03
Hungary	National_Repr_Surv	286	0.38	0.97	0.10	0.26	0.02	0.05
Ireland	NANS_2012	226	0.30	0.91	0.08	0.24	0.01	0.05
Italy	INRAN_SCAI_2005_06	518	0.17	0.53	0.04	0.13	0.01	0.02
Netherlands	VCPBasis_AVL2007_2010	173	0.39	1.35	0.08	0.24	0.02	0.05
Netherlands	VCP-Elderly	739	0.30	0.98	0.06	0.18	0.01	0.03
Romania	Dieta_Pilot_Adults	128	0.28	0.80	0.07	0.20	0.01	0.04
Sweden	Riksmaten 2010	367	0.51	1.81	0.07	0.21	0.01	0.04

Appendix E. Main food categories contributing to the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316)

Table E1: Main food categories contributing to the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) according the regulatory maximum level exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing.

FCS category number	FCS category description	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
8.3.1, 8.3.2	Heat- and non-heat-treated- meat products, meat products	41.8-100 (6)	44.6-100 (10)	49.7-100 (18)	58.7-94.7 (17)	54.5-100 (17)	52.1-100 (14)
9.2	Processed fish and fishery products, only preserved and semi-preserved fish products	9.4-57.8 (3)	13.7-55.4 (7)	5.3-50.3 (17)	5.3-39.8 (17)	5.87-38.2 (13)	5.4-42.0 (11)
9.3	Fish roe	19.0 (1)	10.2 (1)	6.4 (1)	5.1 (1)	6.1-7.3 (2)	5.4-5.9 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

Table E2: Main food categories contributing to the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from its use as a food additive according to the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is a contributor.

FCS category number	FCS category description	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
8.3.1, 8.3.2	Heat- and non-heat-treated-meat products, meat products	96.6-100 (6)	96.6-100 (10)	97.5-100 (18)	98.3-99.9 (17)	98.3-100 (17)	98.0-100 (14)
9.2	Processed fish and fishery products including molluscs and crustaceans	0.3-3.4 (3)	0.4-3.0 (7)	0.1-2.5 (17)	0.1-1.7 (17)	0.2-1.7 (13)	0.2-2.0 (11)
9.3	Fish roe	0 (1)	0 (1)	0 (1)	0 (1)	0 (2)	0 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

Table E3: Main food categories contributing to the combined exposure to erythorbic acid (E 315) and sodium erythorbate (E 316) from its use as a food additive according to the non-brand-loyal exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is a contributor.

FCS category number	FCS category description	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
8.3.1, 8.3.2	Heat- and non-heat-treated- meat products, meat products	87.1-100 (6)	88.3-100 (10)	90.3-100 (18)	93.2-99.4 (17)	93.0-100 (17)	92.1-100 (14)
9.2	Processed fish and fishery products including molluscs and crustaceans	1.1-12.9 (3)	1.7-11.7 (7)	0.6-9.7 (17)	0.6-6.8 (17)	0.7-7.0 (13)	0.6-7.9 (11)
9.3	Fish roe	0 (1)	0 (1)	0 (1)	0 (1)	0 (2)	0 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific population.

Appendix F. Summary of the available *in vitro* and *in vivo* genotoxicity studies

Reliability (validity):

1. reliable without restriction (valid without restriction)
2. reliable with restrictions (valid with restrictions or limited validity)
3. insufficient reliability (insufficient validity)
4. reliability cannot be evaluated (validity cannot be evaluated)
5. reliability not evaluated since the study is not relevant and/or not required for the risk assessment

The reliability criteria are based on Klimisch et al. (1997) as recommended by the Scientific Committee in its scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011). The relevance of the study result is based on its reliability and on the relevance of the test system (genetic endpoint): High, limited or low.

In vitro studies

Test System	Test Object	Test material	Concentration	Result	Reference	Reliability/Comments	Relevance of the test System concerning the genetic endpoint	Relevance of the result
Bacterial gene mutation	*S.* Typhimurium TA1535; TA1537; TA1538	Erythorbic acid	0.25 and 0.5%	Negative	Litton Bionetics, 1974	**Reliability: 3** This study was limited, because it uses an old method with a limited number of strains and only one dose.	High	Low
Yeast gene mutation	*Saccharomyces cerevisiae* strain D4	Erythorbic acid	2 and 4%	Negative		**Reliability: 4** The study is limited in the protocol and the assay did not receive further validation and is presently considered obsolete.	Low	Low
Bacterial gene mutation	*S.* Typhimurium Strains: No information	Na erythorbate	No information	Weakly mutagenic	Zeiger, 1993	**Reliability: 4** (No information on strains and protocol)	High	Low
Bacterial gene mutation	*S.* Typhimurium TA92; TA94; TA100; TA1535;	Erythorbic acid	Up to 50 mg/plate	Weak positive for TA100 With and without S9	Ishidate et al. (1984)	**Reliability: 3** A weak positive result was obtained at an excessive dose level (50 mg/plate), however, the OECD guideline no. 471 recommends a maximum dose-level of 5 mg/plate. Additionally, results at lower	High	Low

Test System	Test Object	Test material	Concentration	Result	Reference	Reliability/Comments	Relevance of the test System concerning the genetic endpoint	Relevance of the result
	TA1537					dose levels of erythorbic acid were not reported and results obtained with sodium erythorbate up to 5 mg/plate was negative.		
		Sodium erythorbate	Up to 5 mg/plate	Negative		**Reliability: 2** Reporting deficiencies and not all strains used as recommended in the current OECD guideline 471.	High	Limited
Chromosomal aberration	Chinese hamster fibroblast cell line (CHL)	Na erythorbate	Up to 0.25 mg/mL	Negative		**Reliability: 2** The study was only performed in the absence of S9 metabolism.	High	Limited
Bacterial gene mutation	*S.* Typhimurium TA1530; TA1535; TA1536; TA1537; TA1538	Na erythorbate	Single dose 100 mg/plate	Negative	Newell et al., 1974	**Reliability: 3** Very limited in its protocol, choice of strains, only one dose, absence of repetition of negative results.	High	Low
Yeast gene mutation	*Saccharomyces cerevisiae* strain D3	Na erythorbate	5%	Negative		**Reliability: 4** The study is limited in the protocol and the assay did not receive further validation and is presently considered obsolete.	Low	Low
Bacterial gene mutation	*Salmonella* TA98 and TA100	Na erythorbate	No information	Negative	Kawachi et al., 1980	**Reliability: 3** No information on metabolic activation and range of concentrations used were available.	High	Low
Bacterial gene mutation	*Salmonella* TA98; TA100	Na erythorbate	No information	Negative	Peters et al., 1983, as reported by SCF, 1997	**Reliability: 3** The study is poorly documented and no information on the range of concentrations is available.	High	Low

Test System	Test Object	Test material	Concentration	Result	Reference	Reliability/Comments	Relevance of the test System concerning the genetic endpoint	Relevance of the result
Bacterial gene mutation	No information	Erythorbic acid	No information	Positive	Hayashi et al. (1988)	**Reliability: 3** Results obtained and details on the protocol have been already published in the paper by Ishidate et al., (1984)..	High	Low
Chromosomal aberration	Chinese hamster cells with and without metabolic activation	Na erythorbate	Up to 2 mg/L	Negative	Matsuoka, et al. 1979	**Reliability: 3** The study is poorly documented. Three concentrations tested but results available only for the high concentration.	High	Low
Chromosomal aberration	Human fibroblasts (HE2144 cells)	Na erythorbate	No information	Negative	Kawachi et al., 1980; Sasaki et al., 1980, as reported by SCF, 1997	**Reliability: 4** The information on this test is limited. The publication cited by the SCF is not available.	High	Low
Sister-chromatid exchanges (SCEs)	Human fibroblasts (HE2144 cells)	Na erythorbate	No information	Negative	Kawachi et al., 1980; Sasaki et al., 1980, as reported by SCF, 1997	**Reliability: 4** The information on this test is limited. The publication cited by the SCF is not available. Three concentrations tested but results available only for the high concentration.	Low	Low
Primary DNA Damage	*Bacillus subtilis* Rec strains H17 and M45	Erythorbic acid	No information	Positive	Nonaka, 1989	**Reliability: 4** The information on this test is limited. Test generally not used for genotoxicity risk assessment.	Limited	Low

In vivo studies

Test System	Test Object	Test material	Route	Dose	Result	Reference	Reliability / comments	Relevance of the test system concerning the genetic endpoint	Relevance of the result
Host-mediated	Mouse *Salmonella* Typhimurium strain TA1530; *Saccharomyces cereviae* D3	Na erythorbate	Oral: gavage	0.2, 1 or 5 g/kg bw/day 1 or 5 days	Negative	Newell et al. 1974	**Reliability: 4** Not a validated test	Limited	Low
Dominant lethal assay	Rat (40 males/group)	Na erythorbate	Oral: gavage	0, 0.2, 1.0 and 5.0 g/kg bw either once or on five successive days	Negative		**Reliability: 1** Occasional statistically significant differences did not suggest a time or dose-dependent effect.	High	High
Heritable translocation test	Mice (40 males/group)	Na erythorbate	Diet	2 and 10 g/kg bw/ day	Negative		**Reliability: 1** No increase in reciprocal translocations was observed. Poorly sensitive test	High	High
Micronucleus assay	Mice bone marrow(6 /group)	Erythorbic acid	i.p.	0, 187.5, 375, 750 and 1,500 mg/kg bw; In addition to single-dose administration, a multidose study with 750 mg erythorbic acid/kg bw administered 4 times at 24- h intervals was carried out.	Negative	Hayashi et al., 1988	**Reliability: 2** No mortalities in response to the treatment after 1 treatment and 2/6 after 4 × 750 mg/kg bw. A clear decrease in the percentage of polychromatic erythrocytes was noted at the top dose. There was no statistically significant induction of micronuclei in the bone	High	Limited

Test System	Test Object	Test material	Route	Dose	Result	Reference	Reliability / comments	Relevance of the test system concerning the genetic endpoint	Relevance of the result
							marrow of mice in the single or multidose study. The Panel noted the low number of PCE examined and only one sampling time after single treatment.		
Chromosomal aberration	Rat bone marrow	Na erythorbate	No information	No information	Positive	Kawachi et al., 1980	**Reliability: 3** Difficult to interpret the results because the study is not described in detail.	High	Low
Dominant lethal assay	Male rat	Na erythorbate	Gavage as a single dose and also with 5 consecutive daily doses	Unspecified	Negative	Jorgenson et al., 1978a	**Reliability: 3** The test was briefly reported and no information on dose-levels tested in this study was provided.	High	Low
Comet assay	Male ddY mice	Erythorbic acid/ Na erythorbate	Oral (gavage) Sampling after 3 and 24 h	20,00 mg/kg	Negative	Sasaki et al., 2002	**Reliability: 1** Eight organs –glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow – were analysed. Use isolated nuclei method	High	High

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANOVA	analysis of covariance
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
CONTAM	EFSA Panel on Contaminants in Food Chain
DNA	deoxyribonucleic acid
EC	European Commission
EDTA	ethylenediaminetetraacetic acid
EINECS	European Inventory of Existing Commercial chemical Substances
EMCESA	Embutidos del centro, SA
FAO	Food and Agriculture Organization of the United Nations
FASEB	Federation of American Societies for Experimental Biology
FCS	food categorisation system
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FEEDAP	Scientific Panel on Additives and Products or Substances used in Animal Feed
HILIC	hydrophilic interaction liquid chromatography
HPLC	high-performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
LEL	lowest effect level
LOD	limit of detection
LOQ	limit of quantification
MPL	maximum permitted level
NOAEL	no observed adverse effect level
OEDC	Organisation for Economic Co-operation and Development
OIV	International Organisation of Vine and Wine
SCF	Scientific Committee on Food
TEM	triethylenemelamine
TemaNord	Nordic Working Group on Food Toxicology and Risk Assessment
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UV	ultraviolet
WHO	World Health Organization