European Food Safety Authority The EFSA Journal (2007) 537, 1-21

Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the safety of alpha-cyclodextrin

(Request N° EFSA-Q-2006-319)

(Adopted on 6 July 2007)

SUMMARY

A request has been submitted under the Novel Food Regulation (EC) No. 258/97 for the placing of α-cyclodextrin (α-CD) on the market as a novel food ingredient to be added as dietary fibre to a variety of foods. The applicant intends to add the ingredient to bakery products, beverages, ready-to-eat breakfast cereals and other grain products, yoghurts and other dairy products, snacks, soups and sweets.

Alpha-cyclodextrin is a non-reducing cyclic saccaharide consisting of six α-1,4-linked glucopyranosyl units. It is manufactured by the enzyme action of an α-cyclodextrin glucosyltransferase (α-CGTase) on food grade liquefied starch. During the production process 1-decanol is added to form an insoluble complex with the α-CD and the α-CD is obtained as a white crystalline powder following steam distillation to liberate the 1-decanol. A specification has been provided in which the content of α-CD is > 98 %.

Ingested α-CD is resistant to the action of digestive enzymes and is not hydrolysed to a significant extent during small intestine passage. A very small fraction of the α-CD (< 1 %) is absorbed and excreted with the urine. About 99 % reaches the larger intestine where the α-CD ring is readily opened by microbial enzymes. The resulting linear malto-oligosaccharides are then further hydrolysed and fermented to absorbable and metabolisable short-chain fatty acids. Overall the metabolic fate of ingested α-CD resembles that of other non-digestible but fermentable carbohydrates such as resistant starch or inulin.

Anticipated daily intake levels for consumers aged 2-5 years, 6-12 years, 13-19 years and 20 years and over in the US have been provided by the applicant on the basis that users will regularly consume all the categories of food that contain α-CD at the maximum level. The mean value for users of all age groups combined is 10.7 g/person/day (0.20 g/kg bw/day) and 18.8 g/person/day (0.42 g/kg bw/day) at the 90th percentile. In its consideration of the safety of α-CD, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) calculated for European consumers a total intake of 65 g/person/day from both additive and ingredient use. An intake assessment provided by Food Standards Australia New Zealand to JECFA is 16 g and 37 g/person/day at the mean and 95th percentile respectively. The calculations made by JECFA were based on Food Balance Sheets, whereas the US and Australian/New Zealand estimates were based on individual food consumption data.

Two human studies using both single and repeated dosing with α-CD showed that α-CD at the applied dose levels - 25 g at the single dose and 15 g per 2,200 kcal for repeat dosing - is well tolerated and does not affect markers of carbohydrate and fat metabolism. Concerns that cyclodextrins might impair the bioavailability of vitamins and minerals are unwarranted.

In 28- and 90- day feeding studies there were no signs of toxicity in rats and dogs. At very high doses (20 % of the diet corresponding to 13.9 g/kg bw/day in rats and 10.4 g/kg bw/day in dogs) caecal enlargement was observed most likely due to the high concentration of an osmotically active substance in the large intestine. Studies of embryotoxicity and teratogenicity in mice, rats and rabbits fed diets containing α-CD at levels up to 20 % did not indicate any adverse effects. Alpha-cyclodextrin showed no effects in assays for genotoxicity *in vitro* and *in vivo* and was demonstrated not to be an irritant or sensitizer after dermal application.

The Panel considers that the margin between the maximum intake of α-CD for dogs that shows no signs of toxicity and the maximum estimated daily intake (EDI) for humans is sufficient.

On the basis of all the information reviewed the Panel concluded that there are no safety concerns for the proposed use levels and anticipated consumption.

KEY WORDS

Alpha-cyclodextrin, dietary fibre, novel food ingredient

BACKGROUND

On 19 October 2004, Dr. Bär, Bioresco (on behalf of Wacker Chemie) submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97 to the competent authorities of Belgium for placing on the market 'α-Cyclodextrin' (α-CD) as a novel food ingredient.

On 24 August 2005, the competent authorities of Belgium forwarded to the Commission their initial assessment report, which concluded that the use of α-CD as recommended by the applicant was acceptable.

On 28 September 2005, the Commission forwarded the initial assessment report to the other Member States. Several of these Member States submitted additional comments/objections.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the competent authorities of Member States can be summarised as follows:

- The limit for arsenic (3 mg/kg) in the specification is too high.
- The analysis for α-CD in foods is not sufficiently sensitive.
- The EDI values for α-CD are based on US data; consumption may be higher than indicated.
- The tolerance to repeated doses of α-CD has not been examined in humans and data on the tolerance of children are lacking.
- Children may be a potentially sensitive group of consumers with regard to intestinal tolerance.
- The consequences of α-CD intake on human carbohydrate and lipid metabolism, as well as on gastrointestinal transit time and the intestinal microbiota should be investigated.
- The absence of interference of α-CD with the absorption of lipophilic nutrients and minerals should be confirmed experimentally.
- The use of α-CD may be problematic for diabetics, a potentially sensitive group of consumers.
- The safety margin is less than 100.
- Alpha-CD should be regarded as dietary fibre only if there is evidence of physiological benefits that are typical of dietary fibre.
- The energy value of α-CD should be in line with Directives 2001/13/EC and 90/496/EC.
- Could unidentified proteins, which might be present in α-CD at up to 5 mg/kg have an allergenic potential?

In view of these questions and the Community interest in this matter the European Commission has decided to seek the opinion of the European Food Safety Authority.

In addressing Member States' comments and assessing the overall safety of α-CD the Panel has used information from the original dossier provided by the applicant, the initial assessment carried out by the Belgian Competent Authority, the comments/concerns raised by

the Member States, the responses to these concerns provided by the applicant and the safety assessment of α-CD as a food ingredient by JECFA (WHO, 2006).

Existing authorisations and evaluations

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated the safety of α-CD added to food for food-technological purposes only and allocated an acceptable daily intake (ADI) "not specified" (WHO, 2002). The use of α-CD as a dietary fibre has also been considered by JECFA and an ADI "not specified" allocated (WHO, 2006).

In Australia/New Zealand, α-CD has been reviewed as a novel food (FSANZ, 2004) and approval was recommended by Food Standards Australia New Zealand with no specific conditions to limit its use. In the US a Generally Recognized as Safe (GRAS) Notice has been submitted to the FDA (GRAS Notice No. 155).

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002 the European Food Safety Authority is asked to carry out the additional assessment for 'α-Cyclodextrin' in the context of Regulation (EC) No 258/97.

In particular, EFSA is asked to consider the elements of a scientific nature in the comments/objections raised by the other Member States.

ASSESSMENT

The products belong to class 1, sub-category 1.2 (pure chemicals or simple mixtures from non-GM (genetically modified) sources with no history of food use in the Community), as defined in the recommendations from the Scientific Committee on Food (SCF) concerning the assessment of novel foods (European Commission, 1997b). Accordingly, information related to the structured schemes I, II, III, IX, XI, XII, XIII has been submitted.

I. Specification of the novel food (NF)

Alpha-cyclodextrin (α-CD) is a non-reducing cyclic saccharide consisting of six α-1,4-linked glucopyranosyl units (CAS-number: 10016-20-3; formula: $(C_6H_{10}O_5)_{6;}$ molecular weight: 972.85 Daltons).

The specifications provided by the applicant are shown in Table 1.

Table 1. Specifications of α-CD

Assay:	≥ 98 %[a]
Ash:	≤ 0.1 %
Reducing sugars:	≤ 0.5 %
Heavy metals:	≤ 5 mg/kg
Lead:	≤ 0.5 mg/kg
Volatile organics:	≤ 20 mg/kg[b]

[a]on anhydrous basis; potential impurities: residual starch, maltooligosaccharides, glucose, β-cyclodextrin.

[b]residues of 1-decanol used as complexant in the course of the production process.

The specification provided by the applicant in the originally submitted dossier contained a value for arsenic (≤ 3 mg/kg). In response to an objection expressed by one of the Member States, the applicant proposed not to include arsenic in the specifications (in analogy to the specifications accepted by JECFA), because neither the source material from which α-CD is produced nor the manufacturing process indicate that such a limit is necessary. According to the applicant, in four batches of α-CD arsenic was not detectable (limit of detection: 1 mg/kg).

Protein (determined by polyacrylamide gel electrophoresis; limit of detection: 5 mg/kg α-CD) and DNA from the recombinant source of the α-CGTase used in the production process (quantitative PCR; limit of detection: 0.005 ng DNA/reaction) are not detectable in the final α-CD preparation.

Several batches of α-CD were tested and shown to be within the specification.

Stability

Alpha-CD is water-soluble and crystallizes with about 6 mol water per mol α-CD. It is stable under the temperature and pH-conditions typically encountered in food storage and processing. With increasing temperature bound water is lost; the melting point is above 250°C. Hydrolysis occurs under strong acidic conditions (half-life in 1N HCl at 60°C: 6.2 h).

Alpha-CD is hydrolyzed by α-amylases of fungal or bacterial origin. Salivary (human) and pancreatic (porcine, human) amylases are not able to hydrolyze α-CD to a significant degree.

Analysis in foods

According to the information provided by the applicant, a validated HPLC method for the measurement to determine α-CD in foods has a detection limit of 2.2 % and a recovery rate of

98.9 %. The applicant indicated that the development of an official method with a lower limit of detection is in progress.

II. Effect of the production process applied to the NF

Alpha-CD is produced by the action of an α-cyclodextrin glucosyltransferase (α-CGTase) on food-grade, liquefied starch. The α-CGTase is obtained from a genetically modified strain of *Escherichia coli* K-12. The gene encoding the enzyme was obtained from *Klebsiella oxytoca*. Both host and donor strains are non pathogenic and non toxigenic.

During the enzymatic production process, 1-decanol is added to form an insoluble complex with the formed α-CD. The precipitate is transferred to a decanting centrifuge and purified by dissolution and re-precipitation. 1-Decanol is removed from the complex by steam distillation; upon cooling α-CD is obtained as a white, crystalline powder.

The starting starch material and sodium hydroxide (used for pH-adjustment during the production process) are food-grade. The complexing agent 1-decanol has a purity ≥ 98 %. The α-CGTase preparation complies with general purity criteria for enzyme preparations (heavy metals: < 5 mg/kg; total aerobic counts: <100 CFU/g; coliforms: absent; pathogens: absent; source organism: 0 CFU/ml).

Critical control points and control standards of the production process have been provided by the applicant.

III. History of the organism used as the source of the NF

The applicant states that α-CD is produced from food grade liquefied starch and consequently that no information is required under this heading.

IX. Anticipated intake and extent of use of the NF

The applicant intends to add α-CD as a dietary fibre to a variety of foods, viz. bakery products, beverages, ready-to-eat breakfast cereals and other grain products, yoghurt and other dairy products, snacks, soups and sweets. The added α-CD would increase the dietary fibre of these foods by about 2.4-5.6 g per serving (Table 2). At these levels, this ingredient is close to the maximum that can be added for organoleptic reasons.

Estimates of daily intake levels of α-CD for consumers in different EU Member States on the basis of the intended use levels have not been attempted by the petitioner "because of the variation of food consumption patterns in the different Member States". However it is argued that since the use of processed foods is higher in the United States than in most European countries, EDI calculations based on US food intake data may be used as valid, or even conservative, substitutes for European ones. The calculation model relies on food consumption data from the US Continuing Surveys of Food Intakes by Individuals (CSFII) conducted in 1994-96 and 1998 (USDA, 1994-96, 1998). Each individual from representative samples was surveyed for two non-consecutive days using 24 hour recall interviews. The EDI of α-CD averaged over the two observation days and expressed in g/day and g/kg bw/day was calculated for each food category in which α-CD could be used and for all the categories combined.

For users aged 2-5 years, the EDI from all proposed uses combined was 9.9 and 15.7 g/day at the mean and 90th percentile respectively (corresponding to 0.60 and 0.96g/kg bw/day); for users aged 6-12 years, 11.4 and 18.3 g/d (0.37 and 0.63 g/kg bw/day); for users aged 13-19 years, 11.8 and 20.6 g/d (0.19 and 0.36 g/kg bw/day); and for users aged 20 and over, 10.5 and 18.9 g/day (0.14 and 0.27 g/kg bw/day). The intake of α-CD from all its intended uses in food was estimated at 10.7 g/person/day (mean of users of all age groups combined) and 18.8 g/person/day at the 90th percentile corresponding to 0.20 and 0.42 g/kg bw/d. However this is a conservative estimate because it assumes that users will consume all the categories of food that contain α-CD on a regular basis and this is unlikely.

In its consideration of the safety of α-CD as a food ingredient, JECFA has made intake calculations for European consumers assuming that α-CD would be added to all possible food categories at the maximum proposed use levels and using "European diet" food consumption data in the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) database. The Committee calculated a total intake of α-CD of 65 g/person/day from both additive and ingredient use. This estimate is also considered very conservative for the reasons given above and the Committee suggested that intakes were likely to be 30-50 % of this value.

An intake assessment provided by Australia and New Zealand to JECFA, based on a national 1-day recall survey and assuming that α-CD would be present at the highest proposed levels in all the food categories, came up with 16 g/person/day and 37 g/person/day for the mean and 95th percentile consumer, respectively.

The Panel noted that the GEMS/Food database is based on Food Balance Sheets of annual food production as well as on import and export for individual countries, aggregated into clusters according to similar consumption behaviour. Waste at the household and individual level is not usually considered and intake values are given mostly for the whole raw agricultural commodity. These data reflect food availability and cannot be compared with estimates based on individual food consumption data, as presented for US and Australia/New Zealand.

Table 2. Proposed uses of α-cyclodextrin

Food Category	Serving Size	Max. Concentration of α-CD in Final Food (%)
Bakery products		
Bread and rolls	50 g	5
Brownies	40 g	7
Cakes (light weight)	55 g	5
Crackers	30 g	10
Grain-based bars	40 g	7
Coffee cakes, crumb cakes, muffins, biscuits & corn bread	55 g	5
Refrigerated dough	-	5
Dry baking mixes	-	5
Beverages		
Dry mixes for beverages	-	1
Carbonated and non-		

carbonated sugar-free soft drinks	240 ml	1
Fruit juices and juice drinks	240 ml	1
Vegetable juice	240 ml	2
Formula diets e.g. meal replacements	240 ml	1
Soy milk	240ml	2
Cereals and other grain products		
Breakfast cereals, ready to eat		
20-43 g/cup	30 g	9
> 43 g/cup	55 g	5
Instant rice	140 g	2
Pastas	140 g	2
Dairy products		
Yogurt	225 g	2.5
Dry mixes for milk-based Beverages	-	2.5
Frozen dairy desserts, reduced fat ice creams and frozen yogurt	½ cup	2.5
Snack foods		
Chips, pretzels, popcorn, extruded snacks, grain-based snack mixes	30 g	3
Soups		
Canned soups	245 g	2
Dry mixes for soups	-	2
Sugar and sweets		
Hard candies	2.5-15 g	15

A number of concerns were raised by Member States concerning the EDI, viz. the reliance on US consumption data rather than European ones; the calculation of the consumption data based on a body weight of 46 to 54 kg rather than a more realistic figure of 60 kg; and the fact that the authorisation of α-CD might double the intake of dietary fibre.

The applicant has responded by agreeing that it would have been preferable to have used European consumption data rather than US ones but that there is no European database that would allow the calculation of EDIs with the same degree of differentiation (per age group, per eating occasion, per body weight, etc) as can be done with the US data. Additionally the use of US data is justified because it represents a "worst case" in that the intake of processed and prepared foods is higher in the US than in most European countries. The Panel noted that in some European countries these differentiated types of data are also available. However, a rough estimate made by the Panel, using intake data of UK adults (Henderson *et al.*, 2002), showed that the average α-CD intake would approach the estimates of US and Australia/New Zealand. The use of an unusual, low body weight was explained by the applicant by pointing out that in the Dossier the EDIs are presented in g/d and in g/kg bw/d for the different age groups separately and for all age groups combined. From these values the average body

weight of each age group can be calculated. In the group of adults aged 20 years and older, it is for example about 75 kg. In the total population of "users" of α-CD containing foods it is however only about 53.5 kg. The reason for this is that the younger age groups are over represented in comparison with the total population because the percentage of users of α-CD containing food is decreasing with age for many food categories e.g. beverages and snack foods. The panel accepts this approach.

On the concern that authorisation of α-CD might double the intake of dietary fibre, the applicant points out that the calculations from the US data are based on the concomitant use of α-CD in all proposed food categories at the highest possible concentrations. Under realistic conditions, the intake of α-CD would be considerably smaller even among consumers who purposely eat high-fibre foods. It should also be noted that α-CD would be expected to partially replace other fermentable fibres such as inulin/FOS, resistant starch or resistant dextrins.

XI. Nutritional information on the NF

Animal studies

In vitro experiments have shown that α-CD is not cleaved by the pancreatic juice of dogs or salivary amylase (Karrer, 1923; French, 1957; Marshall and Miwa, 1981; Kondo *et al*., 1990; McCleary *et al*, 2004).

The metabolism of pure α-CD was examined for the first time by Andersen *et al*. (1963) in an experiment in rats using ^{14}C-labelled α-CD with ^{14}C-labelled potato starch for comparison. After ingestion of ^{14}C-starch, $^{14}C0_2$ appeared rapidly in breath reaching a maximum at 3.5 h after dosing whereas after ^{14}C-α-CD the maximum exhalation rate of $^{14}C0_2$ was reached at about 7.5-9.5 h. The cumulative $^{14}C0_2$ production over the duration of the experiment (16-23 h) for both substrates was about 60 % of the administered dose. It was concluded that α-CD is not digested in the small intestine to a significant extent but is fermented by the intestinal microbiota to absorbable and metabolisable short-chain fatty acids (SCFAs) as shown by the delayed appearance of the ingested ^{14}C in respiratory CO_2.

The absorption, distribution, metabolism and excretion of intravenously and orally administered α-CD in rats were examined in two studies using uniformly ^{14}C-labelled α-CD in rats as a tracer (de Bie and van Ommen, 1994; van Ommen and de Bie, 1995; van Ommen *et al.*, 2004). The first study examined the excretion and blood kinetics after oral administration, and the blood kinetics after intravenous administration. The results with oral administration (about 200 mg/kg bw) were in keeping with those of Andersen *et al*. (1963). The experiment with intravenous administration of α-CD (50 mg/kg bw) suggested a half-life of ^{14}C in the blood about 88 min and within 8 h of dosing about 80 % of the administered dose was excreted as α-CD with the urine (de Bie and van Ommen, 1994).

The second study comprised four experiments in which groups of Wister rats (4 rats/sex/group) received single doses of ^{14}C-α-CD by gavage (3 experiments) or injection in the tail. In the first experiment two groups of rats received single doses of 200 and 1000 mg/kg bw ^{14}C-α-CD by gavage. The results demonstrated that about 60 % of the administered ^{14}C is expired as $C0_2$ within the first 24 h. Its appearance was delayed suggesting microbial fermentation of α-CD in the distal segments of the gut followed by absorption and metabolism of the resulting SCFAs. During the first 8 h after dosing about 1 % of the administered ^{14}C was excreted with the urine. Metabolic profiling by HPLC revealed the presence of α-CD in the 0-4 h urine samples. In the 4-8 h samples less than half of the

radiolabel represented α-CD. No α-CD was detected in subsequent urine samples. The faeces and gastrointestinal (GI) contents together contained about 16 and 18 % of the administered ^{14}C in the low and high-dose groups respectively although only traces of unchanged α-CD were left in these samples. About 16 % of the administered ^{14}C was retained in organs and the carcass after 24 h and this fell to about 10 % after 48 hours, the difference appearing as $^{14}C0_2$ in the 24-48 h after dosing.

In a second experiment one group of rats received a single oral dose of 200 mg/kg bw ^{14}C-α-CD. The primary aim was to examine the blood kinetics of orally administered α-CD. During the first 3 hours the blood concentration of ^{14}C remained low (0.05-0.22 % of the administered dose). ^{14}C levels then increased slowly reaching a maximum at about 12 h. Metabolic profiling of the blood sample after 8 h suggested only a trace of ^{14}C-α-CD. About 1 % of the ^{14}C dose was recovered from urine excreted from 0-4 h, all of it represented as α-CD. From 4-8 h 0.3 % of the ^{14}C was detected in the urine about half of which represented α-CD.

In a third experiment one group of rats received a single dose of 50 mg/kg bw ^{14}C-α-CD by injection in the tail vein. The blood ^{14}C concentration decreased steadily (first order kinetics) with a half life of 26 and 21 min in males and females respectively. HPLC analysis revealed that 94-100 % of the blood ^{14}C activity was due to α-CD. The main route of excretion was with the urine. All of the ^{14}C excreted during the first 8 hours after dosing represented α-CD. Small amounts of ^{14}C were recovered in the faeces and GI contents (0.6-4.5 %) suggesting that minor amounts of α-CD may be excreted with the bile.

In the fourth experiment one group of germfree rats received a single oral dose of 200 mg/kg bw ^{14}C-α-CD by gavage. The absence of a significant intestinal microbiota in the GI tract for the duration of the experiment resulted in a low exhalation of $^{14}C0_2$ during the 24 h observation period (mean 1.3 % of the administered dose; range 1.11-1.43 (n=8)). About 0.34 % (range 0.16-0.74 %) of the administered ^{14}C was excreted with the urine during the first 8 h. An additional 0.93 % (range 0.17-2.14 %) was excreted between 8 and 24 h after dosing. Retention of ^{14}C in the organs was very small (<0.1 %) while the biggest part, 90.7 % was found in the faeces and GI contents, all of it as ^{14}C-α-CD (van Ommen and de Bie, 1995; van Ommen *et al*., 2004).

Taken together these results give a coherent picture of the absorption, distribution, metabolism and excretion of α-CD in rats. Ingested α-CD is resistant to the action of digestive enzymes and is not hydrolysed to a significant extent during small intestinal passage. Because of its relatively high molecular volume and its hydrophilic surface only a very small fraction (about 1 %) is absorbed which is excreted as α-CD with the urine. About 99 % of the ingested α-CD reaches the microbe-colonised segments of the gut where the α-CD ring is readily opened by microbial enzymes. The resulting linear malto-oligosaccharides are then further hydrolysed and fermented via well-established metabolic pathways (Antenucci and Palmer, 1984). Overall the metabolic fate of ingested α-CD resembles that of other non-digestible but fermentable carbohydrates such as resistant starch or inulin.

Human studies

Proof for the low digestibility of α-CD stems from an applicant's study in which 12 healthy male volunteers, after overnight fasting, received single doses of 25 g α-CD, 50 g starch (in the form of about 100 g white bread) and a mixture of 50 g starch and 10 g α-CD. Capillary blood was collected from finger pricks at regular intervals over a 3 h period for analysis of glucose and insulin. The ingestion of 50 g starch produced the expected rise of blood glucose and insulin levels. In contrast, no significant increase in blood glucose and insulin levels was

observed after the intake of 25 g α-CD. After intake of 50 g starch and 10 g α-CD, the glycaemic and insulinaemic responses were delayed and reduced in comparison with those observed after intake of starch alone.

Urine samples were collected from all subjects during the 3 h period after ingestion and the recovered α-CD expressed in percent of the administered dose. After ingestion of 25 g α-CD, 0.05 % (range 0.00-0.18 %, n = 12) was excreted with the urine during the 3 h period. After ingestion of 10 g α-CD, 0.01 % (0.003-0.31 %) was excreted. Assuming that absorbed α-CD is not metabolised by the human body (as in rats) less than 0.1 % of ingested α-CD is absorbed by humans (Gaebert and Korff, 2004).

Member States were concerned about the absence of data from a human tolerance study with repeated dosing of α-CD assessing the potential interaction of α-CD with vitamins and minerals. Such a study has now been conducted in the US by a food manufacturer and the results made available on a confidential basis pending publication of the study (Lefevre *et al*., 2006). In this double blind, randomised cross-over study the tolerance to α-CD when consumed with a regular diet was examined in 14 male and 21 female, mildly hypercholesterolaemic but otherwise healthy volunteers. The subjects consumed four different diets for four weeks each, separated by an approximately one week washout period between the experimental periods. The control diet was an average American diet. One of the test diets was the control diet with a daily α-CD supplement of 15 g per 2200 kcal (diets were provided at 5 caloric values of 1800, 2200, 2600, 3000 and 3400 kcal/d to maintain body weight). Biochemical parameters were measured at the start of the study and at the end of each dietary period. None of the examined parameters (plasma triglycerides, LDL-, HDL- and total cholesterol, fasting glucose and insulin, albumin, alkaline phosphatase, alanine aminotransferase, creatinine phosphokinase, creatinine, uric acid, Ca, K, Mg and Fe) was adversely affected by the α-CD treatments. Complaints about gastrointestinal side effects were not reported.

Regarding the potential interaction of α-CD with lipophilic nutrients, plasma β-carotene and tocopherols were analysed after completion of the study in stored plasma samples. Plasma levels of β-carotene and β-/γ-tocopherol did not differ between the control and the α-CD treatments (Lefevre *et al.*, 2006). The α-tocopherol level was slightly but statistically significantly lower at the end of the α-CD period (by 6.5 %), the relevance of which was questioned by the author. The biological relevance of this minor change is not considered relevant by the Panel as the values are well within the normal range (SCF, 2003). Considering the similar functionality of α-CD and β-CD as complex forming agents, it also seems reasonable to take into account data obtained for β-CD.

When β-CD was first assessed for safety by JECFA there was a concern that ingested β-CD might impair the bioavailability of certain lipophilic essential nutrients by formation of complexes (WHO, 1993). However subsequent studies were conducted and it was concluded that such concern was unwarranted (WHO, 1996). The ingestion of β-CD at dietary concentrations up to 5 % did not influence the plasma levels of vitamins A, D and E and liver concentrations of vitamins A and E in dogs (Bellringer *et al*., 1995).

The formation of inclusion complexes with CDs is reversible (Connors, 1995; Stella *et al*., 1999) and so in the presence of other food components complexed guest molecules would be replaced by other organic compounds that have a higher affinity with the cyclodextrin cavity or are present at higher concentrations. A number of studies have shown that complexes of fat-soluble vitamins or other lipophilic compounds with β or α-CD are bioavailable and indeed may have a higher bioavailability than the free uncomplexed form owing to the

increased water-solubility (Szejtly and Bolla, 1980; Szejtly *et al.,* 1983*;* Horiuchi *et al*., 1988; Bardos *et al*., 1989).

Taking all these aspects into account, an impairment of the bioavailability of vitamins from the use of α-CD in food would not be expected.

Considering its low viscosity and the chemical structure lacking anionic or cationic groups, α-CD is not expected to impair the small intestinal absorption of minerals. There have been a number of reviews that considered an impairment of vitamin and mineral absorption from the consumption of increased levels of dietary fibre and have concluded that dietary fibre at recommended levels of intake does not adversely affect the vitamin and mineral status of the consumer (e.g. Gordon *et al*., 1995; Gorman and Bowman, 1993; Rossander *et al*., 1992; Kelsay, 1990).

A Member State suggested that the consequences of α-CD on the gastrointestinal transit time and the intestinal microbiota should be investigated. However there is no certainty of what a good transit time is and data on the microbial composition of faecal material would not give any indication of risk. It is generally considered that carbohydrate fermentation in the gut is a harmless process.

XII. Microbiological information on the NF

The specification for the α-CD product includes a microbiological level of <1000 colony-forming units (CFU)/g and absence of salmonellae and *Escherichia coli* from 10g. In an analysis of 5 batches of product (4 from pilot scale and 1 from production scale batches), the total viable count of microorganisms was <40 CFU/g for the four pilot scale samples and <10 CFU/g for the production batch. Salmonellae and *E. coli* were absent from 10g samples of all the batches.

XIII. Toxicological information on the NF

Acute toxicity studies

The LD_{50} was calculated from groups of Sprague-Dawley rats given intravenous doses of 576, 900 and 1400 mg/kg bw to be 1000 mg/kg bw (Frank *et al*., 1976). In mice given single doses of 500, 750, 1000 and 2000 mg/kg bw into the tail vein, the intravenous LD_{50} was estimated to be between 750 and 1000 mg/kg bw (Riebeek, 1990a). The same author estimated the LD_{50} for rats given single intravenous doses of 500, 750 and 1000 mg/kg bw to be between 500 and 750 mg/kg bw (Riebeek, 1990b).

In a micronucleus test 15 male and 15 female Swiss mice received a single oral dose of an aqueous α-CD solution by gavage (10 g α-CD/kg bw) while a control group received water only. No signs of toxicity were observed and all animals survived until termination of the experiment (Immel, 1991).

Subacute/subchronic toxicity studies

A 28-day feeding study with α-CD was conducted in Wistar rats. Groups of 5 rats/sex each were fed diets to which 0, 1, 5, 10 or 15 % α-CD was added at the expense of pregelatinised potato starch. Treatment started when the rats were 5-6 weeks old. Body weights were recorded initially and then at 7 day intervals. Food consumption was measured weekly and

water consumption daily. Haematological and clinico-chemical parameters were analysed in blood samples collected on day 28 when the rats were killed. All rats survived to the end of the study. In rats fed 15 % α-CD diarrhoea occurred at day 6 and continued to the end of the study. Body weights of this group were below controls (male – 26 % ($p < 0.01$); females – 7 % (not statistically significant)). Food intake was reduced in males of the 15 % α-CD group (-22 %). Water intake was increased in males and females. There were a few changes of haematological parameters but these were not dose related and/or occurred in one sex only. The same applied to the clinico-chemical parameters except that alkaline phosphatase was increased significantly in males and females of the 15 % α-CD group. The absolute and relative liver weights were significantly decreased in males of the 15 % α-CD group and for relative liver weight in the females. The caecum weights (full and empty) were increased slightly in the 5 % α-CD group and more markedly in the 15 % group. No gross abnormalities were detected at necropsy that could be attributed to the α-CD treatment. Microscopic examination of the main organs only revealed slight changes to the surface epithelial cells of the caecum in 7 out of 10 rats of the 15 % α-CD group but no signs of inflammation or degenerative changes of the intestinal mucosa.

In a subchronic (13 week) oral toxicity study groups of 20 male and 20 female Wistar rats received diets with 0, 1.5, 5 or 20 % (w/w) α-CD. A comparison group received a diet with 20 % lactose. Soft stools were observed during the first few weeks in most animals of the 20 % α-CD group and the 20 % lactose group. Otherwise no signs of treatment related reactions were seen. Mean body weights were slightly but significantly reduced in males but not females of the 20 % α-CD group. Males of the 20 % lactose group also showed reduced body weights. On day 91 the reductions were 6.1 % for the α-CD group and 7.8 % for the lactose group. The haematological and clinico-chemical parameters as well as urine analysis did not reveal adverse changes that could be attributed to the α-CD treatment. Histopathological examination of the organs and tissues at the end of the experiment did not reveal any abnormalities that could be attributed to the treatment. It was concluded that the ingestion of α-CD for 13 weeks at dietary levels up to 20 % (corresponding to intakes of 12.6 and 13.9 g/kg bw/d for male and female rats respectively) which is the NOAEL was well tolerated and did not produce any signs of toxicity (Lina, 1992; Lina and Bär, 2004a).

None of the effects that were observed in the 28 day rat study could be reproduced in the 90 day study even though a higher maximum dose was used. The persistent diarrhoea and its sequelae in the 28 day study may have been due to the composition of the basal diet (pregelatinised potato starch and whole ground wheat in the 28 day study and ground wheat and maize in the 90 day study).

A 90 day toxicity study was also carried out in Beagle dogs. Four groups (4 dogs/sex/group) received diets with 0, 5, 10 or 20 % (w/w) α-CD. All dogs remained in good health during the study. Transient diarrhoea occurred in all treatment groups. No treatment related differences were observed with respect to ophthalmic examination, haematological parameters, clinico-chemical analysis of the plasma and urine analysis. No abnormalities were seen at necropsy that could be attributed to treatment. Organ weight data revealed some caecal enlargement in the 10 and 20 % dose groups. No treatment related effects were observed in any of the various organs and tissues examined microscopically. It was concluded that the intake of α-CD at dietary levels up to 20 % (= 9.8 and 10.4 g/kg bw/d in male and female dogs respectively) which is the NOAEL was tolerated without any toxic effects (Til and van Nesselrooij, 1993; Lina and Bär, 2004b).

Chronic toxicity/carcinogenicity and reproduction studies

No long term studies of toxicity, carcinogenicity or reproductive toxicity have been conducted with α-CD. JECFA concluded that, given the known fate of this compound in the gastrointestinal tract, such studies were not required for an evaluation. In view of the results of the subchronic toxicity studies in animals and the known fate of the compound in the gastrointestinal tract, the Panel agrees with the view of JECFA that such studies are not required.

Embryotoxicity/teratogenicity studies

Studies have been carried out by feeding α-CD in ground feed at 0, 5, 10 or 20 % to pregnant Swiss Albino mice (Price *et al*., 1996; NTP, 1994), pregnant Sprague-Dawley rats (Verhagen and Waalkens-Berendsen, 1991; Waalkens-Berendsen and Bär, 2004) and artificially-inseminated New Zealand White rabbits (Waalkens-Berendsen and Smits-van Prooije, 1992; Waalkens-Berendsen *et al*., 2004).

The results indicated that α-CD administered in the diet to pregnant mice at levels corresponding to 49.3 g α-CD/kg bw/day had no adverse effect on embryo/foetal development. Similarly no adverse effects were observed neither in rats at α-CD intakes corresponding to 11 g/kg bw/day nor in rabbits at dietary concentrations corresponding to an intake of 5.9-7.5 g/kg bw/day. The substance was not teratogenic.

Studies on genotoxicity

An Ames test was performed with *Salmonella enterica* var. Typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 using concentrations of α-CD of 0, 0.25, 0.74, 2.22, 6.67 and 20.0 mg/plate with and without metabolic activation using a rat liver S9 fraction. The substance did not induce mutations in the Ames test (Blijleven, 1991). A mouse micronucleus test using mouse bone marrow and a 10 g α-CD/kg bw dose was also negative (Immel, 1991).

Studies on skin sensitisation and skin and eye irritation

The potential of α-CD to induce cutaneous delayed hypersensitivity was examined in guinea pigs (Prinsen, 1992). Dermal irritation and corrosion and eye irritation were tested in albino rabbits (Prinsen, 1991a and 1991b). The results demonstrated that α-CD does not provoke signs of hypersensitivity and is not a sensitizer, is not irritating or corrosive to the skin and that solutions of α-CD are not irritating or corrosive to the eye. However dry α-CD powder is irritating but not corrosive to the eye.

Human studies

In an early metabolic study of α-CD, two type-2 diabetic subjects received 50 g α-CD/day with a low carbohydrate diet. Nausea was noted in one subject on one of two experimental days about 10-12 min after ingestion. Other side effects did not occur (von Hoesslin and Pringsheim, 1923).

In subsequent experiments a purified CD preparation (mainly α-CD with some β and γ-CD) was consumed by diabetic patients at doses of 50-100 g/d. Some but not all volunteers reported nausea, and, occasionally, diarrhoea (von Hoesslin and Pringsheim, 1927).

The applicant provided a study in which the gastrointestinal tolerance of α-CD was examined in 12 healthy male volunteers. A single 25 g bolus dose of α-CD (dissolved in 250 ml water) was administered to overnight fasted subjects. One subject reported diarrhoea and three others abdominal discomfort. However, these effects were rated "mild" and did not prevent the volunteers from further participation in the study. The ingestion of 10 g α-CD (dissolved in 250 ml water) together with 100 g fresh white bread was not associated with any side effects in any of the subjects.

One Member State expressed concerns regarding diabetics and children as vulnerable groups in the population that require special consideration.

With regard to diabetics it was argued that any novel carbohydrate must be assessed for suitability for consumption by diabetics and attention was drawn to two early studies (von Hoesslin and Pringsheim, 1923 and 1927) on diabetic subjects. There was a concern by a Member State about a decrease in urinary glucose excretion after intake of α-CD in untreated diabetics. The applicant indicated that ingested α-CD (50-100 g), unlike white bread, does not increase the urinary glucose excretion which is not surprising since α-CD is not digested to absorbable glucose. The Panel accepts the applicant's response.

On the question of children, the concern was that the mean EDI was four times higher on a per kg body weight basis per eating occasion than that of adults (children aged 2-5 years, 0.17 g/kg bw; 6-12 years, 0.12 g/kg bw; and adults aged 20 and over, 0.08 g/kg bw per eating occasion i.e. about 2 and 1.5 times that of adults). The applicant responds that in absolute terms the intake per eating occasion does not exceed 12 g even for the 90th percentile consumer of the 6-12 age group. Intakes of polyols e.g. sorbitol, of this order of magnitude are usually well tolerated by children (Akerblom *et al*., 1982; Steinke *et al*., 1961) and since α-CD has about a 6 times lower osmotic activity than these polyols it would be unlikely that α-CD would elicit undesirable intestinal effects.

One Member State expressed concern that the safety margin between the NOAEL of a 13-week toxicity study in dogs (10 g/kg bw/day) and the EDI for the 90th percentile in the youngest age group (1 g/kg bw/day) is a factor of 10. In the view of the applicant a safety margin of 10 is sufficient because α-CD is not absorbed to any significant extent and therefore is not subject to metabolism nor influences the metabolism of the human body. Considering that ingested α-CD is metabolised only by the intestinal microbiota, the variability of the degradation products is expected to be small. JECFA has accepted this view and considers a safety margin of 10 to be sufficient for accepting the safety of α-CD (WHO, 2006). The Panel agrees with this view.

The question of potential allergenicity of residual protein in α-CD, which, according to the specification may reach 5 mg/kg, was raised by a Member State. This level is the limit of detection. The production methods are such that it is likely that there is no, or only a trace, of protein from the plant source or from the *E. coli* derived CGTase preparation. Directive 2003/89/EC applies to the labelling requirements.

DISCUSSION

The applicant intends to add α-CD to a variety of foods so that each serving would provide an additional 2.4-5.6 g of dietary fibre. The data regarding the absorption, metabolism and excretion of α-CD are consistent with its use as dietary fibre.

The EDI data of α-CD supplied by the applicant were based on US rather than European data because they allowed a greater degree of differentiation between age groups, eating occasions, body weights etc. In addition they were justified on the basis that the intake of processed and prepared foods is higher in the US than in most European countries and therefore represented a worst-case scenario. The intake of α-CD was estimated at 10.7 g and 18.8 g/person/day for the mean and 90th percentile respectively (0.20 and 0.42 g/kg bw/day). Calculations by JECFA based on European data gave an EDI of 65 g/person/day for α-CD (JECFA suggested that the real value would be 30-50 % of the estimated value) while Australia/New Zealand calculations gave values closer to those from the US, of 16 g and 37 g/person/day for the mean and 95th percentile (0.27 g and 0.62 g/kg bw/day for a 60 kg consumer). However, the JECFA estimate is based on Food Balance Sheets rather than on individual food consumption data. All the estimates of intake assumed that α-CD would be added to all possible food categories at the maximum proposed use levels and that the real values would be less than the estimates.

The intake of α-CD in animal studies even at high doses (up to 20 % of the diet, the highest dose tested) has been demonstrated to be well tolerated and to produce no signs of toxicity. The NOAEL from a 13 week study in dogs was 10 g/kg bw/day and 13 g/kg bw/day in rats. It is considered by the Panel that this gives a sufficient margin of safety since these doses are 10-100 times higher than the different estimates of potential intake by humans and since ingested α-CD is metabolised only by the intestinal microbiota and the variability of the degradation products is expected to be small.

Alpha-cyclodextrin showed no effects in assays for genotoxicity *in vitro* or *in vivo*. No long term studies of toxicity, carcinogenicity, or reproductive toxicity were carried out with α-CD. In view of the results of the subchronic toxicity studies in animals and the known fate of the compound in the gastrointestinal tract the Panel agrees with the view of JECFA that such studies are not required.

Although concerns have been raised that α-CD might impair the absorption of fat-soluble vitamins and minerals there is sufficient evidence from analogy with β-cyclodextrin and human studies with α-CD to discount these effects. Some interactions of α-CD with other food components that might affect the absorption of certain vitamins and minerals cannot be totally ruled out. However, the available evidence together with the reported effects of α-CD on serum α-tocopherol levels, like other effects of different dietary sources of fibre on the availability of nutrients, are too small to be considered of nutritional significance.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of all the information reviewed the Panel concluded that there are no safety concerns for the proposed use levels and anticipated consumption.

DOCUMENTATION PROVIDED TO EFSA

Dossier on α-Cyclodextrin for the placing on the market as a novel food ingredient to be added as dietary fibre to a variety of foods. Application pursuant to Regulation (EC) N° 258/97 submitted by Bioresco (Switzerland), October 2004.

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ACKNOWLEDGEMENT

The Scientific Panel on Dietetic Products, Nutrition and Allergies wishes to thank Karl-Heinz Engel and Annette Pöting for their contributions to the draft opinion.