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Re-evaluation of β-cyclodextrin (E 459) as a food additive

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Abstract

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of β-cyclodextrin (E 459) as a food additive. β-Cyclodextrin is a non-reducing cyclic oligosaccharide consisting of seven α-1,4-linked D-glucopyranosyl units. The Scientific Committee on Food (SCF) allocated an acceptable daily intake (ADI) of 5 mg/kg body weight (bw) per day to β-cyclodextrin (E 459) in 1996. β-Cyclodextrin is poorly absorbed following oral administration in animals and humans. It is hydrolysed to maltose and glucose by the gut microflora and endogenous amylases in the colon; consequently, β-cyclodextrin levels in tissues and serum are low (< 1%). β-Cyclodextrin has a low acute oral toxicity. Short-term and subchronic toxicity studies were available in rats and dogs. In rats, the main reported effect was an adaptive enlargement of the caecum, resulting from consumption of poorly digestible carbohydrates. From a 6-month study in rats, a no observed adverse effect levels (NOAEL) of 600 mg/kg bw per day was identified and from a 52-week dogs study, the NOAEL was 466 and 476 mg/kg bw per day in males and females, respectively. The Panel considered that there was no indication for genotoxicity of β-cyclodextrin. From a chronic toxicity studies in rats, a NOAEL of 654 and 864 mg/kg bw per day in males and females, respectively, was identified. Carcinogenicity studies in mice and rats were available and no evidence for carcinogenicity was found. The Panel concluded that, based on the available toxicological database, there is no reason to revise the current ADI of 5 mg/kg bw per day for β-cyclodextrin. Based on the available reported use and use levels, the Panel also concluded that the ADI was exceeded in the refined *brand-loyal scenario* (considered the most relevant scenario) in all population groups except for infants at the mean and in all population groups at the 95th percentile.

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Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of β-cyclodextrin (E 459) when used as a food additive.

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

β-Cyclodextrin (E 459) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

The Scientific Committee on Food (SCF) evaluated β-cyclodextrin (E 459) in 1996 and established an acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) per day based on a no observed adverse effect level (NOAEL) of 1.25% in the diet, equivalent to an intake of 466 mg/kg bw per day (based on urinalysis findings) in a 1-year dog study and applying an uncertainty factor of 100. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated β-cyclodextrin (E 459) in 1993 and 1995 and in the latest evaluation established an ADI of 0–5 mg/kg bw per day from the 1-year study in dogs.

In animals and humans, β-cyclodextrin (E 459) is hydrolysed by the gut microflora and endogenous amylases in the colon to maltose and glucose, which can be absorbed. Therefore, concentrations of β-cyclodextrin in tissues and serum are low (< 1%). Urinary excretion varies with species but is in most cases less than 5% of the oral dose. Some unhydrolysed β-cyclodextrin is excreted in the faeces, but this excretion takes several days and is dose-dependent and low (1–6% of dose in dogs; 3–5% of dose in rats).

β-Cyclodextrin shows very low acute oral toxicity with oral median lethal dose (LD_{50}) values for mice, rats and dogs; all greater than 3,000 mg/kg bw.

In 28- and 90-day dietary studies in rats, the main effect was an adaptive effect on the caecum (enlargement) and the Panel identified NOAELs for β-cyclodextrin of 3,297 and 5,439 mg/kg bw per day (the highest doses tested) in these studies, respectively. A 6-month study in rats revealed an adverse effect on body weight gain in animals treated with β-cyclodextrin, but no other consistent treatment-related adverse effects were observed and the Panel identified a NOAEL of 600 mg β-cyclodextrin/kg bw per day. Two 13-week studies and a 28-week study in dogs were available but they had some limitations and were not considered for this assessment. In dog studies, the NOAELs following 52 weeks of dietary exposure in dogs were 456 and 476 mg/kg bw per day in males and females, respectively, and 861 and 988 mg/kg bw per day in males and females, respectively.

β-Cyclodextrin gave negative results in a bacterial reverse mutation assay, in a hypoxanthine-guanine phosphoribosyltransferase (HPRT) assay using V79 Chinese hamster cells, in an *in vitro* chromosomal aberration and an *in vivo* micronucleus test, which were of limited reliability. However, based on these data, the Panel considered that there was no indication for genotoxicity for β-cyclodextrin.

There was one chronic toxicity study in rats and the NOAEL (52-week dietary exposure) was 654 and 864 mg/kg bw per day for males and females, respectively, based on mild hepatotoxicity. Carcinogenicity studies were also available in mice and rats and there was no evidence that β-cyclodextrin had carcinogenic properties in any of the studies. In mice, the Panel considered the 225 mg/kg bw per day as the NOAEL based on the inflammatory changes observed in the caecum, colon and rectum at 675 mg/kg bw per day. In the JECFA monograph addendum (1995), it was concluded that the NOAEL for this study was 25 mg/kg bw per day based on the inflammatory effects in the lower gastrointestinal tract. The Panel did not agree with this conclusion since the inflammatory effects in the lower intestinal tract were only seen in animals of the highest dose. Therefore, the Panel considered the dose of 225 mg/kg bw per day as the NOAEL for this study. The Panel noted that unlike JECFA (1995), the SCF (1997) considered that the inflammatory effects observed in mice were not observed in studies in rats and dogs, and concluded that they were due to the high doses of undigestible compound tested and were not relevant for humans. The Panel noted that inflammation, localised in the caecum and colon, was seen in one mouse study. However, in several other studies in rats and dogs no inflammation was reported in the caecum and large intestine. In line with the SCF, the Panel considered that the effect reported in mice was species specific. Therefore, the NOAEL from this study was considered not relevant for the human risk assessment of β-cyclodextrin.

From the multigeneration reproductive toxicity studies, the Panel considered that dose levels up to 10,000 mg β-cyclodextrin/kg in the diet (equal to 1,108–1,531 mg/kg bw per day for males and

655–1,525 mg/kg bw per day for females) did not affect reproductive parameters and parental toxicity. At higher dose levels, maternal weight gain, food consumption and pup weight were decreased. Three prenatal developmental toxicity studies in rats that tested β-cyclodextrin did not show any evidence that β-cyclodextrin had an adverse effect at doses up to 2,500 and 5,000 mg/kg bw per day on developmental parameters.

The dietary exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) was calculated based on (1) maximum permitted levels (MPLs) set out in the EU legislation and maximum reported use levels for the three food categories in which β-cyclodextrin (E 459) is authorised at *quantum satis* (*QS*) (*maximum level exposure assessment scenario*) and (2) the reported use levels (the *refined exposure assessment scenario*).

The exposure estimates in the *maximum level exposure assessment scenario* exceeded the ADI of 5 mg/kg bw per day for all population groups at the 95th percentile and also at the mean level except for infants. Similar observation was noted for the *refined brand-loyal* exposure estimate. For the *non-brand-loyal scenario*, the ADI was exceeded in toddlers and children at the mean and in all populations groups at the 95th percentile of exposure. The main contributing food categories for all population groups were flavoured drinks, coffee, tea, herbal and fruits infusions and food supplements supplied in a solid form in both scenarios.

The Panel considered that the uncertainties identified in its estimates for the food categories taken into account in this assessment would, in general, result in an overestimation of the exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) in European countries for the regulatory maximum level exposure scenario and for the refined exposure scenarios. However, the Panel is aware that other potentially important sources of exposure (in particular according to Annex III Part 1 and 5) of β-cyclodextrin were not considered in this assessment because no data were available.

β-Cyclodextrin (E 459) is authorised in a limited range of foods and with reported data provided by industry; according to the Panel, it is expected that brand-loyalty will result in higher exposure in the general population due to these food uses either from Annex II (e.g. flavoured drinks, food supplements) or from Annex III (part 4) (coffee, tea, herbal and fruits infusions). The Panel therefore selected the *brand-loyal refined scenario* as the most relevant exposure scenario for this food additive.

Concerning the presence of trichloroethylene, classified as carcinogenic to humans (Group 1), as residual solvent in β-cyclodextrin (E 459), the Panel noted that a range of benchmark dose 95% lower confidence limit ($BMDL_{05}$) values have been derived from animal carcinogenicity studies (from 1.4 to 53 mg/kg bw per day) without detailed consideration of any other non-carcinogenic endpoint. The Panel also noted that based on the maximum permitted residual level of trichloroethylene in the EU specifications of β-cyclodextrin (E 459) (1 mg/kg), the exposure to trichloroethylene associated to the use of β-cyclodextrin as a food additive at the ADI of 5 mg/kg bw per day would be 5 ng/kg bw per day and this would result in a margin of exposure (MoE) of 280,000 even with the lowest BMDL value (1,4 mg/kg bw per day).

The Panel concluded that based on the available toxicological database there is no reason to revise the current ADI of 5 mg/kg bw per day for β-cyclodextrin (E 459).

Based on the available reported use and use levels, the Panel also concluded that the ADI was exceeded in the refined *brand-loyal scenario* (considered the most relevant scenario) in all population groups except for infants at the mean and in all population groups at the 95th percentile.

The Panel recommended that:

- microbiological specifications should be included in the EU specification for β-cyclodextrin (E 459);
- the maximum limits for the impurities of toxic elements (lead and arsenic) in the EU specification for β-cyclodextrin (E 459) should be revised in order to ensure that β-cyclodextrin (E 459) as a food additive will not be a significant source of exposure to those toxic elements in food;
- analytical data and more information on use and use levels should be provided in order to perform an adequate exposure assessment;
- the presence of trichloroethylene, classified as carcinogenic to humans (Group 1) (IARC, 2014), as a residual solvent in β-cyclodextrin (E 459) should be reduced to the lowest level possible according to the SCF recommendations.

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1. Introduction

The present opinion deals with the re-evaluation of β-cyclodextrin (E 459) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008[1] 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[2]. 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[3] of 2001. The report 'Food additives in Europe 2000[4]' 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.

1.1.2. Terms of Reference

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 of the European Parliament and of the Council on food additives.

1.2. Information on existing evaluations and authorisations

β-Cyclodextrin (E 459) is a food additive authorised according to Annex II and Annex III of Regulation (EC) No 1333/2008 and specific purity criteria have been defined in Commission Regulation (EU) No 231/2012.

In 1996, the SCF evaluated the safety of β-cyclodextrin as a carrier and stabiliser, manufactured by the action of cycloglycosyltransferase (CGTase) obtained from *Bacillus circulans* on partially hydrolysed starch. An acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) per day was allocated based on a no observed adverse effect level (NOAEL) of 1.25% in the diet, equivalent to an intake of 466 mg/kg bw per day (based on urinalysis findings) in a 1-year dog study and applying an uncertainty factor of 100 (SCF, 1997). Two further opinions concluded that β-cyclodextrin produced using CGTase obtained from *Paenibacillus macerans* (SCF, 2000b) and CGTase obtained from *Bacillus licheniformis* (SCF, 2000a) should also be included in the ADI of 0–5 mg/kg bw per day.

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[2] 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.
[3] COM(2001) 542 final.
[4] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

β-Cyclodextrin (derived from *Bacillus macerans*, *Bacillus circulans* or related *Bacillus* strains) was evaluated in 1993 and 1995 by JECFA. In 1993, the toxicological data were considered insufficient to derive an ADI, as chronic toxicity testing was still in progress and additional information on the effects of β-cyclodextrin on the bioavailability of lipophilic nutrients and on the production methods was requested. Therefore, a temporary ADI of 0–6 mg/kg bw per day was allocated, based on a NOEL of 2.5% in the diet, which was equal to 1,230 mg/kg bw per day, from a 13-week preliminary study in dogs (HRC, 1992a [Documentation provided to EFSA n. 15]), and an uncertainty factor of 200 (JECFA, 1993).

In an addendum to the monograph (JECFA, 1995), additional data from 1-year studies in the dog and rats, a three-generation reproductive study in the rat, carcinogenicity studies in rat and mice, and information on the effects of β-cyclodextrin on the bioavailability of lipophilic nutrients was evaluated. An ADI of 0–5 mg/kg bw per day was allocated based on the no observed effect level (NOEL) of 1.25% in the diet (equal to 470 mg/kg bw per day from the 1-year study in the dog (HRC, 1994b [Documentation provided to EFSA n. 18]) and applying an uncertainty factor of 100.

TemaNord summarised the findings of JECFA and the SCF and concluded that sufficient data exist for β-cyclodextrin as a food additive, and that a re-evaluation was not necessary (TemaNord, 2002).

β-cyclodextrin is permitted as an absorbent and chelating agent in cosmetic products (European Commission database-CosIng[5]).

The European Medicines Agency (EMA) carried out a background review for cyclodextrins used as excipients (EMA, 2014).

2. Data and methodologies

2.1. Data

The Panel was not provided with a newly submitted dossier. EFSA launched a public call for data,[6,7] to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to July 2016. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based, however not always these were available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[8]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of β-cyclodextrin (E 459) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety of β-cyclodextrin (E 459) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001).

When the test substance was administered in the feed or in drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is expressed as 'equivalent'.

Dietary exposure to β-cyclodextrin (E 459) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum levels permitted in the EU legislation and/or reported use levels

[5] Available online: http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.simple
[6] Call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Published: 15 February 2012. Available online: http://www.efsa.europa.eu/en/dataclosed/call/120215
[7] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption – Extended deadline: 30 September 2014. Published: 9 March 2014. Available from: http://www.efsa.europa.eu/en/dataclosed/call/datex140310
[8] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012[9], the food additive E 459 is identified as:
Chemical name: Cycloheptaamylose

- Description: Virtually odourless white or almost white crystalline solid
- EINECS Number: 231-493-2
- Chemical formula: $(C_6H_{10}O_5)_7$
- Molecular weight: 1,135 g/mol
- Solubility: An aqueous solution of the β-cyclodextrin (E 459) is clear and colourless. It is sparingly soluble in water; freely soluble in hot water; slightly soluble in ethanol.

According to Roquette (2012), β-cyclodextrin (E 459) is white or almost white, amorphous or crystalline powder. Under powder form, the typical particle size distribution (via laser scattering) is as follows: mean size 728 nm, median size 599 nm, and the absence of particles with a size < 100 nm (Roquette, 2016 [Documentation provided to EFSA n. 28]). No melting point is defined; a dehydration step is observed at around 100°C and a decomposition point at 286°C. Regarding its solubility, in addition to the information indicated in the Commission Regulation (EU) No 231/2012, it is mentioned that β-cyclodextrin is practically insoluble in anhydrous ethanol and in dichloromethane. The octanol/water partition coefficient is not known.

Synonyms: cycloamylose; cycloheptaglucan; cycloheptaglucosan; dextrin, β-cyclo, Schardinger β-dextrin; β-cycloheptaamylose; kleptose B; rhodocap N, Betadex (SciFinder,[10] software).

CAS Registry Number: 7585-39-9 (SciFinder, software) (Figure 1).

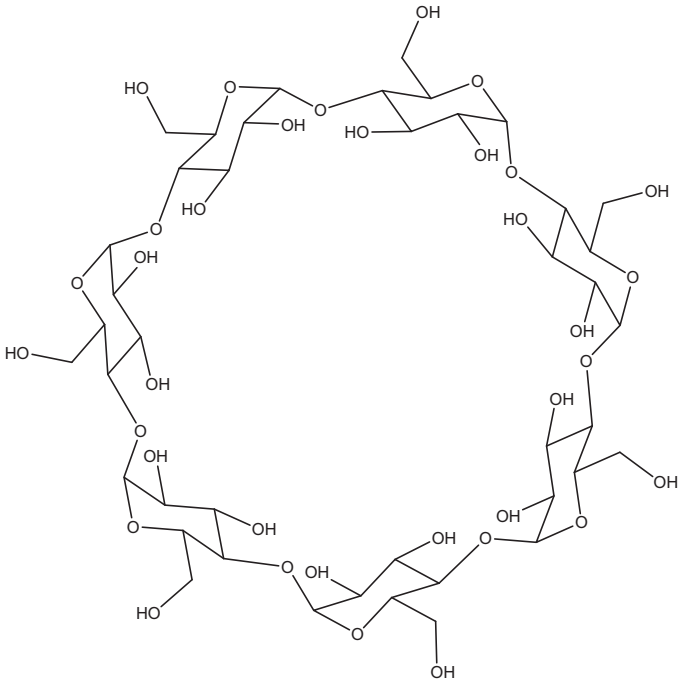


Figure 1: Structural formula of β-cyclodextrin (ChemBioDraw Ultra 13.0, software)

[9] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.
[10] SciFinder® the choice for chemistry researchTM.

3.1.2. Specifications

The specifications for β-cyclodextrin (E 459) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006) are listed in Table 1.

Table 1: Specifications for β-cyclodextrin (E 459) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Definition	β-Cyclodextrin is a non-reducing cyclic saccharide consisting of seven α-1, 4-linked D-glucopyranosyl units. The product is manufactured by the action of the enzyme cycloglycosyltransferase (CGTase) obtained from *Bacillus circulans*, *Paenibacillus macerans* or recombinant *Bacillus licheniformis* strain SJ1608 on partially hydrolysed starch	A non-reducing cyclic saccharide consisting of seven α-1,4-linked D-glucopyranosyl units manufactured by the action of cyclodextrin transglycolase on hydrolysed starch followed by purification of the β-cyclodextrin; purification is by preparation of a β-cyclodextrin/solvent inclusion compound followed by steam stripping of the solvent before final purification
Assay	Content not less than 98.0% of $(C_6H_{10}O_5)_7$ on an anhydrous basis	Not less than 98.0% of $(C_6H_{10}O_5)_7$ on an anhydrous basis
Description	Virtually odourless white or almost white crystalline solid. Appearance of the aqueous solution is clear and colourless	Virtually odourless, slightly sweet tasting white or almost white crystalline solid
Identification		
Solubility	Sparingly soluble in water; freely soluble in hot water; slightly soluble in ethanol	Sparingly soluble in water; freely soluble in hot water; slightly soluble in ethanol
Specific rotation	$[\alpha]_D^{25}$: between + 160° and + 164° (1% solution)	$[\alpha]_D^{25}$: between + 160° and + 164° (1% solution)
pH value	5.0–8.0 (1% solution)	–
Purity		
Water content	Not more than 14% (Karl Fischer method)	Not more than 14% (Karl Fischer method)
Other cyclodextrins	Not more than 2% on an anhydrous basis	Not more than 2% on an anhydrous basis
Solvent residues	Not more than 1 mg/kg for each of toluene and trichloroethylene	Not more than 1 mg/kg for each of toluene and trichloroethylene
Sulfated ash	Not more than 0.1%	Not more than 0.1%
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 1 mg/kg	Not more than 1 mg/kg
Reducing substances	–	Not more than 1% (as glucose)

The Panel noted that, according to the EU specifications for β-cyclodextrin, (E 459), impurities of the toxic elements, lead and arsenic, are accepted up to concentration of 1 mg/kg for both. Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009, 2010).

Considering that β-cyclodextrin is produced by enzymatic hydrolysis (see Section 3.1.3), the Panel considered that microbiological specifications should be included in the EU specifications of β-cyclodextrin.

3.1.3. Manufacturing process

Commission Regulation (EU) No 231/2012 states that β-cyclodextrin (E 459) is manufactured by the action of CGTase obtained from *B. circulans*, *P. macerans* or recombinant *B. licheniformis* strain SJ1608 on partially hydrolysed starch.

The Panel noted that for the production of β-cyclodextrin (E 459) only the bacterial species listed in Commission Regulation (EU) No 231/2012 can be used.

According to Roquette (2012) [Documentation provided to EFSA n. 27], β-cyclodextrin (E 459) is manufactured from starch subject to an initial enzymatic hydrolysis step which is followed by a second enzymatic hydrolysis and cyclisation step using trichloroethylene as a solvent. The enzyme CGTase obtained as previously stated can be used. β-Cyclodextrin exists as a suspension in the reaction solution and the crude product is isolated by physical methods. Purification is achieved by filtration and crystallisation. After washing, the purified β-cyclodextrin is dried and sieved to yield the commercial food additive.

3.1.4. Methods of analysis in food

Kinalekar et al. (2000) developed a method for the simultaneous determination of α-, β- and γ-cyclodextrin using liquid chromatography with a refractive index detector. Optimisation of the solvent system led to the use of acetonitrile:water (70:30) mix. The limit of detection (LOD) for β-cyclodextrin was 0.0462 mg/mL and the limit of quantitation (LOQ) was 0.154 mg/mL. The method was applied to samples from a continuous production process to monitor levels of the products which may cause inhibition of the enzyme.

López et al. (2009) developed a sensitive liquid chromatographic method with refractive index detection, for the identification and quantification of β-cyclodextrin in milk, cream and butter. Linearity was in the range of 0.01–4 mg/mL ($r = 0.9998$). Repeatability of the method was assessed; the coefficients of variation were 1.24, 3.01 and 5.36% for milk, cream and butter, respectively. The LOD in milk was determined to be > 0.03 mg/L.

An internal analytical method based on ion exchange chromatography with amperometric detection has been developed by Roquette for the identification of β-cyclodextrin in foods (Roquette (2012) [Documentation provided to EFSA n. 27]). Full details of the method were provided as well as examples of the use of the analytical methods to identify and quantify the β-cyclodextrin content of typical food products.

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

Cyclodextrins are cyclic oligosaccharides with hydrophilic outer surface and a lipophilic central cavity. They can form complexes with different guest molecules improving the water solubility. In aqueous solutions, cyclodextrins are able to solubilise lipophilic compounds by taking up some lipophilic moiety of the molecule into the central cavity, i.e. through formation of hydrophilic inclusion complexes.

According to data in literature, cyclodextrins in general do not undergo any thermal degradation below 220–230°C and up to that temperature range they retain their complexing power (Cravotto et al., 2006). By analysing β-cyclodextrin hydrates by differential thermal gravimetry and differential scanning calorimetry, it was observed that the substance loses the hydration water in a temperature range between 64 and 100°C (Kohata et al., 1993; Wang et al., 2014) and that fully dehydrated β-cyclodextrin starts to decompose at 265°C (Kohata et al., 1993).

Tsutsumiuchi et al. (2011) heated model foods consisting of carbohydrates, asparagine, albumin and sodium chloride at 180°C and monitored acrylamide production. Using glucans, such as β-cyclodextrin, starch and cellulose, led in some cases to an increased production of acrylamide. The results of heating studies on glucans alone showed the formation of several carbonyl compounds such as acetone and acetaldehyde. The authors concluded that acrylamide was formed through the reaction of asparagine and the volatile carbonyl compounds from pyrolysis of the glucans, and not through reaction with glucose formed by hydrolysis of the glucans. The Panel noted that this observation is only relevant when β-cyclodextrin (E 459) is used in foods subjected to heat treatment (e.g. snacks).

According to Roquette (2012) [Documentation provided to EFSA n. 27], β-cyclodextrin has demonstrated a stability of 100% in the presence of α-amylase, 96% in the presence of β-amylase of fungal origin and 90% in the presence of α-amyloglucosidase (1% solution of the enzyme in 570°C).

3.2. Authorised uses and use levels

Maximum levels of β-cyclodextrin (E 459) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives. In this opinion, these levels are named maximum permitted levels (MPLs).

Table 2 summarises the food categories that are permitted to contain β-cyclodextrin (E 459) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 2: MPLs of β-cyclodextrin (E 459) in food categories according to Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
0	Food additives permitted in all categories of foods	Only foods in tablet and coated tablet form, excluding the foods listed in Table 1 of Part A of this Annex	*Quantum satis*
05.2	Other confectionery including breath freshening microsweets	Only foods in tablet and coated tablet form	*Quantum satis*
14.1.4	Flavoured drinks	Only flavoured powdered instant drinks	500
17.1[(a)]	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Only foods in tablet and coated tablet form	*Quantum satis*

FCS: food categorisation system; MPL: maximum permitted level.
(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

β-Cyclodextrin (E 459) is also authorised according to Annex III to Regulation (EC) No 1333/2008 as a carrier for all food additives (Part 1) up to 1,000 mg/kg in the final food. According to Part 4 of Annex III to Regulation (EC) No 1333/2008, β-cyclodextrin (E 459) is authorised for use in encapsulated flavourings up to 500 mg/L in 'Flavoured teas and flavoured powdered instant drinks' (FCS 14.1.5) and up to 1,000 mg/kg in 'Flavoured snacks' (FCS 15.1), and according to Part 5 section A in nutrients up to 100,000 mg/kg in their preparation and 1,000 mg/kg in final food.

3.3. Exposure data

3.3.1. Reported use levels of β-cyclodextrin (E 459)

Most food additives in the EU are authorised in a food category at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to *quantum satis* (QS), as is the case for 3 of the 4 food categories (including FCS 0) in which the use of β-cyclodextrin (E 459) is authorised according to Annex II to Regulation (EC) No 1333/2008 (Table 2).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[7] for occurrence data (usage level and/or concentration data) on β-cyclodextrin (E 459). In response to this call, information on the use levels of β-cyclodextrin (E 459) in foods was submitted to EFSA by industry. No analytical data were submitted to EFSA.

3.3.1.1. Summary on reported use levels of β-cyclodextrin (E 459) in foods provided by industry

Information on the actual uses and use levels of β-cyclodextrin (E 459) were made available by FoodDrinkEurope (FDE; n = 1), the International Chewing Gum Association (ICGA; n = 1) and Intertek Scientific & Regulatory Consultancy (n = 9) on behalf of a manufacturer of β-cyclodextrin (E 459). This manufacturer collected information regarding use and use levels from its customers, and these data were reported to EFSA via Intertek Scientific & Regulatory Consultancy.

In summary, industry provided EFSA with nine use levels in foods belonging to the food categories 14.1.4 and 17.1 in which β-cyclodextrin (E 459) is authorised according to Annex II to Regulation (EC) No 1333/2008. In addition, one use level on 'Chewing gum', as food in tablet and coated tablet form

and therefore authorised through the FCS 0, and one on 'Potato-, cereal-, flour-, or starch-based snacks', authorised according to Annex III to Regulation (EC) No 1333/2008 for all food additives (Part 4), were also provided. Use levels reported for the FCS 14.1.4 were also considered relevant for the category 14.1.5. No information on the possible use of β-cyclodextrin (E 459) as a carrier for food additives (Part 1) and nutrients (Part 5) were made available to EFSA.

Appendix A lists the data on the use levels of β-cyclodextrin (E 459) in foods as reported by industry.

3.3.2. Summarised data extracted from the Mintel GNDP

The Mintel GNPD[11] is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products with more than 800,000 of those on the European food market. The Mintel GNPD started covering European food markets in 1996, currently having 20[12] out of its 28 member countries presented in the Mintel GNPD.

For the purpose of this Scientific Opinion, the Mintel GNPD was used to identify food products that contain β-cyclodextrin (E 459) by checking food label information. The Mintel GNPD contains the compulsory ingredient information present on food product labels.

According to the Mintel GNPD, β-cyclodextrin (E 459) was labelled on only a few food products (n = 17) belonging to food supplements, confectionary, bakery wares and processed fish. Of these 17 food products, only 11 were marketed between 2011 and 2016. It was possible to know from the food label information available in the Mintel GNPD if the presence of β-cyclodextrin (E 459) in bakery wares and processed fish products was due to a carry-over from other ingredients of these food products.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by 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 recently[13] added in the Comprehensive database were also taken into account in this assessment.[8]

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparison should be made with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to 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.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Table 3: Population groups considered for the exposure estimates of β-cyclodextrin (E 459)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Netherlands, Italy, Spain
Children[(a)]	From 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden

[11] http://www.gnpd.com/sinatra/home accessed on 23/11/2015.
[12] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[13] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

Population	Age range	Countries with food consumption surveys covering more than 1 day
Adolescents	From 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	From 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

(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, 2011a).

Consumption records in the EFSA Comprehensive Database were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx food classification system has been linked to the food categorisation system (FCS) as presented in Annex II to Regulation (EC) No 1333/2008, part D and in Annex III to Regulation (EC) No 1333/2008 (Part 4), to perform exposure calculations.

3.3.3.2. Food categories considered for the exposure assessment of β-cyclodextrin (E 459)

The food categories in which the use of β-cyclodextrin (E 459) is authorised according to Annex II to Regulation (EC) No 1333/2008 and Annex III to Regulation (EC) No 1333/2008 (Part 4) were selected from the nomenclature of the EFSA Comprehensive Database at the most detailed level possible of FoodEx (up to FoodEx Level 4) (EFSA, 2011b).

It was not possible to identify which food categories could contain β-cyclodextrin (E 459) as a carrier for food additives (Part 1) and nutrients (Part 5), since no use levels were supplied for these uses by industry. These possible sources of exposure were therefore not considered in the assessment of dietary exposure leading to a possible underestimation of the exposure estimates.

The restriction that β-cyclodextrin (E 459) should only be used in foods in tablet and coated tablet form could also not be taken into account since no information on this is present in the Comprehensive database. Therefore, the whole FCS 05.2 'Other confectionery including breath freshening microsweets' and 17 'Food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children' were considered in the exposure assessment. In addition, the FCS 05.3 'Chewing gum' was included in the assessment since chewing gum can be consumed in tablet form (FCS 0) and use levels were reported by industry.

The assumption that all foods categorised in FCS 05.2 'Other confectionery including breath freshening microsweets', 17 'Food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children' and 05.3 'Chewing gum' (considered from the authorised FCS 0) are consumed in tablet form may have resulted in an overestimation of the exposure. The restriction related to 'Only flavoured powdered instant drinks' for the category 14.1.4 'Flavoured drinks' and FCS 14.1.5 'Flavoured teas and flavoured powdered instant drinks' was considered as leading to an overestimation.

In the EFSA Comprehensive database, no information is provided on the type of food supplements consumed by infants and young children. In the exposure assessment, even if this food category refers in the EU regulation to foods supplements excluding infants and young children, it was in this opinion therefore assumed that the food supplements consumed in these population groups were the same as those consumed in the older population groups for which concentration data were supplied, resulting in an overestimation of the exposure to β-cyclodextrin (E 459) in these two population groups.

3.4. Exposure to β-cyclodextrin (E 459) from its use as a food additive

The Panel estimated chronic exposure to β-cyclodextrin (E 459) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to β-cyclodextrin (E 459) was calculated by multiplying β-cyclodextrin (E 459) concentrations for each food category (Appendix B) with their respective consumption amount per kilogram 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. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The exposure was estimated 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 of exposure were calculated per survey and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large 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.

Exposure assessment to β-cyclodextrin (E 459) was carried out by the ANS Panel based on: (1) MPLs as set down in the EU legislation and maximum use levels for three food categories in which β-cyclodextrin (E 459) was authorised at QS (defined as the *regulatory maximum level exposure assessment scenario*); and (2) the reported use levels (defined as the *refined exposure assessment scenario*). These two scenarios are described in detail below.

3.4.1. 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 Annex III to Regulation (EC) No 1333/2008 (Part 4). As β-cyclodextrin (E 459) is authorised according to QS in most of the food categories, a *maximum level exposure assessment scenario* was estimated based on the maximum reported use levels as provided by industry (EFSA ANS Panel, 2014) in the case of FCS 05.3 'Chewing gums' and FCS 17.1 'Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms'. In the case of FCS 05.2, 'Other confectionery including breath freshening microsweets', the maximum use level of 30,000 mg/kg reported for FCS 05.3 'Chewing gums' was used. For both food categories FCS 14.1.4 'Flavoured drinks' and 14.1.5 'Flavoured teas and flavoured powdered instant drinks', the MPL of 500 mg/L was used, and for food category 15.1 'Potato-, cereal-, flour-, or starch-based snacks', an MPL of 1,000 mg/kg. Appendix B summarises the concentration levels of β-cyclodextrin (E 459) used in the *regulatory maximum level exposure scenario*.

The exposure estimates derived following this scenario should be considered as the most conservative, as this scenario assumes that a consumer will be continuously exposed to β-cyclodextrin (E 459) present in food at the maximum reported use levels and MPLs.

3.4.2. Refined exposure assessment scenario

The refined exposure assessment scenario is only based on use levels reported by industry since no analytical data were submitted. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix B summarises the concentration levels of β-cyclodextrin (E 459) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to β-cyclodextrin (E 459) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to β-cyclodextrin (E 459) present at the mean reported use level in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

3.4.3. Dietary exposure to β-cyclodextrin (E 459)

Table 4 summarises the dietary exposure to β-cyclodextrin (E 459) from its use as a food additive in five population groups (Table 3) according to the different exposure scenario's. Detailed results per population group and survey are presented in Appendix C.

Table 4: Summary of dietary exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4), in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	0.7–4.5	1.9–19.1	2–24.4	1.7–16.2	1–7.7	0.9–6.8
95th percentile	2.6–16.2	8.7–56.6	8–73.1	6.3–56.8	4–26.3	3.4–15.2
Refined estimated exposure scenario						
Brand-loyal scenario						
Mean	0.2–4	0.4–9.2	1–9.3	1–6.8	0.8–5.1	0.7–6.4
95th percentile	1.3–13.9	5.6–28.6	3.8–20.5	4.5–14.7	2.9–12.9	2.8–14.3
Non-brand-loyal scenario						
Mean	0.1–2.3	0.4–5.4	0.6–5.8	0.6–4.1	0.5–3.5	0.5–4.3
95th percentile	0.7–8.7	3.2–17.2	2.6–13	2.4–9.1	1.8–8.4	1.7–10.1

Using the maximum level exposure assessment scenario, the mean exposure to β-cyclodextrin (E 459) ranged from 0.7 to 24.4 mg/kg bw per day across all population groups (Table 4). The 95th percentile of exposure to β-cyclodextrin (E 459) under this scenario could be as high as 73.1 mg/kg bw per day in children.

The refined exposure to β-cyclodextrin (E 459) ranged, under the brand-loyal scenario, from 0.2 to 9.3 mg/kg bw per day and from 1.3 to 28.6 mg/kg bw per day at the mean and 95th percentile, respectively. Corresponding estimates for the non-brand-loyal scenario were from 0.1 to 5.8 mg/kg bw per day and from 0.7 to 17.2 mg/kg bw per day at the mean and 95th percentile, respectively (Table 4).

3.4.4. Main food categories contributing to exposure to β-cyclodextrin (E 459) according to the maximum level exposure assessment scenario (Table 5)

Table 5: Main food categories contributing to exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) using maximum usage levels and MPLs (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
5.2	Other confectionery including breath freshening microsweets	7.3–64.7 (3)	16.5–85.1 (10)	29.4–78.4 (18)	11.4–80.2 (17)	9.9–64.1 (16)	6.7–55.9 (11)
14.1.4	Flavoured drinks – Only flavoured powdered instant drinks	–		10 (1)	6.4–10.1(2)	–	–
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	5.8–88 (5)	8.1–25.4 (5)	6.5–42.8 (9)	8.4–55.4 (11)	12.6–69.6 (16)	18.1–78.8 (14)
15.1	Potato-, cereal-, flour-, or starch-based snacks	8.6 (1)	5.3–20.8 (3)	5.1–12.4 (3)	5.6–8 (3)	5–8.4 (2)	–
17.1	Food supplements supplied in a solid form	41–100 (3)	15.8–78.7 (4)	6.1–15.3 (3)	7.2–7.2 (1)	5.1–33.7 (11)	5.5–47.2 (8)

MPL: maximum permitted level.

(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.

3.4.5. Main food categories contributing to exposure to β-cyclodextrin (E 459) using the refined exposure assessment scenario (Tables 6 and 7)

Table 6: Main food categories contributing to exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) using the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
5.3	Chewing gum	–	–	8.4–28.2 (2)	5.5–27.9 (4)	–	–
14.1.4	Flavoured drinks – Only flavoured powdered instant drinks	31.7–38.5 (3)	7.5–98.6 (8)	24.3–95.6 (18)	29–85.6 (17)	13.2–77.3 (17)	6–40.2 (12)
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	6.2–91.4 (5)	5.8–52.3 (7)	5.3–61.5 (12)	5–64 (14)	17.7–79.2 (16)	23.6–92.2 (14)
15.1	Potato-, cereal-, flour-, or starch-based snacks	5.3–9.6 (3)	5.3–100 (7)	5.3–27.7 (10)	6.8–21.9 (8)	5.4–13.3 (3)	–
17.1	Food supplements supplied in a solid form	45.7–100 (3)	30.1–95 (4)	11.9–71.4 (5)	5.4–38.2 (6)	9.1–58.6 (11)	6.2–70.3 (8)

(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 7: Main food categories contributing to exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) using the non-brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
5.3	Chewing gum	–	–	6.3 (1)	6.8 (1)	–	–
14.1.4	Flavoured drinks – Only flavoured powdered instant drinks	28.4–31.3 (3)	5–97.3 (8)	17.9–93.5 (18)	29.4–80.6 (17)	10.7–71.9 (17)	5.7–38.4 (12)
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	30.6–87.7 (4)	5.1–47 (7)	6.4–58.3 (12)	6.2–59.1 (14)	15.4–75.7 (16)	17.9–90.5 (14)
15.1	Potato-, cereal-, flour-, or starch-based snacks	8–13.2 (4)	9–100 (7)	5.1–41.5 (16)	6–35.5 (15)	6.2–20.9 (5)	6.7 (1)
17.1	Food supplements supplied in a solid form	53.7–100 (3)	6.7–96.3 (5)	5.3–77.5 (6)	7.9–47.6 (6)	12.9–67.5 (11)	5.3–76.6 (9)

(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.

3.4.6. Uncertainty analysis

Uncertainties in the exposure assessment of β-cyclodextrin (E 459) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure

assessment (EFSA, 2007), the sources of uncertainties have been summarised and evaluated in Table 8.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate of β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4)

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 to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer	+/–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 5 in both exposure scenarios out of 6 food categories for which use level were provided by industry)	+
Food categories included in the exposure assessment: data not available for certain food categories which were therefore excluded from the exposure assessment (n = 1 for the refined scenarios out of 6 food categories)	–
Presence of β-cyclodextrin (E 459) as a carrier for food additives (Part 1) and nutrients (Part 5) not considered in the assessment of dietary exposure leading of a possible underestimation of the exposure estimates	–
Concentration data:	
– levels considered applicable to all items within the entire food category	+
Maximum level exposure assessment scenario:	
– use of the maximum reported use level as provided by industry in the case of QS (n = 3 out of 6 food categories)	+/–
– use of MPL for food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008 (n = 1 food category) and Annex III Part 4 (n = 2 food categories)	+
Refined exposure assessment scenarios:	
– exposure calculations based on the maximum or mean use levels (reported use by industry)	+/–
Uncertainty in possible national differences in use levels of food categories	+/–

(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, in general, result in an overestimation of the exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) in European countries for the regulatory maximum level exposure scenario and for the refined exposure scenarios considering only food categories for which data have been reported. However, the Panel is aware that other potentially important sources of exposure (in particular according to Annex III Part 1 and 5) of β-cyclodextrin were not considered in this assessment because no data were available.

3.4.7. Exposure via other sources

β-Cyclodextrin is also permitted in cosmetic products and used as an excipient in medicinal products (Martindale: The Complete Drug Reference, online). However, quantification of exposure via these sources is not known and could therefore not be taken into account in this opinion.

3.5. Exposure to trichloroethylene as an impurity of β-cyclodextrin (E 459)

Trichloroethylene is classified as carcinogenic in humans (Group 1) (IARC, 2014). The Panel also noted that a maximum limit for trichloroethylene of 1 mg/kg in the EU specifications for β-cyclodextrin (E 459) is included.

The Panel calculated the exposure to trichloroethylene from the use of β-cyclodextrin (E 459) as a food additive considering the maximum limit as indicated in the EU specifications for this food additive (Table 9). The exposure was calculated for six population groups following the approach presented in Section 3.4.

Table 9: Exposure to trichloroethylene (μg/kg bw per day) from the use of β-cyclodextrin (E 459) as a food additive in six population groups (minimum–maximum)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	0.0007–0.0045	0.0019–0.0191	0.002–0.0244	0.0017–0.0162	0.001–0.0077	0.0009–0.0068
95th percentile	0.0026–0.0162	0.0087–0.0566	0.008–0.0731	0.0063–0.0568	0.004–0.0263	0.0034–0.0152
Refined estimated exposure scenario						
Brand-loyal scenario						
Mean	0.0002–0.004	0.0004–0.0092	0.001–0.0093	0.001–0.0068	0.0008–0.0051	0.0007–0.0064
95th percentile	0.0013–0.0139	0.0056–0.0286	0.0038–0.0205	0.0045–0.0147	0.0029–0.0129	0.0028–0.0143
Non-brand-loyal scenario						
Mean	0.0001–0.0023	0.0004–0.0054	0.0006–0.0058	0.0006–0.0041	0.0005–0.0035	0.0005–0.0043
95th percentile	0.0007–0.0087	0.0032–0.0172	0.0026–0.013	0.0024–0.0091	0.0018–0.0084	0.0017–0.0101

3.6. Biological and Toxicological data

3.6.1. Absorption, distribution, metabolism and excretion

3.6.1.1. *In vitro* studies

Evidence of hydrolysis of β-cyclodextrin to glucose by bacterial enzymes in the colon was provided in a study which investigated its digestibility *in vitro* by colonic microflora (Szabo et al., 1981). Rat caecal contents were incubated with β-cyclodextrin (1.66 mg/L) under anaerobic conditions at 37°C for unspecified time intervals. The carbohydrate content was then determined. The results from this study confirmed that β-cyclodextrin can be converted into glucose in the colon.

Antenucci and Palmer (1984) tested 32 *Bacteroides* strains from the human colon for their ability to degrade α- and β-cyclodextrin *in vitro*. Of the strains tested, 24 strains were able to degrade β-cyclodextrin, as evidenced by their ability to grow on β-cyclodextrin as their sole source of carbon. A more detailed investigation of cyclodextrinase (CDase) activity was tested in two strains, *Bacteroides ovatus* 3524 and *Bacteroides distasonis* C18-7. The organisms were grown on a minimal medium containing α- and β-cyclodextrin (0.5%) and the CDase activity was assayed by measuring the increase in reducing sugar (as glucose) when the cyclodextrins were incubated at 37°C for 4 h with a crude enzyme preparations. The products of cyclodextrin hydrolysis by the crude enzyme preparations from the two strains were different. *B. ovatus* 3524 CDase catalysed the production of glucose only, while the *B. distasonis* C18-7 CDase catalysed the production of series of malto-oligomers. However, the hydrolysis mixtures were found to be free of malto-oligomers with a degree of polymerisation above 4 (DP > 4). CDase was stable and active under expected conditions of the colon environment (pH 7.0; 37°C). From the study, the authors concluded that cyclodextrins (including β-cyclodextrin) will be extensively hydrolysed in the human colon.

3.6.1.2. *In vivo* studies

Rats

In a study by Andersen et al. (1963), fasted Wistar rats (2 males) were administered a single dose of 2.5 mL of a 2.5% aqueous solution (no dose in mg/kg bw given) of either ^{14}C-uniformly labelled α-cyclodextrin, β-cyclodextrin or gelatinised potato starch by gavage. Rats were placed in a metabolism chamber for 16–23 h and respiratory carbon dioxide (CO_2) was measured. Urine, faeces and carcasses were also analysed for radioactivity. The proportion of β-cyclodextrin excreted in expired air as CO_2 was 48.6% and 66.8% of the dose at 17 and 23 h, respectively. This was similar to the proportion of excreted CO_2 from the starch in expired air (64.3% and 58.6% at 17 and 23 h, respectively). However, initially β-cyclodextrin was metabolised much slower than starch, with a maximum output of CO_2 after 8–9 h for β-cyclodextrin and 1 h for starch. Also, small amounts of CO_2 were still being excreted after 20 h following dosing with β-cyclodextrin, but CO_2 output had reached background levels by 8 h after dosing with starch. The study result did not differentiate between the

metabolism of β-cyclodextrin in the gut by the gut microflora or following absorption, but, according to the authors it supported that β-cyclodextrin was degraded slowly in the colon by intestinal microflora, thus forming linear dextrin and glucose that could be absorbed and metabolised in the same manner as starch. The study also showed that the tissues distribution of radioactivity was similar for β-cyclodextrin and starch at 17 and 23 h after dosing. Apart from the gastrointestinal tract, the liver had the highest proportion of the administered dose (up to 3%), but other tissues, such as the heart, lungs, kidneys, sex organs and spleen each had less than 0.5% of the administered dose at 17 and 23 h after dosing with β-cyclodextrin or starch. According to the authors, this study demonstrated that the cyclic dextrins were metabolised but at a slower initial rate than starch.

Absorption of β-cyclodextrin from the small intestine of rats was studied using everted sacs of jejunum–ileum segments in order to block active transport mechanisms (Szabo et al., 1981). Five rats (CFY-derived; sex not stated) with ligated gut loops were administered β-cyclodextrin at single oral doses of 50 and 100 mg/kg bw (no further details on methodology given). Passive absorption was shown to be concentration dependent and the active transport blocker, phloretin, did not alter absorption.

In a study by Gerloczy et al. (1985), ^{14}C-uniformly labelled β-cyclodextrin was administered by gavage (24 h) to fasted female CFY rats: one rat received the test substance at a dose of 36.1 mg/kg bw, a second rat at a dose of 313.5 mg/kg bw and three other rats at a dose of 1.88 mg/kg bw. For comparison purposes, ^{14}C-glucose was administered by gavage at a dose of 12.5 mg/kg bw to one female CFY rat and to one Wistar R (Amsterdam) x Long Evans (Chester Beatty) F_1 male rat. For the rats dosed with ^{14}C-labelled β-cyclodextrin, 49–64% of the radioactivity was recovered as $^{14}CO_2$ from the exhaled air within 24 h. Fifty percent of the exhaled total radioactivity (as $^{14}CO_2$) was recovered at 7.3 h (for the 36.1 mg/kg bw dose) and at 6.3 h (for the 1.88 mg/kg bw dose). The highest radioactivity exhalation from the rats dosed with ^{14}C-glucose was measured in the first 2 h after treatment. Within this interval, 24.4% of the administered radioactivity was recovered. Two percent was exhaled between 10 and 12 h, and only 0.45% between 22 and 24 h; in 24 h altogether, 58.5% of the total radioactivity was recovered. It was found that the 36.1 mg/kg bw and the 1.88 mg/kg bw dose of ^{14}C-labelled β-cyclodextrin as well as the 12.5 mg/kg bw dose with ^{14}C-glucose are metabolised to a very similar extent, although the rate of exhalation was different: with ^{14}C-labelled β-cyclodextrin the maximum exhaled radioactivity appeared 4–6 h later than with ^{14}C-glucose. The radioactivity of the urine and faeces as percentage of the administered radioactivity was also measured 10 h after treatment. Independent of the dose, no significant difference was found in the radioactivity of the urine between ^{14}C-labelled β-cyclodextrin (4.2–4.8%) and ^{14}C-glucose treated animals (2–3.6%). Radioactivity of the faeces could not be compared because rats treated with the ^{14}C-labelled β-cyclodextrin excreted no faeces within the observed time interval.

In the same study by Gerloczy et al. (1985), the distribution of the radioactivity in 15 organs and in fat and muscle tissue, was measured 24 h after treatment, for the animals treated with ^{14}C-labelled β-cyclodextrin (doses 1.88 and 36.1 mg/kg bw) and with ^{14}C-glucose (dose 12.8 mg/kg bw); for the animals treated with ^{14}C-labelled β-cyclodextrin at a dose of 313.5 mg/kg bw, measurement was performed 10 h after treatment. No significant difference was found between all the treated animals. No specific accumulation was observed in the organs of animals treated with ^{14}C-labelled β-cyclodextrin. However, 24 h after treatment, a considerable difference (10–15% vs 2%) was found between the radioactivity of the large intestine contents of the ^{14}C-labelled β-cyclodextrin (doses 1.88 and 36.1 mg/kg bw; 10–15% radioactivity) treated animals and the ^{14}C-glucose treated animals (dose 12.8 mg/k bw). At the highest dose (313.5 mg ^{14}C-labelled β-cyclodextrin/kg bw), about 55% total administered radioactivity was recovered from the large intestine 10 h after administration. The authors concluded that the β-cyclodextrin as such was not absorbed significantly from the stomach or small intestine, but that it was hydrolysed to open chain dextrin/glucose in the large intestine by gut microflora and endogenous amylases. The maltose and glucose thus formed could then be absorbed. The Panel noted that the short post dose collection time for excreta was a significant limitation in the study design.

Frijlink et al. (1990) studied the pharmacokinetics of β-cyclodextrin in Wistar rats. In a preliminary study, two (16 h-fasted) male rats were administered through a stomach catheter 100 mg β-cyclodextrin (corresponding to 250 mg/kg bw calculated based on body weight of the animals, 400–410 g) and then placed in a metabolism cage to collect urine 0–24 and 24–48 h after administration. Blood was also collected (time intervals not clearly stated). In the main study, Wistar rats (eight females/group of which four animals/group were fasted) were orally administered by gavage 50, 100 or 150 mg β-cyclodextrin dissolved in 5 mL water (calculated based on the body weight of the animals, 160–180 g, corresponding to 294, 590 and 890 mg/kg bw, respectively) and

then placed in metabolism cages to collect urine for 24 h after treatment. In the preliminary study, β-cyclodextrin was only detectable in plasma between 10 and 60 min after administration, and the concentrations (2–11 μg/mL) were near the LOD (20 μg/mL) and considered too low to allow a reliable estimation of bioavailability. In the main study, it was shown that the proportion of the dose that was absorbed increased up to a dose of 100 mg, but that there was no further increase for the 150 mg dose. In all cases, the absorption was approximately 1% or less. In the same study, the authors reported that after intravenous administration of different doses of β-cyclodextrin (0, 25, 100 and 200 mg/kg), the plasma concentration was below the LOD (20 μg/mL) after 3 h. In addition, the elution pattern from the urine sample was identical to that of the injected β-cyclodextrin, which according to the authors suggested that no significant metabolism of intravenously administered β-cyclodextrin occurred. The Panel agreed with this conclusion.

In a 52-week dietary study, Sprague–Dawley rats (20 animals/sex per group) were fed diets containing β-cyclodextrin at doses of 0, 12,500, 25,000 or 50,000 mg/kg diet (equal to 0, 654, 1,313 or 2,655 mg/kg bw per day for males and to 0, 864, 1,743 or 3,614 mg/kg bw per day for females) (HRC, 1994a [Documentation provided to EFSA n. 17]). According to the authors: 'in week 13, β-cyclodextrin was detected, at below the limit of accurate detection (250 mg/kg), in the faeces of 5/10 males receiving 50,000 mg/kg diet (2655 mg/kg bw per day) and 5/10 females (3614 mg/kg bw/day). In 2/10 males receiving 50,000 mg/kg diet (2655 mg/kg bw per day) β-cyclodextrin was detected at 330 mg/kg or 560 mg/kg. No β-cyclodextrin was detected in the faeces of control rats or in the remaining female and male rats receiving 50,000 mg/kg diet. In week 52, no β-cyclodextrin was detected in the faeces of female and male rats control or receiving 50,000 mg/kg diet (either 2,555 or 3,614 mg/kg bw/day)'.

Kubota et al. (1996) investigated the absorption, distribution and excretion of intravenously and orally administered β-cyclodextrin or glycosyl-β-cyclodextrin, in groups of male Wistar rats (number of animals not stated). Via intravenous administration, the rats received a dose of 50 mg/kg bw of the test substances dissolved in normal sterile saline. At appropriated intervals, blood samples (0.3 mL) were taken. After sacrifice, the kidney and liver were analysed. In the same study, (16 h-fasted) rats received via gavage a single dose of 500 mg/kg bw of the test substances. Samples of the plasma, urine, kidney, liver and faeces were analysed. Within l0 h after intravenous administration of the test substances, about 90% of each dose was recovered unchanged in the urine. From this, the authors concluded that no significant metabolism of intravenously administered β-cyclodextrin occurs. After oral administration of the test substances (dose 500 mg/kg bw), the maximum plasma concentrations (4.45 ± 0.26 μg/mL) were observed within 40 min and 0.6% of the total accumulated dose of β-cyclodextrin and 0.3% of glycosyl-β-cyclodextrin appearing in the urine (collected for 120 h after oral administration). The distribution of the test substances in the kidneys and liver was analysed and 4 h after administration, approximately 0.011% and 0.002% of the administered dose was detected in the kidney and liver, respectively. This percentage was reduced to approximately 0.002% and 0.0005%, in the kidney and liver, respectively, 120 h after administration.

In a review, Stella and He (2008) stated that following oral administration in rats, only insignificant amounts of intact cyclodextrins are absorbed from the gastrointestinal tract because of their bulky and hydrophilic nature. Any absorption was by passive diffusion. According to the authors, the parent compounds α-cyclodextrin and β-cyclodextrin were practically resistant to stomach acid and salivary and pancreatic enzyme digestion and the absorbed cyclodextrins were essentially excreted in the urine without undergoing significant metabolism.

Dogs

In a 13-week study (HRC, 1992a [Documentation provided to EFSA n. 15]), pure-bred Beagle dogs (two animals/sex per dose) were administered β-cyclodextrin in the diet at doses of 0, 12,500, 25,000, 50,000 or 100,000 mg/kg diet (equal to mean intakes of 0, 570, 1,234, 2,479 or 4,598 mg/kg bw per day). The study was conducted according to good laboratory practice (GLP). The highest dose group received β-cyclodextrin at a concentration of 50,000 mg/kg diet for the first week, after which the concentration was increased up to 100,000 mg/kg diet. It was found that serum concentrations of β-cyclodextrin (up to 6 h) increased in a dose-dependent manner, although none was detected in the serum of the low-dose group (570 mg/kg bw per day), and only low concentrations were detected in the other dose groups. In the 1,234, 2,479 and 4,598 mg/kg bw per day groups, the serum β-cyclodextrin concentrations were 1–5 mg/L, 4–8 mg/L and 8–46 mg/L, respectively. In the urine, approximately 0.6–1.7% of the mean 24-h dose was excreted in 24 h, without a dose-dependency. In week 13, faecal excretion of β-cyclodextrin was 1–2% of the daily dose (570–2,479 mg/kg bw per day),

except for the highest dose group (4,598 mg/kg bw per day), which was 6.5% of the daily dose. According to the authors, these results indicated that, in the dog, when administered at dietary levels of up to 4,598 mg/kg bw per day a proportion of β-cyclodextrin may be absorbed from the gastrointestinal tract.

In a 52-week study (HRC, 1994b [Documentation provided to EFSA n. 18]), pure bred Beagle dogs (32 animals/4 groups/4 animals/sex per group) were administered β-cyclodextrin at doses of 0, 6,200, 12,500 or 50,000 mg/kg diet (equal to 229, 456 or 1,831 mg/kg bw per day for males and to 224, 476 or 1,967 mg/kg bw per day for females). The study was conducted according to GLP. Clinical signs, body weight and food consumption were monitored throughout the study. Unchanged β-cyclodextrin was observed in urine, and to a lesser extent in faeces. During a 24-h period in a metabolism cage, males and females dogs excreted in their urine 1.3–6.2% and 1.4–3.3% of the daily dose of β-cyclodextrin, unchanged, in week 13 and 52, respectively.

3.6.1.3. Human studies

Flourie et al. (1993) assessed the fate of β-cyclodextrin in the human gastrointestinal tract in four subjects (two males and two females) with ileostomies and in 10 healthy volunteers (three males and seven females). Ileal effluent was collected after oral administration of β-cyclodextrin during fasting (10 g of β-cyclodextrin) and postprandially (10 g of β-cyclodextrin three times daily with meals). In 10 human volunteers, the amount of β-cyclodextrin passing into the colon was determined by breath hydrogen analysis. Faeces were collected after oral administration during fasting and postprandially. In patients with ileostoma, 91 ± 5% and 97 ± 10% (mean ± SD) of β-cyclodextrin was recovered from the small intestine ingested during fasting and with meals, respectively. In healthy volunteers, hydrogen excretion in the breath was low and only traces of β-cyclodextrin were recovered in stools. The study showed that β-cyclodextrin was poorly digested and absorbed in the human small intestine, but that it is fermented by colonic microflora. The authors concluded that these properties were similar between fasted subjects and subjects who consumed a meal before administration of β-cyclodextrin. Therefore, the intestinal transit time did not affect the extent of absorption in the small intestine.

Overall, the Panel noted that β-cyclodextrin as such is poorly absorbed following oral administration in animals and in humans. Percentage of absorptions were not always indicated, but in the study by Frijlink et al. (1990) the proportion of absorbed β-cyclodextrin following administration of an oral dose was 0.5–1.1%. However, in animals (Andersen et al., 1963; Szabo et al., 1981; Gerloczy et al., 1985) and humans (Flourie et al., 1993), β-cyclodextrin was hydrolysed by gut microflora and endogenous amylases in the colon, to maltose and glucose, which were absorbed. Consequently, the proportion of intact β-cyclodextrin excreted in the faeces was dose-dependent and low, with 1–2% of the daily doses (up to 2,479 mg/kg bw per day) and 6.5% of for the highest dose group of (4,598 mg/kg bw per day) being excreted into faeces in dogs (HRC, 1992a [Documentation provided to EFSA n. 15]). Similar results were observed in rats for which 3.6–5.1% of an oral dose was excreted in faeces (Andersen et al., 1963). Accordingly, tissues and serum concentrations of intact β-cyclodextrin are extremely low (< 1%) (Kubota et al., 1996; HRC, 1992a [Documentation provided to EFSA n. 15]). Urinary excretion varies between species but in most cases, it was less than 5% of the oral dose.

3.6.2. Acute toxicity

Mifune and Shima (1977) reported oral acute toxicity values for male and female mice of > 12,500 mg/kg bw, and for male and female rats of > 12,000 mg/kg bw.

Oral acute toxicity studies were conducted in Cleavase Fragment Length Polymorphism (CFLP) mice, CFY rats (a type of Sprague–Dawley rat) and mongrel dogs (Chinoin Pharmaceutical and Chemical works Ltd., 1980 [Documentation provided to EFSA n. 5]). Mice and rats (10 animals/sex) were given 3,000 and 5,000 mg β-cyclodextrin/kg bw, respectively, by gavage, and were observed for 14 days. Similarly, mongrel dogs (three animals/sex) were given 5,000 mg β-cyclodextrin/kg bw by gavage and were observed for 14 days. There were no deaths in any of the species groups.

The Panel considered that β-cyclodextrin has a low acute toxicity.

3.6.3. Short-term and subchronic toxicity

Rats

In a 28-day dietary study in Wistar rats (4 groups/5 animals/sex per group) β-cyclodextrin was administered at a concentration of 5% in the diet (TNO-CIVO Institutes, 1987 [Documentation

provided to EFSA n. 29]). The study was stated to be conducted according to the OECD test guideline 407 and to the GLP. Control animals received diets without added β-cyclodextrin. Based on food consumption data, the authors of the study calculated that the intakes of β-cyclodextrin were 4,856, 4,454, 4,192 and 3,297 mg/kg bw per day for weeks 1–4 for males, and 4,667, 4,314, 4,048 and 3,619 mg/kg bw per day for females. In males, body weights were statistically significantly decreased compared with controls in the first week only ($p < 0.01$). However, the Panel noted that the observed reduction in body weight was less than 10% compared to that of the controls and not consistent across the study. The Panel considered the effects on body weight not of toxicological relevance. A number of intestinal effects, such as diarrhoea in all animals for the first 7 days of treatment only, swollen 'belly' in one female in week 1, and one male and one female having enlarged caeca at the end of week 4, were observed. Overall, the authors of the study concluded that these intestinal changes were not of toxicological significance but rather adaptive changes associated with the ingestion of large quantities of a poorly digestible carbohydrate. A few changes in haematology (decreased red blood cell count, increased mean corpuscular volume and mean corpuscular haemoglobin) and clinical chemistry (decreased γ-glutamyl-transferase activity; increased glutamic oxalacetic transaminase and glutamic pyruvic transaminase activities) parameters were also observed, which the authors of the study concluded to be generally slight and of doubtful toxicological significance. The Panel considered that no adverse effects were observed at the doses tested.

In a 90-day dietary study with Sprague–Dawley rats (6 groups/20 animals/sex per group) were fed diets supplemented with β-cyclodextrin at concentrations of 1.25, 2.5, 5 or 10% (equal to 668, 1,335, 2,676 or 5,439 mg/kg bw per day and to 738, 1,488, 3,045 or 6,074 mg/kg bw per day for males and females, respectively) (Olivier et al., 1991). One group received a diet with 10% lactose (mean 90-day intake 5,235 mg/kg bw per day for males and 5,875 mg/kg bw per day for females). A negative control group received a diet without supplementation with β-cyclodextrin or lactose. One animal died in course of the study, but according to the authors there was no indication that the death was compound-related. There were no dose-related adverse effects pertaining to haematology, blood or urine chemistry. A small fraction of β-cyclodextrin was recovered in the urine of animals fed 5% and 10% β-cyclodextrin, amounting to 0.1–0.3% of the highest administered dose. The absolute relative filled caecal weight of the rats that were fed β-cyclodextrin or lactose was increased due to incomplete digestion in the small intestine and subsequent degradation in the large intestine. Histopathological examination did not demonstrate treatment-related effects indicative of a toxicological response. The only treatment-related effect was a statistically significant increase in filled caecal weights for both sexes. The authors stated that caecal enlargement was a generic response to poorly digestible sugars and other carbohydrate in rats and mice and the caecal enlargement was considered by the authors of the study an adaptive effect. Based on these findings, the Panel considered that the NOAEL of this study was 5,439 mg/kg bw per day for males and 6,074 mg/kg bw per day for females (the highest doses tested).

Rao et al. (2000) evaluated in a 90-day dietary study in male and female albino rats (Wistar-CFT strain, 40 g; 8 animals per group), the safety of residual β-cyclodextrin in eggs treated for cholesterol removal. The animals were fed a standard control diet without any supplementation (control diet) and diets supplemented either with 250 mg/kg egg yolk alone, or diets supplemented with 250 mg/kg egg yolk together with β-cyclodextrin at levels of 20 mg β-cyclodextrin/kg diet, 125 mg β-cyclodextrin/kg diet and 250 mg β-cyclodextrin/kg diet. Daily food intake, body weight gain, relative organ weights, histopathology, haematology and serum enzyme activities were studied. The study did not reveal any toxicity at any of the levels tested. On this basis, the authors concluded that β-cyclodextrin-treated egg samples do not cause any toxicity at the doses tested.

As reported by JECFA (1993) in the Makita et al. (1975) study, groups of 17 (19 at the highest dose) male and female Sprague–Dawley rats (4-week-old), were given β-cyclodextrin by gavage in aqueous suspension at daily dose of 0, 100, 400 or 1,600 mg/kg bw per day. After 3 months, an interim sacrifice was made as follows: control, five males and four females; 100 mg/kg bw per day group, three males and five females; 400 mg/kg bw per day group, five males and four females; 1,600 mg/kg bw per day group, two males and four females. The remaining animals were maintained on the same dosing regimen for 6 months. Food and water intake and body weights were determined weekly. At termination, urinalysis was carried out on five animals of each sex in each group; haematological and serum biochemical analyses were carried out on all survivors. At necropsy, the brain, pituitary, thyroid, thymus, heart, lung, liver, kidneys, adrenals, spleen, pancreas, testes or ovaries were weighed, and these organs and the stomach and intestinal tract were examined histologically. A total of 18 animals died during the study spread over the different doses groups; these were claimed to be due to misdosing. A small decrement of weight gain of both sexes was observed,

in the top-dose group only. No other treatment-related effects were observed. Serum biochemical indices generally were within the normal range although some significant differences from controls were observed, notably a dose-related increase in alk-P-ase in males and a decrease in blood glucose in females of the top two dose groups. Gross and histopathological examination did not reveal any treatment-related abnormalities. The deficit in weight gain at the top-dose level was considered an adverse effect in the absence of other pathological changes, and therefore, JECFA considered that the NOAEL for this study was 400 mg/kg bw per day. The Panel noted that the deficit in weight gain was lower in females than in males but from the available graph (article in Japanese) this deficit appears to be lower than 10%. Given the limitations in this study (e.g. a low number of animals, high mortality), the Panel did not consider this study for the assessment of β-cyclodextrin as a food additive.

β-Cyclodextrin, as a suspension in 1% methylcellulose, was administered by gavage to Long-Evans rats (15 animals/sex per dose) for 6 months at doses 0, 200, 400 or 600 mg/kg bw per day (Chinoin Pharmaceutical and Chemical Works Ltd., 1982 [Documentation provided to EFSA n. 6]). Satellite groups of rats (six animals/sex per group) were administered β-cyclodextrin at the same doses and under the same conditions, but these animals were retained for an additional 2-month treatment-free, recovery period. Five deaths (one male from the low-dose group, one female from each of the low- and high-dose groups and two males from the mid-dose group) due to respiratory disease occurred that were not related to the test compound. There were no clinical signs of toxicity. In the high-dose males, there was a 10% reduction in weight gain during weeks 6–21 compared with the control group. There were no statistically significant differences between group mean body weights at any stage in the study. There were no statistically significant reductions in food consumption for any group. Some statistically significant differences (compared with controls) for haematology and clinical chemistry parameters were either sporadic or within normal ranges for these parameters, and the authors of the study did not judge them to be toxicologically significant. A statistically significant increase in blood glucose concentrations was observed in females of all doses ($p < 0.001$) and males in the high-dose group ($p< 0.01$). In addition, the glucose concentrations were within the normal range. There were statistically significant decreases in relative spleen weights (relative to body weight) of males in the high-dose group ($p < 0.05$) and females of all groups compared with the control group ($p < 0.01$, $p < 0.05$ and $p < 0.01$ for the low-, mid- and high-dose groups, respectively). However, these decreases were within normal limits and there were no corresponding microscopic findings (Mészûros and Vetési, 1982 [Doc provided n. 25]). During the recovery period, the reduced body weights in the high-dose group males returned to be comparable with those of the control animals. Blood glucose concentrations were higher in all treated males and high-dose females compared with controls, but remained within the normal range. There were also some sporadic organ weight changes that were not dose-dependent or consistent between the sexes, and did not reveal any corresponding microscopic findings. Therefore, these were not considered to be of toxicological significance. The Panel considered 600 mg/kg bw per day, the highest dose tested, as the NOAEL because the decreased body weight observed at this dose only in males was transient and reversible.

Dogs

β-Cyclodextrin was administered to groups of Beagle dogs (three animals/sex per dose) in boluses (made from egg yolk and dried breadcrumbs) before the standard diet, for 24 weeks (Institut of Obstetrics, 1978 [Documentation provided to EFSA n. 21]). The doses administered were 100, 250 or 500 mg/kg bw per day. Control animals were given the boluses without β-cyclodextrin. The Panel noted that the study was limited due to the poor condition of some of the animals having parasitic infection.

β-Cyclodextrin was administered in the diet of pure-bred Beagle dogs (2 animals/sex per dose) for 13 weeks at doses of 0 (control), 12,500, 25,000, 50,000 or 100,000 mg/kg diet (equal to 0, 570, 1,234, 2,479 or 4,598 mg/kg bw/day) (HRC, 1992a [Documentation provided to EFSA n. 15]). The highest dose group received β-cyclodextrin at a concentration of 50,000 mg/kg diet for the first week, after which the concentration was increased to 100,000 mg/kg diet. The study was performed in compliance with GLP principles. There were no treatment-related deaths. Liquid faeces were observed in all groups but more frequently in the high-dose group. Body weight gain over the exposure period was statistically significantly reduced in the high-dose group compared with the control group ($p < 0.01$). There were no treatment-related effects on food consumption or adverse findings in the ophthalmoscopy examination. For clinical chemistry and urinalysis, results from males and females were combined. At 6 and 13 weeks, there were reductions in plasma cholesterol concentrations (week 6: $p < 0.01$ for the two highest doses; week 13: $p < 0.05$ for highest dose only), high-density

lipoprotein concentrations (week 6: $p < 0.05$ and $p < 0.01$ for 2,479 or 4,598 mg/kg bw per day group, respectively; week 13: $p < 0.05$ for the high-dose group only) and β-lipoprotein concentrations ($p < 0.05$ for the two highest doses at 6 and 13 weeks). Plasma total protein, albumin, calcium and phospholipid concentrations were also slightly, but statistically significantly ($p < 0.05$) reduced in animals of the high-dose group at weeks 6 and 13. Sodium concentrations were also slightly, but statistically significantly reduced for the high-dose group ($p < 0.05$ at week 6 and $p < 0.01$ at week 13). Urinary protein concentrations were statistically significantly increased in week 6 in the high-dose group ($p < 0.05$) and in week 13 in the two highest doses ($p < 0.01$) compared with the control group. One dog from each of the two high-dose groups had a slightly increased relative liver weight. There were no such findings in any of the other animals. The thymus weight in the high-dose group was reduced compared with the controls ($p < 0.05$). There were no abnormal macroscopic or microscopic findings. The authors of the study concluded that in the absence of microscopic findings, the minor effects on organ weights were not of toxicological significance. The authors noted that the observed effects were indicative of a 'mild toxic response' as there was an absence of treatment-related macro- and microscopic adverse findings. The Panel noted that this study was a dose range-finding study with only a low number of animals tested and has limited value for risk assessment.

β-Cyclodextrin was administered in the diet to Beagle dogs (six males) at a dose of 50,000 mg/kg diet (equivalent to 2,350 mg/kg bw per day) for the first week, followed by 100,000 mg/kg diet (equivalent to 3,970 mg/kg bw per day) for the remaining 11 weeks (CIT, 1996 [Documentation provided to EFSA n. 7]). The study was performed in compliance with GLP principles. Animals were examined for clinical signs of toxicity, food and water consumption were measured daily and body weights weekly. Blood biochemistry analysis was performed weekly, from 2 weeks prior to the start of treatment, then in weeks 3, 6, 9 and 12 of treatment. Urinalysis was performed from 2 weeks prior to the start of treatment and then weekly during treatment. Macroscopic examinations of the euthanised animals were conducted; the kidneys were examined microscopically. There were no adverse findings from any of the observations or examinations undertaken. There was no evidence of kidney toxicity. The Panel, however, noted that only a single high dose was tested and therefore considered this study to be limited.

In a dietary study (HRC, 1991 [Documentation provided to EFSA n. 14]), pure bred Beagle dogs (four animals/sex per group) were fed diets containing β-cyclodextrin at levels of 10,000, 25,000 or 50,000 mg/kg diet for 52 weeks (equal to 342, 861 or 1,729 mg/kg bw per day for males and 383, 988 or 2,075 mg/kg bw per day for females). Control animals received untreated diets. The study was conducted in compliance with GLP. There were no treatment-related deaths, clinical signs of toxicity, effects on haematology or clinical chemistry parameters or organ weights, and there were no adverse ophthalmoscopy macroscopic or microscopic findings. In weeks 6 and 13, mean urinary protein concentration was statistically significantly increased in animals in the high-dose group (males and females and sexes combined, except for females at week 6; $p < 0.05$ for all except week 13 combined for which $p < 0.01$). However, in the absence of macroscopic or microscopic findings, the toxicological significance of this finding is doubtful. The authors concluded that the no observed effect levels (NOELs) were 1,729 and 2,075 mg/kg bw per day (the highest doses tested) for males and females, respectively. However, the Panel considered that, based on the increase in urinary protein that was seen at the highest dose tested, the NOAEL was 861 and 988 mg/kg bw per day for males and females, respectively.

Bellringer et al. (1995; HRC, 1994b [Documentation provided to EFSA n. 18]) administered β-cyclodextrin via the diet to pure bred Beagle dogs (four animals/sex per group) at levels of 6,200, 12,500 or 50,000 mg/kg diet (equal to 229, 456 or 1,831 mg/kg bw per day for males and 224, 476 or 1,967 mg/kg bw per day for females) for 52 weeks. Control animals were fed a basic diet without β-cyclodextrin. In addition to the normal investigations conducted, serum concentrations of vitamin A, D and E were also determined. The study was carried out in compliance with GLP principles. There were no treatment-related deaths, and body weight, food consumption, ophthalmoscopy, haematology, bone marrow smears, organ weights and macroscopic examinations were similar for treated and control groups. The only clinical sign that was observed slightly more frequently in treated dogs than in controls was intermittent liquid faeces. Serum chemistry, haematology and urinalysis parameters were measured 1 week prior to the start of treatment, then after 13, 26, 39 and 52 weeks of treatment. There were no effects on serum chemistry that showed a dose response. Only females that received the highest dose of β-cyclodextrin showed a slight reduction in serum vitamin A, but without a dose response. Serum vitamin D and E concentrations in treated animals were comparable with those of the controls. At all-time points, urinary total protein concentrations were increased for males and females of the 50,000 mg/kg diet group compared with controls (only statistically significant at

week 26; $p < 0.01$). Calcium concentrations were also increased in males and females of the high-dose group, but this finding only reached statistical significance in males at weeks 13 ($p < 0.05$), 39 ($p < 0.05$) and 52 ($p < 0.01$), and was not observed in females at week 52. There were no treatment-related microscopic findings. The authors of this study concluded that the NOAELs were 1,831 and 1,967 mg/kg bw per day for males and females, respectively. However, the Panel considered that the NOAEL was 456 and 476 mg/kg bw per day in males and females, respectively, based on the increase in total protein concentration in the urine.

Overall, the Panel concluded that there were several short-term and subchronic toxicity studies on β-cyclodextrin in rats. In 28- and 90-day dietary studies in rats the main effect was an adaptive effect on the caecum, which the study authors concluded to be a result of consuming poorly digestible carbohydrates (TNO-CIVO Institutes, 1987 [Documentation provided to EFSA n. 29]; Olivier et al., 1991). The Panel considered that the NOAEL in these studies was 3,297 and 5,439 mg β-cyclodextrin/kg bw per day, the highest doses tested, for the 28- and 90-day studies, respectively. A 6-month gavage study in rats revealed an adverse effect on body weight gain in animals treated with β-cyclodextrin, but no other consistent treatment-related adverse effects were observed and the Panel identified a NOAEL of 600 mg β-cyclodextrin/kg bw per day from this study (Chinoin Pharmaceutical and Chemical Works Ltd., 1982 [Documentation provided to EFSA n. 6]).

Based on the observed urinalysis findings (Bellringer et al., 1995), the Panel considered the NOAEL for β-cyclodextrin in the dog to be 456 and 476 mg/kg bw per day in males and females, respectively.

3.6.4. Genotoxicity

3.6.4.1. *In vitro*

β-Cyclodextrin was tested in a bacterial reverse mutation assay with *Salmonella Typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation, in two separate experiments in compliance with GLP (Hazleton-IFT, 1987a [Documentation provided to EFSA n. 11]). β-Cyclodextrin, a white powder, was tested as solution in water at concentrations of 0.1, 0.5, 1.0, 2.0 or 4.0 mg/plate, in triplicate. Positive (standard positive controls depending on the strain) and negative vehicle controls were also conducted. β-Cyclodextrin was clearly negative for gene mutation with and without metabolic activation. The Panel noted that β-cyclodextrin was not tested in TA102 or *Escherichia coli* WP2. However, no oxidising or cross-linking properties are expected based also on the absence of relevant structural alerts for genotoxicity (OECD (Q)SAR Toolbox[14]) and, therefore, the Panel considered the negative results reliable with limitations.

In another test conducted according to GLP principles (Hazleton-IFT, 1987b [Documentation provided to EFSA n. 12]), β-cyclodextrin was incubated as an extract with *S. Typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation in two separate experiments. The test was performed using the ultrafiltered supernatant of a suspension of β-cyclodextrin (brown powder) prepared as follows: 1 g of β-cyclodextrin powder was added to 50 mL of water, heated for 20 min at 60°C and ultrafiltered. Aliquots of 0.5, 1, 5, 10 and 50 μL of supernatant were added to each plate. β-Cyclodextrin was clearly negative for gene mutation with and without metabolic activation. However, the Panel noted that the β-cyclodextrin concentration in the test extract was not determined and, therefore, the outcome of the study was considered by the Panel of insufficient reliability.

In a host-mediated assay, Ra(x) LE(H) F1 hooded male rats (two animals/dose) were orally administered β-cyclodextrin at concentrations of 100 or 1,000 mg/kg bw (no additional details given) (National Institut of Radiobiology, 1978 [Documentation provided to EFSA n. 24]). One hour after administration, the rats were euthanised and liver specimens acquired. Liver samples were incubated separately with two strains of bacteria (*S. Typhimurium* TA1538, and *E. coli* Bs WP2 uvrA trp-) for 60 min at 37°C, then washed and spread on to test plates. Colonies representing survivors were counted after 24 h, and mutant colonies were counted after 48 h. Untreated animals were used to determine the spontaneous mutation frequency. β-Cyclodextrin did not increase the spontaneous mutation frequency of base-pair exchange (*E. coli* Bs WP2 uvrA trp-) or spontaneous frameshift mutations (*S. Typhimurium* TA1538). The Panel noted that this assay does not belong to the assays recommended for regulatory purposes (EFSA Scientific Committee, 2011). Accordingly, the relevance of the study result is low.

In a hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutation assay (6-thioguanine resistance) using V79 Chinese hamster cells, the cells were exposed to β-cyclodextrin (white powder),

[14] OECD QSAR Toolbox, version 3.3.5.17 (July 2015).

at concentrations of 10, 30, 100, 300 or 1,000 μg/mL, for 3 h in the presence and absence of metabolic activation (Institut Pasteur de Lille, 1990 [Documentation provided to EFSA n. 22]). The test was conducted using two replicate cultures for each concentration in two separate experiments. The positive controls were benzo(a)pyrene and methylmethanesulfonate with and without metabolic activation, respectively. There were also solvent negative controls. No substantial, reproducible, and concentration-dependent increase in the mutation frequency was observed. Therefore, the Panel considered the study reliable with minor limitations and agreed with the conclusion of the authors of the study report that β-cyclodextrin was not mutagenic in this assay.

β-Cyclodextrin was tested in a chromosomal aberrations assay in human lymphocytes *in vitro*. The study was conducted in compliance with GLP (Institut Pasteur de Lille, 1991 [Documentation provided to EFSA n. 23]). Lymphocytes were taken from two healthy volunteers (one male and one female). These samples were tested separately with 100, 300 or 1,000 μg β-cyclodextrin/mL, positive control (without metabolic activation: mitomycin C at 0.25 μg/mL; with metabolic activation: cyclophosphamide at 50 μg/mL), or solvent control, in the presence or absence of metabolic activation. In the absence of S9 metabolic activation, a continuous treatment until harvesting was used, while in its presence, cultures were treated only for 1 h. Cultures were incubated at 37°C for 24 h and colcemid (0.1 μg/mL) was added during the last 2 h to accumulate cells in metaphase. A number of 100 metaphases were examined for induction of chromosomal aberrations for each replicate culture. Under the conditions of this study, β-cyclodextrin was negative for clastogenic activity *in vitro*. The Panel noted that the study was in compliance with the OECD test guideline No 473 (OECD, 1983a) applicable at the time when the study was performed. However, compared to the current version of the OECD guideline (OECD, 2014a) there are some limitations (e.g. the length of treatment time in the presence of S9 (1 h vs 3–6 h) and the absence of a short treatment (3 h) in the absence of S9 metabolic activation, selection of the high concentration, number of cells scored). On this basis, the results of the study were considered of limited reliability by the Panel.

3.6.4.2. *In vivo*

A micronucleus test was conducted in compliance with GLP principles (Hazleton-IFT, 1988 [Documentation provided to EFSA n. 13]). IFFA CREDO mice (five animals/sex) were administered 100 mg β-cyclodextrin/kg bw by a single intraperitoneal injection based on the results of preliminary toxicity trials where mortality and clinical signs were observed up to 200 mg/kg bw at 48 and 72 h. Negative control animals received the vehicle only (carboxymethylcellulose hydrogel; 1%). Positive control animals received cyclophosphamide. Animals were euthanised after 24, 48 or 72 h; bone marrow smears were prepared (two smears/animal/group) from the femoral bone marrow. For each smear, a minimum of 1,000 polychromatic erythrocytes (PCE) were examined for the presence of micronuclei. At the same time, the number of normochromatic erythrocytes (NCE) was also recorded in order to assess bone marrow toxicity. Results were expressed as the ratio of NCE to PCE. No increases in the NCE/PCE ratio were observed at the three sampling times. The number of PCE with micronuclei was not significantly increased in groups treated with β-cyclodextrin, compared with the concurrent negative controls. The positive control gave a clear positive result. The Panel noted that the study was in compliance with the OECD test guideline No 474 applicable at the time (OECD, 1983b) when the study was performed. However, compared to the current version of the OECD guideline (OECD, 2014b) there are some limitations (e.g. one dose level was assayed and only 1,000 PCE were scored) and, therefore, the results obtained were considered of limited reliability by the Panel.

Overall the Panel noted that β-cyclodextrin gave negative results in a bacterial reverse mutation assay, in a HPRT assay using V79 Chinese hamster cells, an *in vitro* chromosomal aberration assay and in an *in vivo* micronucleus test which were of limited reliability. However, based on these data, the Panel considered that there was no indication for genotoxicity of β-cyclodextrin.

3.6.5. Chronic toxicity and carcinogenicity

Mice

The carcinogenic potential of β-cyclodextrin was investigated in a life-time feeding study, which was performed using a protocol comparable to the current OECD guidelines TG 451 (OECD, 2009) and Council Recommendation 83/571/EEC[15] (Gur et al., 1993a [Documentation provided to EFSA n. 9];

[15] Council Recommendation of 26 October 1983 concerning tests relating to the placing on the market of proprietary medicinal products (83/571/EEC). OJ L 332, 28.11.1983, p. 11–32.

Waner et al., 1995). Four groups of CD-1 mice (50 animals/sex per group) were fed diets containing β-cyclodextrin to provide doses of 0, 25, 75, 225 and 675 mg/kg bw per day for 104 week. However, as recommended by the guidelines when males of the control group reached a survival rate of 25% (in week 93), terminal kill of all survivors was carried out. Terminal sacrifice of the females was undertaken during weeks 104 and 105. Mortality was similar among all groups. Treatment at all doses was well tolerated with no clinical signs of reaction. Body weight, food intake and water consumption were similar in all groups throughout the entire study period. No effects on white blood cells examined at 12 and 18 months and prior to sacrifice were noted. There were no palpable masses attributed to the treatment. Treatment-related lesions (both macro- and microscopic) were noted only in decedent animals, except for one male mouse of the high-dose group, sacrificed at terminal kill. In the decedent mice, these changes were limited to the large intestines (mainly caecum) and included desquamation of surface epithelium, mucous secretion containing exfoliated cells in the caecum, colon and rectum. Acute catarrhal inflammation, mucosal flattening and atrophy of the intestinal glands were only seen in the caecum. The incidence of these changes was significant in both sexes treated with the highest dose. Mucosal flattening and atrophy were also observed in one male mouse of the 225 mg/kg bw per day group and two male mice of the 75 mg/kg bw per day. The authors concluded that, under conditions of this study, β-cyclodextrin via a diet up to 675 mg/kg bw per day was not considered to be carcinogenic.

In the JECFA monograph addendum (1995), it was concluded that the NOAEL for this study was 25 mg/kg bw per day based on the inflammatory effects in the lower gastrointestinal tract. The Panel did not agree with this conclusion since the inflammatory effects in the lower intestinal tract were only seen in animals of the high-dose group. Therefore, the Panel considered the dose of 225 mg/kg bw per day as the NOAEL.

Rats

β-Cyclodextrin was administered via the diet to Sprague–Dawley rats (20 animals/sex per group) at levels of 12,500, 25,000 or 50,000 mg/kg diet (equal to 654, 1,313 or 2,655 mg/kg bw per day for males and 864, 1,743 or 3,614 mg/kg bw per day for females) for 52 weeks (HRC, 1994a [Documentation provided to EFSA n. 17]). Control animals were fed a basic diet without β-cyclodextrin. The study was carried out in compliance with GLP. There were no treatment-related deaths or clinical signs of toxicity, and body weight, food consumption, ophthalmoscopy, haematology, urinalysis, bone marrow smears, organ weights and macroscopic tissue examinations were similar for treated and control groups. Serum chemistry parameters were measured 1 week prior to the start of treatment, then after 13, 26, 39 and 52 weeks of treatment. At week 13, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were statistically significantly increased ($p < 0.01$) in males and females in the high- and mid-dose groups as compared to the controls. The increase continued but appeared to be slightly reversible, particularly at week 39 when the increase was only observed in the high-dose females, until week 52 when both enzyme concentrations were elevated in the high-dose group ($p < 0.01$; males and females), and ALT was increased in males of all treated groups ($p < 0.05$ for the low-dose group; and $p < 0.01$ for the mid- and high-dose group). Ornithine transcarbamoylase (OTC) concentrations were statistically significantly elevated in female rats at week 13 in the high-dose group only, compared with controls. However, elevated concentrations of OTC in males were observed at 13 ($p < 0.01$), 26 (mid-dose: $p < 0.05$; high-dose: $p < 0.01$) and 52 ($p < 0.05$) weeks in the high- and mid-dose groups. A reduction in triglyceride concentrations was noted for males and females of the high-dose group, compared with the controls, from week 26 onwards. The reduction only occasionally reached statistical significance ($p < 0.01$ for males in week 26 and 52; $p < 0.05$ for females in week 39). There were treatment-related microscopic findings in the liver and kidneys. In males and females of the high-dose group, there was a statistically significant increase in the incidence of single cell necrosis, centrilobular hepatocellular hypertrophy and portal inflammatory cell infiltration compared with the controls ($p < 0.01$ in most cases). Females of the mid- and high-dose groups also had a statistically significant increase in the incidence of basophilic hepatocytes ($p < 0.01$) compared with controls. In the mid-dose group, females had an increased incidence of single cell necrosis ($p < 0.01$) and males had portal inflammatory cell infiltration ($p < 0.05$). Female rats of the high- and mid-dose groups were also observed to have a statistically significantly increased incidence of minimal/trace amounts of pigment in the epithelium of the kidney cortical tubules compared with the control group rats ($p < 0.01$). The authors explained that β-cyclodextrin appeared to exacerbate normally spontaneous ageing in the liver of female rats (foci of basophilic hepatocytes), and that the centrilobular hepatocellular hypertrophy is a common adaptive

response. However, the single cell necrosis and inflammatory responses should be considered as mild hepatotoxicity, which is consistent with the elevated concentrations of serum ALT, AST and OTC and reduced concentrations of triglycerides. The slight increases in ALT and AST in males of the low-dose group were not associated with microscopic changes and were, therefore, not considered to be toxicologically significant. The authors of the study considered the hepatotoxicity observed in the mid- and high-dose groups treatment-related and concluded that the NOAEL for this study was equal to 654 and 864 mg β-cyclodextrin/kg bw per day in male and female rats, respectively. The Panel agreed with this conclusion.

In the study by Toyoda et al. (1997), Fischer 344 rats (50 animals/sex per dose) were administered β-cyclodextrin in their diets at concentrations of 2.5% or 5% (equal to 981 or 2,016 mg/kg bw per day for males and 1,134 or 2,344 mg/kg bw per day for females) for 104 weeks. Control rats received a basic diet. Following the 104-week exposure period surviving rats were given a basic diet for a further 5 weeks, and then killed. Survival was not affected by treatment with β-cyclodextrin, and no clinical signs of toxicity were observed. There was, however, a dose-dependent reduction in body weight gain in male rats of both groups during weeks 20 and 4, respectively, and for female rats of the 5% group from week 16. At week 104, mean body weight was decreased by 6 and 8% in the low- and high-dose male groups, respectively, compared with the control group (p values not stated). Body weight decreases of 2% and 9% were observed in the low- and high-dose female groups, respectively, compared with the controls (p values not stated). However, there was no effect on food consumption in any of the groups. There were no treatment-related changes in organ weights, or haematology and clinical chemistry parameters. Observed tumours were similar in incidence, organ distribution and histological characteristics to those known to occur spontaneously in this strain of rat. There were no treatment-related non-neoplastic microscopic findings in any of the treatment groups. The Panel considered that although statistically significant, the body weight losses observed in males and females at 104 weeks were not toxicologically significant as they were less than 10%. The Panel considered the NOAEL for this study to be 2,016 and 2,344 mg/kg bw per day, the highest doses tested, for males and females, respectively.

A carcinogenicity study was performed in Fischer F344 rats (50 animals/sex per group; approximately 4–5 weeks of age) exposed to 0, 25, 75, 225 or 675 mg β-cyclodextrin/kg bw per day via diet for 122 weeks in males and 129–130 weeks for females, according to the OECD guideline TG 451 (Gur et al., 1993b [Documentation provided to EFSA n. 10]; Waner et al., 1995). As recommended by the guideline, when males of the control group reached a survival rate of 25%, terminal kill of all survivors was carried out. Control animals received basic diet without β-cyclodextrin. No clinical and haematological data were recorded excepted formula of white blood cells for control and highest dose. Treatment did not affect survival of the rats, and no treatment-related clinical signs of toxicity were observed in any of the groups. Body weight, food and water consumption and organ weights were not affected by treatment. There were no treatment-related macroscopic findings. None of the observed microscopic lesions were attributable to treatment with β-cyclodextrin. There was a slight increase in the incidence of primary tumours in treated male rats; however, there was no dose–response relationship, and the incidence was within the limits of the historical controls from the laboratory conducting the study and background data from the literature. The authors concluded that under the conditions of this study β-cyclodextrin is assessed as showing no evidence of carcinogenicity in the rat. The Panel concluded that the NOAEL for this study was 675 mg/kg bw per day (the highest dose tested).

Overall, the Panel considered the NOAEL from chronic toxicity in rats 654 and 864 mg β-cyclodextrin/kg bw/day for males and females, respectively, based on the observed mild hepatotoxicity. In carcinogenicity study in mice, the Panel considered 225 mg/kg bw per day as the NOAEL based on the inflammatory changes observed in the caecum, colon and rectum at 675 mg/kg bw per day. Unlike JECFA (1995), the SCF (1997) considered that the inflammatory effects observed in mice (Gur et al. (1993a [Documentation provided to EFSA n. 9]) were not observed in studies in rats, and concluded that they were due to the high doses of undigestible compound tested and were not relevant for humans. The Panel noted that inflammation, localised in the caecum and colon, was seen in one mouse study. However, in several other studies in rats and dogs, no inflammation was reported in the caecum and large intestine. In line with the SCF (1997), the Panel considered that the effect reported in mice was species specific. Therefore, the NOAEL from this study was considered not relevant for the human risk assessment of β-cyclodextrin.

Based on the data available, the Panel noted that there was no evidence that β-cyclodextrin has carcinogenic properties.

3.6.6. Reproductive and developmental toxicity

3.6.6.1. Reproductive toxicity studies

In a two-generation reproductive toxicity study (HRC, 1992b [Documentation provided to EFSA n. 16]), β-cyclodextrin was administered in the diet of Sprague–Dawley rats (32 animals/sex per group) at concentrations of 10,000, 25,000 or 50,000 mg/kg diet (equal to 1,108, 2,713 or 5,444 mg/kg bw per day for males and 655, 1,584 or 3,164 mg/kg bw per day for females of the F_0 generation in the first week of treatment and for the F_{1a} generation equal to 1,531, 3,882 or 7,996 mg/kg bw per day for males and 1,525, 3,815 or 7,819 mg/kg bw per day for females). The study was conducted in compliance with the OECD guideline 416 (OECD, 1983c) and according to GLP. Following 10 weeks of treatment for males and 2 weeks of treatment for females, animals were mated and pregnant females were allowed to give birth to the F_1 generation. Pups (28 pups/sex) from the F_1 generation were selected and mated following 12 weeks of treatment with dietary β-cyclodextrin. These animals were allowed to give birth to their pups (F_2 generation) and wean them on day 21, after which pups were examined macroscopically. The F_0 animals were also remated, but they were euthanised on gestation day (GD) 20 and the fetuses (F_{1b}) examined for visceral and skeletal abnormalities. At the highest concentration, there were marginally lower body weight gains during gestation for the F_0 and F_{1a} females ($p < 0.05$). There was slightly longer median precoital time for the F_{1a} only. There were two total litter losses from the F_0 first mating, one of which was associated with maternal death not related to treatment. There were also three total resorptions among F_{1a} dams. Embryonic deaths (mainly early) were slightly increased ($p < 0.05$) in the F_{1b} litters. There were also slightly lower fetal weights ($p < 0.05$) for the F_{1b} litters and slightly lower pup body weight gain ($p < 0.05$) for the F_{1a} litters. In F_{1b} fetuses, there was a higher incidence of variant sternebrae ($p < 0.05$) (unossified and/or reduced). Following administration of 25,000 mg β-cyclodextrin/kg diet, marginally lower body weight gain during gestation in the F_0 (second mating only) and F_{1a} females were observed ($p < 0.05$). There was also a slightly longer median precoital time for the F_{1a} mating. There were two total litter losses; one in the F_0 and one in the F_{1a} generations, and one possible total resorption in an F_{1a} dam. Embryonic deaths (mainly early) were slightly increased in the F_{1b} litters ($p < 0.05$). Pups of the F_{1a} litters had slightly lower pup body weight gain ($p < 0.05$). All parameters for the 10,000 mg/kg diet group were comparable with the control group (equal to 1,108–1,531 mg/kg bw per day for males and 655–1,525 mg/kg bw per day for females). The authors of the study indicated that at 50,000 mg/kg diet and to a lesser extent at 25,000 mg/kg diet there was some evidence of adverse effects on the parent animals and offsprings although, in general, the effects were slight and only a few were consistent through both generations. The authors therefore concluded that a dietary concentration of 10,000 mg/kg (equal to 1,108–1,531 mg/kg bw per day for males and 655–1,525 mg/kg bw per day for females) was the NOAEL for either the parent treated animals or their offsprings in term of growth development, fertility and general performance. The Panel agreed with this conclusion.

Barrow et al. (1995) administered β-cyclodextrin to rats in a three-generation reproductive toxicity study. In the study, Ico:OFA.SD.(IOPS Caw) rats (30 animals/sex per dose) were administered β-cyclodextrin in the diet at concentrations of 0%, 1.25%, 2.5% or 5% (groups 1, 2, 3 or 4). Control animals received a basic diet. Two subsequent generations (25 animals/sex per dose) randomly selected from the F_{1b} and F_{2b} litters received dietary concentrations of 0%, 0.31%, 0.62% or 1.25% β-cyclodextrin (groups 1, 2, 3 or 4). Pup weight was decreased in the 5% group (F_1). Concentrations were reduced 3 weeks before mating of the F_1 generation to ensure identification of a NOAEL. There were no adverse findings for reproductive parameters. The authors considered that the NOAEL for reproductive toxicity was 1.25% β-cyclodextrin. Due to the modification in dose levels between the groups, the dose in concentration the figures in mg/kg bw per day are only presented for the last phase of the study and were considered to be equal to 636–1,891 mg/kg bw per day for males and 819–2,922 mg/kg bw per day for females, depending on the phase of the study. The Panel considered that due to modification in dose levels this study cannot be used to identify a NOAEL.

3.6.6.2. Developmental studies

Rats

In a prenatal developmental toxicity study, Wistar rats (26–28 sperm positive females; 22–25 pregnant dams/group) were administered β-cyclodextrin (in 1.25% aqueous methylcellulose) by

gavage at GD 7–16 at doses of 100, 500 or 2,500 mg/kg bw per day (Institut of Drug Research, 1985a [Documentation provided to EFSA n. 19]). Control animals received 1.25% aqueous methylcellulose by gavage. The study was conducted according to a protocol similar to the current OECD guideline 414 (OECD, 2001) but not according to GLP. The pregnant rats were euthanised at GD 21, and dams and fetuses examined. The numbers of corpora lutea, implantations, preimplantation loss, early and late embryonic death, as well as fetal deaths were recorded. Fetuses and placentas were weighed and examined for visceral and skeletal anomalies. There were no adverse effects on dam health, and there was no treatment-related developmental toxicity. The Panel considered that the NOAEL observed in this study for general systemic toxicity and developmental toxicity was 2,500 mg/kg bw per day, the highest dose tested.

In a prenatal developmental toxicity study conducted according to a protocol similar to the OECD guideline 414 (OECD, 2001) and to GLP, pregnant Sprague–Dawley rats (at least 20/group) were administered β-cyclodextrin (in 1% methylcellulose) by gavage at doses of 1,250, 2,500 or 5,000 mg/kg bw per day at GD 7–16 (Biotoxicology Laboratory Roquette Fréres, 1991 [Documentation provided to EFSA n. 4]). Control animals received 1% methylcellulose. The dams were euthanised at GD 21. Dams and fetuses were examined. Dams in the highest dose group had a slight, but statistically significantly decreased body weight gain (GDs 8, 9 and 21: $p < 0.05$; GDs 10–16: $p < 0.01$). This reduced body weight gain was associated with reduced food consumption. There were no other signs of toxicity relating to general systemic toxicity or development toxicity in any of the groups. The Panel considered that the NOAEL in this study was 5,000 mg β-cyclodextrin/kg bw per day (the highest dose tested) for developmental toxicity and 2,500 mg β-cyclodextrin/kg bw per day for maternal toxicity.

In a prenatal developmental toxicity study by Barrow et al. (1995), Ico:OFA.SD.(IOPS Caw) rats (at least 20 pregnant dams/group) were administered β-cyclodextrin (in 1% aqueous methylcellulose) by gavage at GD 6–15 at doses of 1,250, 2,500 and 5,000 mg/kg bw per day. Control animals received 1% aqueous methylcellulose by gavage. The study was conducted according to a protocol similar to the current guideline OECD 414 (OECD, 2001) but not according to GLP. The pregnant rats were euthanised at GD 20, and dams and fetuses examined. The numbers of corpora lutea, implantations, preimplantation loss, early and late embryonic death, as well as fetal deaths were recorded. Fetuses and placentas were weighed and examined for visceral and skeletal anomalies. There were no adverse effects on dam health, and there was no treatment-related developmental toxicity. The Panel considered that, under the conditions of this study, the NOAEL for general systemic toxicity and developmental toxicity was 5,000 mg/kg bw per day, the highest dose tested.

Rabbits

In a prenatal developmental toxicity study, New Zealand white rabbits (12–14 pregnant dams/group) were administered β-cyclodextrin (in 1.25% aqueous methylcellulose) by gavage at GD 7–19 at doses of 150, 300 and 600 mg/kg bw per day (Institut for Drug Research, 1985b [Documentation provided to EFSA n. 20]). Control animals received 1.25% aqueous methylcellulose only by gavage. The study was conducted according to the guideline OECD 414 (OECD, 2001), but not to GLP. The dams were euthanised at GD 28, and the dams and fetuses were examined. The numbers of corpora lutea, implantations, preimplantation losses, early and late embryonic deaths, as well as fetal deaths were recorded. Fetuses and placentas were weighed and examined for external, visceral and skeletal anomalies. The authors reported that, in all groups, animal infections of the kidneys and liver were observed. Furthermore, five animals with purulent processes were observed. The authors concluded that there were no treatment-related adverse effects on the dams or the fetuses in any of the treatment groups. However, the Panel noted that at necropsy at GD 28 only fetuses of eight out of the 21 treated control, three out of the 18 low-dose group, six out of the 19 mid-dose group and six out of the 17 high-dose group were evaluated. Therefore, the Panel considered that this study had too many limitations to be used for risk assessment.

Overall, from the multigeneration reproduction toxicity studies, the Panel concluded that dose levels up to 10,000 mg β-cyclodextrin/kg in the diet (equal to 1,108–1,531 mg/kg bw per day for males and 655–1,525 mg/kg bw per day for females) did not affect reproductive parameters and parental toxicity. At higher dose levels, maternal weight gain, food consumption and pup weight were decreased. Three prenatal developmental toxicity studies in rats did not show any evidence that β-cyclodextrin has an adverse effect at doses up to 2,500 and 5,000 mg/kg bw per day on developmental parameters.

3.6.7. Other studies

3.6.7.1. Human studies

JECFA in 1993 evaluated the results of a placebo-controlled, double blind study by van Dokkum and Van de Beek (Van Dokkum and Van der Beek, 1990; as referred to by JECFA (1993)), in which healthy male volunteers (n = 18) aged 23 ± 2 years, were given 0, 24 or 48 g β-cyclodextrin/day (corresponding approximately to 0, 343 or 686 mg/kg bw per day, assuming an average body weight of 70 kg) for three consecutive periods of 1 week. β-Cyclodextrin was administered in a chocolate drink equally divided over three normal meals. To avoid drastic effects on bowel function, doses were gradually increased over the first 3 days in subjects in the high-dose group. Subjects filled in a subjective questionnaire regarding abdominal complaints. At the end of each 7-day period, breath hydrogen concentration was measured. One of the subjects had to be replaced after 3 days of treatment due to abdominal cramps, nausea and diarrhoea. However, it was not clear what dose of β-cyclodextrin this subject received or whether the symptoms preceded treatment. The only statistically significantly increased abdominal effect was flatulence ($p < 0.05$) in the high-dose group (assumed to be compared with the control group, but this is not explicitly stated). Breath hydrogen did not change significantly (assumed to be compared with the control group, but this is not explicitly stated). The authors of the study concluded that the dose of 24 g β-cyclodextrin/day (approximately 343 mg/kg bw per day) was well tolerated by humans on a short-term basis.

3.6.7.2. Special studies on nephrotoxicity

The JECFA monograph (JECFA, 1993) summarised three nephrotoxicity studies in rats following subcutaneous injections (Frank et al., 1976; Hiasa et al., 1981) or intraperitoneal injections (Perrin et al., 1978). These studies are not directly relevant to the toxicity of β-cyclodextrin as a food additive due to the routes of exposure used and therefore not described in any detail here. However, they support that the minor changes in urinalysis observed in dogs following 52 weeks of dietary exposure (HRC, 1991 [Documentation provided to EFSA n. 14]; Bellringer et al., 1995), which were considered by the authors as of no toxicological importance, might be of toxicological relevance in the cases of extremely high oral doses when some absorption might occur.

3.7. Discussion

In animals and humans, β-cyclodextrin (E 459) is hydrolysed by gut microflora and endogenous amylases in the colon to maltose and glucose, which can be absorbed. Therefore, concentrations of β-cyclodextrin in tissues and serum are low (< 1%). Urinary excretion varies with species but is in most cases less than 5% of the oral dose. Some unhydrolysed β-cyclodextrin is excreted in the faeces, but this excretion takes several days and is dose-dependent and low (1–6% of dose in dogs; 3–5% of dose in rats).

β-Cyclodextrin shows very low acute oral toxicity with oral median lethal dose (LD_{50}) values for mice, rats and dogs, all greater than 3,000 mg/kg bw (Chinoin Pharmaceutical and Chemical works Ltd., 1980 [Documentation provided to EFSA n. 5]).

There were a number of short-term and subchronic toxicity studies on β-cyclodextrin in rats and dogs. In 28- and 90-day dietary studies in rats, the main effect was an adaptive effect on the caecum (enlargement), which the study authors concluded to be a result of consuming slowly digestible carbohydrates (TNO-CIVO Institutes, 1987 [Documentation provided to EFSA n. 29]; Olivier et al., 1991). The Panel considered that the NOAELs for β-cyclodextrin in these studies were 3,297 and 5,439 mg/kg bw per day (the highest doses tested) for the 28- and 90-day studies, respectively. A 6-month study in rats revealed an adverse effect on body weight gain in animals treated with β-cyclodextrin, but no other consistent treatment-related adverse effects were observed and the Panel identified a NOAEL of 600 mg β-cyclodextrin/kg bw per day (Chinoin Pharmaceutical and Chemical Works Ltd., 1982 [Documentation provided to EFSA n. 6]). Two 13-week and a 28-week studies in dogs were available but they had some limitations and were not considered for this assessment. In two dog studies, the NOAELs following 52 weeks of dietary exposure in dogs were 456 and 476 mg/kg bw per day in males and females, respectively (Bellringer et al., 1995), and 861 and 988 mg/kg bw per day in males and females, respectively (HRC, 1991 [Documentation provided to EFSA n. 14]).

The Panel noted that β-cyclodextrin gave negative results in a bacterial reverse mutation assay (Hazleton-IFT, 1987a [[Documentation provided to EFSA n. 11]), in a HPRT assay using V79 Chinese hamster cells (Institut Pasteur de Lille, 1990 [Documentation provided to EFSA n. 22]), an *in vitro*

chromosomal aberration assay (Institut Pasteur de Lille, 1991 [Documentation provided to EFSA n. 23]) and in an *in vivo* micronucleus test (Hazleton-IFT, 1988 [Documentation provided to EFSA n. 13]) which were of limited reliability. However, based on these data, the Panel considered that there was no indication for genotoxicity of β-cyclodextrin.

One chronic toxicity study was conducted in rats and the NOAEL (52-week dietary exposure) was 654 and 864 mg/kg bw per day for males and females, respectively, based on mild hepatotoxicity, (HRC, 1994a [Documentation provided to EFSA n. 17]). Carcinogenicity studies were also available in mice (Gur et al., 1993a [Documentation provided to EFSA n. 9]), and rats (Gur et al., 1993b [Documentation provided to EFSA n. 10]; Toyoda et al., 1997). There was no evidence that β-cyclodextrin had carcinogenic properties in any of the studies. In mice, the Panel considered the 225 mg/kg bw per day as the NOAEL based on the inflammatory changes observed in the caecum, colon and rectum at 675 mg/kg bw per day. In the JECFA monograph addendum (1995), it was concluded that the NOAEL for this study was 25 mg/kg bw per day based on the inflammatory effects in the lower gastrointestinal tract. The Panel did not agree with this conclusion since the inflammatory effects in the lower intestinal tract were only seen in animals of the highest dose. Therefore, the Panel considered the dose of 225 mg/kg bw per day as the NOAEL for this study. The Panel noted that unlike JECFA (1995), the SCF (1997) considered that the inflammatory effects observed in mice were not observed in studies in rats and dogs, and concluded that they were due to the high doses of undigestible compound tested and were not relevant for humans. The Panel noted that inflammation, localised in the caecum and colon, was seen in one mouse study. However, in several other studies in rats and dogs, no inflammation was reported in the caecum and large intestine. In line with the SCF (ref), the Panel considered that the effect reported in mice was species specific. Therefore, the NOAEL from this study was considered not relevant for the human risk assessment of β-cyclodextrin.

From the multigeneration reproductive toxicity studies, the Panel considered that dose levels up to 10,000 mg β-cyclodextrin/kg in the diet (equal to 1,108–1,531 mg/kg bw per day for males and 655–1,525 mg/kg bw per day for females) (HRC, 1992b [Documentation provided to EFSA n. 16]) did not affect reproductive parameters and parental toxicity. At higher dose levels, maternal weight gain, food consumption and pup weight were decreased. Three prenatal developmental toxicity studies in rats that tested β-cyclodextrin (Institut for Drug Research, 1985a [Documentation provided to EFSA n. 19]; Biotoxicology Laboratory Roquette Fréres, 1991 [Documentation provided to EFSA n. 4]; Barrow et al., 1995) did not show any evidence that β-cyclodextrin had an adverse effect at doses up to 2,500 and 5,000 mg/kg bw per day on developmental parameters.

Overall, based on the available toxicological database, the Panel considered that there is no reason to revise the current ADI of 5 mg/kg bw per day for β-cyclodextrin (E 459). The current ADI was established by the SCF in 1997 based on a NOAEL of 1.25% in the diet, equivalent to an intake of 466 mg/kg bw per day (based on urinalysis findings) in a 1-year dog study and applying an uncertainty factor of 100.

The dietary exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) was calculated based on (1) MPLs set out in the EU legislation and maximum reported use levels for the three food categories in which β-cyclodextrin (E 459) is authorised at QS (defined as the *maximum level exposure assessment scenario*) and (2) the reported use levels (defined as the *refined exposure assessment scenario*).

The Panel noted that the refined exposure estimates are based on information provided on the reported level of use of β-cyclodextrin (E 459). If actual practice changes, this refined estimates may no longer be representative and should be updated.

The exposure estimates in the *maximum level exposure assessment scenario* exceeded the ADI of 5 mg/kg bw per day for all population groups at the 95th percentile and also at the mean level except for infants. Similar observation was noted for the *refined brand-loyal* exposure estimate. For the *non-brand-loyal scenario*, the ADI was exceeded in toddlers and children at the mean and in all populations groups at the 95th percentile of exposure. The main contributing food categories for all population groups were flavoured drinks, coffee, tea, herbal and fruits infusions and food supplements supplied in a solid form in both scenarios.

The Panel considered exposure estimates obtained according to the *maximum level exposure assessment scenario* as conservative, as this scenario assumes that all foods and beverages in which β-cyclodextrin (E 459) is authorised contain this food additive at the maximum reported use level. The refined exposure assessment approach results in more realistic long-term exposure estimates, since this approach is based on use levels provided by the industry and assumes that people, in the long-term, consume foods and beverages that contain the food additive at a mean concentration level for

all products (*non-brand-loyal scenario*) or that one product contains the food additive at the maximum concentration level (*brand-loyal scenario*) and the remaining products contain the food additive at a mean concentration level. In case of β-cyclodextrin (E 459), the Panel considered that the uncertainties identified in its estimates for the food categories taken into account in this assessment would, in general, result in an overestimation of the exposure to β-cyclodextrin (E 459) from its use as a food additive according to Annex II and Annex III (Part 4) in European countries for the regulatory maximum level exposure scenario and for the refined exposure scenarios. However, the Panel is aware that other potentially important sources of exposure (in particular according to Annex III Part 1 and 5) of β-cyclodextrin were not considered in this assessment because no data were available.

β–Cyclodextrin (E 459) is authorised in a limited range of foods and with reported data provided by industry; according to the Panel, it is expected that brand-loyalty will result in higher exposure in the general population due to these food uses either from Annex II (e.g. flavoured drinks, food supplements) or from Annex III (Part 4) (coffee, tea, herbal and fruits infusions). The Panel therefore selected the *brand-loyal refined scenario* as the most relevant exposure scenario for this food additive.

Concerning the presence of trichloroethylene, classified as carcinogenic to humans (Group 1) (IARC, 2014), as residual solvent in β-cyclodextrin (E 459), the Panel noted that a range of benchmark dose 95% lower confidence limit ($BMDL_{05}$) values have been derived from animal carcinogenicity studies (from 1.4 to 53 mg/kg bw per day according to EPA (2011)) without detailed consideration of any other non-carcinogenic endpoint. The Panel also noted that based on the maximum permitted residual level of trichloroethylene in the EU specifications of β-cyclodextrin (E 459) (1 mg/kg), the exposure to trichloroethylene associated to the use of β-cyclodextrin as a food additive at the ADI of 5 mg/kg bw per day would be 5 ng/kg bw per day and this would result in a margin of exposure (MoE) of 280,000 even with the lowest BMDL value (1,4 mg/kg bw per day).

4. Conclusions

The Panel concluded that based on the available toxicological database there is no reason to revise the current ADI of 5 mg/kg bw per day for β-cyclodextrin (E 459).

Based on the available reported use and use levels, the Panel also concluded that the ADI was exceeded in the refined *brand-loyal scenario* (considered the most relevant scenario) in all population groups except for infants at the mean and in all population groups at the 95th percentile.

5. Recommendations

The Panel recommended that:

- microbiological specifications should be included in the EU specification for β-cyclodextrin (E 459);
- the maximum limits for the impurities of toxic elements (lead and arsenic) in the EU specification for β-cyclodextrin (E 459) should be revised in order to ensure that β-cyclodextrin (E 459) as a food additive will not be a significant source of exposure to those toxic elements in food;
- analytical data and more information on use and use levels should be provided in order to perform an adequate exposure assessment;
- the presence of trichloroethylene, classified as carcinogenic to humans (Group 1) (IARC, 2014), as a residual solvent in β-cyclodextrin (E 459) should be reduced to the lowest level possible according to SCF recommendations (SCF, 2002a,b,c,d, as referred to by EFSA Scientific Committee (2005)).

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ALT	alanine aminotransferase
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
AST	aspartate aminotransferase
BMDL	benchmark dose lower confidence limit
$BMDL_{05}$	benchmark dose 95% lower confidence limit
bw	body weight
CAS	Chemical Abstracts Service
CDase	cyclodextrinase
CFLP	Cleavase Fragment Length Polymorphism
CGTase	cycloglycosyltransferase

CIT	Centre International de Toxicologie
CO_2	carbon dioxide
CONTAM	EFSA Panel on Contaminants in Food Chain
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EFEMA	European Food Emulsifiers Manufacturing Associtation
EPA	Enviromental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FCS	food categorisation system
FDE	FoodDrinkEurope
GD	gestation day
GNPD	Global New Products Database
GLP	good laboratory practice
GPT	glutamate-pyruvate-transaminase
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HRC	Huntingdon Research Centre
IARC	International Agency for Research on Cancer
ICGA	International Chewing Gum Association
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
MoE	margin of exposure
MPL	maximum permitted level
NCE	norchromatic erythrocytes
NOEL	no observed effect level
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OTC	ornithine transcarbamoylase
PCE	polychromatic erythrocytes
QS	*quantum satis*
SCF	Scientific Committee on Food
SD	standard deviation
TemaNord	is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion
TLC	thin-layer chromatography
WHO	World Health Organization

Appendix A – Summary of reported use levels of β-cyclodextrin (E 459) in foods provided by industry

FCS category number	FCS food category	N samples	Reported use levels (mg/L or mg/kg as appropriate)		Data provider
			Typical mean (min–max)	Maximum mean (min–max)	
5.3	Chewing gum	1	3,000	30,000	International chewing gum Association
14.1.4	Flavoured drinks – Only flavoured powdered instant drinks	1	260	260	Food Drink Europe (FDE)
14.1.4/14.1.5[(a)]	Flavoured drinks – Only flavoured powdered instant drinks	6	262.5 (225–300)	375 (250–500)	Intertek Scientific & Regulatory Consultancy
15.1	Potato-, cereal-, flour-, or starch-based snacks	1	1,000	1,000	Intertek Scientific & Regulatory Consultancy
17.1	Food supplements supplied in a liquid form	2	60,000 (50,000–70,000)	61,250 (52,500–70,000)	Intertek Scientific & Regulatory Consultancy

FCS: food categorisation system.
(a): Reported as 'Flavoured drinks' (14.1.4) and 'Flavoured teas and flavoured powdered instant drinks' 14.1.5.

Appendix B – Concentration levels[(a)] used in the exposure scenarios (mg/kg)

FCS category number	FCS food category description	MPL	Maximum level[(a)]	Concentration levels used in the refined exposure assessment		Data source/comments
				Mean	Maximum	
5.2	Other confectionery including breath freshening microsweets	QS	30,000[(b)]	0	0	No usage levels reported to EFSA
5.3	Chewing gum[(c)]	QS	30,000	3,000	30,000	Usage levels
14.1.4	Flavoured drinks – Only flavoured powdered instant drinks	500	500	262.5	500	Usage levels from 14.1.4 and 14.1.5
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products[(a)]	QS	500			
15.1	Potato-, cereal-, flour-, or starch-based snacks[(a)]	QS	1,000	1,000	1,000	Usage levels
17	Food supplements defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children	QS	70,000	60,000	70,000	Usage levels

FCS: food categorisation system; MPL: maximum permitted level; QS: *quantum satis*.
(a): Authorised according to Annex III to Regulation (EC) No 1333/2008 as a carrier (Part 4).
(b): Maximum usage levels from category 5.3.
(c): As food in tablet and coated tablet form and therefore authorised through the FCS 0.

Appendix C – Summary of total estimated exposure of β-cyclodextrin (E 459) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw/day)

	Number of subjects	MPL scenario		Brand-Loyal scenario		Non-Brand-Loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Infants							
Bulgaria (NUTRICHILD)	659	2.2	8.2	1.1	4.5	2.1	8.0
Germany (VELS)	159	4.5	16.2	2.3	8.7	4.0	13.9
Denmark (IAT 2006_07)	826	0.7	2.7	0.1	0.7	0.2	1.3
Finland (DIPP_2001_2009)	500	1.3	2.6	1.1	2.2	1.3	2.6
United Kingdom (DNSIYC_2011)	1366	0.9	4.2	0.6	3.0	0.8	3.8
Italy (INRAN_SCAI_2005_06)	12	1.4		1.0		1.4	
Toddlers							
Belgium (Regional_Flanders)	36	10.9		4.4		8.3	
Bulgaria (NUTRICHILD)	428	6.6	26.9	1.9	5.6	3.1	8.8
Germany (VELS)	348	19.1	48.8	5.4	16.9	9.2	28.6
Denmark (IAT 2006_07)	917	11.0	38.0	0.9	3.2	1.6	5.6
Spain (enKid)	17	1.9		0.4		0.4	
Finland (DIPP_2001_2009)	500	2.0	8.7	1.4	6.4	1.7	7.6
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	10.3	36.4	3.7	17.2	5.4	25.1
United Kingdom (DNSIYC_2011)	1314	4.0	19.4	1.9	7.2	2.7	11.5
Italy (INRAN_SCAI_2005_06)	36	2.0		1.1		1.5	
Netherlands (VCP_kids)	322	18.5	56.6	2.9	11.3	5.0	18.6
Children							
Austria (ASNS_Children)	128	9.8	31.9	2.6	6.4	4.1	11.2
Belgium (Regional_Flanders)	625	10.1	28.7	3.5	9.6	6.4	18.1
Bulgaria (NUTRICHILD)	433	7.3	30.6	2.1	5.8	3.6	10.3
Czech Republic (SISP04)	389	12.6	50.7	2.9	8.2	5.1	14.7
Germany (EsKiMo)	835	16.2	48.9	3.8	10.7	5.7	16.0
Germany (VELS)	293	19.6	47.2	4.9	12.2	8.2	20.0
Denmark (DANSDA 2005-08)	298	19.0	47.4	2.2	5.6	4.0	10.0
Spain (enKid)	156	6.4	27.7	1.0	4.8	2.1	9.9
Spain (NUT_INK05)	399	4.6	17.8	0.9	3.3	1.5	6.1
Finland (DIPP_2001_2009)	750	17.4	56.3	2.9	9.0	3.7	11.1
France (INCA2)	482	5.1	18.9	1.2	4.1	2.2	7.5
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	11.6	37.8	3.4	8.7	5.2	13.7
Greece (Regional_Crete)	838	2.1	9.1	0.6	2.6	1.0	3.8
Italy (INRAN_SCAI_2005_06)	193	2.0	8.0	0.7	2.9	1.2	4.4
Latvia (EFSA_TEST)	187	7.2	21.4	2.8	6.1	4.7	10.1
Netherlands (VCP_kids)	957	19.4	48.9	2.9	8.5	5.0	14.7
Netherlands (VCPBasis_AVL2007_2010)	447	24.4	60.0	5.8	13.0	9.3	20.5
Sweden (NFA)	1,473	23.7	73.1	4.0	8.8	6.7	15.0
Adolescents							
Austria (ASNS_Children)	237	4.9	15.7	1.9	5.8	3.2	9.5
Belgium (Diet_National_2004)	576	6.6	18.5	2.4	6.3	4.3	11.3

	Number of subjects	MPL scenario		Brand-Loyal scenario		Non-Brand-Loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Cyprus (Childhealth)	303	2.2	7.4	1.0	3.1	1.7	5.4
Czech Republic (SISP04)	298	8.1	34.0	2.3	6.1	3.9	10.4
Germany (National_Nutrition_Survey_II)	1,011	7.0	28.1	2.0	6.5	3.5	11.4
Germany (EsKiMo)	393	12.2	35.5	3.1	9.1	4.9	13.7
Denmark (DANSDA 2005-08)	377	11.8	31.7	1.8	4.3	3.2	7.5
Spain (AESAN_FIAB)	86	3.2	15.1	0.6	2.4	1.0	4.5
Spain (enKid)	209	5.1	21.7	0.8	3.4	1.6	6.5
Spain (NUT_INK05)	651	3.8	16.6	0.9	3.0	1.5	5.1
Finland (NWSSP07_08)	306	11.5	41.8	1.5	4.6	2.1	6.4
France (INCA2)	973	2.9	10.9	0.8	2.8	1.5	5.0
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	7.3	22.9	2.5	5.5	4.1	9.4
Italy (INRAN_SCAI_2005_06)	247	1.7	6.3	0.8	2.7	1.3	4.7
Latvia (EFSA_TEST)	453	4.2	10.8	2.1	4.7	3.4	7.3
Netherlands (VCPBasis_AVL2007_2010)	1,142	14.8	40.2	4.1	8.8	6.8	14.7
Sweden (NFA)	1,018	16.2	56.8	2.6	5.8	4.3	9.8
Adults							
Austria (ASNS_Adults)	308	3.3	12.3	1.6	5.0	2.6	9.2
Belgium (Diet_National_2004)	1,292	3.8	12.4	1.6	5.0	2.8	8.9
Czech Republic (SISP04)	1,666	2.1	5.9	1.1	2.9	1.7	4.8
Germany (National_Nutrition_Survey_II)	10,419	5.7	20.2	2.1	5.8	3.5	10.6
Denmark (DANSDA 2005-08)	1,739	6.8	21.0	1.3	4.3	2.3	7.6
Spain (AESAN)	410	1.7	6.1	0.9	2.9	1.5	5.5
Spain (AESAN_FIAB)	981	1.5	5.5	0.5	1.8	0.8	3.2
Finland (FINDIET2012)	1,295	6.8	26.3	2.3	6.6	3.1	9.5
France (INCA2)	2,276	2.1	7.9	0.8	3.3	1.6	6.2
United Kingdom (NDNS-RollingProgrammeYears1-3)	1,266	5.6	14.1	3.0	6.7	4.7	10.9
Hungary (National_Repr_Surv)	1,074	2.3	6.5	1.2	3.4	2.1	5.6
Ireland (NANS_2012)	1,274	6.3	17.7	3.5	8.4	5.1	11.7
Italy (INRAN_SCAI_2005_06)	2,313	1.0	4.0	0.5	1.8	0.8	2.9
Latvia (EFSA_TEST)	1,271	1.9	5.0	0.9	2.5	1.7	4.5
Netherlands (VCPBasis_AVL2007_2010)	2,057	7.7	21.2	3.1	7.9	5.0	12.9
Romania (Dieta_Pilot_Adults)	1,254	1.8	5.8	0.7	2.3	1.1	3.6
Sweden (Riksmaten 2010)	1,430	6.9	26.3	3.0	4.3	4.0	7.7
The elderly							
Austria (ASNS_Adults)	92	2.1	7.9	1.1	4.1	2.0	7.9
Belgium (Diet_National_2004)	1,215	1.6	6.6	0.6	2.7	1.1	5.1
Germany (National_Nutrition_Survey_II)	2,496	3.3	11.3	1.5	4.8	2.7	9.0
Denmark (DANSDA 2005-08)	286	3.3	13.5	0.8	3.3	1.4	6.2
Finland (FINDIET2012)	413	3.9	13.3	2.1	5.4	2.6	6.4
France (INCA2)	348	1.3	5.2	0.6	2.6	1.1	4.8
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	5.6	13.5	3.1	6.8	4.9	11.6
Hungary (National_Repr_Surv)	286	1.9	4.4	1.0	2.4	1.7	4.2
Ireland (NANS_2012)	226	6.8	15.2	4.3	10.1	6.4	14.3
Italy (INRAN_SCAI_2005_06)	518	0.9	3.4	0.5	1.7	0.7	2.8

	Number of subjects	MPL scenario		Brand-Loyal scenario		Non-Brand-Loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Netherlands (VCPBasis_AVL2007_2010)	173	4.6	12.0	2.2	6.1	3.4	10.0
Netherlands (VCP-Elderly)	739	5.2	13.7	2.6	6.6	4.0	9.6
Romania (Dieta_Pilot_Adults)	128	2.1	5.2	0.8	1.9	1.3	3.1
Sweden (Riksmaten 2010)	367	1.9	7.6	0.6	2.2	1.1	3.8

bw: body weight; MPL: maximum permitted level.