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November 1, 2016

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, Maryland 20740

Subject: Notice of a GRAS Exclusion for Alpha-Cyclodextrin

Dear Sir/Madam:

In accord with 21 C.F.R. part 170, subpart E, Wacker Chemical Corporation hereby submits the enclosed notice that the general use of its alpha-cyclodextrin in processed and ultra-processed foods (other than beverages) at a level of up to 3% (w/w) as consumed, and in certain beverages at a level of up to 1.05% (w/v) as consumed, is excluded from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

Sincerely,

(b) (6)

Ricardo Carvajal

RC/sas
Enclosures

GRAS NOTICE FOR ALPHA-CYCLODEXTRIN
SUBMITTED BY WACKER CHEMICAL CORPORATION

Part 1 – Signed statements and certification

(1) Applicability of 21 C.F.R. part 170, subpart E

We submit this GRAS notice in accordance with 21 C.F.R. part 170, subpart E.

(2) Name and address of the notifier

Company: Wacker Chemical Corporation
Name: Helmut Reuscher Ph.D.
Address: 3301 Sutton Road, Adrian, Michigan 49221-9397
Phone: (517) 264-8794
Fax: (517) 264-8795

(3) Name of the notified substance

Alpha-cyclodextrin

(4) Applicable conditions of use of the notified substance

(a) Foods in which the substance is to be used

As explained in detail in this notice, the substance is to be used in (1) processed foods other than beverages (products that are directly derived from whole foods and recognized as such), (2) ultra-processed foods other than beverages (products that are formulated mostly or entirely from substances derived from whole foods), and (3) certain types of beverages. Some of these foods may constitute meat or poultry products falling under the jurisdiction of the U.S. Department of Agriculture. The substance is not intended to be used in infant foods.

(b) Levels of use in such foods

The substance may be used in processed and ultra-processed foods other than beverages at a level of up to 3% (w/w) as consumed, and in diet soft drinks at a level of up to 1.05% (w/v) as consumed. These use levels will supersede the corresponding use levels specified in GRN 000155. The use levels for the other types of beverages specified in GRN 000155 would remain unchanged, and are listed in Table 1.

Table 1 Food categories and use levels for alpha-cyclodextrin

	Maximum use level (%)	
	GRN 000155[a]	Present GRN
Processed and ultra-processed foods (as consumed)		3
Breads, rolls, cakes, baking mixes, refrigerated dough	5	3
Brownies and bars	7	3
Crackers (sweet and non-sweet)	10	3
Coffee whiteners (dry), formula diets, meal replacements, nutritional supplements	1	3
Ready-to-eat breakfast cereals	2 to 9	3
Instant rice, pasta, and noodles (prepared)	2	3
Condiments	3	3
Reduced fat spreads	20	3
Dressings and mayonnaise	5	3
Yogurt, milk beverage mixes, and frozen dairy desserts	2.5	3
Pudding mixes (dry)	1	3
Snack foods	1	3
Canned and dry soups (prepared)	2	3
Hard candy	15	3
Chewing gum	10	3
Beverages (as consumed)		
Diet soft drinks	1	1.05
Beverage mixes, fruit juices, instant coffees and teas	1	1
Vegetable juices, soy milk and non-soy (imitation) milk	2	2
[a] As per Agency Response Letter GRN 000155.		

(c) Purpose for which the substance is used

The substance is for general use in foods.

(d) Description of the population expected to consume the substance

The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

(5) Basis for the GRAS determination

The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with 21 C.F.R. §§ 170.30(a) and (b).

(6) Exclusion from premarket approval

The notified substance is not subject to the premarket approval requirements of the FDC Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

(7) Availability of data and information

If the Food and Drug Administration (FDA) asks to see the data and information that are the basis for our conclusion of GRAS status either during or after FDA's evaluation of our notice, we will agree to make the data and information available to FDA. Further, upon FDA's request, we will allow the Agency to review and copy the data and information during customary business hours at the above address, and will provide FDA with a complete copy of the data and information either in an electronic format that is accessible for the Agency's evaluation or on paper.

(8) Applicability of FOIA exemptions

None of the data and information in Parts 2 through 7 of our GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. § 552.

(9) Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(b) (6)

Name: Helmut Reuscher Ph.D.
Title: Director

10-31-16
Date

Please address correspondence to:

Ricardo Carvajal
Hyman, Phelps & McNamara, P.C.
700 13th Street NW, Suite 1200
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Phone: 202-737-5600
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Part 2 – Identity, method of manufacture, specifications, and physical or technical effect

(1) Identity of the notified substance

(a) Chemical name

Alpha-cyclodextrin; α-cyclodextrin; α-CD; alpha-dextrin; α-dextrin

(b) Chemical Abstracts Service (CAS) Registry Number

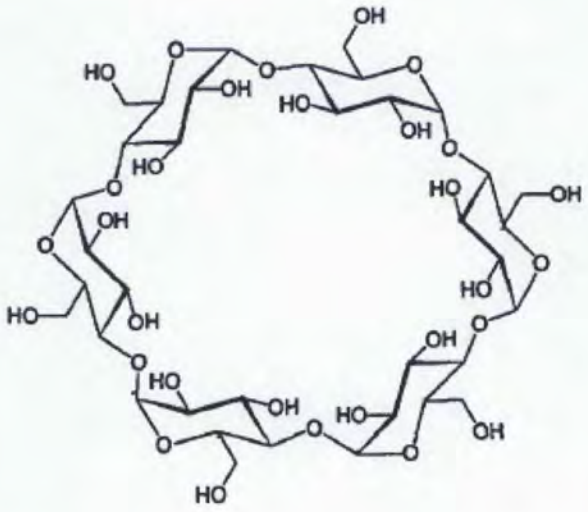
10016-20-3

(c) Empirical formula

$(C_6H_{10}O_5)_6$

(d) Structural formula

Alpha-cyclodextrin (hereinafter "α-CD") is the cyclic polymer of six α-1,4-linked glycopyranosyl units.



(e) Characteristic properties

Cyclodextrins are cyclic α-(1-4)-linked maltooligosaccharides. α-CD is a ring-shaped molecule made up of six glucose units linked by α-1,4-bonds. The circular molecules of α-, β- and γ-CD are shaped like a hollow truncated cone or torus. Because the hydrogen atoms and the oxygen atoms of the glycosidic bonds are facing the inner side of the torus, while the hydroxyl groups are located on the outer side, cyclodextrins have a hydrophobic cavity and, at the same time, a hydrophilic outer surface which

makes them water-soluble. The hydrophobic cavity enables cyclodextrins to form inclusion complexes with a variety of organic compounds. The diameter of the cavity provides for a certain selectivity of the complexation of "guest" molecules, i.e., the bigger ring of the 8-membered γ-CD can accommodate a wider variety of guest molecules than the smaller rings of α- and β-CD. Large guest molecules may complex with more than one cyclodextrin molecule (Le Bas & Rysanek, 1987).

Cyclodextrins were first isolated by Villiers in 1891 from a culture medium of *Bacillus amylobacter* (*Clostridium butyricum*) grown on a medium containing starch. During studies on microbial food spoilage, Schardinger isolated *Bacillus macerans*, a heat-resistant cyclodextrin-producing microorganism. In recognition of his detailed investigations on cyclodextrins (from 1903-1911), these substances are referred to as "Schardinger dextrins" in the early literature (French, 1957). Meanwhile, many bacteria have been found to produce cyclodextrins from starch. On a commercial scale, cyclodextrins are produced today from starch using cyclodextrin glucosyltransferases, a group of bacterial amylolytic enzymes.

The formation of an inclusion complex with a guest molecule is the basis for many applications of cyclodextrins in food, cosmetics, and pharmaceutical preparations (Hedges et al., 1995; Nagatomo, 1985; Loftsson & Masson, 2001). The formation of complexes between cyclodextrins and guest molecules is reversible, and excess water would in most cases result in a dissociation of the complex (Hedges et al., 1995; Nagatomo, 1985; Loftsson and Masson, 2001). The suitability of the different cyclodextrins for these applications varies in relation to the size of the "guest" molecule, which the cyclodextrin ring should accommodate.

(f) Any known toxicants that could be in the source

The potential impurities of α-CD are residues of the cyclodextrin-glycosyltransferase (CGTase, EC 2.4.1.19, CAS 9030-09-5) preparation, residual starch, linear maltooligosaccharides, maltose, glucose and β-cyclodextrin.

The CGTase preparation is obtained from a recombinant strain of *Escherichia coli* K12. *E. coli* K12 is a nonpathogenic and nontoxigenic host organism which has been used for the production of other food ingredients such as chymosin or γ-cyclodextrin and which is recognized as safe (FDA, 1990, 2000). The *E. coli* K12 strain belongs to risk group 1 in the classification of human etiologic agents (NIH, 2002), and is also recommended as a safe host organism by the EU Commission (EU Commission, 1997). The gene coding for the α-CGTase stems from *K. oxytoca*, strain M5a1, which has been well characterized (Randriamahefa,

1994) and has been used for many years in biotechnological research and applications. The vector used for introduction of the α-CGTase gene in the *E. coli* K-12 host is derived from the pJF118EH vector which is derived from pBR322, a widely used mobilization-defective vector that is considered to be safe (EU Commission, 1997). The α-CGTase preparation has been subjected to Ames tests in *S. typhimurium* strains TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 uvrA with and without metabolic activation, as well as in a chromosome aberration test in cultured human lymphocytes, without any evidence of a genotoxic effect (Bär, et al. 2004). In a 13-week subchronic toxicity study in rats, the α-CGTase preparation did not produce any adverse effects at levels of up to 260 mg TOS/kgbw/d, which was the highest dose level tested (Bär et al., 2004).

α-CD does not contain any CGTase activity because the enzyme is inactivated by heat and is removed completely during the α-CD production process. DNA from the CGTase source organism (*E. coli* K12) could not be detected either using sensitive polymerase chain reaction (PCR) techniques. Any non-proteinaceous, hydrophilic or lipophilic by-products present in the CGTase preparation would also be removed by the applied purification steps. The absence of protein was demonstrated by appropriate analytical tests.

The complexant, 1-decanol, is efficiently removed during purification. Analysis of five α-CD pilot batches showed 1-decanol residues of <15 ppm. 1-Decanol has FEMA GRAS status (FEMA No. 2365) (Hall and Oser, 1965) and is approved FDA for use in foods as a synthetic flavoring substance (21 C.F.R. § 172.515) and as a synthetic fatty alcohol (21 C.F.R. § 172.864). In addition, 1-decanol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as a flavoring substance and determined to not pose safety concerns at estimated daily intakes (for eaters only) of 7 and 28 μg/person/day in the United States and Europe, respectively (WHO, 1998). In the EU, 1-decanol is listed in the Register of Flavoring Substances under FL No 02.024 (Commission Decision 2002/113/EC).

(2) Description of method of manufacture

α-, β- and γ-Cyclodextrins (CDs) are formed by the action of cyclodextrin-glycosyltransferases (CGTase, EC 2.4.1.19, CAS 9030-09-5) on starch. CGTases are amylolytic enzymes which are produced naturally by different strains of Bacilli and other species of bacteria (Sicard & Saniez, 1987; Schmid, 1989, 1991; Starnes, 1990; Tonkova, 1998). CGTases degrade starch by a cyclization reaction. There is evidence that the enzyme recognizes the 6,7 or 8 glucose units from the non-reducing end of an amylose molecule, attacks the adjacent α-1,4-linkage, and transfers it to the C-4 position of the non-reducing end to produce α-, β- or γ-CD

(Schmid, 1989). Typically, mixtures of α-, β- and γ-CD are formed by the action of CGTases on starch, with the β-form being predominant for thermodynamic reasons. Different CGTases produce α-, β-, and γ-CD in different proportions during the initial phase of the reaction. The ratio of the formed cyclodextrins is also influenced by other conditions such as the reaction time, temperature, and presence of ethanol (Goel & Nene, 1995).

Cyclodextrins are isolated from the enzymatic reaction mixture either by the "solvent process," in which a suitable organic substance is added to form an insoluble complex with the cyclodextrins, or the "non-solvent process," in which chromatographic separation techniques are applied (Sicard & Saniez, 1987; Schmid, 1991; Rendleman, 1993).

α-CD is produced using CGTase from a recombinant strain of *Escherichia coli* K12, harboring the CGTase gene of *Klebsiella oxytoca*, and applying the solvent process for separation of the obtained α-CD.

In the first step of α-CD production, food-grade, liquefied starch is treated with CGTase under controlled pH and temperature conditions. 1-Decanol is added as a complexant to precipitate formed α-CD. The complex is removed and purified by dissolution in water and re-precipitation. The complexant is separated from α-CD by decantation and steam distillation. Obtained by crystallization, α-CD is a white powder with a purity of ≥ 98.0%.

(3) Specifications for food-grade material

The specifications for food-grade α-CD are provided below:

Assay:	≥ 98% of α-CD (on an anhydrous basis)
Ash (residue on ignition):	≤ 0.1%
Reducing sugars:	≤ 0.5%
Heavy metals (as Pb):	≤ 5 ppm
Lead:	≤ 0.5 ppm
Volatile organics:	≤ 20 ppm

The "volatile organics" are residues of the complexant 1-decanol. Additional purity criteria are included in the company's internal specifications, such as microbiological purity and optical density.

(4) Data and information bearing on physical or other technical effect

Our GRAS notice does not include data and other information bearing on physical or other technical effect because such data and other information are not necessary to demonstrate safety.

Part 3 – Dietary exposure

(1) Introduction

As described in detail in Part 6 of this notice, α-CD is the subject of GRN 000155, to which FDA responded with a "no questions" letter in 2004.[1] In 2012, the GRAS uses of α-CD were extended to encompass use as a flavor adjunct (carrier, stabilizer) pursuant to a self-determination. Since 2012, further food technological benefits of α-CD were discovered (Li et al., 2014). Alpha-CD was found, for example, to be a useful whipping agent for preparing fat-reduced mousses, confectionary and bakery fillings, fruit-based desserts, baked snacks and certain dairy-based products such as yogurt. It also can be used as an emulsifier over a wide range of fat levels to prepare products such as mayonnaises, dressings and cake icings. Other possible applications include use in baked goods such as cakes, muffins, pancakes and waffles. Some but not all of these applications are included in the list of proposed uses specified in GRN 000155 (see Table 1 in Part 1 of this GRN, and also in Appendix 2). Furthermore, it is possible that additional useful food applications of α-CD will be discovered in future. Therefore, it is necessary to (a) overhaul the list of intended uses of alpha-cyclodextrin that was considered in GRN 000155, and (b) revise the calculation of the estimated intakes that may result from the new range of intended uses.

Considering furthermore that not only the composition of foods is changing over time but also new types of food are occasionally developed, a mere update of the existing list of foods that might contain added α-CD may be a short-lived solution. Therefore, we here apply a different, more flexible, yet with regard to the safety assessment still conservative approach for estimating the intake of α-CD that may result from the extended range of uses.

(2) Consumption of processed and ultra-processed foods

The common denominator of all foods that contain added α-CD, be it as an ingredient of the basic matrix of the food or be it as an ingredient of one of the components of the food (such as, for example, its glazing or filling), is that such foods by definition are "processed" or even "ultra-processed."

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[1] Because only a few of the proposed uses encompassed by GRN 000155 actually materialized, the present intake of α-CD by the US population is orders of magnitude below the estimated intakes that were presented in GRN 000155 (i.e., 0.21 and 0.43 g/kg bw/d for the mean and the 90th percentile consumer, respectively).

According to the 2010 Dietary Guidelines for Americans Report, a processed food is "any food other than a raw agricultural commodity that has been subject to washing, cleaning, milling, cutting, chopping, heating, pasteurizing, blanching, cooking, canning, freezing, drying, dehydrating, mixing, packaging, or other procedures that alter the food from its natural state. Processing also may include the addition of other ingredients to the food, such as preservatives, flavors, nutrients, and other food additives or substances accepted for use in food products, such as salt, sugars, and fats (USDA, 2010)."

Definitions of processed and ultra-processed foods have been published and examples were provided (Moubarac et al., 2014).[2] Of note is that each of these

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2 "Processed foods are ready-to-consume products which are manufactured to make them durable and more palatable and attractive. They are directly derived from foods and recognizable as versions of the original foods. Generally produced to be consumed as part of meals or dishes, or may be used, together with ultra-processed products, to replace food-based freshly prepared dishes and meals. Processes include canning and bottling using oils, sugars or syrups, or salt and methods of preservation such as salting, salt-pickling, smoking and curing. Examples of processed food are canned or bottled vegetables and legumes preserved in brine; peeled or sliced fruits preserved in syrup; tinned whole or pieces of fish preserved in oil; salted nuts; un-reconstituted processed meat and fish such as ham, bacon, smoked fish; cheese. Ultra-processed foods are formulated mostly or entirely from substances derived from foods. Typically they contain little or no whole foods. They are durable, convenient, accessible and highly or ultra-palatable. Typically they are not recognizable as versions of foods, although they may imitate the appearance, shape and sensory qualities of foods. Many ingredients are not available in retail outlets. Some ingredients are directly derived from foods, such as oils, fats, flours, starches and sugar. Others are obtained by further processing of food constituents. They contain preservatives; stabilizers, emulsifiers, solvents, binders, bulkers; sweeteners, sensory enhancers, colors and flavors; processing aids and other additives. Their bulk may come from added air or water. Micronutrients may 'fortify' the products. Most are designed to be consumed by themselves or in combination as snacks. They displace food-based freshly prepared dishes, meals. Processes include hydrogenation, hydrolysis; extruding, molding. Examples are chips (crisps), many types of sweet, fatty or salty snack products; ice-cream, chocolates, candies (confectionary); French fries (chips), burgers and hot dogs; poultry and fish 'nuggets' or 'sticks' ('fingers'); breads, buns, cookies (biscuits); breakfast cereals; pastries, cakes, cake mixes; 'energy' bars; preserves (jams), margarines: desserts; canned, bottled, dehydrated, packaged soups, noodles; sauces; meat, yeast extract; soft, carbonated, cola, 'energy' drinks; sugared, sweetened milk drinks, condensed milk, sweetened including 'fruits' yoghurts; fruit and fruit 'nectar' drinks; instant coffee, cocoa drinks; no-alcohol wine, beer; pre-prepared meat, fish, vegetables, cheese, pizza, pasta dishes; infant formulas, follow-on milks, other baby products; 'health', 'slimming' products such as powdered or 'fortified' meal and dish substitutes."

categories (including the category of unprocessed/minimally processed foods) comprises liquid foods (i.e., beverages), semi-liquid foods, and solid foods.

When these definitions were applied to the food intake data of the 2007/2008 National Health and Nutrition Examination Survey (NHANES), it was determined that 64.7% of the ingested dietary energy was provided in the United States by ultra-processed foods and 4.9% by processed foods.[3] Furthermore, it was found that the combined value (i.e., 69.6%) was slightly higher for the US than for Canada (62.0%) and the UK (63.4%) (Baraldi et al., 2013; Moubarac et al., 2013b).

More recent data from the UK which are based on the UK National Diet & Nutrition Survey (2008-2012) are in keeping with this observation (Adams & White, 2015).[4]

Conceivably, a saturation point is reached for the consumption of ultra-processed foods when they account for about two thirds of the daily energy intake (Solberg, 2014). In turn this means that unprocessed and minimally processed foods (as defined in Moubarac et al., 2014) provide still about one third of the daily energy intake, even in affluent Western societies.

Since foods to which α-CD has been added are by definition processed or ultra-processed foods, this food consumption data provides a basis for estimating the maximum daily intake of α-CD regardless of the exact type of food or food component in which α-CD is used at present or might be used in future.

(3) Intake of α-CD from its combined uses in processed and ultra-processed foods (other than beverages)

The daily intake of α-CD from its use at a given level in processed and ultra-processed foods may be estimated from the total energy intake from such foods. The total energy intake with foods of any type is about 50 kcal/kg bw/d for the 90th percentile adult subject (Bär & Würtzen, 1990). This value was first proposed and applied twenty-five years ago in Europe. As it has been demonstrated by

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3 Based on the more recent NHANES 2009-2010 data, ultra-processed and processed food account for 57.9 and 9.4% of the ingested dietary energy, respectively (Martinez Steele et al., 2016).

4 An analysis of purchases of packaged foods (including beverages) showed that 15.9 and 61.0% of the food energy was provided by moderately and highly processed products, respectively (Poti et al., 2015). However, since food waste was not taken into account and foods without a bar-code were not included (such as fresh fruits), this analysis tends to overestimate the consumption of processed foods.

Swinburn et al. (2009) and is illustrated in **Figure 1** at Appendix 1, this value is still fully applicable also to the current U.S. population with a higher than European average body weight.

Figure 1 presents the data points of the daily energy expenditure and the body weight of 1399 U.S. adults. The black line is the corresponding regression line.[5] The red line depicts the relationship between the daily metabolizable energy (ME) intake with food (i.e., solid and liquid foods together) and the body weight for a fixed arbitrary value of 50 kcal/kg bw. As may be seen from the distribution of these data points, more than 90% of U.S. adults have a daily ME intake below 50 kcal/kg bw.

For the purpose of the present report, a person's daily energy expenditure is considered to be identical to its daily ME intake (Seale et al., 1990).[6]

The 90th percentile ME intake of 50 kcal/kg bw/d stems from the combined intake of both solid and liquid foods (such as soft drinks and milk which contribute a significant amount of energy to the total daily energy intake of U.S. residents). On average, indeed 21% of the per capita energy intake was contributed by beverages in 2002 (Duffey & Popkin, 2007).[7] Accordingly, the 90th percentile consumer of food energy who consumes 50 kcal/kg bw/d obtains about 40 kcal/kg bw/d from solid and semi-solid foods and 10 kcal/kg bw/d from beverages.

Applying an average energy density of 2 kcal/g to foods other than beverages (Hansen, 1979; Kant & Graubard, 2005)[8], this daily ME intake of 40 kcal/kg bw corresponds to a food intake of 20 g/kg bw/d. If 70% of this amount, i.e., 14 g/kg bw/d, is obtained from processed or ultra-processed food (Baraldi et al., 2013; Moubarac et al., 2013a) and if all of that food contained α-CD at the highest technologically feasible concentration of 3%, an α-CD intake of about 420 mg/kg bw/d would result for the 90th percentile consumer.

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5 Reading example: For a person with a body weight of 100 kg, the average energy expenditure is about 3260 kcal, i.e., 32.6 kcal/kg bw.

6 While in people with increasing body weight the energy intake is slightly higher than the energy expenditure, this difference is small on a daily basis.

7 A somewhat smaller contribution (15%) of beverages to the total daily energy intake was reported from a more recent analysis of US food purchase data (Poti et al., 2015). However, since food wasting was not considered in that study (which is likely bigger for foods than beverages), this data does not invalidate the earlier reported value of 20% which is derived from food consumption data (Duffey & Popkin, 2007).

8 A somewhat lower value of about 1.5 kcal/g has been proposed by others for foods including milk products (Douglass et al., 1997).

Applying the same calculation to the mean consumer who ingests 28.5 kcal/kg bw/d corresponding to 11.4 g food (excluding beverages)/kg bw/d and thus 7.98 g/kg bw/d processed and ultra-processed food, an α-CD intake of about 239 mg/kg bw/d is obtained. For the mean and 90th percentile adult consumer with an average body weight of 70 kg, daily α-CD intakes of 16.7 g and 29.4 g, respectively, would result from such a ubiquitous use of α-CD at the highest feasible concentration (3%) in each and every processed and ultra-processed food (except beverages). These calculated α-CD intakes are clearly overestimates because they are based on the assumption that 80% of the total daily energy intake is provided by solid or semi-solid foods of which 70% is processed or ultra-processed and contains α-CD at the highest feasible concentration (3%).[9]

(4) Intake of α-CD from its use in certain types of beverages

For estimating the α-CD intake with beverages, the ingested volumes rather than the ingested energy must be considered and taken as a basis, because both the energy density of beverages varies widely (from about 0–70 kcal/100 mL) and the liquid intake varies widely (from about 15–40 mL/kg bw/d). With such widely varying parameters, it is difficult to make a reasoned "worst case" estimation of the daily α-CD intake from its use in beverages. Instead, the food consumption data that were presented in GRN 000155 may be applied. The description of the beverages in which α-CD is intended to be used is still valid and so are the projected maximum use levels, except for diet soft drinks in which the maximum is to be adjusted from 1% to 1.05%.[10] The projected intakes of α-CD from its use in beverages were estimated from U.S. food consumption data as described in GRN 000155. The two-day average intake per user of such products was estimated at 3.5 g/d (mean) and 7.5 g/d (90th percentile) for users (age 2 years and older) of such products. The increase of the maximum use concentration of α-CD in soft drinks from 1% to 1.05% would increase these values by not more than about 0.1-0.2 g/d.

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9 In comparison to the estimated daily α-CD intakes of 11.4 g for the mean and 19.8 g for the 90th percentile consumer, which were regarded to be GRAS as per GRN 000155, here the projected intakes are about 2-times higher.

10 According to GRN 000155, α-CD is intended to be used in beverage mixes, diet soft drinks, fruit juices, instant coffee, instant tea, and formula diets at a level of 1% (beverage as consumed) and in vegetable juices as well as soy and non-soy imitation milk at a level of 2%.

(5) Intake of α-CD from its combined use in processed and ultra-processed foods and in certain types of beverages

The intakes of α-CD from its use in solid foods and semi-solid foods on the one hand in certain types of beverages on the other hand are additive.

For the mean adult consumer, an α-CD intake of 16.7 g/d would result from its use at the highest intended concentration in all processed and ultra-processed foods and an intake of 3.6 g/d would result from its use in the specified types of beverages. For the 90th percentile adult consumer, an α-CD intake of 29.4 g/d from all processed and ultra-processed food and 7.9 g/d from specified beverages is estimated.

Quite clearly, these are hypothetical figures which in reality will never be attained because there exists other ingredients available that may substitute for α-CD in formulating certain types of food and beverages. In some corresponding earlier cases, market share adjustments have therefore been made and were accepted in some GRAS Notices [e.g., for D-psicose (10% share) in GRN 000400, isomaltulose (5-10% share) in GRN 000184, etc.]. However, while the market reality demonstrates the existence of competition among food additives of similar functionality, the estimation of market shares is somewhat speculative and is, therefore, not applied in this report.

The presented intake estimates of α-CD pertain to adult consumers. This raises the question about the projected intakes among children. Since the fractional consumption of processed and ultra-processed is similarly high (but not higher) in children and since the energy intake per kg body weight is also not much higher than in adults, the α-CD intake on a per kg body weight basis is expected to also be similar. Only for beverages (soft drinks) a slightly higher intake (on a per body weight basis) may be expected. On the other hand, it should be recognized that α-CD is not absorbed as such but is, like most other soluble dietary fibers, degraded by the intestinal microbiota to common end-products of fermentation. Therefore, there is no systemic exposure to α-CD and a comparative estimation of intakes per kg body weight for children vs. adults would be quite misleading. Any concern about an "over-consumption" of α-CD among children from the intended uses of α-CD is, therefore, not warranted.

Part 4 – Self-limiting levels of use

The levels at which α-CD would become self-limiting are above the levels specified in Table 1 in Part 1 of this notice.

Part 5 – Experience based on common use in food before 1958

Because the statutory basis for our conclusion of GRAS status is not through experience based on common use in food, our notice does not include evidence of a substantial history of consumption of the notified substance for food use by a significant number of consumers prior to January 1958.

Part 6 – Narrative

(1) Introduction

Alpha-CD is a cyclic oligomer consisting of six glucose units joined "head-to-tail" by α-1,4-glycosidic bonds. It is produced from food-grade starch by means of cyclodextrin glycosyltransferase ("CGTase"), an amylolytic enzyme which hydrolyzes the amylose molecule and at the same time ties the resulting oligomers to a ring. Alpha-CD formed in this way is separated from the reaction mixture by complexation with 1-decanol, isolation of the insoluble complex, disintegration of the recovered complex at elevated temperature in the presence of water, re-precipitation of the complex, removal of 1-decanol from the recovered complex by steam stripping and isolation of α-CD from the aqueous phase by crystallization. Alpha-CD is produced in accordance with current Good Manufacturing Practice (cGMP) for food ingredients and complies with the Food Chemicals Codex ("FCC") monograph for α-CD.

Wacker introduced α-CD as a food ingredient in the U.S. food market in 2005 after having received a "no further questions" letter from FDA in response to its GRAS notice, GRN 000155 (FDA, 2004)(Appendix 2). The conditions under which the use of α-CD was generally recognized as safe for the first time, based on scientific procedures, are specified in GRN 000155 and the FDA's subsequent reply (FDA, 2004). These uses comprise the then expected application of α-CD for the fiber supplementation of foods and beverages and its use as carrier or stabilizer for flavors (i.e., as a flavor adjuvant), carrier, stabilizer or solubilizer of colors, vitamins and fatty acids as well as for improving the 'mouthfeel' of beverages. The maximum levels of α-CD considered at that time for defined categories of processed foods were shown in Section 3 (b) of GRN 000155. The FDA's response letter to GRN 000155 is attached to this report as Appendix 2 for reference.

In early 2012, interest arose in the use of α-CD as flavor adjunct, i.e., as carrier and stabilizer of flavors, in certain types of food that were not covered by GRN 000155. The use level of α-CD as flavor adjunct was given with $\leq$ 750 mg/kg food (as consumed). The range of foods in which the use of flavors with α-CD was considered feasible comprised any type of processed food except:

(a) liquid foods, i.e., beverages which were already contained in GRN 000155 (i.e., diet soft drinks, beverage mixes, fruit juices, instant coffees and teas, vegetable juices, soy milk and non-soy (imitation) milk, soups and milk beverage mixes), and
(b) foods with a standard that would preclude the use of added flavors or α-CD as a flavor adjunct.

The foods in which α-CD was intended to be used as a flavor adjunct were, therefore, described collectively as "solid foods" and "semi-liquid foods such as yoghurts, frozen dairy desserts, sauces, and relishes", i.e., any processed food other than liquid foods as described above under item (a).

For assessing the safety of these additional potential uses of α-CD, Wacker convened a panel of scientific experts, to assess the safety of α-CD as a flavor adjunct in foods. The maximum additional intake of α-CD that would result from its use as a flavor adjunct in foods other than liquid foods was estimated at not more than 0.75 and 1.4 g/d for the 70-kg mean and 90th percentile consumer, respectively. This estimated additional intake of α-CD was considered small in comparison to the estimated daily intakes of 11.4 g for the mean and 19.8 g for the 90th percentile consumer that were calculated from food consumption data of the 1994-1996 and 1998 Continuing Surveys of Food Intakes by Individuals and that were considered GRAS as per GRN 000155. Based on the assessment of the pivotal, then publicly available safety studies of α-CD and considering that single (bolus) α-CD doses of 10 - 25 g α-CD were well tolerated by adult human subjects, the proposed additional uses of α-CD as a flavor adjunct in foods other than liquid foods were considered to be generally recognized as safe based on scientific procedures.

(2) Safety assessment of α-CD used as an ingredient of processed and ultra-processed foods (other than beverages) at a level of up to about 3% (in food as consumed) and in certain types of beverages at a level of up to about 1.05% (as consumed)

A substantial body of evidence is available for the safety assessment of α-CD as a food ingredient. The pivotal, publicly available safety studies have been reviewed earlier by Wacker's GRAS Expert Panel (Anderson et al., 2004) and authoritative food safety assessment bodies (WHO, 2002, 2005; FSANZ, 2004; EFSA, 2007). Since then, additional studies with α-CD have been published. The pertinent studies were retrieved from a comprehensive search of the scientific literature conducted through March 16, 2016 and their results are included in this report.

Investigations on the metabolic fate of ingested α-CD demonstrate that α-CD is not digested to glucose in the GI-tract to any relevant degree; i.e., its disposition in the GI tract is similar to that of other soluble dietary fibers (Diamantis & Bär, 2002; van Ommen et al., 2004).

The toxicity of α-CD was examined in standard in-vitro and in-vivo toxicity tests including cytotoxicity[11] and genotoxicity tests, subchronic (3-month) oral toxicity studies in rats (Lina & Bär, 2004a) and dogs (Lina & Bär, 2004b), and oral embryotoxicity/teratogenicity studies in mice, rats and rabbits (Price et al., 1996; NTP, 1994a, b; Waalkens-Berendsen & Bär, 2004; Waalkens-Berendsen et al., 2004). α-CD was administered with the daily diet to rats and mice at dietary concentrations of up to 20% and to dogs at concentrations of up to 5%. In all these studies, α-CD was well tolerated and did not elicit adverse effects.[12]

The human intestinal tolerance to oral doses of α-CD was demonstrated in a study on the glycemic impact of a single dose of α-CD. In this study, twelve healthy, fasted, 23–24 year old, male volunteers consumed a bolus dose of 25 g α-CD together with 100 g white bread without experiencing any untoward intestinal effects (Diamantis & Bär, 2002). In other human studies in which the effects of α-CD on the glycemic impact of starch or sucrose were examined, single bolus doses of 2–10 g α-CD were similarly well tolerated (Jarosz et al., 2013; Buckley et al., 2006; Gentilcore et al., 2011). The tolerance to repeated oral doses of α-CD was demonstrated by two studies with α-CD intakes of 6 g/d for periods of 2 months in healthy adults (Comerford et al., 2011) and 3 months in obese diabetic subjects (Grunberger et al., 2007) and in one study at intakes of 30 g/d for 7 days (Park et al., 2012).

An interaction of ingested α-CD with the absorption of soluble vitamins or other lipophilic nutrients is not expected because the cavity of the α-CD molecule is too small to accommodate molecules of this size. Furthermore, there is direct evidence for the absence of complex formation of α-CD with vitamins D_3, E and K_1 from an in-vitro experiment (Okada et al., 1988). An interference with the absorption of other lipophilic nutrients is also not expected because ingested α-CD will efficiently be degraded by the intestinal microbiota.

The intake of α-CD of 29.4 g/d that would result for the 70-kg, "heavy" (i.e., 90th percentile) consumer from its proposed use as an ingredient in processed and ultra-processed foods (excluding beverages) at the technologically maximally feasibly level of 3% is higher than the 90th percentile intake (19.8 g/day for the "heavy"

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11 The results of recent tests of the in-vitro cytoxicity of α-CD and its derivatives on Caco-2 cells and human erythrocytes and on immortalized murine microvascular endothelial cells are in line with the results of earlier similar studies that have been taken into consideration in previous safety assessments of α-CD already (Roka et al., 2015; WHO 2002; EFSA, 2007; FSAZ, 2004).

12 A recent study in which dogs received α-CD in two daily doses of 6 g α-CD each provides further support for the gastrointestinal tolerance of this substance (Guevara et al., 2015).

consumer) that has been determined to be "generally recognized as safe" in GRN 000155. However, this higher intake is still within the range of α-CD single doses (≤ 15 g) and daily doses (≤ 30 g) that were well tolerated by adult human subjects.

Thus, the pivotal, publicly available safety studies in animals and tolerance studies in humans of α-CD demonstrate that the proposed uses of α-CD as an ingredient in processed and ultra-processed foods (excluding beverages) and in certain types of beverages as described in Table 1 in Part 1 of this GRN do not present a risk to human health.

(3) Conclusions

In summary, the GRAS determination based on scientific procedures of the proposed uses of α-CD as an ingredient of processed and ultra-processed food as defined herein (but excluding infant foods) at levels of up to 3% (in the foods as consumed) and in certain specified types of beverages at a concentration of up to 1.0–1.05% relies on:

(1) the nature of α-CD – a soluble dietary fiber in the form of a cyclic oligomer of six units of glucose that is not absorbed from the gastro-intestinal tract (it is degraded by intestinal microbiota to usual end-products of fermentation); and

(2) published animal toxicity studies and human tolerance studies.

It is further supported by the corresponding conclusion of internationally recognized experts, e.g., FAO/WHO (JECFA) ("ADI not specified"), EFSA, and FSANZ. Finally, an independent panel of scientific experts, qualified by training and experience to evaluate the safety of food ingredients, concluded that under the conditions of intended use in foods, Wacker's α-CD is GRAS through scientific procedures. The panel's opinion is included at Appendix 3.

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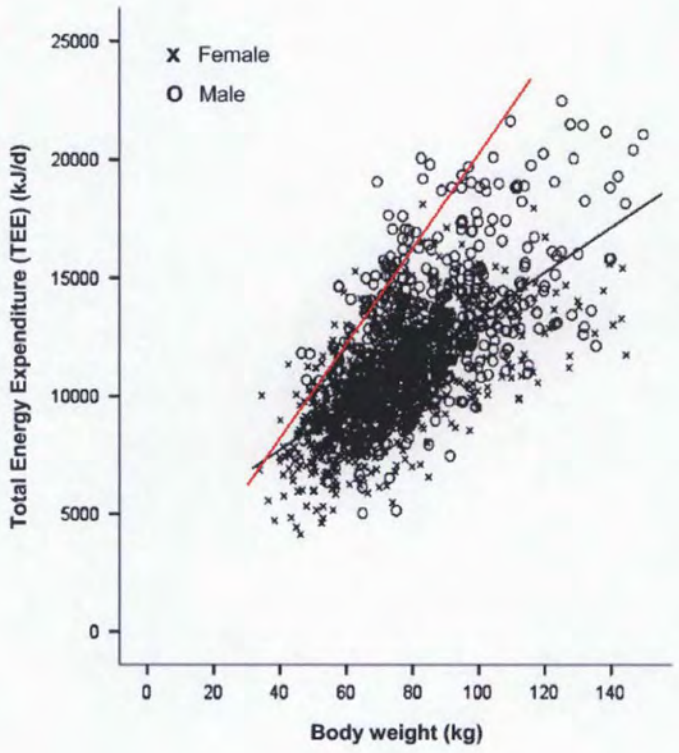
Appendix 1

Figure 1: Relation between body weight and energy expenditure in US adults



Relation between body weight and energy flux in US adults [energy flux = total energy expenditure (TEE) measured by the doubly labeled water technique]. (Pearson's correlation r = 0.65, P < 0.0001; n = 1399). Black line: linear regression (Swinburn et al., 2009). Red line: Energy expenditure = 50 kcal/kg bw (inserted by authors of this report).

Appendix 2

Agency Response Letter GRAS Notice No. GRN 000155

Return to inventory listing: **GRAS Notice Inventory (http://www.fda.gov/grasnoticeinventory)**

See also **Generally Recognized as Safe (GRAS) (/Food/IngredientsPackagingLabeling/GRAS/default.htm)** and **about the GRAS Notice Inventory (/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm)**

CFSAN/Office of Food Additive Safety

December 22, 2004

Diane McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth Street, N.W.
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Washington, D.C. 20005-5929

Re: GRAS Notice No. GRN 000155

Dear Ms. McColl:

The Food and Drug Administration (FDA) is responding to the notice, dated June 25, 2004, that you submitted on behalf of Wacker Chemical Corporation (Wacker) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on June 28, 2004, filed it on June 30, 2004, and designated it as GRAS Notice No. GRN 000155.

The subject of the notice is alpha-cyclodextrin. The notice informs FDA of the view of Wacker that alpha-cyclodextrin is GRAS, through scientific procedures, for use in selected foods for fiber supplementation, as a carrier or stabilizer for flavors (flavor adjuvant), colors, vitamins and fatty acids, and to improve mouth-feel in beverages. These uses are described in Table 1.

Table 1
Food categories and use levels for alpha-cyclodextrin

Food category	Maximum use level percent (w/w)
Breads, rolls, cakes, baking mixes, refrigerated dough	5
Brownies and bars	7
Crackers (sweet and non-sweet)	10
Diet soft drinks, beverage mixes, fruit juices, instant coffees and teas, coffee whiteners (dry), formula diets, meal replacements, and nutritional supplements	1
Vegetable juices, soy milk and non-soy (imitation) milk	2
Ready-to-eat breakfast cereals	2 to 9 [a]
Instant rice, pasta, and noodles (prepared)	2
Condiments	3
Reduced fat spreads	20
Dressings and mayonnaise	5
Yogurt, milk beverage mixes, and frozen dairy desserts	2.5
Pudding mixes (dry)	1
Snack foods	1
Canned and dry soups (prepared)	2
Hard candy	15
Chewing gum	10

[a] The notifier states that use level in ready-to-eat cereals will vary based on weight of serving size (i.e., if less than 20 g/cup the level is 2 percent ; 20-43 g/cup the level is 9 percent; greater than or equal to 43 g/cup the level is 5 percent).

As part of its notice, Wacker reports that a panel of individuals (Wacker's GRAS panel) has evaluated the data and information that are the basis for Wacker's GRAS determination. Wacker considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. Wacker's GRAS panel report discusses the following information concerning alpha-cyclodextrin: 1) the manufacturing process and specifications; 2) estimated daily intake; 3) absorption, distribution, metabolism, and excretion; 4) published toxicological studies conducted in animals; 5) published studies that concern cellular and genetic effects; and 6) published studies conducted with humans. Wacker's GRAS panel concludes that, based on scientific procedures, alpha-cyclodextrin meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is GRAS under the conditions of intended use.

The alpha-cyclodextrin is intended for use in selected solid, semi-liquid, and liquid foods. According to the notifier, alpha-cyclodextrin has nutritional properties similar to fermentable dietary fiber, is stable under food processing conditions, and has a low viscosity in aqueous solutions. Structurally, alpha-cyclodextrin is shaped like a hollow truncated cone or torus. The

cavity of alpha-cyclodextrin is hydrophobic and the outer surface is hydrophilic. Alpha-cyclodextrin is water soluble and can form inclusion complexes with lipophilic substances. The formation of reversible inclusion complexes is the basis for alpha-cyclodextrin applications.

Alpha-cyclodextrin (CAS Registry No. 10016-20-3), also known as cyclohexa-amylose, cyclomalto-hexose or alpha-dextrin, has an empirical formula of $(C_6H_{10}O_5)_6$ and molecular weight of 973 Daltons. Structurally, alpha-cyclodextrin is a cyclic polymer of six alpha-1,4-linked glycopyranosyl units.

The notifier describes their manufacturing process for alpha-cyclodextrin. In the first step of alpha-cyclodextrin production, food-grade, liquefied starch is treated with a cyclodextringlycosyltransferase (CGTase, EC 2.4.1.19, CAS 9030-09-5) under controlled pH and temperature conditions. The CGTase is obtained from a recombinant strain of *Escherichia coli* K12, harboring the CGTase gene of *Klebsiella oxytoca*. Alpha-cyclodextrin is precipitated from the enzymatic reaction mixture by addition of 1-decanol. The precipitate is further purified by dissolution in water and re-precipitation. The 1-decanol is separated from alpha-cyclodextrin by decantation and steam distillation. The final alpha-cyclodextrin product is obtained by crystallization and is a white powder with a purity of ≥ 98.0 percent. The notifier provides additional specifications including limits on the maximum levels of volatile organic compounds, heavy metals, and lead (less than 0.5 parts per million[(1)]).

Using the foods and use levels summarized in Table 1 and the two day consumption data from the United States Department of Agriculture (USDA) Continuing Survey of Food Intakes by Individuals 1994-96, 98 (CSFII), the notifier calculated an estimate of the daily intake (EDI) of alpha-cyclodextrin. The notifier estimates the mean and 90th percentile intake (users only and of all age groups combined) of alpha-cyclodextrin from the intended uses in Table 1 (except chewing gum) to be 11.4 and 19.8 g/person/day. The calculation also includes a mean "per eating occasion" intake of 3.9 g/person. The notifier used a separate survey on chewing gum use in the United States to provide an additional EDI for alpha-cyclodextrin ingested from chewing gum as 0.9 g/person/day.

The notifier reports that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated alpha-cyclodextrin in June 2001 for technological uses in food. On the basis of the available safety studies on alpha-cyclodextrin and studies on the related beta- and gamma-cyclodextrin, JECFA allocated an Acceptable Daily Intake (ADI) of "not specified[(2)]" for alpha-cyclodextrin. The notifier reports that the 63rd meeting of JECFA in 2004 also determined an ADI of "not specified" for alpha-cyclodextrin.

Wacker's GRAS panel discusses published studies regarding absorption, distribution, metabolism, excretion, bioavailability, toxicity and mutagenicity conducted with alphacyclodextrin in humans and various animal species. In summary, alpha-cyclodextrin is not digested by the mammalian digestive enzymes; however, it is completely fermented by the intestinal microbiota. Less than 1 percent of the alpha-cyclodextrin is absorbed; however, this amount is not metabolized and is excreted unchanged in the urine. Two 13-week toxicity studies with rats and dogs provide no evidence for adverse reactions in the gastrointestinal-tract, the kidneys, the liver or any other organs or tissues at alpha-cyclodextrin intakes of up to 20 percent of the diet (13 g/kg bw/day in rats and 10 g/kg bw/day in dogs). The notifier states that the EDI calculations indicate that the intake of alpha-cyclodextrin per eating occasion (3.9 g/person) would be below the doses that were tolerated by adult volunteers who experienced no side-effects (10 g/person) or minimal intestinal symptoms (25 g/person). Based on these studies, the GRAS panel concludes that alpha-cyclodextrin is safe for its intended uses.

Based on the information provided by Wacker, as well as other information available to FDA, the agency has no questions at this time regarding Wacker's conclusion that alpha-cyclodextrin is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of alpha-cyclodextrin. As always, it is the continuing responsibility of Wacker to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in the notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

(1)The lead specification is a maximum level of 0.5 ppm; however, batch analysis records included in GRN 000155 indicate a lead level of less than 1 ppm. FDA notes that a maximum lead level of 1 ppm is within the specifications described in the Food Chemicals Codex (5th edition) for beta- and gamma-cyclodextrin.

(2)ADI 'not specified' is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

More in GRAS Notice Inventory (/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm)

Appendix 3

Expert Panel Consensus Statement concerning the Generally Recognized as Safe (GRAS) status of alpha-Cyclodextrin for Use in Processed and Ultra-processed Foods and Certain Beverages Based on Scientific Procedures

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Prepared for:
Hyman, Phelps & McNamara, P.C.
700 Thirteenth Street NW, Suite 1200
Washington, D.C. 20005

April 08, 2016

1. INTRODUCTION

The undersigned, an independent panel of experts qualified by their scientific training and national and international experience to evaluate the safety of food and food ingredients (the "Expert Panel"), was specially convened by Wacker Chemical Corp. (formerly Wacker Biochem Corp.) and asked to evaluate the safety and Generally Recognized as Safe (GRAS) status of alpha-cyclodextrin (hereinafter "alpha-CD") for general use in processed and ultra-processed foods as defined herein (excluding beverages) at levels of up to 3%, and in certain specified types of beverages at concentrations up to 1.05%.

Pivotal, publicly available safety studies were critically reviewed previously by Wacker's GRAS Expert Panel (Anderson et al., 2004) and authoritative food safety assessment bodies (WHO, 2002, 2005; FSANZ, 2004; EFSA, 2007). At the request of Wacker, Albert Bär Ph.D. performed a comprehensive search of the scientific literature through 16 March 2016 relating to the safety/toxicity and dietary intake/consumption of alpha-CD and summarized the results of the pertinent studies and other information for consideration by the Expert Panel in a dossier, "Alpha-Cyclodextrin", dated March 2016. The Expert Panel critically evaluated that summary and other available data and information believed to be pertinent to the safety of alpha-CD under the intended conditions of use.

Following an independent, critical and collaborative evaluation of the data and information, the Expert Panel independently and jointly unanimously concluded that Wacker's alpha-CD, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is safe and Generally Recognized As Safe (GRAS) based on scientific procedures for use in processed and ultra-processed foods as defined herein (but excluding beverages) at levels up to 3% in the foods as consumed, and in certain specified types of beverages as consumed at a concentration up to 1.05% of alpha-CD.

2. BACKGROUND

Wacker's GRN 000155 (submitted on 25 June 2004) provided the FDA with a summary of the basis for Wacker's GRAS determination for the then-intended uses of alpha-CD. The safety of these uses of alpha-CD was supported by data on its absorption, distribution, metabolism and excretion. The toxicological studies included acute toxicity studies in mice and rats, subchronic oral toxicity studies in rats and dogs, embryotoxicity/teratogenicity studies in mice, rats and rabbits, standard genotoxicity tests, and a study in humans which provided data on the gastrointestinal tolerance of 25-g single doses of alpha-CD. Data on the safety of 1-decanol were also considered because trace amounts of this processing aid may be present in alpha-CD (not more than 20 ppm as per *FCC* specifications).

Detailed reports of the pivotal safety studies on which the Panel based its report are publicly available (Lina & Bär 2004a, b; Waalkens-Berendsen & Bär, 2004; Waalkens-Berendsen et al., 2004).

Following review of Wacker's GRAS Notice (GRN 000155), FDA issued a response letter (22 December 2004) stating that the agency had no questions regarding Wacker's conclusion that the intended uses of alpha-CD described in the GRAS Notice are GRAS based on scientific procedures (FDA, 2004).

The safety of alpha-CD has also been critically reviewed and summarized by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA") which allocated an ADI "not specified" on two separate occasions for alpha-CD for use as a food-technological additive and as a nutritive substance (dietary fiber) resulting in an estimated daily intake of alpha-CD of 11.4 and 19.8 g/d for the mean and 90^{th} percentile consumer, respectively (WHO, 2002, 2005). Food Standards Australia/New Zealand (FSANZ) performed its own safety assessment of alpha-CD (FSANZ, 2004), confirmed JECFA's ADI "not specified" and authorized alpha-CD as a novel food in Australia/NZ.

The latest assessment of the safety of alpha-CD was conducted by the European Food Safety Authority (EFSA) (EFSA, 2007). The EFSA Panel also concluded that there are no safety concerns for the consumption of alpha-CD which was subsequently authorized as a novel food without limitations of use by the European Commission (Commission Decision 2008/413/EC). As of March 16, 2016, no data were identified in the scientific literature that would conflict with EFSA's conclusion on the safety of alpha-CD.

3. EXPANDED USE OF ALPHA-CD IN PROCESSED AND ULTRA-PROCESSED FOODS

In view of proposed new applications of alpha-CD in foods that are not covered by GRN 000155 and considering the more flexible authorization of alpha-CD as a novel food in the European Union, Wacker requested the Expert Panel to assess the safety of the use of alpha-CD in any kind of processed and ultra-processed foods (except beverages) at levels of up to 3% and in certain specified beverages at concentration of up to 1.05%.

Based on publicly available data on the consumption of defined processed and ultra-processed foods in the US (Baraldi et al., 2013; Moubarac et al., 2013) coupled with published data on (a) the food intake per kg bw for the $\geq 90^{th}$ percentile of US consumers (Swinburn et al., 2009), (b) an average energy density of 2 kcal/g food (other than beverages) (Hansen 1979; Kant & Graubard, 2005), and (c) an approximately 80:20 ratio of energy intake from foods and beverages (Duffey & Popkin, 2007), the intake of alpha-CD from its combined use in all processed and ultra-processed foods at a level of 3% was estimated at 420 mg/kg bw/d or 29.4 g/d for the 90^{th} percentile consumer with a body weight of 70 kg. The additionally proposed use of alpha-CD in beverages specified in GRN 000155 at a level of 1.05% would result in an estimated alpha-CD intake of 7.5 g/d for the 90^{th} percentile consumer of such beverages (calculation based on intake data presented in GRN 000155).

Single doses of 25 g and repeated doses of 2 x 15 g/d alpha-CD for 7 days were well tolerated by humans (Diamantis & Bär, 2002, Park et al., 2012). The weight of evidence including extensive toxicological data and human data and the opinions of international experts including JECFA, FSANZ AND EFSA, support the safety of the intended uses of alpha-CD.

4. CONCLUSION

We, the Expert Panel, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of Wacker's alpha-CD summarized in a dossier ("Alpha-Cyclodextrin") and other information deemed pertinent, and unanimously conclude that Wacker's alpha-CD, produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is safe for use as an ingredient for general use in processed and ultra-processed foods as defined herein (but excluding beverages) at levels up to 3% in the foods as consumed, and in certain specified types of beverages as consumed at a concentration up to 1.05%.

We further conclude that the proposed use of Wacker's alpha-CD is Generally Recognized As Safe (GRAS) based on scientific procedures for use as an ingredient for general use in processed and ultra-processed foods as defined herein (but excluding beverages) at levels up to 3% in the foods as consumed, and in certain specified types of beverages as consumed at a concentration up to 1.05%.[1]

It is our opinion that other qualified experts would concur with these conclusions.

By (b) (6)

12 April 2016

Joseph F. Borzelleca, Ph.D. Date
Professor Emeritus Pharmacology & Toxicology
Virginia Commonwealth University School of Medicine
Richmond, Virginia

By: (b) (6)

14 April 2016

Robert Nicolosi, Ph.D. Date
Professor Emeritus Department of Clinical
Laboratory & Nutritional Sciences,
UMass Lowell, Lowell MA 01075

[1] The Expert Panel considered the safety, but not the suitability, of the proposed use of alpha-CD as an ingredient in processed and ultra-processed meat and poultry products.

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SUBMISSION END

From: Ricardo Carvajal
To: McMahon, Carrie
Subject: RE: REGARDING: GRN 678 (alpha-cyclodextrin)
Date: Tuesday, January 10, 2017 1:35:26 PM
Attachments: image001.png

Dear Ms. McMahon:

Thank you for the information in your email below. At present, Wacker wishes to exclude uses of alpha-cyclodextrin in meat and poultry products falling under the jurisdiction of USDA. Given the method that Wacker used for estimating daily intake, the exclusion of uses in meat and poultry products has no impact on the dietary exposure analysis in Part 3 of the GRN. Please let me know if you have any further questions about this issue.

Best regards,
Ricardo

Ricardo Carvajal, J.D., M.S.
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From: McMahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
Sent: Friday, December 30, 2016 10:38 AM
To: Ricardo Carvajal
Subject: REGARDING: GRN 678 (alpha-cyclodextrin)

Dear Mr. Carvajal,

REGARDING: GRAS Notice No. GRN 678 (alpha-cyclodextrin)

According to Wacker Chemical Corporation (Wacker), alpha-cyclodextrin is to be used in a varieties of processed and ultra-processed foods, as well as certain beverages. Wacker further explains that some of these foods may constitute meat or poultry products falling under the jurisdiction of the U.S. Department of Agriculture (USDA).

We noted that Wacker's GRAS Notice does not contain data or information supporting the suitability of alpha-cyclodextrin in meat or poultry products falling under USDA jurisdiction. Consequently, prior to forwarding the GRAS Notice to USDA for their evaluation, we contacted USDA's Risk, Innovations, and Management Staff to ask about the necessity of suitability data/information.

USDA advised that data/information supporting the suitability of alpha-cyclodextrin for use in USDA-regulated products is needed in order for USDA to conduct their evaluation. They asked that we refer you to the Compliance Guidance (available online here: https://www.fsis.usda.gov/wps/wcm/connect/c64d8f3b-56aa-49c9-91f3-daf0caaba6bd/New-Technology-Protocols-042015.pdf?MOD=AJPERES).

So that FDA and USDA can determine how to proceed, please confirm whether or not Wacker is able to provide suitability data to support the use of alpha-cyclodextrin in products falling under USDA jurisdiction.

Please contact me if you have any questions.

Regards,

Carrie McMahon, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1202
Carrie.McMahon@fda.hhs.gov

From:	Ricardo Carvajal
To:	McMahon, Carrie
Cc:	Albert Bar
Subject:	RE: GRN 678 - alternate FDA contact for Feb 26-Mar3
Date:	Monday, March 06, 2017 1:45:47 PM
Attachments:	Annex 5 - Wacker Alpha-CD GRAS Dossier 2004 (00304108).pdf Annex 4 - Wacker Alpha-CD GRAS Dossier 2004 (00304106).pdf Annex 3 - Wacker Alpha-CD GRAS Dossier 2004 (00304104).pdf Annex 2 - Wacker Alpha-CD GRAS Dossier 2004 (00304100).pdf Annex 1 - Wacker Alpha-CD GRAS Dossier 2004 (00304099).pdf Wacker Alpha-CD GRAS Dossier 2004 (00304088).pdf

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Dear Carrie:

As we discussed, attached is the GRAS dossier and annexes prepared in support of Wacker's GRAS determination in 2004. We will follow up with the answers to FDA's questions by Wednesday.

Best regards,
Ricardo

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α-CYCLODEXTRIN

(α-CD)

Dossier prepared on behalf of Wacker Chemie GmbH, Munich, Germany, for the safety assessment of α-cyclodextrin

Author: Albert Bär

Date: June 01, 2004

CONTENTS

Abbreviations

ADI	Acceptable daily intake
CD(s)	Cyclodextrin(s)
CGTase	Cyclodextrin glycosyltransferase
d.s.	Dry substance
DE	Dextrose equivalent
DM-β-CD	Dimethyl-β-cyclodextrin
EDI	Estimated daily intake
FCC IV	Food Chemicals Codex, 4th Edition
GC	Gas chromatography
GI	Gastro-intestinal
HP-β-CD	Hydroxypropyl-β-cyclodextrin
HPLC	High performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg bw	kilogram body weight
n.s.	not significant
PEG	Polyethylene glycol
PUFA(s)	Polyunsaturated fatty acid(s)
RI	Refractive index
SCF	Scientific Committee on Food
SCFA(s)	Short-chain fatty acid(s)
TOS	Total organic solids

1 NAME AND CHEMICAL STRUCTURE, PHYSICOCHEMICAL AND CHEMICAL PROPERTIES, OCCURRENCE IN NATURE

1.1 Name and chemical structure

Common or usual name:	α-cyclodextrin
Synonyms:	cyclohexa-amylose cyclomalto-hexaose α-dextrin[1]
CAS Registry Number:	10016-20-3
Empirical formula:	$(C_6H_{10}O_5)_6$
Molecular weight:	973 daltons
Structural formula:	α-CD is the cyclic polymer of six α-1,4-linked glycopyranosyl units (see Fig. 1)

1.2 Physicochemical properties

Solubility in water (g/100ml at 25°C): 13.0 (range: 12.8-18.0)[2]

Specific rotation $[\alpha]_D^{25}$ (1%, H_20): 148 ± 3[3]

[1] Freudenberg & Rapp, 1936; Samec & Blinc, 1939

[2] 12.8 (Jozwiakowski & Connors, 1985); 13.0 (Nagatomo, 1985); 14.5 (French et al., 1949); 18.0 (Okada et al., 1988)

[3] 136 ± 3 (FLUKA, Catalogue of chemicals and biochemicals, 1995); 148.8 (Okada et al., 1988); 150.5 (French et al., 1949; Andersen et al., 1963)

Water of hydration (wt%):	6.0 – 10.9[4]
Melting point (°C):	ca. 250-278°C (decomposition)[5]
Acid dissociation (pK_a at 25°C)	12.33[6]

1.3 Other properties relevant for the use of α-CD as a food ingredient

1.3.1 Hygroscopicity

α-CD crystallizes with about 6 mol water per mol α-CD (Nakai et al., 1986). At low environmental relative humidity (R.H. < 20%), some of this water is lost (Nakai et al., 1986). On the other hand, additional water is taken up in a humid atmosphere (up to a total water content of about 6.6 mol/mol α-CD at R.H. ≥ 79%). However, the product remains a dry, pourable powder (Nagatomo, 1985; Nakai et al., 1986; Tanada et al., 1996). A non-stoichiometric hydrate with 7.57 mol of water of hydration[7] has also been described (Maggi et al., 1998).

[4] 6.0-6.6 (Nakai et al., 1986); 6.9-10.9 (Nagatomo, 1985)

[5] 250-260 °C (Linssen et al., 1991); 278 °C (Aldrich Chemical Company, Catalogue/handbook of fine chemicals, 1999/2000)

[6] according to Gelb et al., 1982

[7] syn: water of crystallisation

1.3.2 Chemical stability

Under the pH conditions encountered in foods, α-CD is stable. Strong acids hydrolyze α-CD, but the hydrolysis rate is slower than that of linear maltooligosaccharides. The half-life of ring opening in 1N HCl at 60 °C is 6.2 hours (Uekama & Irie, 1987). No degradation occurs in an alkaline environment.

Since α-CD has no reducing end, it does not undergo Maillard reactions. Because it carries no reactive functional groups, it does not chemically react with other food components.

1.3.3 Thermal stability

Under the temperature conditions applied during the processing and storage of food, α-CD is stable. With increasing temperature, α-CD looses bound water (Nakai et al., 1986). Thermal decomposition occurs at about 250-278°C (melting point).

1.3.4 Enzymatic stability

α-CD is hydrolyzed by α-amylases of fungal or bacterial origin (Freudenberg & Rapp, 1936; Jodal et al., 1984; Saha & Zeikus, 1992; McCleary, 2002). Salivary (human) and pancreatic (porcine, human) α-amylases, on the other hand, are unable to hydrolyze α-

CD to a significant extent (Kondo et al., 1990; Marshall & Miwa, 1981; McCleary, 2002).

1.3.5 Complexation

The circular molecules of α-, β- and γ-CD are shaped like a hollow truncated cone or torus (Fig. 2). Because the hydrogen atoms and the oxygen atoms of the glycosidic bonds are facing the inner side of the torus, while the hydroxyl groups are located on the outer side, cyclodextrins have a hydrophobic cavity and, at the same time, a hydrophilic outer surface which makes them water-soluble. The hydrophobic cavity enables cyclodextrins to form complexes with a variety of organic compounds. The diameter of the cavity provides for a certain selectivity of the complexation of "guest" molecules, i.e., the bigger ring of the 8-membered γ-CD can accommodate a wider variety of guest molecules than the smaller rings of α- and β-CD. Large guest molecules may complex with more than one cyclodextrin molecule (Le Bas & Rysanek, 1987). Typical equilibrium constants rank between 10^0-10^4 Mol^{-1} (Connors, 1995).

The formation of inclusion complexes is the basis for certain applications of cyclodextrins in food, cosmetics, and pharmaceutical preparations (Hedges et al., 1995; Nagatomo, 1985; Loftsson & Masson, 2001). However, it must be noted that the formation of complexes between cyclodextrins and guest molecules is reversible. Consequently, there is an equilibrium between free and complexed forms in solution. In food and biological fluids, many

factors will influence this equilibrium. For example, other molecules may compete for the binding of the guest molecule, or for inclusion in the cyclodextrin ring.

1.4 Occurrence in Nature

Cyclodextrins were first isolated by Villiers in 1891 from a culture medium of Bacillus amylobacter (Clostridium butyricum) grown on a medium containing starch. More detailed work on cyclodextrins was done by Schardinger between 1903-1911. During studies on microbial food spoilage, he isolated Bacillus macerans, a heat-resistant, α-CD producing microorganism (Schar-dinger, 1903; French, 1957). Since then, many other bacteria have been described which are able to produce cyclodextrins from starch. Investigations on the CD metabolism of K. oxytoca suggest that CDs play a role in a starch utilization pathway with extra-cellular conversion of starch into CD by CGTase, subsequent uptake of the CD by a specific transport system, intracellular ring opening by a cyclodextrinase, and finally hydrolysis and further catabolism of the formed maltooligosaccharides (Feederle et al., 1996; Fiedler et al., 1996; Pajatsch et al., 1998).

However, there is no known significant intake of naturally occurring cyclodextrins with food.

2 MANUFACTURING PROCESS

2.1 General aspects of cyclodextrin production

α-, β-, and γ-Cyclodextrin, i.e., cyclohexa-, hepta- and octoamylose, are formed by the action of cyclodextrin-glycosyltransferase (CGTase, EC 2.4.1.19, CAS 9030-09-5, syn: cyclodextringlucanotransferase).

CGTases are amylolytic enzymes which are produced naturally by different strains of Bacilli (e.g., B. macerans, B. circulans, B. lentus, B. firmus, B. subtilis, B. megaterium) and organisms of different other species (e.g., Klebsiella pneumoniae, K. oxytoca, Micrococcus sp., Thermoanaerobacter sp., Brevibacterium sp.) (Bikbulatova et al., 2000; Tonkova, 1998; Sicard & Saniez, 1987; Schmid, 1989; Bender, 1977).

CGTases degrade starch by a cyclization reaction. There is evidence that the enzyme recognizes the 6 to 8 glucose units from the non-reducing end of an amylose molecule, attacks the adjacent α-1,4-linkage, and transfers it to the C-4 position of the non-reducing end to produce α-, β- or γ-CD (Schmid, 1989). Typically, mixtures of α-, β- and γ-CD are formed by the action of CGTases on amylose[8]. However, different CGTases produce α-, β-, and γ-CD

[8] CDs with 9-12 glucose units have been described as well (Pulley & French, 1961).

in different proportions during the initial phase of the reaction (Yim et al., 1997). On this basis, a distinction between α-, β-, and γ-CGTases has been made. The CGTase from K. oxytoca primarily produces mainly α-CD and is, therefore, regarded as α-CGTase.

For isolation of cyclodextrins from the enzymatic reaction mixture, two different procedures are available. In the "solvent process", a suitable organic substance is added which forms an insoluble complex with the desired cyclodextrin. In the "non-solvent process", chromatographic techniques are applied to separate the formed cyclodextrins (Sicard & Saniez, 1987).

By adding, in the solvent process, an appropriate complexing agent, the yield of that cyclodextrin is favored which forms an insoluble complex with the complexant. 1-Decanol has been identified as selective complexant for enhancing the yield of α-CD (Flaschel et al., 1982, 1984; Armbruster & Jacaway, 1972).

2.2 Manufacturing process of α-CD

2.2.1 Description of process

A scheme of the production process (batch process) of α-CD used by Wacker Chemie GmbH is shown in Figure 3. The critical control points and control standards are shown in Annex 3.

In a first step, food-grade, liquefied starch with a low dextrose equivalent (DE 5.5-7.5) is treated in a closed stainless steel vessel with α-CGTase (prepared as described in section 2.2.2) under controlled pH and temperature conditions (pH 6.0-7.0; 35-45°C). During the process, 1-decanol is added in stoichiometric quantity in order to precipitate formed α-CD as α-CD/1-decanol complex (data on 1-decanol are provided in section 2.2.3). After completion of the reaction, the insoluble α-CD/1-decanol complex is continuously mixed with water and separated from the reaction mixture (containing residual starch and the enzyme preparation) in a decanting centrifuge. The recovered precipitate is resuspended in water, and dissolved by heating to 110-130°C (30 min). On subsequent cooling of the two-phase system to 45-75°C, the complex precipitates again. The precipitate is recovered by centrifugation and 1-decanol is stripped off by steam distillation at 100-130°C. Upon cooling to a temperature of 15-40°C, α-CD crystallizes from the solution. The crystals are removed by filtration and dried. α-CD is obtained as a white, crystalline powder with a water content of less than 11%.

In summary, the different products entering the process are the following:

Product	**Function**	**Specifications**
Liquefied starch solution	Raw material	Food-grade, 31 – 34% d.s., DE 5.5-7.5
Sodium hydroxide	Processing aid	Food-grade; 21 CFR § 184.1763
α-CGTase preparation	Enyzme	Complies with general purity criteria for enzyme preparations (FCC IV) Enzymatic Activity: > 500 U/ml; Heavy metals: <5 ppm Total aerobic counts: <100 CFU/g Coliforms: absent Pathogens: absent Source organism: 0 CFU/ml
Water (deionized)	Solvent	
1-Decanol	Complexing agent	Purity: > 98%

2.2.2 Production and safety of the applied enzyme (CGTase) preparation

2.2.2.1 Source organism

Klebsiella oxytoca M5a1 (formerly K. pneumoniae M5a1, formerly Aerobacter aerogenes M5a1) was found to secrete a catalytically very efficient α–CGTase (Bender, 1977). The gene coding for the CGTase was isolated from this strain and characterized (Binder et al., 1986), cloned in pHE3 (Henneke et al., 1982), isolated again and inserted in the expression vector pJF118EH. The characteristics of the expression vector pJF118EH are described in the literature (Fürste et al., 1986). It carries a controllable *tac* promoter.

A strain of Escherichia coli K12 served as host. The applied strain is characterized by its Hfr genotype and "leaky" phenotype. Its secretion capacity was enhanced by selection for D-cycloserin resistence as described elsewhere (Böck et al., 1994). Furthermore, the tra-A gen (coding for the pilin protein) was deleted in order to ensure that the strain has no ability for conjugation. The efficacy of this procedure was confirmed by mating tests.

The source organism of α-CGTase (E. coli WCM105xpCM703) has been deposited at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany).

2.2.2.2 Production

For production of α-CGTase, the recombinant E.coli strain is cultured in a standard medium containing food-grade glucose, food-grade mineral salts, a nitrogen source (casein hydrolysate), micro-nutrients, food-grade vitamin B_1, and yeast extract. The pH of the fermentation broth is adjusted to the desired value by adding food-grade ammonia or phosphoric acid.

The E. coli inoculum is prepared in a scale-up cascade of four steps (agar plates, shake flasks, lab-scale fermenter, pilot fermenter). The culture media used in the scaling-up procedure, contain tetracycline at a concentration of 20 mg/l. With the inoculum, tetracycline is transferred to the medium used in the production fermenter (concentration: 1.3 mg/l).

After the E. coli has grown to a certain density, enzyme production is initiated by addition of isopropyl thiogalactoside (IPTG) (78 mg/l). When the desired enzyme activity has been reached in the fermentation broth, bacterial cells and particles are removed by centrifugation. The supernatant is cooled to 10°C and filtered first through modular filters and then through membrane filters with a nominal pore size of 0.2 μm. The filtrate is concentrated by cross-flow ultrafiltration using membranes with an nominal cut-off molecular weight of 20,000 daltons. For preservation,

sorbic acid may be added to the concentrate at a concentration of 5 g/l.

The identity of the recombinant E. coli strain grown in the production fermenter is verified by culturing a sample on agar plates. The α-CGTase preparation which has the appearance of a brownish liquid, is analyzed for enzyme activity (specifications: > 500 U/ml) and microbial contaminations (specifications: recombinant E. coli 0 CFU/ml, total aerobic microorganisms < 10,000 CFU/ml). The crude, liquid enzyme preparation has a dry matter content of about 2-3% (TOS is about 15-25 g/l).

Routine quality control of the α-CGTase production process include checks of the identity of the recombinant E. coli strain, the compliance with specifications of the ingredients used in the culture media, the sterilization of vessels and media before use, the relevant fermentation parameters (pH, temperature, stirring rate, optical density, glucose concentration and aeration), as well as the integrity of the filters applied for the concentration process.

2.2.2.3 Safety of applied source organism

The gene coding for α-CGTase stems from Klebsiella oxytoca M5a1 (formerly K. pneumoniae M5a1) (Bender, 1977; Binder et al., 1986). The morphological and biochemical characteristics of K.

oxytoca and K. pneumoniae are identical except that the former metabolizes L-tryptophane to indole.

Klebsiella oxytoca is a gram-negative, facultative anaerobe which belongs to the family of the Enterobacteria. It is a normal member of the fecal microbiota (found in 30-40% of humans), but it also was isolated from aquatic sources and from the rhizosphere of rice where it acts as an associative nitrogen fixer. Like for E. coli, there exist pathogenic strains of K. oxytoca which have been associated with hemorrhagic colitis, urinary tract infections and septicemia. Nonetheless, K. oxytoca belongs to risk group 1 in the classification of human etiologic agents as laid down in the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH, 2002). Group 1 comprises agents that are not associated with disease in healthy adult humans.

Pullulanase (EC 3.2.1.41), an enzyme used in starch processing [21 CFR § 172.892(i)] and fruit processing is obtained from different microorganisms including Klebsiella pneumoniae, Klebsiella planticola and Klebsiella aerogenes (FAO, 1992; Munch, 1999).

The particular strain which served as donor of the α-CGTase gene, K. oxytoca M5a1, is well characterized (Randriamahefa, 1994). Unlike most other strains of K. oxytoca, it does not possess capsule material. It is deposited at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) under No. 7342. The strain has been used for many years in biotech-

nological research and applications. In the Compendium of Guidance of the UK Advisory Committee on Genetic Modification (ACGM) it is cited as a "disabled or non-colonizing host", i.e., as a strain with a history of safe use and a negligible capacity to persist in humans (ACGM, 2000).

The vector used is derived from the pJF118EH vector which is described completely in the literature (Fürste et al., 1986).[9] pJF118EH is derived from pBR322, a widely used mobilization-defective vector which is considered to be safe (EU Commission, (COM(95)640 final, OJ C 356, 22.11.1997, p. 19). The cloned DNA fragment of K. oxytoca contains only the CGTase gene as demonstrated by sequence analysis.

As a host for the α-CGTase expression and secretion vector, a strain derived from E. coli K12 was used. E. coli K12 belongs to risk group 1 in the classification of human etiologic agents (NIH, 2002). It is used already for the production of Chymosin A which has been determined to be safe for direct use in food (55 Fed. Reg. 10932, p. 32-34, March 23, 1990). E. coli K12 also has been recommended as a safe host organism by the EU Commission (COM(95)640 final, OJ C 356, 22.11.1997, p. 19).

[9] This vector introduces a tetracycline resistance gene in the source organism. However, this is not of concern because this gene is not present in the enzyme preparation in a transferable form, i.e. a transfection is not possible. Furthermore, the source organism is not released in the environment. The CGTase preparation does not contain any source organism (specifications: E.coli, 0 CFU/ml). Finally, it should be noted that 31% of naturally occurring E.coli strains are highly resistant to tetracycline (Bryan et al., 2004).

2.2.2.4 Safety of crude CGTase preparation

The α-CGTase preparation produced as described in section 2.2.2.2, has been subjected to in-vitro genotoxicity tests (Ames tests, in-vitro chromosome aberration test) and a subchronic (13-week) oral toxicity study in rats.

The batch which was applied for the genotoxicity tests, had a dry substance content of 1.5%, a total protein content of 0.50% (enzyme activity 625 U/ml) and a total ash content of 0.55%. The α-CGTase batch which was applied for the 13-week oral toxicity study in rats, had a dry substance content of 1.9%, a total protein content of 0.356% (enzyme activity 530 U/ml) and a total ash content of 0.60%.

Bacterial reverse mutation tests (Ames tests)

The potential of the α-CGTase preparation to induce gene mutations in vitro was examined in the histidine-requiring Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100 and in the tryptophan-requiring Escherichia coli strain WP2uvrA.

The study was conducted in duplicate by direct plate incorporation as there was no indication of an unspecific promoting effect

of the CGTase preparation on bacterial growth. The tests were performed in the absence and presence of metabolic activation (S9-mix). In the first test, the α-CGTase preparation was tested at five concentrations ranging from 62-5000 µg/plate; in the second test, it was tested at five concentrations ranging from 313 to 5000 µg/plate.

The results of the two tests demonstrate that α-CGTase in the absence and presence of S9-mix did not cause a more than two-fold increase of the mean number of revertant colonies. The mean number of his^+ and trp^+ revertant colonies of the negative controls were within the usual range. The positive control treatment produced the expected increase in the number of revertant colonies. It was, therefore, concluded that the tested α-CGTase preparation is not mutagenic under the conditions of this assay (Krul & van den Wijngaard, 2004; Bär et al., 2004).

In vitro chromosome aberration test

The clastogenic potential of the α-CGTase preparation was tested in cultured human peripheral blood lymphocytes. Two independent tests were performed both in the absence and presence of metabolic activation (S9-mix).

In the first test, the α-CGTase preparation was tested at concentrations of 0, 39, 78, 156, 313, 625, 1250, 2500 and 5000 µg/ml.

Mitomycin C and cyclophosphamide were used as positive control substance in the absence and the presence of S9-mix, respectively. In the absence of S9-mix, the treatment times were 4 hours (pulse treatment) and 24 hours (continuous treatment) in culture medium with FCS. In the presence of S9-mix, the treatment time was 4 hours (pulse treatment) in culture medium without FCS. In both the absence and presence of S9-mix, the harvest time of the cells was 24 hours after onset of the treatment.

In the second test, the α-CGTase preparation was tested at concentrations of 0, 1000, 2000, 3000, 4000 and 5000 µg/ml. In the absence of S9-mix, one group of cultures was treated continuously for 24 hours and another group continuously for 48 hours. In the presence of S9-mix, one group of cultures was pulse-treated for 4 hours and harvested 24 hours after start of the treatment. A second group was pulse-treated for 4 hours and harvested 48 hours after start of the treatment.

Two hours before the end of the 24 and 48-hour incubation periods, the cultured lymphocytes were arrested in the metaphase stage of their mitosis by adding colcemid (0.1 µg/ml). The cells were harvested and processed for determination of the mitotic index, chromatid and chromosome-type aberrations and other anomalies.

The mitotic index was decreased under certain conditions of treatment at α-CGTase preparation when tested at concentrations

of $\geq$ 2500 µg/ml. However, in none of these cases was the miotic index reduced below the level that would be indicative of a toxic effect of the treatment (60% of the vehicle control values).

No chromosome aberrations were induced by the α-CGTase preparation when tested at concentrations of up to 5000 µg/ml. All treatments of the cultures, in the absence and the presence of S9-mix, resulted in frequencies of cells with structural aberrations (excluding gaps) which were similar to those observed in concurrent negative controls and fell within historical negative control (normal) ranges. Cells with numerical chromosome aberrations (polyploids or others) were not detected on any slide. It was concluded that, under the conditions of this study, the tested αCGTase preparation was not clastogenic to cultured human lymphocytes (de Vogel, 2004; Bär et al., 2004).

Subchronic (13-week) toxicity test

Groups of 20 males and 20 female Wistar rats received the α-CGTase preparation by gavage at doses of 5, 10 or 20 ml/kg bw/day. Control animals were dosed with tap water (20 ml/kg bw/day). Body weights, food and water consumption, ophthalmoscopic observations, as well as hematological and urinary parameters did not differ between treated groups and controls. Clinicochemical analyses performed after 30, 60 and 91-95 days of treatment revealed significantly decreased plasma cholesterol levels (day 60) and triglycerides (at termination) in males of the high-

dose group. Results of a battery of tests for detecting neurological, behavioral or physiological dysfunctions revealed no abnormalities. Organ weights were not affected by the treatment. Histopathological examination revealed a number of slight alterations in the lungs, namely an increased incidence of alveolar hemorrhages with hemoglobin crystals in males (dose-related, significant in high-dose group), and an increased incidence of septal cellularity in all treated groups (not dose-related, significant in the males of the low- and high-dose group). An accumulation of alveolar macrophages was seen more frequently in treated males than in controls but the difference was not statistically significant and a dose-response relationship was not apparent. In females, accumulation of alveolar macrophages was seen more often in the low- and high-dose group (difference to controls not significant). Since the incidence and severity of these changes were not dose-related, it was considered that they represent an unspecific response to the gavage of the test solution. Microaspiration of the protein-containing test solution or vagally mediated reflex bronchospasms due to the gavage could lead to the observed changes (Conybeare & Leslie, 1988). An association between gastro-esophageal reflux and an accumulation of alveolar macrophages has been observed in children (Nussbaum et al., 1987). The authors concluded, therefore, that no adverse effect in response to the α-CGTase treatment was seen up to the highest dose level tested (20 ml/kg bw/d, corresponding to 260 mg TOS/kg bw/d) (Jonker, 1994; Bär et al., 2004).

Even if all the organic substances of the enzyme preparation were carried over into the α-CD, which they are not, the human expo-

sure would be at least 1000-times below the NOAEL. The 100-fold safety margin, which is considered adequate for this type of study, is therefore exceeded by far (Pariza & Johnson, 2001).

2.2.2.5 Regulatory status

The α-CGTase is removed completely during the production process as shown by the absence of protein in α-CD (see Section 3.1.2 and Annex 4). The enzyme, therefore, falls outside the definition of a "food additive" ([FD&C Act Sec. 201(s)] under the conditions of intended use in the production of α-CD.

Nonetheless, by applying the principles used for the safety assessment of microbial enzyme preparations (Pariza and Johnson, 2001), α-CGTase was determined to be acceptable for use in production of α-CD (Figure 4).

2.2.3 Safety of applied complexant

2.2.3.1 General description

1-Decanol (syn.: decan-1-ol, decyl alcohol, capric alcohol) (CAS 112-30-1) is a colorless liquid with a melting point of 7°C and a boiling point of 220-235°C. It has a very low water solubility of 37 mg/l at 25°C. Having a floral, waxy and fruity odor, 1-decanol is used as a flavoring substance at very low concentrations. The commercial product has a purity of ≥98%.

2.2.3.2 Biological studies

No data are available on the absorption, distribution, metabolism and excretion of 1-decanol. However, it is generally assumed that ingested aliphatic primary alcohols are absorbed and oxidized to the corresponding aldehyde which is then rapidly oxidized to the acid. Even-numbered acids are metabolized via β-oxidation to acetyl-CoA which then enters the citric acid cycle (WHO, 1998).

2.2.3.3 Toxicological studies

The safety of 1-decanol has been examined in a number of studies including genotoxicity tests, acute oral toxicity tests and two embryotoxicity/teratogenicity studies (inhalation and oral administration).

A gene mutation assay was conducted with B. subtilis H17 (rec^+) and M45 (rec^-) using 17 μg 1-decanol/disk. A negative result was obtained (Oda et al., 1978 cited in WHO, 1998).

The acute oral toxicity of 1-decanol was examined in two studies with rats. LD_{50} values of >5 and 12.8 g/kg bw were reported (Henkel KGaA, unpublished data, Archive No 281; Bär & Griepentrog, 1967). In mice, a LD_{50} of 6.5 g/kg bw was observed (RTECS HE 4375000).

An embryotoxicity /teratogenicity study was conducted with Sprague-Dawley rats. The dams were exposed to 1-decanol by inhalation (100 mg/m^3; 6h/d) from day 1-19 of gestation. No maternal toxicity was observed. The reproductive outcome (number of resorptions, litter size, fetal weights) was not adversely affected by the treatment, and there were no signs of fetotoxicity or teratogenicity (Nelson et al., 1990).

An embryotoxicity/teratogenicity study with oral administration of a series of primary alcohols including 1-decanol was conducted in not specified random-bred albino rats. A group of 10 female rats received daily doses of 400 mg decanol mixed with 600mg water by gavage from day 1-15 of pregnancy. This dose corresponds to about 2.3 g/kg bw/d at start of the treatment. A control group of 20 rats received 1 ml water/d by gavage. Signs of maternal toxicity were not reported. Pre- and post-implantation losses were significantly increased with decanol but the size and weight of the fetuses were not impaired. Teratogenic activity was not observed. It was concluded that all the tested primary alcohols (C1, C2, C4, C9, C10) increased the number of pre- and post-implantation losses. In this respect, decanol and nonanol were clearly less active than ethanol or methanol. Retardation of fetal development was observed with all the tested alcohols, except decanol. None of the tested alcohols had a teratogenic activity (Barilyak et al., 1991).

2.2.3.4 Regulatory status

1-Decanol has been evaluated by JECFA as a flavoring substance and was determined to not pose safety concerns. The actual daily intake (for eaters only) was estimated at 7 and 28 μg/person/day in the US and Europe, respectively (WHO, 1998).

In the US, 1-decanol has a FEMA GRAS status (FEMA No. 2365) and is listed as a synthetic flavoring substance in 21 CFR § 172.515. Belonging to the group of "fatty alcohols", its use in food of synthetic 1-decanol is regulated in 21 CFR § 172.864. This includes in particular its use in the synthesis of food additives and other substances permitted in food.

In the EU, 1-decanol is listed in the Register of Flavoring Substances under FL No 02.024 (Commission Decision 2002/113/EC).

3 SPECIFICATIONS

3.1 Potential impurities

In this section, the sources and identity of potential impurities of α-CD are described. For those impurities which are relevant from a safety or quality point of view, limit values have been established in the specifications (see section 3.2 and Annex 1). The tests by which compliance with these purity criteria is examined, are described in Annex 1 or, for standard tests, are contained in FAO Food and Nutrition Paper 5 Rev. 2 (1991). Analytical data of five representative batches of α-CD, produced at pilot-plant scale, are shown in Annex 2.

3.1.1 Impurities from raw material

Residues of the food-grade liquefied starch would be expected to be negligible given the multi-step nature of the process. Malto-oligosaccharides, maltose and glucose which originate from the raw material or are formed from starch as a by-product of the amylolytic action of α-CGTase, are detected by the test for reducing sugars. Remaining inorganic salts are detected as ash.

3.1.2 Impurities from α-CGTase preparation

The crude α-CGTase preparation contains all the material that has passed the 0.2 μm filter (i.e., cell and cell debris are removed) and has a molecular weight of more than about 20,000 daltons. It also contains the components of the fermentation broth with a molecular weight below 20,000 daltons but only at about the concentrations at which they are present in the fermentation broth (the bulk of it is removed by ultrafiltration). The high-molecular weight fraction consists mainly of protein (α-CGTase and ultrafilterable components of spontaneously lysed E. coli).

The high-molecular components of the enzyme preparation are separated from α-CD during the first precipitation step with complexant. Any remaining amounts of protein will be denatured by heat during the α-CD purification process (re-precipitation and steam stripping at temperatures of 110-130°C) (see section 2.2.1.).

In order to examine the efficacy of the purification procedure, three batches of α-CD were analyzed for protein using polyacrylamide gel electrophoresis (PAGE). The detection limit of this method is 5 mg protein per kg of α-CD. No protein could be detected with this test (report by F. Lottspeich of July 7, 2002, see Annex 4). For one batch, the result was confirmed by subjecting an acid-hydrolyzed sample of α-CD to amino acid analysis which would detect protein at levels of ≥10 mg/kg α-CD (report by F. Lottspeich of June 7, 2001, see Annex 4).

In order to analyze α-CD for the presence of DNA from the recombinant source of the CGTase, a quantitative PCR method was applied. One batch of α-CD was analyzed. DNA could not be detected in the sample. The limit of detection was 0.005 ng DNA/reaction. This corresponds to a limit of detection of 0.01% GMO in a standard GMO test (report by Genetic ID Laboratory of Oct 10, 2001, see Annex 4).

The low-molecular weight fraction consists mainly of nutrients of the fermentation broth (amino acids, peptides, glucose, minerals, vitamin B_1) as well as small amounts of tetracycline, IPTG and sorbic acid. These impurities are separated from α-CD during the first and second precipitation step and during crystallization. Remaining inorganic salts would be detected as ash. Remaining glucose would be detected by the test for reducing substances.

3.1.3 Residues of complexant

Impurities of the complexant are removed from α-CD by the two precipitation steps. The complexant (1-decanol) is removed by steam stripping. Analysis of five batches of α-CD produced at pilot plant scale demonstrates that 1-decanol is removed efficiently by the applied procedure (residues of $<$ 15 ppm were found by HPLC analysis)(Annex 2). Any remaining complexant is detected by the test for volatile organic compounds (see Annex 1).

3.2 Specifications

The specifications prepared at the 57th meeting of JECFA and published in FAO Food and Nutrition Paper 52 Add. 9 are presented in Annex 1. It is intended to submit these specifications for inclusion in the Food Chemicals Codex, with the difference, however, of a lower value for lead (0.5 instead of 1 ppm).

For the purpose of the safety assessment, the following purity criteria are relevant.

Assay:	Not less than 98% of α-CD (on an anhydrous basis)[10]
Ash (residue on ignition):	Not more than 0.1%
Reducing sugars:	Not more than 0.5%
Heavy metals (as Pb):	Not more than 5 ppm
Arsenic:	Not more than 3 ppm
Lead:	Not more than 0.5 ppm
Volatile organics:	Not more than 20 ppm[11]

Additional purity criteria are included in the company's internal specifications such as microbiological purity and optical density (for limits and actual values see Annex 2).

[10] Potential impurities of α-CD are residual starch, maltooligosaccharides, glucose and β-cyclodextrin.
[11] "Volatile organics" are residues of complexant (1-decanol).

4 INTENDED USES IN FOODS

4.1 Use for a nutritional purpose as soluble dietary fiber

4.1.1 α-CD is a dietary fiber in physiological terms

To date, there is no definition of "dietary fiber" for the purposes of food labeling. For verification of compliance with nutrition labeling regulations, FDA has specified that dietary fiber in food will be quantified by AOAC International Official methods of analysis for dietary fiber (Prosky, 2001). However, in recognition of the nutritional benefits of dietary fiber, recent definitions rely no longer on the chemical-analytical approach only, but include a reference to the physiological effects of fiber as a further definitional criterion.

The American Association of Cereal Chemists (AACC) developed a definition according to which

"dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (AACC, 2001).

At about the same time, the Food and Nutrition Board of the Institute of Medicine presented another proposal according to which

1. *Dietary Fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants.*
2. *Functional Fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans.*

Total Fiber is the sum of Dietary Fiber and Functional Fiber

(Institute of Medicine, 2002).

The relative merits and/or limitations of these two proposals may be debated. However, there does appear to be consensus that "dietary fiber" should be defined in nutritional functional terms (namely non-digestibility coupled with beneficial physiological effects) rather than solely chemical analytical terms.

In animals (rats) and man, α-CD is not digested to absorbable glucose in the small intestine to a significant extent (see section 7.1 for data). However, α-CD is fermented by the microbiota of the large intestine. Therefore, α-CD has the nutritional properties of a fermentable dietary fiber, similar to so-called "resistant starch". Some physiological effects that are typical for dietary fiber, such as increased fecal bulk and decreased levels of plasma triglycerides and cholesterol, were seen in feeding studies with α-CD in rats (Kaewprasert et al., 2001; Shizuka et al., 1996; Lina, 1992). An attenuation of the glycemic response

to bread consumption was observed in healthy human volunteers (Diamantis & Bär, 2002) (see Section 7.4 for details).

In a 90-day oral toxicity study, five groups of Wistar rats (20 animals/sex/group) were fed diets with 0, 1.5, 5 and 20% α-CD, and - for comparison - 20% lactose. At the end of the treatment period, plasma triglycerides and phospholipids were significantly below control levels in the males of the 20% α-CD group. Total cholesterol was also reduced but the difference to controls was not significant. In females, a similar trend was observed for all three parameters. However, the differences to controls were smaller and not statistically significant (Lina, 1992).

In a two-week feeding study, male SD rats received diets with 5 or 10% cellulose, pectin, α-CD, β-CD, γ-CD, or three different, chemically modified CDs (6 rats/group). A control group with un-supplemented diet was not included. At the end of the treatment period, plasma total lipids, triglycerides and phospholipids were significantly lower in the 10% α-CD group than in the 10% cellu-lose group. At the 5% dose level, the difference was significant for phospholipids only. Pectin produced similar effects as α-CD (in comparison to cellulose). Plasma cholesterol levels were not different between α-CD and cellulose fed animals (Shizuka et al., 1996).

The effect of dietary α-, β- and γ-CD on liver and serum lipids, and organic acid concentrations in cecal contents was examined in male Wistar rats. Diets with 5% additions of the different CDs

were administered for a period of 7 days. The cecal concentrations of acetate and propionate were significantly increased in the α-CD and β-CD groups. Butyrate and the other end-products of bacterial fermentation were also present at higher concentrations but the differences to the control group were not statistically significant. Total serum cholesterol was significantly lower in the α-CD and β-CD groups than in controls. A significantly higher lipid concentration in the liver of the α-CD fed rats was not matched by a corresponding increase in the β-CD group[12] (Kaewprasert et al., 2001).

4.1.2 α-CD is a dietary fiber in analytical terms

For analyzing the dietary fiber content of food, starch is typically removed by enzymatic digestion. In all AOAC International Official Methods, heat-resistant amylase of microbial origin (Termamyl), with or without the addition of glucoamylase, is used. Since this aggressive treatment results in a partial degradation of resistant starch, which under physiological conditions would escape digestion, resistant starch is only incompletely detected as dietary fiber (McCleary, 2001). The same is true for α-CD which also is hydrolyzed by Termamyl but not by salivary or pancreatic amylase (Marshall & Miwa, 1981; Kondo et al., 1990).

[12] Since the liver triglyceride and cholesterol contents did not differ between the treatments, the higher liver lipid concentration of α-CD fed rats was ascribed to a higher content of phospholipids. Phospholipids were calculated by difference (total lipids - triglycerides - cholesterol).

For the measurement of resistant starch in starch samples and plant materials, a method has recently been adopted by the AOAC Methods Committee (First Action Method) (AOAC No. 2002.2). In this method, the digestible starch is removed by treatment under controlled conditions with pancreatic α-amylase and amyloglucosidase. The not digested resistant starch is then precipitated with ethanol (50% v/v final concentration) and quantitated enzymatically, after alkali-catalyzed hydrolysis to glucose. The method has been used successfully for the measurement of resistant starch in cornflakes and canned beans (Megazyme, 2001).

Applying this method to a food which contains α-CD (with or without resistant starch being present at the same time), α-CD would remain intact in the supernatant of the 50% ethanol precipitation step. While it has been shown that α-CD is not hydrolyzed under the conditions of the applied digestive step with pancreatic amylase and amyloglycosides (Mc Cleary, 2002, 2004), α-CD is not precipitated by 50% ethanol.[13] Therefore, α-CD remains, together with glucose (formed by digestion of non-resistant starch) and other solubles, in the supernatant. After desalting of the supernatant, as described in AOAC Method 2001.13 for resistant maltodextrin, α-CD could be quantitated specifically by applying the validated HPLC method described in Section 4.3.

However, it should be noted that α-CD unlike other naturally occurring or synthetic dietary fibers (beta-glucans, polydextrose, resistant dextrin, fructooligosaccharides, etc.) is a single sub-

[13] Also by 78% ethanol -CD is not precipitated completely.

stance and not a mixture of substances with different degrees of polymerization and/or chemical structure.

Therefore, α-CD can be quantitated directly by HPLC and does not require the application of a complex analytical method.

In this respect, it is also noteworthy that α-CD is not expected to interfere with the existing methods of fiber analysis because it is degraded by Termamyl [applied in AOAC 985.29 (the "gold-standard" method for dietary fiber) and AOAC 2001.03 (resistant dextrin)] and because it is not precipitated by 50% ethanol [applied in AOAC method 2002.02 (resistant starch)]. Therefore, the value obtained from the specific HPLC method for α-CD may be added to the value obtained by one of the official methods for fiber analysis. A risk of double counting of dietary fiber components does not exist.

4.1.3 Use levels of α-CD for the fiber supplementation of foods

Recommendations for adult dietary fiber intake generally fall in the range of 20-35 g/day (ADA, 2002). For the purpose of nutrition labeling a Daily Reference Value (DRV) of 25 g is used [21 CFR § 101.9(c)(9)]. For children older than 2 years a daily fiber intake of age (in y) + 5 g has been recommended (for references, see ADA, 2002).

Despite the manifold physiological benefits of an adequate fiber intake, the actually consumed amounts (14-16 g/d) fall short from recommended levels (Environ, 2000; Alaimo et al., cited in ADA, 2002). The addition of α-CD can help close this gap particularly well since it is chemically stable, has a low viscosity, is water soluble, and is taste- and odorless.

A nutritionally meaningful fortification of a nutrient requires the addition of at least 10% of its RDV per serving [21 CFR § 101.54(e)]. Similarly, a food must contain at least 10% of the RDV per serving in order to qualify for a "good source" type of claim [21 CFR § 101.54(c)]. The proposed use levels of α-CD in bakery products, beverages, ready-to-eat breakfast cereals, instant rice, pasta, yoghurt, dry mixes for milk-based beverages, reduced-fat spreads, soups, snacks and formula diets correspond to this requirement (Table 4). In most cases, these concentrations approach the technically feasible maximum that may be used without distorting the texture and/or flavor profile of these foods, i.e., there is a self-limiting effect for food technological reasons of the use of α-CD in food.

4.2 Use for a food-technological purpose

The ability to form complexes with certain organic molecules coupled with a relatively high water solubility makes α-CD a useful food ingredient that can fulfill different food-technological

functions. However, pure α-CD has not hitherto been available to the food industry at feasible cost. Therefore, most of the earlier application work on cyclodextrins has been carried out with β-CD (Pszczola, 1988; Linssen et al., 1991; Allegre & Deratani, 1994). Yet, data obtained for β-CD are in many cases also applicable for α-CD, because the basic functionality of cyclodextrins, namely the formation of inclusion complexes, is the same. Whether, in a given case, α- or β-CD is more suitable, depends on a number of factors including the size and lipophilicity of the guest molecule and the water-solubility of the formed complex.

The use levels which are required for α-CD to fulfill a useful food-technological function, are far below those needed for fiber supplementation.

4.2.1 Use as carrier/stabilizer for flavors (flavor adjuvant)

By forming inclusion compounds, α-CD can protect certain flavors in dry food preparations from degradation or evaporation. Flavors are premixed with α-CD, typically in a ratio of about 5-20 parts of flavor with 100 parts of α-CD. A stable preparation is obtained which is then added to the dry food (e.g., breakfast cereals, crackers and savory snacks, compressed tablets and candies, chewing gum, as well as dry mixes for the preparation of cakes, soups, puddings, milk beverage mixes, etc.).

Flavor-retention during the manufacturing of extruded snacks is improved when the flavors are added in the form of CD-complexes (Kollengode & Hanna, 1997).

α-CD may also be used as carrier with a stabilizing effect for coffee extract as well as garlic and mustard oil (Ohta et al., 1995, 1999).

In some applications, α-CD improves the quality of foods or food ingredients by suppressing undesirable flavor notes or odors [e.g., masking of sulfurous odor of thioctic acid (syn: α-lipoic acid)].

4.2.2 Use as carrier/stabilizer/solubilizer for colors (coloring adjunct)

By forming inclusion compounds, α-CD can stabilize certain sensitive colors, such as anthocyanins (Ukai et al., 1996; Tamura et al., 1997).

4.2.3 Use as carrier/stabilizer/solubilizer of fatty acids

α-CD can form complexes with fatty acids (Schlenk & Sand, 1961; Park et al., 2002). Polyunsaturated fatty acids (PUFAs) are

thereby protected from oxidation (rancidity) (Park et al., 2002; Yoshii et al., 1996, 1997; Regiert et al., 1996; Imagi et al., 1992). This application is useful for the preparation of formula diets in powder form which should contain the recommended daily allowance of PUFAs. Complexes of PUFAs with α-CD may also be used for the production of PUFA-fortified foods (Yoshii et al., 1996, 1997).

The water solubility of fatty acids is increased by the complexation with α-CD (Artiss & Zak, 1985).

A stabilizing effect of α-CD has been noted in oil-in-water emulsions (o/w = 60/40) (Yu et al., 2001). It may, therefore, be used in salad dressings, marinades and reduced-fat spreads.

4.2.4 Use as carrier/stabilizer/solubilizer of certain vitamins

α-CD appears to form weak complexes with retinol acetate, vitamin K_1 and β-carotene[14]. Retinol, vitamin D and E do not appear to form complexes with α-CD presumably because the size of its cavity is too small (Figure 2) (Pitha, 1981; Kobayashi et al., 1992). Complexation with α-CD slightly increases the water solu-

[14] Since complexation is weak and reversible, it does not affect the bioavailability of these nutrients (see section 7.2).

bility of the complexed vitamins and increases the stability of β-carotene (Pitha, 1981; Szente et al., 1998).

Riboflavin forms a 1:2 inclusion complex with α-CD. Riboflavin was found to have a higher stability to photo-degradation in the complexed form (Loukas, 1997).

Vitamin C (sodium ascorbate) forms a 1:1 inclusion complex with α-CD. The ascorbate-α-CD complex can be incorporated in liposomes. The complexed ascorbate is less susceptible to degradation by UV light than free ascorbate (Loukas et al., 1996).

4.2.5 Use for an improved mouth-feel in low-calorie soft-drinks

Low-calorie soft drinks lack the "mouthfeel" of regular soft drinks in which surcrose and/or fructose yields not only sweetness but also an adequate viscosity. At present, polysaccharides are often used to increase the viscosity of low-calorie soft drinks. α-CD could serve the same purpose but it would offer the advantage of high stability, purity and more "sugar-like" viscosity because of its lower molecular weight.

4.2.6 Use for suppression of halitosis in hard candies, breath mints and chewing um

By complexing the volatile organic substances that account for malodor (halitosis), cyclodextrins contribute to the breath-freshening properties of hard candies, breath mints and chewing gum (Yajima, 1981). The amount of α-CD that may be incorporated in such products, is limited for technological reasons (mechanical stability of comprimates, transparency of hard boiled candies, texture of chewing gum, etc.). Pure α-CD has a limited suitability for direct compression (Maggi et al., 1998).

4.3 Determination of α-CD in foods

α-CD can be quantitated efficiently by HPLC with a RI (refractive index) detection unit.

For extraction of water-soluble α-CD from the food matrix, and for separation from the bulk of other components (solids, fat-soluble components) standard techniques may be used.

The HPLC method for measurement of α-CD has been validated. The method has a detection limit of 2.2% and a recovery rate of 98.9%. The retention times of α-, β- and γ-CD are sufficiently distinct to achieve complete resolution of the three CDs (Heusler & Wenders, 1997). The method is identical to the one which has been used successfully for the determination of γ-CD in food (Gaebert, 1998).

5 ESTIMATED DAILY INTAKE

The estimated daily intake (EDI) of α-CD from its different projected uses in food, excluding chewing gum, but otherwise as specified in Table 4, has been calculated for the US population by ENVIRON (Arlington, VA) using the dietary survey approach (Annex 5). This calculation model relies on food consumption data from the 1994-96 and 1998 Continuing Surveys of Food Intakes by Individuals (CSFII 1994-96 and CSFII 1998). In 1994-1996, CSFII data were collected from a representative sample of individuals residing in households in the US. The CSFII 1998 was a supplemental survey of children (0-9 year old). Each individual was surveyed for two non-consecutive days using 24-hour recall interviews. The foods consumed were coded according to a system which contains about 6,000 different categories.

For the purpose of the present EDI calculation it was assumed that each food (or food component) which may contain α-CD, indeed contained α-CD at the highest, feasible concentration (as specified in Table 4). Where α-CD was used in a component of the food (e.g., in the dressing), the intake of that component was calculated from data on food composition.

The EDI of α-CD averaged over the two observation days and expressed in g/day and g/kg bw/d was calculated for each food category in which α-CD may be used, and for all these food categories combined. Mean and 90^{th} percentile intakes were calculated for

users of the following age groups: preschoolers 2-5 years of age; elementary school children 6-12 years of age; teenagers 13-19 years of age; the adult U.S. population 20+ years of age, and all age groups combined. "Users" were defined as individuals who consumed food in the concerned category on at least one occasion. Since food intake was recorded in the CSFII surveys by time of day and by eating occasion (breakfast, brunch, lunch, dinner, supper, snack, and extended eating occasion), the α-CD intake could also be calculated per eating occasion of each observation day. The detailed results of these calculations are presented in Annex 5.

For users of age ≥ 2 years, the estimated exposure to α-CD from all proposed uses combined (except chewing gum) is 11.4 and 19.8 g/day at the mean and 90th percentile, respectively. This corresponds to intakes of 0.21 and 0.43 g/kg bw/d for the 2-day average of the mean and 90th percentile consumer, respectively. The subpopulation with the highest estimated exposure to α-CD, is preschoolers (0.61 and 0.98 g/kg bw/d for the 2-day average of the mean and 90th percentile user) (Table 5 and Annex 5).

A comparison between the intakes from the various food categories and the total intake from all sources demonstrates that many uses are mutually exclusive. In other words, a consumer is unlikely to eat ready-to-eat cereals and soup at the same eating occasion. Consequently, the α-CD intake from all sources is much smaller than the arithmetic sum of intakes from the different food categories (Annex 5).

The estimated α-CD intakes per eating occasion demonstrate that the consumption of α-CD is evenly distributed over the day (Table 6). At no eating occasion, levels of α-CD intake are reached which might produce intestinal symptoms (see section 8.11).

The intake of α-CD from chewing gum could not be included in Table 4 and Annex 5 because the intake of chewing gum was not recorded in CSFII (Annex 5). Intakes of α-CD from chewing gum had, therefore, to be calculated separately.

The estimation of the α-CD intake from chewing gum is based on the Market Facts Chewing Gum Survey in which 1044 households reported their one-day intake of regular and sugarfree gum by mail. The survey, which was conducted in 1995, reveals that the average number of pieces of gum consumed per day varies between 2.1 for preschoolers and 3.8 for teenagers (3.0 pieces of gum/day for the total population). The weight of a piece of gum varies between 2-3 g. Hence, the chewing gum intake varies between 6-9 g/d. Since α-CD may be present in gum at levels of up to 10%, the α-CD intake from chewing gum (3 pieces/day) is estimated at about 0.6 - 0.9 g/d for the average consumer(see Annex 5 for detailed results and intake estimates for different age groups).

In summary, the intake of α-CD from all its intended uses in food (except chewing gum) is estimated at about 11.4 g/person/day (mean of users of all age groups combined). Additional amounts of α-CD may be ingested with chewing gum (0.9 g/person/day). How-

ever, as explained above, this estimate is based on very conservative assumptions.

While an α-CD intake of the projected magnitude would just about cover the gap between actual and recommended fiber intakes, different fibers are available for the fiber enrichment of food. Depending upon the food concerned, different fibers (or mixtures thereof) will be applied, taking the different cost, and technological or organoleptic properties into account. Therefore, the conservative assumption which underlies the EDI calculations shown in Tables 5 and 6, namely that α-CD is used simultaneously in all foods at the highest feasible concentrations, is not realistic. It follows that the average daily intake of α-CD that can realistically be expected to result from its intended uses, will be far below the estimates presented in Tables 5 and 6.

6 CURRENT REGULATORY STATUS

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the safety of α-CD at its 57th meeting (WHO, 2002). Considering the use of α-CD for food-technological purposes only which results in an estimated daily intake of 1.7 and 3 g for the mean and 90th percentile adult consumer, respectively, the Committee allocated an ADI "not specified"[15]. The use of α-CD as a dietary fiber, with an EDI from its combined uses as a nutritive substance and a food additive of 11.4 and 19.8 g/d for the mean and 90th percentile consumer, respectively, will be considered by the Committee at its 63rd meeting (WHO, 2004).

Products consisting of a mixture of α-, β- and γ-CD have been sold in Japan for food use for several years. These products are considered to be food (i.e., not a food additive) and explicit approval was, therefore, not required. The products [Dexy Pearl K50 and K100 (Bio-Research Corp.) and Celdex Gamma 10 (Nihon Shokuhin Kako Co., Ltd.)] are produced by a non-solvent process and contain about 5-70% α-CD. They are sold in a total amount of more than 100 metric tons/year and are used in a variety of food applications.

In Australia/NZ, α-CD is currently under review as a novel food. The draft assessment report has been published by Food Standards Australia New Zealand (FSANZ) on May 26, 2004 (FSANZ, 2004). Ap-

[15] ADI "not specified" is used to refer to a substance which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its intended uses and from its acceptable background levels in food, does not represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary (WHO, 2002).

proval of the use of α-CD as a novel was recommended by FSANZ with no specific conditions that would limit the use of the substance.

7 BIOLOGICAL STUDIES

7.1 Absorption, disposition, metabolism, and excretion

7.1.1 Digestibility in vitro

In early in-vitro experiments on the digestibility of cyclodextrins by amylases, it was found that pancreatic juice of dogs does not cleave α-CD (Karrer, 1923)[16] and that salivary amylase leaves α-CD intact, hydrolyses β-CD only very slowly, but hydrolyses γ-CD at a rate of about 1% that of starch (French, 1957).

In more recent in vitro studies, it was confirmed that human salivary amylase as well as human or porcine pancreatic amylase are unable to hydrolyze α-CD and β-CD to any measurable extent but hydrolyze γ-CD readily (Marshall & Miwa, 1981; Kondo et al., 1990; McCleary, 2002, 2004).

[16] At that time, α-CD was called "diamylose" and "tetraamylose" (French, 1957).

7.1.2 ADME studies in animals

The metabolism of pure α-CD and β-CD was examined for the first time in 1962 (Andersen et al., 1963)[17]. ^{14}C-labelled α-CD and β-CD were prepared from uniformly ^{14}C-labelled potato starch. Groups of Wistar rats (2 rats/group, fasted for 18 hours prior to the experiment) received similar (not exactly specified) amounts of ^{14}C-α-CD, ^{14}C-β-CD and ^{14}C-starch. The rats were placed in metabolism cages and respiratory CO_2 was collected for 16-23 hrs after dosing. ^{14}C was determined in the collected CO_2, urine, feces and the carcass at termination. After ingestion of ^{14}C-starch, $^{14}CO_2$ appeared rapidly in breath reaching the maximum expiration rate at 1 hour after dosing. In contrast, $^{14}CO_2$ exhalation after ingestion of ^{14}C-α-CD and ^{14}C-β-CD started only about 3.5 hours after dosing and reached the maximum exhalation rate at about 7.5 - 9.5 hours. The cumulative $^{14}CO_2$ production over the duration of the experiment was similar for all three substrates (about 60% of the administered dose). The excretion of ^{14}C-labelled products with urine varied considerably between the two animals of each group. The amounts of ^{14}C recovered from the GI contents and feces tended to be bigger for ^{14}C-α-CD and ^{14}C-β-CD than for ^{14}C-starch. The retention of ^{14}C in the carcass varied between about 16.5% (for ^{14}C-α-CD) and 20.3% (for ^{14}C-starch) (Andersen et al., 1963). Despite the small number of animals tested, it may be concluded from this study that α-CD and β-CD are not digested in the small intestine to a significant extent. Instead, they are fermented

[17] In earlier metabolism studies, α-CD and β-CD preparations of unknown purity were used (von Hoesslin & Pringsheim, 1923).

almost completely by the intestinal microflora to absorbable and metabolizable short-chain fatty acids (SCFA) as shown by the delayed appearance of about 60% of the ingested ^{14}C in respiratory CO_2.

Seemingly at variance with this conclusion are the results of a series of experiments in which rats (JCL:SD strain) received single doses of 1.5 g α-CD or 1.2-1.5 g β-CD (Suzuki & Sato, 1983, 1985). The experiments differed with regard to the time after dosing (1-60 hours) at which feces and/or gastro-intestinal contents were collected for analysis of saccharides and CDs by HPLC. The number of animals varied between 4-6 rats/treatment group and time of sacrifice. In one experiment a substantial fraction (50-65%) of α-CD was recovered unchanged (i.e., not digested and not fermented) in the GI-tract after 8 hours. Another experiment revealed that 60-106% of the administered α-CD dose was excreted unchanged with the feces after 60 hours. The reasons for the unexpectedly low fermentation rate are not clear. However, it should be noted that the experiments were conducted under somewhat unusual conditions (Suzuki & Sato, 1985). The rats were meal-fed and had access to the feed for 2 x 1 hour per day only. This regime may have led to a significant malnutrition of the rats. In parallel, it may have resulted in a reduced number of intestinal microorganisms that would be able to degrade ingested CDs. Furthermore, the administered CD dose was unreasonably high. In fact, the volume that could reasonably have been applied by gavage (volume was not specified by the authors), most likely has been insufficient for dissolving the CDs (particularly β-CD which has a solubility of only 1.8 g/100 ml water at 25°C). Unfortu-

nately, the report does not state whether the rats had ad libitum access to drinking water after dosing and if so, what amounts had been consumed. It is, therefore, conceivable that a substantial fraction of the administered CDs was not dissolved in the intestinal lumen and consequently was not readily available for microbial breakdown. Be that as may, the finding by Suzuki and Sato of a non-fermentability of α-CD is in conflict with the results of several other experiments and may, therefore, be dismissed.

The absorption, disposition, metabolism and excretion of intravenously and orally administered α-CD in rats was examined in two studies using uniformly ^{14}C-labelled α-CD as a tracer (de Bie and van Ommen, 1994; van Ommen & de Bie, 1995; van Ommen et al., 2004).

The first study was a pilot study in which the excretion and blood kinetics after oral administration, and the blood kinetics after intravenous administration were examined in only one rat per dose and route of administration. The results of the two experiments with oral administration of α-CD (about 200 mg/kg bw) were in keeping with those reported by Andersen and collaborators (1963). The experiment with intravenous administration of α-CD (50 mg/kg bw) followed by blood sampling in regular intervals for up to 6 hours after dosing suggested a half-life of ^{14}C in the blood of about 88 minutes. Within 8 hours of dosing about 80% of the administered dose was excreted as α-CD with the urine (de Bie & van Ommen, 1994).

The second (main) study comprised four experiments in which groups of Wistar rats (4 rats/sex/group) received single doses of ^{14}C-α-CD by gavage (three experiments) or injection in the tail vein (one experiment).

In the first experiment, two groups of Wistar rats (fasted overnight) received single doses of 200 and 1000 mg/kg bw ^{14}C-α-CD by gavage. The rats were placed in metabolism cages for 24 hours. Feed and water were provided ad libitum after dosing. Respiratory CO_2 was collected in 30 min intervals. Urine was collected at 4, 8 and 24 hours. The animals of the low-dose group were sacrificed 24 hours after dosing. The animals of the high dose group were kept for another 24 hours in larger metabolism cages for collection of CO_2, urine and feces, whereupon they were killed as well. At termination, plasma, blood cells, contents of GI-tract, different organs and the residual carcass were analyzed for ^{14}C. The urine, feces and contents of the GI-tract were pooled (for the 4 rats of each group and sex) and subjected to HPLC with RI and ^{14}C detection. The results demonstrate that about 60% of the administered ^{14}C is expired with respiratory CO_2 within the first 24 hours. There was no difference in this regard between males and females or between the two dose levels tested. The appearance of $^{14}CO_2$ in breath was delayed suggesting microbial fermentation of α-CD in the distal segments of the gut followed by absorption and metabolism of the formed SCFAs. During the first 8 hours after dosing, about 1% of the administered ^{14}C was excreted with the urine (average of the data of the high and low-dose group and of males and females). Metabolite profiling by HPLC revealed the presence of α-CD in the 0-4 h urine samples. In the 4-8 h sam-

ples, less than half of the radiolabel represented α-CD. No α-CD was detected in the subsequent urine samples. The feces and GI-contents together contained about 16 and 18% of the administered ^{14}C in the low and high-dose group, respectively. However, metabolite profiling indicated that only traces, if any, 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 hours. This value dropped to about 10% after 48 hours. The difference of 6% appeared as $^{14}CO_2$ in the period of 24-48 hours after dosing.

In the second experiment, one group of Wistar rats received a single oral dose of 200 mg/kg bw ^{14}C-α-CD. The animals were placed for 48 hours in metabolism cages with ad libitum access to food and water. Blood samples (0.2 ml) were drawn in regular intervals from a canula which was placed in the jugular vein (12 samples/48 hours). Urine, feces and CO_2 were collected as well. The primary aim of the study was to examine the blood kinetics of oral α-CD. During the first 3 hours, the blood concentration of ^{14}C remained low (range over all rats and measurements: 0.05 - 0.22% of the administered dose). After that time, blood ^{14}C levels increased slowly reaching a maximum at about 12 hours after dosing. Metabolite profiling of the blood sample collected after 8 hours suggested the presence of a trace of ^{14}C-α-CD. About 1% of the ^{14}C dose was recovered from urine excreted from 0-4 hours (mean of males and females). Metabolite profiling demonstrated that all of this radioactivity represented α-CD. In the urine collected from 4-8 hours, 0.3% of the administered ^{14}C was de-

tected about half of which represented α-CD. The amounts of ^{14}C contained in feces and GI contents, or retained in organs and the carcass, were very similar to those found in the first experiment.

In the third experiment, one group of Wistar rats received a single dose of 50 mg/kg bw ^{14}C-α-CD by injection in the tail vein. The animals were placed in metabolism cages. Samples were collected as described for the second experiment, except that blood was drawn from the tail vein over the first 8 hours after dosing. After 24 hours the animals were killed. The blood ^{14}C concentrations decreased steadily (first order kinetics) with a $t_{½}$ of 26 and 21 minutes in male and females, respectively[18]. HPLC analysis of the blood samples revealed the presence of ^{14}C-α-CD which accounted for 94-100% of the blood ^{14}C activity. The main route of excretion was with the urine. All of the ^{14}C excreted with urine during the first 8 hours after dosing represented ^{14}C-α-CD. The recovery of small amounts of ^{14}C in the feces and GI-contents (0.6 - 4.5%) suggests that minor amounts of α-CD may be excreted with the bile[19]. Fermentation of biliary α-CD by the intestinal microorganisms explains the appearance of respiratory $^{14}CO_2$ and the incorporation ^{14}C in organs and tissues. About 11% (range: 5 - 25%) of the administered ^{14}C-α-CD dose may be excreted with the bile (calculated as sum of respiratory, fecal, intestinal, organ,

[18] In a preliminary experiment with one human volunteer, a $t_{1/2}$ of about 60-90 minutes was observed after intravenous infusion of 3.88 mg α-CD (administered over a period of 2 hours) (Hammes et al., 2000).

[19] Excretion via the salivary glands has been suggested as an alternative route of elimination which could explain the appearance of CDs in the GI-tract after intravenous administration (Antlsperger & Schmid, 1996).

and carcass ^{14}C). Unfortunately, the interpretation of the results of this experiment is somewhat hampered by the incomplete recovery of ^{14}C in the majority of rats (less than 85% of the administered dose was recovered in 5 out of 8 rats). The investigators suggested that the urine sampling may have been incomplete because of manipulation of the animals during blood sampling.

In the fourth experiment, one group of germfree Wistar rats received a single oral dose of 200 mg/kg bw ^{14}C-α-CD by gavage. The rats were placed in metabolism cages for 24 hours. CO_2, urine, feces, etc. were collected as described for the first experiment. While precautions were taken to avoid contamination of the animals with microorganism, completely germfree conditions could not be maintained in the metabolism cages. However, the absence of a significant microbial flora in the GI-tract of these rats was demonstrated by a low exhalation of $^{14}CO_2$ during the 24-hour 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 hours after dosing. An additional 0.93% (range 0.17 - 2.14%) was excreted between 8 - 24 hours after dosing. Retention of ^{14}C in the organs (except the GI tract) was very small (<0.1%). The biggest part, namely 90.7% of the administered dose corresponding to 93.2% (range 89-96%) of the total recovered dose was found in the feces and GI contents (stomach, small intestines, cecum, colon). All of the ^{14}C activity in the feces represented ^{14}C-α-CD. In the pooled contents of the small intestine of the male rats a ^{14}C-labelled degradation product of ^{14}C-α-CD was found. This indicates that microbial colonization of that gut segment had started

at the time of killing. However, only ^{14}C-α-CD and no degradation products were found in the contents of the other intestinal segments (cecum, colon) (van Ommen & de Bie, 1995, van Ommen et al., 2004).

Taken together, the results of these four experiments give a coherent picture of the absorption, disposition, metabolism and excretion of α-CD. In line with the results of in vitro experiments with salivary and pancreatic amylase it was found that ingested α-CD is resistant to the action of the digestive enzymes and is not hydrolyzed to a significant extent during small intestinal passage. Because of its relatively high molecular volume and its hydrophilic surface, only a very small fraction of ingested α-CD is absorbed. Since intravenously administered α-CD is not metabolized to a significant extent by the enzymes of the liver or other tissues, the fraction excreted with the urine after oral administration directly reflects the degree of intestinal absorption.[20] In the three experiments with oral administration of ^{14}C-α-CD, not more than about 1% of the administered ^{14}C-α-CD was recovered from the urine produced during the first 8 hours after dosing (i.e., during the period of small intestinal transit).[21] All the not digested and not absorbed α-CD, i.e., about 99% of the ingested amount, reaches the microbially colonized segments

[20] The excretion of a small fraction (about 10% of absorbed α-CD) with the bile is disregarded in this context.

[21] A higher absorption (15%-19%) was observed in the rat ligated-loop model when sodium taurocholate was added to the luminal perfusate. However, this increase could be completely inhibited by the addition of calcium chloride (Irie et al., 1988).

of the gut where the α-CD ring is readily opened by microbial enzymes (certain amylases and cyclodextrinase). The resulting linear malto-oligosaccharides are then further hydrolyzed and fermented via well established metabolic pathways (Antenucci & Palmer, 1984).

Overall, the metabolic fate of ingested α-CD resembles, therefore, that of other non-digestible yet fermentable carbohydrates, such as resistant starch or inulin.

7.1.3 Digestibility in humans

It has been shown that more than 90% of an oral β-CD dose can be recovered from the ideal effluent of ileostomic subjects (Flourié et al., 1993). Since β-CD and α-CD are similarly resistant to the hydrolytic action of pancreatic amylase in vitro, it is expected that the digestibility in vivo of α-CD is similarly low as that of β-CD (i.e., less than 10%).

Direct proof for the low digestibility of α-CD stems from a study in which 12 healthy male volunteers 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 the finger pricks in regular intervals over a 3-hour 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 of blood glucose and

insulin levels was noted after the intake of 25 g α-CD [Diamantis & Bär, 2002; see also section 7.4 for a more complete description of this study].

This result is in line with an early experiment on α-CD in two diabetic subjects. The consumption of 50 g/d α-CD (of unknown purity) together with a low-carbohydrate diet was not associated with an increase of the urinary glucose excretion as was observed after consumption of 50 g white bread (von Hoesslin & Pringsheim, 1923). This result was confirmed in a subsequent series of experiments in which diabetic subjects (including at least two type-1 diabetics) received daily doses of 50-100 g mixed CDs (consisting mainly of α-CD with some β- and γ-CD) (von Hoesslin & Pringsheim, 1927).

7.1.4 Absorption in humans

In the study of the glycemic effect of α-CD, urine was collected from all subjects during the 3-hour period after ingestion of 25g α-CD or 10g α-CD and 100g white bread. The urine volume was measured. An aliquot was permethylated and analyzed by GC. The recovered α-CD was expressed in percent of the administered dose. After ingestion of 25g α-CD, 0.05% (range 0.00-0.18%, n = 12 subjects) were excreted during 3 hours with the urine. After ingestion of 10g α-CD, 0.01% (range 0.003-0.031%) were excreted. Assuming that absorbed α-CD is not metabolized by the human body (like in rats) and assuming that there has been no significant

excretion with the bile as its suggested by a rat study with i.v. administration of α-CD (see Section 7.1.2), less than 0.01% of ingested α-CD is on average absorbed in intact form by humans (Gaebert, 2004).

7.2 Interaction with the absorption of lipophilic nutrients

When the safety of β-CD was assessed for the first time, there was an initial concern that ingested β-CD might impair the bioavailability of certain lipophilic essential nutrients or drugs by formation of complexes (WHO, 1993). However, further data were developed subsequently, and it was concluded that such concern was not warranted (WHO, 1996). The ingestion of β-CD at dietary concentrations of 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). In other chronic feeding studies with β-CD, no clinical effects were seen that were indirectly suggestive of an impaired absorption of vitamins or essential fatty acids (vitamin levels were not measured in these studies) (Bellringer et al., 1995; Toyoda et al., 1997).

Considering the similar functionality of β-CD and α-CD as complex forming agents, it would appear that the conclusion of non-interference with vitamin absorption of β-CD applies equally to α-CD. Furthermore, the possibility of an impaired vitamin absorption due to the ingestion of α-CD can also be dismissed on basis of the following considerations and data.

First, it must be noted that the formation of inclusion complexes with CDs is reversible (Connors, 1995; Stella et al., 1999). It follows from this that in the presence of other food components, or stomach and intestinal contents, complexed guest molecules will be replaced by other organic compounds which have a higher affinity to the cyclodextrin cavity, or are present at higher concentrations. In this way, the expelled guest molecules will become readily available for absorption.

Second, a number of studies have shown that complexes of fat-soluble vitamins or other lipophilic compounds with β- or γ-CD are bioavailable and indeed may have a higher bioavailability than the free, i.e., not complexed form (Szejtly & Bolla, 1980; Szejtli et al., 1983; Horiuchi et al., 1988; Uekama et al., 1988; Bárdos et al., 1989). This higher bioavailability is best explained by the higher water-solubility of the cyclodextrin complex. For retinol acetate and vitamin K_1 a higher water solubility in the presence of the α-CD has been demonstrated (Pitha, 1981).

Third, α-CD is not known to form complexes with retinol, vitamin D and vitamin E probably because the size of its cavity is too small (Pitha, 1981).

For these reasons, an impairment of the bioavailability of vitamins is not expected from the use of α-CD in food.

7.3 Interaction with the absorption of minerals

Having a low viscosity and a chemical structure which lacks anionic or cationic groups, α-CD is not expected to impair the small-intestinal absorption of minerals.

The possibility of an impairment of vitamin and mineral absorption by the consumption of increased amounts of dietary fiber has been addressed in several review articles (e.g., Gordon et al., 1995; Gorman & Bowman, 1993; Rossander et al., 1992; Kelsay, 1990). Invariably it was concluded that dietary fiber, at recommended levels of intake, does not adversely affect the vitamin and mineral status of the average consumer. For resistant starch, this was demonstrated recently in a study in which rats and pigs received diets with 6% native starch or retrograded high-amylose starch. The ingestion of the resistant starch did not significantly affect the absorption or retention of Ca, P, Mg and Zn (De Schrijver et al., 1999).

7.4 Attenuation by α-CD of the glycemic response to starch containing food

Considerable experimental evidence demonstrates that the addition of soluble, fermentable dietary fibers attenuates the glycemic

response to starchy food, i.e., reduces their glycemic index (GI). This effect may arise through delayed gastric emptying, reduced activity of digestive enzymes, poorer mixing of intestinal contents with digestive secretions, and/or an increased thickness of the unstirred layer on mucosal cells which would slow the absorption of glucose.

In order to examine the GI-reducing effect of α-CD, 12 healthy male volunteers received, on separate days after overnight fasting, single doses of 25 g α-CD, 50 g starch (in the form of about 100 g fresh white bread) and a mixture of 50 g starch (bread) and 10 g α-CD. Capillary blood was collected in regular intervals over a 3-hour period for analysis of glucose and insulin. The 50-g starch load produced the expected rise of blood glucose and insulin levels. In contrast, no significant increase of blood glucose and insulin levels was noted after the intake of 25 g α-CD. After intake of starch (bread) with α-CD, the glycemic and insulinemic responses were delayed and reduced in comparison to those observed after intake of starch (bread) alone. Taking the GI of the tested white bread as 100%, the mixture of bread plus α-CD had a GI of about 43%. The insulinemic index (II) was reduced from 100% to 45% (Diamantis & Bär, 2002)[22].

These results demonstrate that α-CD has a beneficial physiological effect in humans and, therefore, complies with recent defini-

[22] In a diabetic subject a reduced urinary glucose excretion was observed after supplementation with 50 g α-CD of a low carbohydrate diet (von Hoesslin & Pringsheim, 1923). However, in subsequent experiments with doses of up to 100 g α-CD this observation could not be reproduced and the blood glucose levels were reported to be unaffected by the treatment (von Hoesslin & Pringsheim, 1927).

tions of dietary fiber also from this perspective (AACC, 2001; Institute of Medicine, 2001). Which of the above mentioned mechanism(s) account for this effect remains yet to be determined.

8 TOXICOLOGICAL STUDIES

8.1 Acute toxicity studies

The results of acute toxicity studies with α-CD are summarized in Table 1.

8.1.1 Parenteral administration

In order to determine the LD_{50}, groups of Sprague-Dawley rats received α-CD intravenously at doses of 576, 900 and 1400 mg/kg bw. There were no deaths in the low-dose group. In the mid-dose group, 5 rats died within 24 hours and 3 more rats in the subsequent 24 hour period. In the high-dose group all rats died within 24 hours. The acute LD_{50} of α-CD was calculated at 1 g/kg bw for the intravenous route of administration (Frank et al., 1976).

In a subsequent experiment, groups of 4 rats were given 0.1 or 1.0 g/kg bw/d α-CD by subcutaneous injection for 1, 2, 3, 4 or 7 consecutive days. One rat of the high-dose group died after 2 days of treatment.Light and electron microscopic examination of the kidneys revealed the typical signs of "resorptive vacuolation" in the high-dose group. In the low-dose group, no abnormalities were detected in the kidneys (Frank et al., 1976).

Resorptive vacuolation of renal tubular cells is a well established effect of the parenteral (not oral!), acute or subchronic administration of cyclodextrins (Coussement et al., 1990; Frank et al., 1976; Hiasa et al., 1981). Resorptive vacuolation (inappropriately also called "osmotic nephrosis") is the direct consequence of the presence of high concentrations of cyclodextrin in the primary urine. It is the microscopically visible result of the uptake of urinary solutes by the epithelial cells via pinocytosis followed by fusion of the pinocytotic vesicles with lysosomes. Not only cyclodextrins but also linear carbohydrates of different molecular size, such as inulin, dextran or sucrose, cause identical renal effects (Fillastre et al., 1967; Kief & Engelbart, 1966). Except for compounds which cannot readily be degraded by lysosomal enzymes and which have a destabilizing effect on the membrane of the pinocytotic vesicles, resorptive vacuolation usually is a reversible phenomenon which does not affect the function or integrity of the tubular cells.

Since α- and β-CD appear to be quite resistant to the attack of mammalian amylolytic enzymes, the resorptive vacuolation after parenteral application of massive doses of these cyclodextrins will persist and will entail fatal renal failure. Whether destabilizing effects on the lysosomal membrane contribute to this course of events, and whether there are differences in this regard between α- and β-CD cannot be determined from Frank's experiments because the two cyclodextrins were administered at different doses.

Using α-CD manufactured as described in section 2 of this Dossier, acute toxicity tests with intravenous application were performed in mice and rats (Riebeck, 1990a, b) and with intraperitoneal application in rats (Riebeck, 1990c, Prinsen, 1991a).

In mice, single doses of α-CD were administered by injection in the tail vein at doses of 500, 750, 1000 and 2000 mg/kg bw. The surviving animals were killed after an observation period of 14 days. Deaths occurred in all dose groups. No macroscopic alteration of kidneys were observed on autopsy. Microscopic examinations were not performed. The intravenous LD_{50} was estimated to be between 750 and 1000 mg/kg bw (Riebeek, 1990a).

In rats, single doses of α-CD were administered by injection in the tail vein at doses of 500, 750 and 1000 mg/kg bw. The surviving animals were killed after an observation period of 14 days. Deaths occurred in the mid-dose group (8 out of 10 rats) and high-dose group (2 out of 2 rats). The surviving animals recovered, gained weight and appeared to be healthy at termination of the study. Macroscopic examination at necropsy did not reveal any treatment-related abnormalities. The intravenous LD_{50} was estimated to be between 500 and 750 mg/kg bw (Riebeek, 1990b).

One group of 3 male Wistar rats received a single intraperitoneal application of 4000 mg/kg bw α-CD. All animals died within a few hours after treatment. At necropsy a "white deposition" was observed on all organs in the abdomen (Riebeck, 1990c). Using lower

α-CD doses of 500, 750, 1000, 1500 and 2000 mg/kg bw, the experiment was repeated. There were no deaths in the lowest dose group, and only 1 out of 10 rats died in the 750 mg/kg bw dose group. A higher mortality was observed in all other dose groups and the LD_{50} was determined at between 750-1000 mg/kg bw. There was no observation of the "white deposits" in the abdominal cavity of animals which died after the treatment or of survivors. A pale kidney was noted in some animals but the incidence of this observation was not related to the applied α–CD dose (no abnormalities were seen in the highest dose group) (Prinsen, 1991a).

8.1.2 Oral administration

Acute toxicity tests with oral application have not been performed with α-CD produced as described in section 2 of this Dossier.

An oral LD_{50} of α-CD of 12.5 g/kg bw is mentioned in a review article (Thompson, 1997) but the cited reference does not contain this information.

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). A control group of identical size received water only. Subgroups of 5 mice /sex were killed after 24, 48 and 72 hours. No signs of intoxication were observed in the treated group and all animals survived until scheduled termination (Immel, 1991).

8.2 Short term toxicity studies with oral administration of α-CD

8.2.1 Mice

Groups of mice where given daily doses of 60 mg α-CD or β-CD by gavage for 15 days. An untreated group served as control. Growth of the animals, relative liver weights and fat content of the livers did not differ between the groups (Miyazaki et al., 1979).

8.2.2 Rats

A mixture of linear dextrins, α-CD, β-CD and γ-CD (50:30:15:5) was fed at dietary levels of 0, 19.5, 39, 58.5 and 78.0% to groups of 20 male Sprague-Dawley rats. Additions of the dextrin mixture to the diet were made at the expense of corn starch. The rats received the test diets by meal feeding (2 x 1 hour/day) for a period of up to 110 days. Only animals of the lowest dose group (receiving feed with about 5.8% α-CD and 2.9% β-CD) exhibited a similar growth as the controls. The other treatment groups had a reduced weight gain (significant for the 58.5% and 78% group). The 78%-group gained hardly any weight. Only in this group animals died prior to scheduled termination (50% mortality). Subgroups of 5 rats per treatment group were killed after 30, 40, 60

and 110 days of treatment. The main organs were removed and weighed. The liver was analyzed for total lipid and triglycerides, the serum for triglycerides. The organ weights of the animals of the lowest dose group did not differ from control values at any time of the study. For all other dose groups, comparisons of organ weights could not be made because of substantial (>10 to 60%) differences of body weights. The lipid levels in the liver and plasma decreased with increasing degree of malnutrition (Suzuki & Sato, 1985). Because a mixture of CDs was fed and an unusual meal-feeding practice was applied which led to malnutrition of the animals, this study is not adequate for the safety assessment of α-CD.

A 28-day feeding study with α-CD was conducted in Wistar rats. Four groups of 5 rats/sex each were fed diets to which 0, 1, 5, 10 or 15% α-CD or 5% β-CD were added at the expense of pregelatinized potato starch. Treatment started when the rats were about 5-6 weeks old. The general condition of the rats was checked daily; body weights were recorded initially and then in 7-day intervals. Food consumption was measured weekly and water consumption daily. Hematological and clinico-chemical parameters were analyzed in blood samples collected on day 28 when the rats were killed. Gross necropsy was performed and organ weights were determined. All rats survived to the end of the study. However, transient diarrhea was observed during the first week of treatment in the 5% β-CD groups. In rats fed 15% α-CD, diarrhea did not occur until day 6, but then persisted throughout the study. Accordingly, body weights of this group were below controls [males: -26% ($p < 0.01$); females: -7% (n.s.)]. 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 hematological parameters but these were not related to the dose and/or occurred in one sex only. The same applies to the clinicochemical parameters except that plasma alkaline phosphatase (AP) was increased significantly in males and females of the 15% α-CD group. The plasma levels of aspartate aminotransferase and alanine aminotransferase tended to be slightly elevated in the 5% β-CD group (significant in females only). The absolute and relative liver weights were significantly decreased in males of the 15% α-CD group. A similar trend was observed in females (significant for relative but not absolute liver weight). The cecum weights (full and empty) were increased slightly in the 5% α-CD and 5% β-CD groups and, more markedly, in the 15% α-CD group. A few other changes of organ weights that were seen in males but not in females of the 15% α-CD group, may be attributed to the lower body weights of this group of rats. No gross abnormalities were detected at necropsy that could be attributed to the α-CD treatment. Microscopic examination of the main organs (liver, kidneys, heart, adrenals, spleen, cecum, mesenteric lymph node) revealed slight changes of the surface epithelial cells of the cecum in 7 out of 10 rats of the 15% α-CD group[23]. However, signs of inflammation or degenerative changes of the intestinal mucosa were not observed. A depletion of glycogen in periportal hepatocytes of males of the 15% α-CD group was attributed to their impaired nutritional state. The decreased liver weight of

[23] The changes were described as "pale, hypertrophic enterocytes situated mainly at the tip of the villi".

this group was probably another manifestation of this condition. The increased water consumption in animals of the 15% α-CD group might have been the consequence of the loss of liquid with the watery stool (diarrhea). In the absence of morphological changes in the kidneys, it appears less likely that a renal mechanism would be responsible for the increased water intake (Lina & Bruynties, 1987; Lina & Bär, 2004a).[24]

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% α-CD. A comparison group received a diet with 20% lactose. α-CD and lactose were added to the diets at the expense of pre-gelatinized potato starch. Three satellite groups (10 animals/sex/group) received the control diet, a diet with 20% α-CD, or a diet with 20% lactose. After the treatment period of 13 weeks, the animals of the main groups were killed. The rats of the satellite groups continued the study receiving the control diet for another 4 weeks (recovery period).

Body weights as well as food and water intakes were recorded at regular intervals. In addition to the standard hematological, clinico-chemical and urinary parameters, urinary pH, creatinine and calcium were measured in urine samples collected a few days before the end of the treatment period and the recovery period. A urine concentration test was performed on days 87/88. Feces were collected for determination of fecal pH, dry weight (per 24h) and

[24] The results of the 90-day feeding study provide additional evidence for the absence of a renal effect.

nitrogen content on days 73-74 (treatment period) and days 112-113 (recovery period). Complete histopathological examination of all standard organs and tissues was conducted in all animals of the control and 20% α-CD groups killed at the end of the treatment period. In the rats of the 1.5 and 5% α-CD groups, the histopathological examination was limited to the kidneys, liver, lungs and gross lesions.

All animals (except one female of the 5% dose group which had to be killed because of a prolapse of the vagina) survived until their scheduled termination. Soft stools were observed during the first few weeks in most animals (particularly males) of the 20% α-CD group and the lactose group. Otherwise, no signs of treatment-related reactions were seen. Mean body weights were slightly, at the end of the study (days 84, 91) significantly, reduced in males, but not females of the 20% α-CD group. Males of the 20% lactose group exhibited reduced body weights throughout the study. This reduction of body weights did, however, not exceed 10% at any time (on day 91: -6.1 and -7.8% in the 20% α-CD and 20% lactose group, respectively). Food intakes were increased by 6.7% in males and 5.6% in females of the 20% α-CD group (the difference to controls was significant on a few occasions). Otherwise, food intakes did not differ between treated groups and controls. The food conversion efficiency was calculated for each of the first four weeks of the study. It was about 10-15% below control levels in males of the 20% α-CD and 20% lactose groups (no difference between these two groups). In females, there was no difference of food conversion efficiency between the groups.

Water consumption did not differ between treated groups and controls except for a slight increase on 2(1) days of the first week of treatment in males (females) of the 20% α-CD group, and on 3 days in males of the 20% lactose group. The hematological and clinico-chemical parameters as well as the semi-quantitative urine analyses did not reveal adverse changes that could be attributed to the α-CD treatment.[25] During the urine concentration test, similar urine volumes were produced by the different treatment groups. In fresh urine samples collected from rats who were not deprived of food and water prior to sampling, urinary pH was decreased and calcium increased in males and females of the lactose group. The ingestion of α-CD had a similar effect on these parameters. Fecal pH was decreased, and fecal dry weight and nitrogen excretion were increased in males of the lactose group. The females of this group also exhibited increased feces weights (increase not significant) and increased nitrogen excretion (significant). Corresponding, yet somewhat more pronounced changes of these fecal parameters were observed in the 20% α-CD group.

At the end of the treatment period, the absolute and relative weights of the full and empty cecum were increased in males and females of the 20% lactose group and, to a bigger extent, 20% α-CD group. The rats of the 20% lactose group also exhibited increased relative weights of the spleen and liver (females), and the testes, adrenals and brain (males). For the spleen ($p<0.01$),

[25] A significant decrease of plasma triglycerides and phospholipids was observed in males of the 20% α-CD group. A similar effect was observed in a 2-week feeding study, in which rats received diets with 5 and 10% α-CD (added at the expense of cellulose) (Shizuka et al., 1996).

testes (n.s.) and adrenals (n.s.), a slight increase was noted also in males of the 20% α-CD group. Except for the cecum, none of these organ weight changes persisted to the end of the recovery period.

Histopathological examination of the organs and tissues of rats killed at the end of the treatment period did not reveal any abnormalities that could be attributed to the treatment. Consequently, no histopathological examination was conducted on animals of the satellite groups.

It was concluded that the ingestion of α-CD for 13 weeks at dietary levels of up to 20% (corresponding to intakes of 12.6 and 13.9 g/kg bw/d for male and female rats, respectively) was well tolerated and did not produce any signs of toxicity (Lina, 1992; Lina & Bär, 2004a). The slightly decreased body weights and feed conversion efficiency in males of the 20% α-CD group were not considered to be adverse effects. Since α-CD is metabolically available only in an indirect way after fermentation by the intestinal microflora, its physiological energy values is lower than that of starch for which it substituted in the test diets. For the same reason, body weights and feed conversion efficiency were also decreased in males of the 20% lactose group. Why effects on body weights and feed conversion efficiency were seen only in males but not females, is not known. However, in the absence of data on carcass composition (ratio of lean-to-fat body weight) any interpretation of small changes in body weights would anyhow remain speculative.

The few, treatment-related changes in the 20% α-CD and lactose groups are those that are typically seen in rodents fed high doses of incompletely digested and absorbed carbohydrates (e.g., softer stool, cecal enlargement, slightly lower body weights, increased urinary calcium excretion, lower urinary and fecal pH, increased fecal weight and fecal nitrogen excretion). In quantitative terms, these changes (except for body weights) were somewhat more pronounced in the 20% α-CD group than in the lactose group. This difference may be explained by the different digestibility of α-CD (completely indigestible) and lactose (about 50% digestible in rats).

It is known that cyclodextrins have a slight inhibitory effect on the activity of certain amylases in vitro (Mora et al., 1974; Brock & Brock, 1990; Fukuda et al., 1992)[26]. Considering the relatively small difference between the 20% α-CD and 20% lactose group of parameters related to the non-digestibility of carbohydrates (in particular the cecum weights), it appears unlikely that the presence of α-CD in the chyme reduced the digestion of dietary starch by pancreatic amylase to a relevant extent. But even if some inhibition of amylase activity took place, this should not be a matter of toxicological concern because the fermentation of undigested starch and α-CD yields the same breakdown products (short-chain fatty acids), and because some starch re-

[26] The inhibition constant of α-CD for porcine pancreatic α-amylase using amylase as a substrate is 7mM (corresponding to 6.8 g/l) (Koukiekolo et al., 2001). γ-CD and β-CD have lower values of 3.0 and 1.2 mM, respectively.

mains undigested and is fermented in the colon also under ordinary dietary conditions (Annison & Toping, 1994; Stephen, 1991).

None of the effects that were observed in the 28-day rat study in association with the intake of α-CD could be reproduced in the 90-day study even though a higher top dose (20% in the 90-day study, 15% in the 28-day study) was applied. The possible reasons for the persistent diarrhea in the 15% α-CD group of the 28-day study, and all the sequelae of it such as increased water consumption and reduced body weight gains are not entirely clear. However, a difference in the composition of the basal diet may have played a role. In the 28-day study the main source of carbohydrate was pre-gelatinized potato starch (25% in the top dose group) and whole ground wheat (18%). In the 90-day study, whole ground wheat (28%) and maize (10%) were used.

8.2.3 Dogs

In a subchronic (90-day) toxicity study, four groups of Beagle dogs received diets with 0, 5, 10 or 20% α-CD (4 dogs/sex/group). The test substance was added to the diet at the expense of pre-gelatinized potato starch. Body weights and food consumption were measured weekly. Ophthalmoscopic observations were made before and at the end of the treatment period. Standard hematological and clinico-chemical analyses were conducted in blood samples collected before start of the treatment and in weeks 6 and 12 of the study. Urine was collected for semi-quantitative urinalysis

in week 13. In week 14, the dogs were killed and subjected to gross examination. Organ weights were determined and histopathological examination of all standard organs and tissues was performed on all animals.

All dogs remained in good health during the study. Transient diarrhea occurred in all three treatment groups. The incidence and severity of diarrhea increased with increasing dietary levels of α-CD (slightly less pronounced in females than in males)[27]. Body weights did not differ significantly between the groups but the weight gain of females of the 20% group was somewhat reduced from week 4 onwards. Food intake was slightly increased, particularly in males of the 20% α-CD group. Correspondingly, the calculated food efficiency was slightly (n.s.) decreased in this group.

No treatment-related differences were observed with respect to ophthalmoscopic examinations, hematological parameters, clinicochemical analyses of the plasma, and semi-quantitative urine analyses. Only the urinary pH was slightly below control levels in males (n.s.) and females ($p<0.05$) of the 20% dose group. No abnormalities were seen at necrospy that could be attributed to treatment. The organ weight data revealed some cecal enlargement in the 10 and 20% dose groups (significant only in males). On microscopic examination, no treatment-related effects were observed in any of the various organs and tissues. Transient diarrhea,

[27] In the 5, 10 and 20% α-CD groups, slight to moderate diarrhoea was reported for 26, 63 and 83% of all observation occasions in males and 13, 40 and 82% in females (4 dogs/group x 92 days = 368 observation occasions = 100%). Severe diarrhoea was observed on 4 and 5 observation occasions in males of the 10 and 20% α-CD groups.

slight cecal enlargement and a slightly increased acidity of the urine were the only effects that could be attributed to the α-CD treatment. The intensity of these physiological changes was much less than is observed commonly in dogs in response to the ingestion of low digestible carbohydrates.

It was concluded that the intake of α-CD at dietary levels of up to 20% (corresponding to intakes of approximately 9.8 and 10.4 g/kg bw/d in male and female dogs, respectively) was tolerated without any toxic effects (Til & von Nesselrooij, 1993; Lina & Bär, 2004b).

8.3 Long-term toxicity/carcinogenicity studies

No data available

8.4 Reproduction studies

No data available

8.5 Special studies on embryotoxicity/teratogenicity

8.5.1 Mice

α-CD was administered in ground feed (0, 5, 10, or 20%) to pregnant Swiss Albino mice during the major period of organogenesis (day 6 - 16 of pregnancy). Maternal clinical signs, body weights, and food and water consumption were monitored at regular intervals from day 0 - 17. On day 17, the dams were killed and the fetuses removed from the uterus and examined for evidence of developmental toxicity. In the dams, 20% α-CD increased maternal relative food consumption[28] at the end of the dosing period (day 12 - 16) and thereafter (16 - 17). No α-CD related effects on maternal food intake were noted for the lower doses of α-CD. Based on the average relative food consumption during the dosing period, the 5, 10, and 20% α-CD groups ingested 13.7, 23.1, and 49.3 g/kg bw/day α-CD, respectively. Maternal relative water consumption was unaffected by α-CD administration.

Examination of the uterine contents indicated that α-CD was not developmentally toxic. The percent resorptions/litter, average number of live fetuses/litter, average fetal body weight/litter, and sex ratio/litter did not differ from control values at all dose levels. The incidence of external, visceral, or skeletal

[28] Relative food consumption = g food intake/kg bw.

malformations showed no treatment-related effects. The incidence of variations was similarly unaffected by the treatment.

The results indicated that α-CD administered to pregnant mice at levels as high as 20% in the diet corresponding to 49.3 g/kg/day had no adverse effect on embryo/fetal development (Price et al., 1996; NTP, 1994b).

8.5.2 Rats

α-CD was administered in ground feed (0, 5, 10, or 20%) to pregnant Sprague-Dawley rats during the major period of organogenesis (day 6 - 16 of pregnancy). Maternal clinical signs, body weight, and food and water consumption were monitored at regular intervals from day 0 - 20. On day 20, the dams were terminated and the fetuses removed from the uterus and examined for evidence of developmental toxicity.

In the dams, α-CD caused dose-dependent increases in maternal relative food consumption during the dosing period (day 6 - 16). Relative food consumption[26] remained elevated on day 16 - 18 in the 10% and 20% α-CD groups, but returned to normal by day 18 - 20. Based on the amount of food consumed during the dosing period, the dams in the control 5, 10, and 20% α-CD groups ingested approximately 0, 4.2, 9.0, and 20 g/kg/day α-CD, respectively. Maternal water consumption during the dosing period showed no

distinct dose-related effects; however, sporadic, individually significant increases in water consumption were observed in all α-CD dose groups. After the dosing period, relative maternal water consumption was elevated on day 16 - 18 in the 10% and 20% α-CD groups. In spite of the treatment-related increases in maternal food consumption, average maternal body weight or average maternal body weight gain during or after the dosing period was not affected by α-CD administration. Examination of the uterine contents indicated that α-CD was not developmentally toxic. The percent resorptions/litter, average number of live fetuses/litter, average fetal body weight/litter, and sex ratio/litter were comparable to controls at all α-CD dose levels. The incidence of external, visceral, or skeletal malformations showed no treatment-related effects. The incidence of variations was similarly unaffected by α-CD administration. These results demonstrate that α-CD did not produce any developmental and maternal toxicity at intakes of up to 20 g/kg/day, the highest dose tested, which is the NOAEL (Price et al., 1996; NTP 1994a).

The possible embryotoxicity/teratogenicity of α-CD was examined in Wistar rats. α-CD was fed at dietary concentrations of 0, 1.5, 5, 10 or 20% to groups of 25 pregnant female rats from day 0 to 21 of gestation. A comparison group received a diet with 20% lactose. The additions to the diet of α-CD and lactose were made at the expense of pre-gelatinized potato starch. Body weight as well as food and water intake were recorded during the treatment period. The rats were killed on day 21 and examined for standard parameters of reproductive performance (number of corpora lutea,

implantations, early and late resorptions, live and dead fetuses, ovary weight, uterus weight (full and empty), placenta weight, as well as weight, length and sex of fetuses). The fetuses were subjected to visceral and skeletal examination using standard techniques. Generally, α-CD was well tolerated and no deaths or abortions occurred in any group. Diarrhea did not occur and soft stool was observed only in one rat of the 20% α-CD group on one day (day 11). Maternal weight gains and food consumption were similar in all groups during gestation, except for a slightly increased food intake in the 20% α-CD group from day 6 - 21 and a decreased food intake in the 10% α-CD group from day 16-21. Water intake was increased from day 16-21 in the 20% α-CD group; in the lactose group, water intake was significantly increased from day 0 - 21. Reproductive performance was not affected by the α-CD treatment. All pregnant females had litters with viable fetuses. None of the examined parameters was influenced by the treatment. Necropsy of the maternal rats did not reveal gross changes that could be attributed to the treatment. Examination of the fetuses for external, visceral and skeletal malformations and anomalies did not reveal any fetotoxic, embryotoxic or teratogenic effects of α-CD. A significantly increased incidence of renal pelvic cavitation, a visceral variation, was found in rats fed 20% α-CD (33% of fetuses affected) and 20% lactose (38% of fetuses affected) versus control rats (18% of fetuses affected). The incidence of reduced or incompletely ossified sternebrae was significantly enhanced in all except the 20% α-CD groups (including the 20% lactose group). In conclusion, no adverse effects were observed at α-CD intakes of up to 20% of the diet, corresponding to

about 11 g/kg bw/d, the highest dose tested, which is the NOAEL (Verhagen & Waalkens-Berendsen, 1991; Waalkens-Berendsen & Bär, 2004).

8.5.3 Rabbits

In a standard embryotoxicity/teratogenicity study, α-CD was administered to groups of sixteen, artificially inseminated New Zealand White rabbits at dietary concentrations of 0, 5, 10, or 20%. A comparison group received a diet containing 20% lactose. α-CD and lactose were added to the diets at the expense of pregelatinized wheat starch. Treatment started on day 0 of gestation and ended on day 29 when the animals were killed. Body weights and food consumption were determined during the treatment period. At termination, the standard parameters of reproductive performance were determined (number of corpora lutea, implantation sites, live and dead fetuses, early and late resorptions; weight of ovaries, uterus (full and empty) and placenta; fetal length, weight and sex). The parental animals were subjected to gross necropsy. The placentas of the control, 20% α-CD, and lactose groups were examined for gross abnormalities. The fetuses were examined under the microscope for external, visceral, and skeletal alterations using standard techniques. The skeletal examinations were restricted to the control, 20% α-CD, and lactose groups.

Except for the occurrence of transient mild diarrhea in 1 rabbit of the 20% α-CD group, the treatments were well tolerated. A reduced food intake in the 20% α-CD group during the first week of treatment resulted in a reduced weight gain during week 1 and 2 of the study. After week 2 there were no differences in weight gains between the groups, and at termination of the study body weights were similar in all groups. Even at the highest dose level, which corresponds to an intake of 5.9 - 7.5 g/kg bw/day, no signs of maternal toxicity were observed. Reproductive performance was not affected by the α-CD treatment. Uterine weight, placental weight, fetal weight, number of fetuses, sex ratio, as well as the number of implantation sites, resorptions and corpora lutea did not differ between treated groups and controls. In the 20% lactose group, the incidence of placental cysts was higher than in the control group.

Visceral and skeletal examinations of the fetuses did not reveal any malformations, anomalies or variations that could be attributed to the α-CD treatment. In the lactose group, the incidence of unossified front phalanges and the total incidence of skeletal retardations were significantly reduced. It was concluded that dietary α-CD was well tolerated by pregnant rabbits, had no adverse effect on reproductive performance and was not embryotoxic, fetotoxic or teratogenic at dietary concentrations of up to 20% corresponding to an intake of 5.9 - 7.5 g/kg bw/d, the highest dose tested, which is the NOAEL (Waalkens-Berendsen & Smits-van Prooije, 1992; Waalkens-Berendsen et al., 2004).

8.6 Special studies on genotoxicity

The results of genotoxicity studies with α-CD are summarized in Table 2. It is concluded that α-CD is not mutagenic in standard Ames tests (Blijleven, 1991). In an in vivo mouse bone-marrow micronucleus test, α-CD did not produce any chromosomal damage or damage of the mitotic apparatus (Immel, 1991).

8.7 Special study on skin irritation/sensitization

The potential of α-CD for inducing cutaneous delayed hypersensitivity was examined in guinea pigs. The study comprised a control group (5 animals/sex) and a treated group (10 animals/sex). Induction was made in two steps. First, a 3% α-CD solution was injected intradermally with Freund's Complete Adjuvans (FCA). The controls received water with or without FCA. One week later, a 30% dilution of α-CD in vaseline was applied topically (controls: vaseline alone). After two more weeks, a challenge treatment was made by applying topically vaseline with 0 (control), 10 or 30% α-CD.

The challenge treatment did not provoke signs of hypersensitivity (erythema, edema) at 24 or 48 hours after the challenge. It was concluded that α-CD is not a sensitizer (Prinsen, 1992).

8.8 Special study on skin irritation and corrosion

The potential of α-CD for inducing dermal irritation and corrosion was examined in three albino rabbits. A mixture of α-CD (0.5 g) with water (0.3 g) was firmly applied to a defined area of the shaven skin for a period of 4 hours. Skin irritation scores were recorded at 1, 24, 48 and 72 hours after removal of the test material. No sign of skin irritation were observed at any time in any animal. This results demonstrates that α-CD is not irritating or corrosive to the skin (Prinsen, 1991c).

8.9 Special studies on eye irritation

The ocular effects of dry α-D were examined in 3 albino rabbits. An amount of 0.062 g α-CD was instilled as a dry powder in the conjunctival cul-de-sac of the right eye of each rabbit. The reaction was examined at 1, 24, 48 and 72 hours and 7 and 14 days after administration. Different signs of acute eye irritation were seen starting at 1 hour after treatment. At 7 days after treatment, eye effects had cleared completely in one rabbit, whereas ischemic necrosis of the nictitating membrane, slight redness and slight swelling of the conjunctivae were still observed in the two other rabbits. At 14 days after treatment, these eye effects had also cleared completely. It was concluded

that dry α-CD powder is irritating but not corrosive to the eye (Prinsen, 1990).

Solutions of α-CD (7.25 and 14%, w/v) were instilled in the conjunctival cul-de-sac of the right eye of 2 groups of 3 rabbits each. The ocular reactions were examined after 1, 24, 48 and 72 hours. The treatments caused slight redness and slight swelling of the conjunctivae in some animals. All eye effects had cleared completely at 24 hours after treatment. It was concluded that solutions of α-CD are not irritating and not corrosive to the eye (Prinsen, 1991b).

8.10 Special studies on cell membranes

The interactions between α-, β- and γ-cyclodextrin and membrane phospholipids, liposomes and human erythrocytes were studied in vitro. None of these three cyclodextrins increased the permeability of dipalmityol-phosphatidylcholine liposomes.[29] No effect on the active transport of $^{42}K^{+}$ into erythrocytes was observed at a concentration of 10^{-2} mol/l (37°C). However, the release of $^{42}K^{+}$, $^{86}Rb^{+}$ and $^{137}Cs^{+}$ from erythrocytes by passive transport was increased with β- but not with α- or γ-CD at a concentration of 1.7 x 10^{-2} mol/l (Szejtli et al., 1986).

[29] The effect of CDs on the permeability of liposomes varies with the composition of the liposomes. The permeability of lecithin and dicetylphosphate containing liposomes was increased by α-CD (Miyajima et al., 1987; Nishijo et al., 2000).

The effects of α-, β- and γ-CD on the integrity of human erythrocytes were examined in a series of in vitro experiments. On incubation of erythrocytes with increasing concentrations of cyclodextrines in isotonic buffer for 30 min, hemolysis was initiated at 3 mM β-CD, 6 mM α-CD and 16 mM γ-CD. On the other hand, hemolysis induced by incubation in hypotonic buffer was reduced by the presence of α- and γ-cyclodextrin at concentrations of up to 5 and 10 mM, respectively. The observation that human erythrocytes tolerated α-CD better than β-CD was confirmed in other studies in which α-CD concentrations of between 5 to 10 mM were required for the induction of hemolysis (Leroy-Lechat et al., 1994; Okada et al., 1988; Skiba, 1990; Kimura et al., 2000). The hemolytic effect of the different CDs was ascribed to a cyclodextrin-mediated extraction of cholesterol and other lipids from the erythrocyte membrane (Irie et al., 1982). β-CD forms stable inclusion complexes with cholesterol. α-CD, on the other hand, does not interact with cholesterol but forms complexes with phospholipids (Irie et al., 1982; Ohtani et al., 1989; Debouzy et al., 1998; Nishijo et al., 2000). It appears that the unsaturated side chain(s) of the phospholipids account for the complex formation (Fauvelle et al., 1997; Jouni et al., 2000). In the case of phosphatidylinositol, a complex with the polar headgroup is formed as well (Fauvelle et al., 1997; Debouzy et al., 1998). On this basis it has been proposed that sugars could act as specific anchor for α-CD at the erythrocyte surface (Debouzy et al., 1998).

The higher tolerance of human erythrocytes to α-CD than to β-CD also was confirmed in a study in which the shape of human erythrocytes and the release of K^+ and hemoglobin were examined in the presence of increasing concentrations of these three cyclodextrins. The highest tolerance was found for γ-CD. β-CD, but not α- or γ-CD, at concentrations of ≥1 mM led to a significant release of cholesterol and protein from the erythrocytes. Phospholipids were released in the presence of ≥1 mM α-CD or β-CD. γ-CD had to be present at a concentration of 4 mM to induce a release of phospholipids, and at a concentration of more than 20 mM for a release of cholesterol or protein (Ohtani et al., 1989).

The cytotoxic potential of different cyclodextrins was studied with P388 murine leukemia cells. To growing cultures of these cells in RPMI medium with 10% fetal calf serum, increasing amounts of cyclodextrins were added. After incubation for two hours in the presence of the cyclodextrins, the cells were washed, suspended in culture medium and incubated for 48 hours whereupon the cells were counted. The concentrations of α-, β- and γ-CD which elicited initial cytotoxicity were 11, 2.5 and 55 mMol (1.1, 0.28, 7.1%), respectively (Leroy-Lechat et al., 1994).

The effect of α-, β- and γ-CD on the growth of rat glial cells was examined using concentrations of 0, 0.1, 0.5, 1.0 and 5.0 g/l. The cells were cultured in serum-free medium for 5 days. α-CD had a slight growth promoting effect at concentrations above 0.5 g/l which, however, was smaller than that of 10% fetal calf

serum (Nakama, 1992). A growth promoting effect of α-CD has also been reported from experiments with human lymphoblast and fibroblast cell lines. No cytotoxicity of α-CD was observed up to the highest concentration tested (2 g/l) (Yamane et al., 1981).

The membrane effects of α-, β- and γ-CD also were examined with bacterial cells using a luminous E. coli strain as a model. It was found that β-CD at concentrations of 0.03 and 0.5% (w/w) reduced luminescence by 20 and 50%, respectively. For α-CD, the respective concentrations were 0.16 and 10%. For γ-CD, a concentration of 2% was required to reduce luminescence by 20%. A reduction by 50% could not be achieved even at the highest concentration tested (10%) (Bar & Ulitzur, 1994).

Cultured human ciliated nasal epithelial cells were used to screen the cilio-inhibitory effect of different cyclodextrins. α-CD exhibited a dose-related effect on ciliary beat frequency (CBF). At concentrations of 1 and 2% in the serum-free test medium, α-CD did not inhibit CBF (45 min exposure time). At α-CD concentrations of 3% and 5%, CBF was reduced by 23 and 54%, respectively. At 3%, the inhibition was completely reversible; at 5% it was partially reversible after washing the cells for 10 minutes. γ-CD had no inhibitory effect on CBF in the tested dose range (2-8%). β-CD was not examined (Agu et al., 2000).

8.11. Special studies on CD-mediated changes of intestinal permeability

Interaction of certain CDs with components of cell membranes (cholesterol, phospholipids) changes their fluidity and permeability (Irie et al., 1982). It is conceivable that such changes lead to an enhanced permeability of epithelia by increased transcellular or paracellular permeation (opening of "tight junctions").

The effects of different CDs on the digestion and absorption of two model peptides [glycosylated calcitonin and octreotide (an octapeptide)] were examined in an intestinal cell culture model (Caco-2) and in situ absorption studies in rats. In a first experiment, the effect of CDs on the peptic and tryptic digestion of calcitonin was measured. At a ratio of 1:15 (calcitonin:CD, w/w), α-CD had no effect but all the other CDs slightly inhibited the enzymatic degradation. Using the Caco-2 model, radiolabelled octreotide as test substance, and CD concentrations of 1% in the serum-containing culture medium, an increase of relative permeation rates (from the apical to the basolateral side of the Caco-2 cell monolayers) was observed. It was highest for α-CD (3.51-fold) and lowest for dimethyl-β-CD (2.43-fold). γ-CD (2.86-fold) and β-CD (2.94-fold) took an intermediate position. Using PEG-4000 as test substance, the same rank order was observed. Measurement in the apical medium of lactate dehydrogenase as a marker of cell integrity indicated that the treatment caused no significant damage to the cells. In situ absorption studies with injec-

tion of a solution of test substance and different CDs (0.5 ml saline with 100 μg octreotide + 5 mg CD or 200 μg calcitonin + 3 mg CD) in a ligated segment of the proximal jejunum confirmed the enhancing effect of CDs on the intestinal absorption of these peptides. However, the rank order of efficacy was changed in comparison to the Caco-2 model in that α-CD had no effect on octreotide absorption and the smallest effect on calcitonin absorption (Haeberlin et al., 1996).

Using the Caco-2 cell model and ^{14}C-mannitol as test substance which is thought be absorbed mainly by the paracellular pathway, the permeability enhancing effect of α-CD, γ-CD and hydroxypropyl-β-CD (HP-β-CD) were examined. Contrary to the observations by Haeberlin and collaborators, α-CD had no effect at concentrations of 0.1 and 1% in the serum-containing culture medium. However, at a concentration of 5% it increased the mannitol absorption. γ-CD (at 0.1, 1.0, 5%) and HP-β-CD (at 1%) did not increase mannitol absorption (McAllister & Taylor, 1999). Using the same experimental system, no change of mannitol was observed at an α-CD concentration of 75 mM (7.3%) by others (Ono et al., 2001).

Using the Caco-2 cell model and ^{14}C-PEG-4000 as test substance which is absorbed entirely by the paracellular pathway, the effects of different CDs were evaluated. It was found that α-CD, γ-CD and HP-β-CD at concentrations of 0.25 - 5%, and β-CD at 1.8% (maximal solubility) did not affect the transepithelial electrical resistance (TEER) of Caco-2 cell monolayers. On the other

hand, DM-β-CD reduced the TEER in a dose-dependent manner. Treatment of the monolayers with CDs for 15 and 60 min did not cause trypan blue staining except for DM-β-CD (at 2.5 and 5%). Permeation PEG-4000 was not affected by the presence of α-CD, β-CD, γ-CD and HP-β-CD at the concentrations tested [up to 5% (β-CD: 1.8%)]. However, DM-β-CD had an enhancing effect on PEG permeation which was directly related to the applied dose and duration of exposure. It was concluded that DM-β-CD, but not any of the other tested CDs, opens the "tight junctions" of Caco-2 monolayers upon direct contact at sufficient concentration for a sufficient period of time (Hovgaard & Brondsted, 1995).

Using higher concentrations of α-CD, an effect on the TEER of Caco-2 cell monolayers was observed by others (Ono et al., 2001). An α-CD concentration of 25 mM (2.4%) appeared to be a no-effect level not only with regard to TEER but also for cytotoxicity (determined by intracellular dehydrogenase activity), the transport of Rhodamine 123, protein release and LDH release of Caco-2 cells (Ono et al., 2001). A significant release of phospholipids was observed at an α-CD concentration of 37.5 mM (lower concentrations were not tested).

The effect of α-CD and β-CD on the absorption of sulfanilic acid (SA) was investigated in a series of experiments including absorption from ligated loops (in situ) of rat small intestine (Nakanishi et al., 1992, 1990). Appreciating the protective role of the mucus which covers the intestinal mucosa, the experiments

were performed in the presence or absence of N-acetyl-L-cysteine, a non-toxic mucolytic agent.

In the absorption experiment with in situ ligated loops it was found that pretreatment (perfusion) with 10 mM solutions of α-CD and β-CD did not enhance the absorption of subsequently administered sulfanilic acid. However, after pretreatment with β-CD in combination with acetyl cysteine, the absorption of sulfanilic acid was increased significantly. α-CD in combination with acetyl cysteine had no effect. Analysis of the perfusate for cholesterol and phospholipids showed that β-CD promoted the release of cholesterol and α-CD the release of phospholipids if the protective mucus layer had been removed by acetyl cysteine. Morphological changes of the intestinal mucosa were not observed with any treatment (Nakanishi et al., 1992).

On perfusion for 30 minutes of small intestinal segments of rats with saline or with saline plus α-, β- or γ-CD (18.5 g/l) it was found that β- and γ-CD increased the cholesterol content of the perfusate. α-CD had no effect on this parameter. The phospholipid and protein concentrations of the perfusate remained unaffected by α-CD. The active glucose transport was significantly depressed after β-CD treatment (effects of α-CD and γ-CD on this parameter were not examined) (Mori et al., 2000).

8.12 Studies in human volunteers

In an early metabolic study of α-CD, two type-2 diabetic subjects received 50 g/d α-CD. The substance was given with a low-carbohydrate diet (the authors did not specify whether the 50-g dose was consumed as a bolus dose or whether ingestion was spread over several eating occasions). Nausea was noted in one subject at one out of two experimental days about 10-12 minutes after ingestion. Other side-effects did not occur. The authors attributed this effect to an (unknown) impurity rather than to α-CD itself (von Hoesslin & Pringsheim, 1923).

In a subsequent series of experiments (which included an unspecified number of diabetic patients), a purified CD preparation (consisting mainly of α-CD with some β- and γ-CD) was consumed at doses of 50-100 g/day. Some but not all volunteers (proportion not specified) reported nausea and, occasionally, diarrhea. The results of urine analyses of four diabetic patients (two of which were presumably type-1 diabetics) were presented. The results demonstrate that the ingestion of the CD preparation did not lead to an elevation of the urinary glucose excretion as was seen after the ingestion of bread (von Hoesslin & Pringsheim, 1927).

The gastrointestinal tolerance of α-CD was examined in 12 healthy male volunteers in the context of a study on its glycemic effects. A single 25-g bolus dose of α-CD (dissolved in 250 ml water) was administered to overnight fasted subjects. One subject

reported diarrhea and three other subjects abdominal discomfort. However, these effects were rated as "mild" and did not prevent the volunteers from further participation in the study. The ingestion of 10 α-CD (dissolved in 250 ml water) together with 100 g fresh white bread was not associated with any intestinal side-effects in any of the subjects (Diamantis & Bär, 2002).

The observation of flatulence, bloating, nausea and soft stool upon ingestion of high doses of low digestible carbohydrates, particularly if ingested in liquid form on an empty stomach, is a well-known phenomenon. It is caused in part by an influx of water in the small intestine (for achieving isotonicity) and in part by the ensuing fermentative process in the more distal parts of the gut.

While there is limited data on the intestinal tolerance of α-CD in humans, studies of other low-digestible carbohydrates such as inulin, fructooligosaccharides (FOS), polydextrose, resistant (malto)dextrins and other oligosaccharides provide additional information. This information is, by and large, applicable to α-CD because the typical intestinal symptoms such as flatulence, bloating, and laxation, are the result of the same (unspecific) sequelae of excessive fiber intake. The largest number of studies is probably available for FOS and inulin. The safety data of fructans including data on their intestinal tolerance in children and adults, has been reviewed (Carabin & Flamm, 1999). It has been concluded that abdominal complaints would occur in adults after a single dose of ≥ 20 g. School-age children tolerated sup-

plementation of the diet with FOS at a level of 3-9 g (single dose).

Accordingly, the FDA issued a "no further questions" letter in response to a GRAS notice of fructooligosaccharides with an estimated exposure of 5.2 g/d for 1-13 year old children, and 6.6 g/d for consumer of age 14 and older. The 90^{th} percentile intakes were estimated for these two age groups at 9.9 and 13.9 g/d, respectively (GRAS Notice No. 44).

Other novel fibers (polysaccharides) that are considered GRAS, for which even higher daily intakes were predicted, include pullulan (9.4 and 18.8 g/d for the mean and 90^{th} percentile consumer) (GRAS Notice No. 99) and arabinogalactan (10.5 and 21 g/d) (GRAS Notice No. 84).

9 SUMMARY AND CONCLUSIONS ON SAFETY OF α-CD

9.1 Production, intended uses and safety studies of α-CD

α-CD is a cyclic polymer consisting of six glucose units joined "head-to-tail" by α-1,4-glycosidic bonds. It is produced by the action of an amylolytic enzyme, cyclodextrin glycosyltransferase (CGTase), on food-grade, liquefied starch. The CGTase is obtained from a genetically modified strain of Escherichia coli K-12. E. coli K-12 is considered to be a safe host organism (NIH 2002; 55 Fed. Reg. 10932, p. 32-34, March 23, 1990; EU Commission, COM(95)final, OJ C356, 22.11.1997, p.19). The gene coding for CGTase was obtained from a non-pathogenic and non-toxigenic strain of Klebsiella oxytoca (ACGM, 2000). The vector was derived from a mobilization-defective vector (pBr 322) which is widely used and which is considered to be safe (EU Commission, COM(95)final, OJ C356, 22.11.1997, p.19). During the enzymatic production process, the α-CD formed is removed from the reaction mixture by precipitation with 1-decanol. The precipitate is removed and purified by dissolution and re-precipitation. The isolated α-CD is freed from 1-decanol by steam-stripping and is purified by crystallization.

In vitro studies with salivary and pancreatic amylase demonstrate that α-CD is not digested. α-CD shares this indigestibility with β-CD, and distinguishes it fundamentally from γ-CD which is read-

ily digested by pancreatic amylase. The indigestibility of α-CD was confirmed in a metabolic study with ^{14}C-α-CD in germfree rats: 90.7% (range 85-101%) of ingested ^{14}C-α-CD was excreted with the feces. In conventional rats, α-CD is completely fermented by the intestinal microbiota as shown by the presence of at most traces, if any α-CD in the feces. The resulting short-chain fatty acids are absorbed and metabolized. The ADME studies in germfree and conventional rats demonstrate that the absorption by passive diffusion of intact ^{14}C-α-CD is very low (< 1%). Systemically administered ^{14}C-α-CD was excreted unchanged with the urine and was not metabolized to any significant extent. In a human study with ingestion of 25g α-CD as a bolus dose, 0.01% of the dose was excreted during a 3-hour period following administration.

Being non-digestible by the enzymes of the human alimentary tract, α-CD complies with the most essential criterion of the definition of dietary fiber. α-CD also exhibits some of the physiological benefits of dietary fiber as shown by fecal bulking and cholesterol-lowering effects in rats and a blunting effect on postprandial blood glucose levels in man. α-CD is, therefore, useful for the fiber supplementation of foods. Because of its low viscosity, it may serve this purpose also in foods in which more viscous plant gums and/or insoluble fibers would impair the taste and texture of the product.

Because of its particular structure which enables α-CD to form inclusion complexes with, for example, certain flavors and fatty acids, α-CD also fulfills certain food-technological functions.

Assuming that α-CD is incorporated at maximum levels of use in all foods (except chewing gum) in which it may fulfill a useful nutritional and/or technological function, the average α-CD intake is estimated to be approximately 11.4 g/person/day for the consumers ("users") of such foods (90^{th} percentile consumer: 19.8 g/person/day). Use of α-CD in chewing gum will contribute about 0.9 g/person/day. The consumption of α-CD is spread evenly over the main meals and in-between meal eating occasions over the day. The intake per eating occasion is estimated at 3.9 and 8.0 g for the mean and 90^{th} percentile consumer, respectively. These levels are below those that are tolerated without intestinal symptoms (10 g single dose) or with mild symptoms only (25 g single dose) (Diamantis & Bär, 2002). Children of age 2-5 years exhibit the highest estimated intake (relative to body weight) with values of 10.2 and 16.2 g/d and 0.61 and 0.98 g/kg bw/d for the mean and 90^{th} percentile users. This exposure is at least ten times lower than the NOAEL in rats and dogs (90-day studies). For an essentially not-absorbed substance this appears to be a sufficient margin of safety.

The toxicity of α-CD was examined in standard in-vitro and in-vivo toxicity tests. Ames tests and a micronucleus test demonstrate that α-CD is not genotoxic. Ingestion of a single α-CD

dose of 10 g/kg bw was not associated with mortality in mice. In acute toxicity tests with parenteral administration the LD_{50} of α-CD varied between 500-1000 mg/kg bw (depending upon species and route of administration) (Table 1). In two 13-week oral toxicity tests, rats and dogs received α-CD with the diet at dietary levels of up to 20%. A few mild, physiological effects (including cecal enlargement, transient diarrhea or stool softening were consequences of the indigestibility and microbial, intestinal fermentation of α-CD ("fiber-like effects"). No reactions to the treatment were observed on histopathological examination of tissues and organs. It was concluded that α-CD ingested at dietary levels of up to 20%, corresponding to about 13 g/kg bw/d (rats) and 10 g/kg bw/d (dogs), was tolerated without any adverse effects. Four embryotoxicity/teratogenicity studies in mice, rats and rabbits with oral administration of α-CD at dietary levels of up to 20% also did not reveal any treatment-related, adverse effects (Table 3).

Special studies on skin irritation/sensitization and eye irritation did not reveal any particular adverse effects of α-CD. In comparative studies on the in vitro effects of different cyclodextrins on cell membranes (e.g., erythrocytes) α-CD was significantly better tolerated than β-CD, but not as well as γ-CD. At concentrations of ≥ 1mM, α-CD was able to extract phospholipids from cell membranes but this was not associated with morphological changes or death of the cells.

The observation of a higher permeation through Caco-2 cell monolayers of two peptides in the presence of α-CD lead to the hypothesis that α-CD might temporarily open the tight junctions. Subsequent experiments with other markers (mannitol, sulfanilic acid) which do not form complexes with α-CD, did not support this hypothesis. It also was recognized that the mucus layer which covers the intestinal mucosa, provides additional protection which is lacking in the Caco-2 cell model.

The impurities which may be present in α-CD, also do not raise toxicological concern. The enzyme preparation which is used for the α-CD production, does not contain viable source microorganism nor detectable amounts of protein or DNA derived from it. The enzyme is inactivated by heat during the purification of α-CD. The safety of the crude enzyme preparation has been examined in Ames tests, an in-vitro chromosome aberration test and a 13-week oral toxicity test in rats. The highest dose tested in the rat study did not produce any adverse effect [(NOAEL: 260 mg TOS/kg bw/d (TOS = Total Organic Solids of enzyme preparation)].

1-Decanol which is used as complexant for the precipitation of α-CD, has been used as a flavor for many years (7-28 μg/person/d). Absorbed 1-decanol is oxidized to decanoic acid which is further degraded to acetyl CoA by β-oxidation. 1-Decanol is not genotoxic in gene mutation tests in B. subtilis (H17 and M45). Its acute oral toxicity in rats is very low (LD_{50} 12.8 g/kg bw). In an embryotoxicity/teratotoxicity study, rats received 1-decanol at a

dose of about 2 g/kg bw/d from day 1-15 of pregnancy. There were no signs of maternal toxicity. The pre- and post-implantation losses were increased with 1-decanol: (controls: 1.9 and 4.4%, resp.; 1-decanol: 15 and 18%, resp.). However, this effect was even more pronounced with ethanol (30 and 39%, resp.). There was no evidence of a teratogenic effect.

According to specifications of α-CD, the 1-decanol residues are below 20 ppm. At an estimated α-CD intake of 11.4 g/person/day (mean consumer), the 1-decanol intake would be less than 230 μg/person/day, or less than 4 μg/kg bw/d. It is considered that at this low dose, absorbed 1-decanol would be metabolized quickly and probably already in the intestinal mucosa, i.e., before reaching the circulating blood. The effect of 1-decanol on pre- and post-implantation losses of pregnant rats, if administered at a 5×10^5 times higher dose (which exceeds the metabolic capacity of the intestinal mucosa), has therefore no practical relevance.

9.2 Target organs of CD toxicity - the example of β-CD

The naturally occurring cyclodextrins, i.e., α-, β- and γ-CD, have a very similar chemical structure but have nonetheless quite different physiological and toxicological properties. While γ-CD is readily digested by pancreatic amylase and thus is metabolized like starch, α-CD and β-CD are non-digestible and are metabolized

like other fermentable dietary fibers. Therefore, it was unexpected when some renal toxicity was found in animal studies with β-CD and a low ADI of 0-5 mg/kg bw/d was consequently allocated by JECFA at its 44th meeting (WHO, 1996). In contrast, no adverse effects were observed in toxicological studies with α-CD and γ-CD, neither in the kidneys nor any other organ. Accordingly, α-CD and γ-CD received more recently an ADI "not specified" by JECFA (WHO, 1999, 2002).

Considering the very different toxicological profile of α-, β- and γ-CD, it may be helpful at this stage to review the pertinent toxicological features of β-CD in order to determine the position of α-CD relative to its "sister" compounds with regard to its physiological and toxicological properties.

Kidney

Toxicity of natural or chemically modified CDs, where there is any, has been the consequence of interactions with membranes. In the case of β-CD, for example, resorptive vacuolation was observed in the renal proximal tubuli of dogs which had received doses of 0.45 and 0.90 g/kg bw/d by s.c. injection for 10 and 7 days, respectively, and of rats which had received single or repeated doses of β-CD by s.c. injection (NOEL: 0.1g/kg bw, LOAEL: 0.225 g/kg bw) (Tabata et al., 1991; Hiasa et al., 1981). Transiently increased levels of blood urea nitrogen (BUN) were ob-

served in fasted rats 24 hours after i.p. administration of 75-600 mg β-CD (Perrin et al., 1978).

Resorptive vacuolation is an overdose phenomenon which may be observed with many compounds that are known to be non-toxic. However, increased urinary concentrations of protein in dogs fed diets with 5 and 10% β-CD for 13 weeks, and increased urinary concentrations of protein, GGT and NAG in dogs fed a diet with 5% β-CD for 52 weeks suggests that even small amounts of absorbed β-CD might exert a certain destabilizing effect on the lysosomal membranes of the epithelial cells of renal proximal tubuli (WHO, 1993, 1996).

Applying an absorption rate of 3%[30],the systemic exposure to β-CD was 74 and 57 mg/kg bw/d for dogs of the 5% dose group of the 90-day and 52-week study, respectively. The next lower dose (2.5 in the 90-day study and 1.25% in the 52-week study) was a NOEL[31]. In a 6-month toxicity study of β-CD in rats, the administration of β-CD by gavage at doses of up to 1.6 g/kg bw/d had no effect on BUN levels or the microscopic appearance of the kidneys (Makita et al., 1975). In a 52-week rat study with administration of β-CD at dietary levels of up to 5%, an increased incidence of pigment in the epithelium of the cortical tubuli was the only effect noted in female but not male rats of the 2.5 and 5% dose groups (HRC 1994, cited in WHO, 1996).

[30] Range in dogs: 1.3 - 6.2% in week 13, and 1.4 - 3.3% in week 52.

[31] JECFA did not define a NOAEL; i.e., the Committee left it open whether the increased urinary enzyme and protein excretion represented truly adverse effect.

In its safety assessment of β-CD, JECFA based its conclusions on the NOEL of the 52-week dog study (470 mg/kg bw/d) and allocated an ADI of 0 - 5 mg/kg bw for β-CD (WHO, 1996)[29].

Intestine

For CDs which, unlike γ-CD, are not digested by pancreatic amylase, the intestinal mucosa may be another target organ for interaction. The oral administration of β-CD with the diet produced indeed flattening and desquamation of the epithelium in the cecum, colon, and rectum of some mice of the highest dose group (Gur et al., 1991). With regard to these effects, the NOEL was set at 25 mg/kg bw/d and the LOAEL at 75 mg/kg bw/d by JECFA. However, intestinal changes were not seen in rats and dogs fed diets with up to 5% for 52 weeks, or up to 10% for 90 days (Olivier et al., 1991; WHO, 1996). A significant increase in submucosal lymphoid follicles in the ceca in males and females of the high dose group was considered to be a consequence of the treatment-induced cecal enlargement (Blumenthal et al. 1990, cited in WHO, 1993).

Liver

The liver could be another target for systemic CD-mediated membrane effects. In a 90-day rat study with oral administration of β-CD at dietary levels of up to 10%, dose-dependent increases of serum levels of GOT, GPT and AP were observed in male and female animals. Hepatocellular necrosis was seen in 4 out of 40 rats of the top-dose group (Toyoda et al., 1995). In a 52-week rat study with administration of β-CD at dietary levels of up to 5%, significantly increased incidences of single cell necrosis and portal inflammatory cell infiltration were seen in males and females of the 5% group and males of the 2.5% group (NOEL: 1.25% corresponding to 650 mg/kg bw/d) (WHO, 1996). However, no adverse effects were noted in the livers of rats fed up to 675 mg/kg bw/d for 2 years (WHO, 1996; Waner et al. 1995; Toyoda et al., 1997).

Proposed mechanism of toxicity

All the observed effects of β-CD in the kidneys, liver and intestine may be ascribed to membrane effects which are mediated by the formation of inclusion complexes of β-CD with cholesterol. Cholesterol is considered to act as the main rigidifier in lipid bilayers. It is conceivable that after ingestion of high doses of β-CD, concentrations are reached locally (e.g., in the luminal contents of the GI-tract, periportal hepatocytes and secondary lysosomes of the renal proximal tubuli) which are sufficient for extracting cholesterol from cellular membranes (Irie et al., 1982; Ohtani et al., 1989). Experiments with human erythrocytes

have shown that incubation in the presence of α-CD (5 mM), β-CD (2 mM) and γ-CD (15 mM) released 0, 19.9 and 2.0% cholesterol, respectively (Irie et al., 1982).

In contrast to β-CD, α-CD and γ-CD are unable to form complexes with cholesterol. However, α-CD and, to a slightly lesser degree γ-CD, can form complexes with phospholipids (Debouzy et al., 1998; Fauvelle et al., 1997; Ohtani et al., 1989; Nakanishi et al., 1992). In this way, also α-CD and γ-CD can induce hemolysis in incubations with erythrocytes. However, the concentrations which are needed for achieving this effect are considerably higher for α-CD, and even more so for γ-CD, than for β-CD (Irie et al., 1982; Leroy-Lechat et al., 1994).

9.3 Conclusions on safety of α-CD

In subchronic (13-week) toxicity studies with α-CD in rats and dogs no histopathological changes were observed in the kidneys, liver and intestines (or any other organs and tissue) at all dose levels tested (up to 20% in the diet). There is no reason to expect that effects would be seen with longer exposure times because α-CD, as readily water-soluble substance, does not accumulate in the organism and because the epithelial cells that could be affected (intestinal mucosa, proximal tubular epithelium) have a rapid turn-over time. Morphological changes would arise only if

the noxious action overwhelmed the regenerative capacity.[32] In other words, there is a threshold dose below which no effects are to be expected regardless of the exposure time.

In conclusion, there is a substantial body of evidence to support the safety of α-CD as a food ingredient. The data demonstrate that α-CD is not digested by the mammalian digestive enzymes. However, it is completely fermented by the intestinal microbiota, like resistant starch or other fermentable dietary fibers. Only minute amounts of α-CD are absorbed unchanged (< 1%). Absorbed α-CD cannot be metabolized and is, therefore, excreted unchanged with the urine. Data from two 13-week toxicity studies with rats and dogs provide no evidence for adverse reactions in the GI-tract, the kidneys, the liver or any other organs or tissues at α-CD intakes of up to 13 g/kg bw/d in rats and 10 g/kg bw/d in dogs. It appears on this basis that α-CD is safe for its intended uses in food which result in an aggregated estimated intake of 11.4 g/person/day. The EDI calculations also revealed that the intake of α-CD per eating occasion will be about 3.9 g. This amount is well below the doses which were tolerated by adult volunteers without side-effects (10 g) or minimal intestinal symptoms only (25 g).

[32] For the kidney, this is exemplified by the case of erythritol which at high doses leads to elevated urinary excretion of enzymes but does not produce morphological changes of the kidneys of rats even after 2 years of treatment (Til et al., 1996; Summary and Conclusions of 53rd meeting of JECFA).

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Figure 1: **Structural formula of a-cyclodextrin**

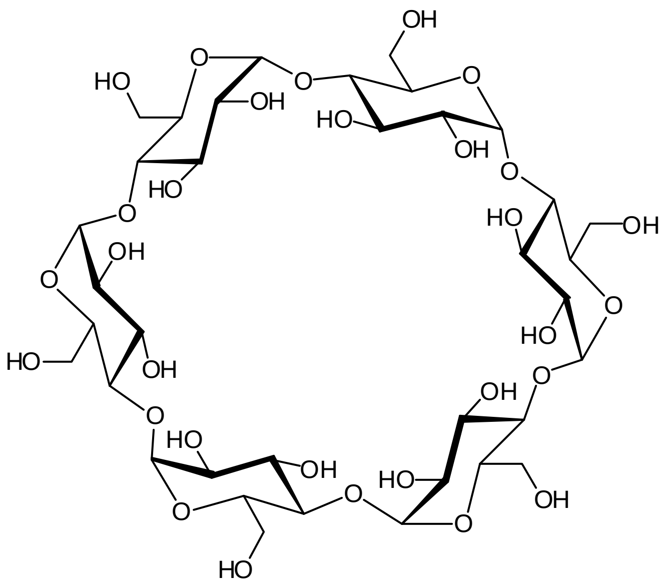
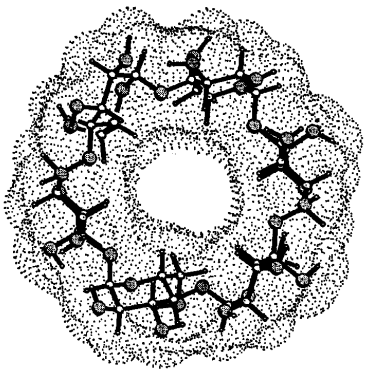
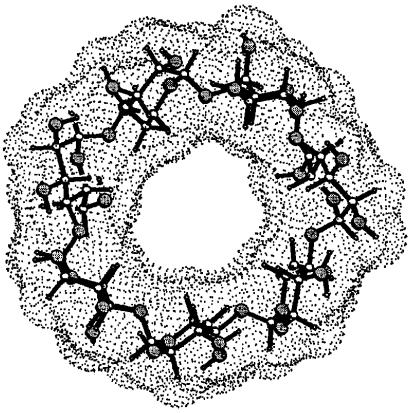


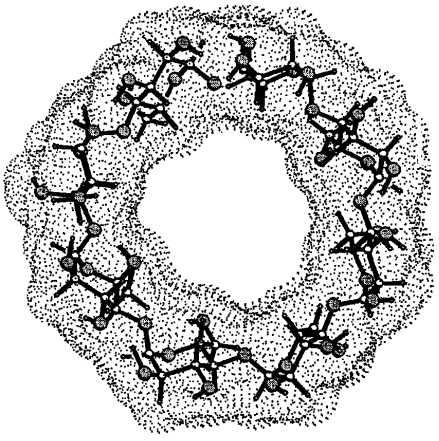
Figure 2 **Models of the torus-like α-, β- and γ-cyclodextrin molecules**



α-cyclodextrin



β-cyclodextrin



γ-cyclodextrin

Figure 3 **Manufacturing process for α-CD**

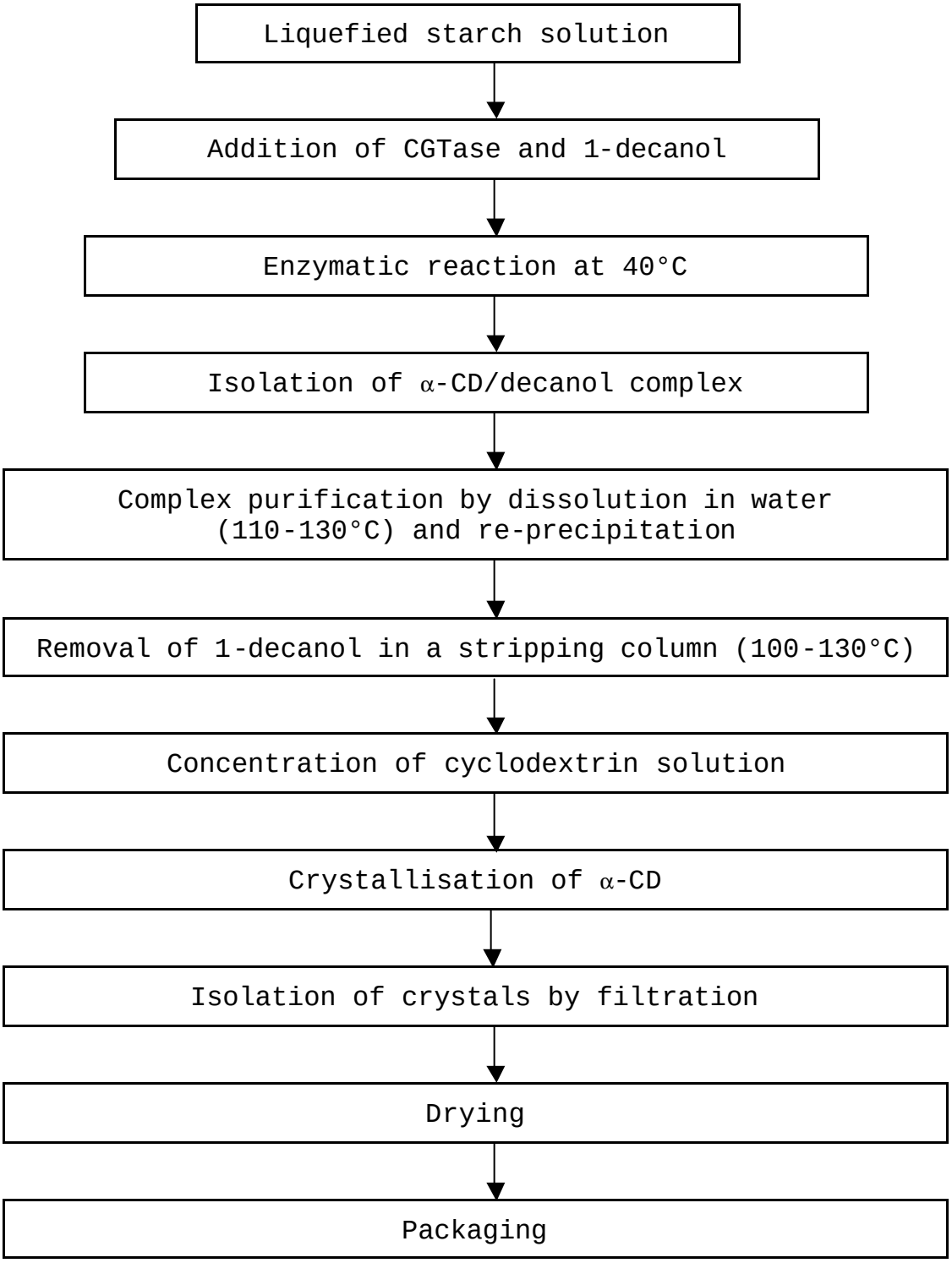


Figure 4 **Safety assessment of a-CGTase using the decision-tree procedure (Pariza & Johnson, 2001)**

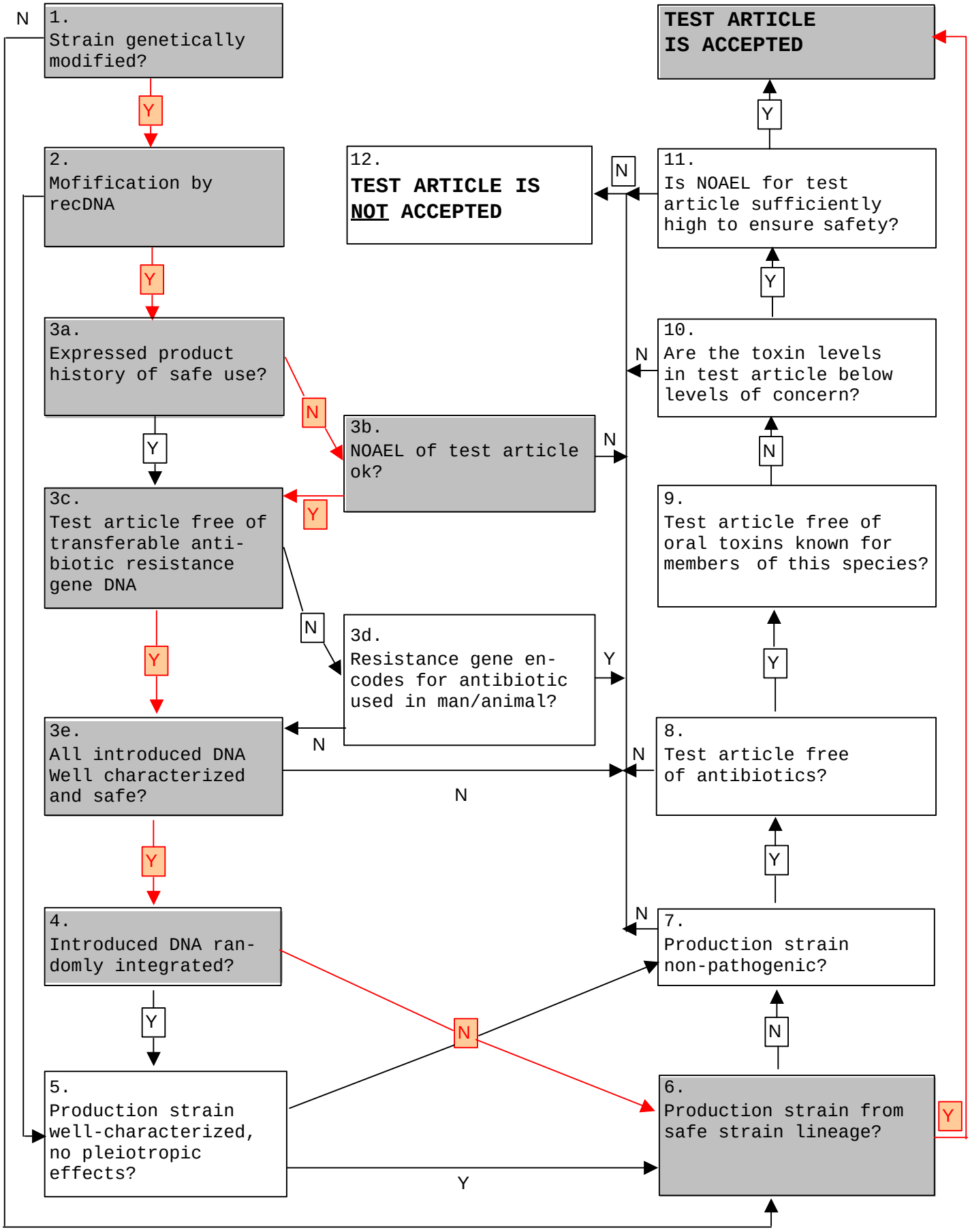


Table 1 Results of acute toxicity studies with a-CD

Species	Sex	Route	LD_{50} mg/kg bw	Reference
Mice	M,F	p.o.	> 10000[a)]	Immel, 1991
Mice	M,F	i.v.	750-1000	Riebeek, 1990a
Rats	?	s.c.	> 1000[a)]	Frank et al., 1976
Rats	M,F	i.v.	1000	Frank et al., 1976
Rats	M,F	i.v.	500-750	Riebeek, 1990b
Rats	M,F	i.p.	750-1000	Prinsen, 1991a

[a)] highest dose tested

Table 2 Results of genotoxicity studies with a-CD

Test	Test system	Concentration	Result	Reference
Ames test[1)]	S.typhimurium TA 1535 TA 1537 TA 1538 TA 98 TA 100	0, 0.25, 0.74, 2.22, 6.67, 20.0 mg/plate	Negative	Blijleven, 1991
Mouse micro-nucleus test	Mouse bone marrow	10g/kg bw	Negative	Immel, 1991

[1)] with and without metabolic activation (rat liver S9 fraction)

Table 3 **Results of standard, oral toxicity studies with α-CD**

Type of study	Species (n)	Dose level (% of diet)	Results	NOAEL	References
Subchronic (4-wk) toxicity test	Wistar rats (5/sex/group)	0, 1, 5, 10, 15, α-CD; 5% β-CD	Persistent diarrhoea associated with reduced body weight gains and increased water consumption	5% (4 g/kg bw/d)	Lina & Bruynties, 1987; Lina & Bär, 2003
Subchronic (13-wk) toxicity study	Wistar rats (20/sex/group)	0, 1.5, 5, 20% α-CD; 20% lactose	Slight cecal enlargement at 20% α-CD; increased urinary Ca excretion and decreased pH at 20% α-CD; increased fecal dry weight and nitrogen excretion and decreased fecal pH at 20% α-CD	20% (m: 12.6 g/kg bw/d; f: 13.9 g/kg bw/d)	Lina, 1992; Lina & Bär, 2003
Subchronic (90-d) toxicity study	Beagle dogs (4/sex/group)	0, 5, 10, 20% α-CD	Slight cecal enlargement in the 10 and 20% dose groups; transient diarrhoea in a few dogs; decreased urinary pH in females of the 20% α-CD group	20% (m: 9.8 g/kg bw/d; f: 10.4 g/kg bw/d)	Til & van Nesselrooij, 1993; Til et al., 2003

Table 3 continued

Type of study	Species (n)	Dose level (% of diet)	Results	NOAEL	References
Embryotoxicity / teratogenicity study	Sprague-Dawley rats	0, 5, 10, 20% (day 6-16)	No differences between treated groups and controls except for an increased food consumption in the 10 and 20% α-CD group and sporadic increases of water consumption in all treated groups	20% (approx. 20 g/kg bw/d)	NTP, 1994a
Embryotoxicity / teratogenicity study	Swiss (CD-1) mice	0, 5, 10, 20% (day 6-16)	No differences between treated groups and controls except for an increase food consumption in the 20% α-CD group. Water intake was unaffected by the treatment	20% (approx. 49 g/kg bw/d)	NTP, 1994b
Embryotoxicity / teratogenicity study	Wistar rats (25f/group)	0, 1.5, 5, 10, 20% α-CD; 20% lactose (day 0-21)	No difference between treated groups and controls	20% (11 g/kg bw/d)	Verhagen & Waalkens-Berendsen, 1991; Waalkens-Berendsen et al., 2003b
Embryotoxicity / teratogenicity study	New Zealand White rabbits (16f/group)	0, 5, 10, 20% α-CD; 20% lactose (day 0-29)	Transient mild diarrhoea in the 10 and 20% α-CD groups	20% (5.9-7.5 g/kg bw/d)	Waalkens-Berendsen & Smits van Prooije, 1992; Waalkens-Berendsen et al., 2003a

Abbreviations: m, male; f, female; bw, body weight; NOAEL, No Observed Adverse Effect Level

Table 4 Intended uses of α-CD[1)]

Food category	maximum use level
1. Bakery products	
Breads and rolls	5%
Brownies	7%
Cakes (light weight)	5%
Crackers (sweet and non-sweet)	10%
Bars (grain based)	7%
Quick breads	5%
Dough (refrigerated)	5%
Baking mixes	5% (dry)
2. Beverages	
Beverage mixes	1% (prepared)
Diet soft drinks	1% (prepared)
Fruit juices	1%
Vegetable juices	2%
Instant coffe/tea	1% (dry)
Coffee whitener	1% (dry)
Formula diets	1% (prepared)
Soy and non-soy (imitation) milk	2% (prepared)
3. Cereals and other grain products	
RTE breakfast cereals	2-9%[3)]
Instant rice	2% (prepared)
Pasta and noodles	2% (prepared)
4. Condiments	
Condiments	3%
5. Dairy products	
Yoghurt	2.5%
Pudding mixes	1% (dry)

Milk beverage mixes	2.5%(prepared)
Frozen dairy desserts	2.5%

6. Fats and oils

Reduced fat spreads	20%
Dressings and mayonnaise	5%

7. Snacks

Salty snacks	1%

8. Soups

Canned soups	2% (prepared)
Dry soups	2% (prepared)

9. Sugars and sweets

Hard candy	15%
Chewing gum	10%[2)]

1) A more detailed list of food categories in which α-CD may be used, is presented in Annex 5.
2) Not included in EDI calculations presented in Tables 5-6. [See Annex 5 (Table 13) for EDI of α-CD from chewing gum].
3) Use level differs with serving size (<20 g/cup, 2%; 20-43 g/cup, 9%; ≥43 g/cup, 5%).

Table 5 Estimated 2-day average intake of a-CD from its proposed uses

Population group	2-day average intake per user			
	g/d		g/kg bw/d	
	Mean	90th perc.	Mean	90th perc.
Children 2 to 5 years old	10.2	16.2	0.61	0.98
Children 6 to 12 years old	11.8	18.7	0.38	0.65
Teenagers 13 to 19 years old	12.4	21.4	0.20	0.36
Adults 20 years and older	11.3	20.2	0.16	0.28
Population 2 years and older	11.4	19.8	0.21	0.43

Note: Details are provided in Annex 5.

Table 6 Estimated intake of a-cyclodextrin per eating occasion

Population group eating occasion	Intake per user			
	g/occasion		g/kgbw/ occasion	
	Mean	90th percentile	Mean	90th percentile
Children 2 to 5 years old				
Breakfast	3.4	6.2	0.20	0.38
Lunch/brunch	3.2	6.2	0.19	0.37
Dinner	2.8	5.7	0.17	0.35
Supper	3.0	6.1	0.17	0.36
Snack	2.2	4.5	0.13	0.27
Unique eating occasion	2.9	5.6	0.17	0.34
Children 6 to 12 years old				
Breakfast	4.3	7.7	0.14	0.25
Lunch/brunch	3.8	7.1	0.12	0.24
Dinner	3.9	7.9	0.12	0.25
Supper	4.0	7.7	0.13	0.26
Snack	2.7	5.9	0.09	0.18
Unique eating occasion	3.6	7.2	0.12	0.24
Teenagers 13 to 19 years old				
Breakfast	5.2	9.7	0.09	0.17
Lunch/brunch	4.7	8.8	0.08	0.16
Dinner	5.1	10.4	0.08	0.17
Supper	4.6	9.2	0.07	0.13
Snack	3.4	7.5	0.06	0.12
Unique eating occasion	4.5	9.2	0.07	0.15
Adults 20 years and older				
Breakfast	4.0	7.8	0.06	0.11
Lunch/brunch	4.6	9.1	0.06	0.13
Dinner	4.7	9.9	0.07	0.14
Supper	4.6	9.6	0.06	0.13
Snack	2.7	5.7	0.04	0.08
Unique eating occasion	4.0	8.3	0.06	0.11
Population 2 years and older				
Breakfast	4.1	7.9	0.08	0.17
Lunch/brunch	4.4	8.7	0.08	0.17
Dinner	4.5	9.6	0.08	0.17
Supper	4.4	9.2	0.08	0.16
Snack	2.8	5.9	0.06	0.13
Unique eating occasion	3.9	8.0	0.07	0.16

Note: Details are provided in Annex 5.

Annex 1

Specifications

Compendium of food additive specifications

Addendum 9

Joint FAO/WHO Expert Committee on Food Additives
57th session
Rome, Italy, 5-14 June 2001

FAO FOOD AND NUTRITION PAPER
52
Add. 9

Food and Agriculture Organization of the United Nations

Rome, 2001

alpha-CYCLODEXTRIN

New specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001). An ADI "not specified" was established at the 57th JECFA (2001).

SYNONYMS α-Schardinger dextrin, α-dextrin, cyclohexaamylose, cyclomaltohexaose, α-cycloamylase

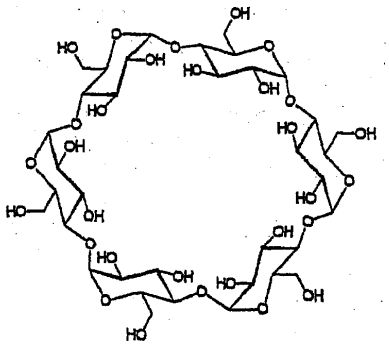
DEFINITION A non-reducing cyclic saccharide consisting of six α-1,4-linked D-glucopyranosyl units produced by the action of cyclodextrin glucosyltransferase (CGTase, EC 2.4.1.19) on hydrolyzed starch. Recovery and purification of α-cyclodextrin may be carried out using one of the following procedures: precipitation of a complex of α-cyclodextrin with 1-decanol, dissolution in water at elevated temperature and reprecipitation, steam-stripping of the complexant, and crystallization of α-cyclodextrin from the solution; or chromatography with ion-exchange or gel filtration followed by crystallization of α-cyclodextrin from the purified mother liquor; or membrane separation methods such as ultra-filtration and reverse osmosis.

Chemical names: Cyclohexaamylose

C.A.S. number: 10016-20-3

Chemical formula: $(C_6H_{10}O_5)_6$

Structural formula



Formula weight: 972.85

Assay: Not less than 98% (dry basis)

DESCRIPTION Virtually odourless, white or almost white crystalline solid

FUNCTIONAL USES Carrier; encapsulating agent for food additives, flavorings, and vitamins; stabilizer; absorbent

CHARACTERISTICS

IDENTIFICATION

Melting range (FNP 5): Decomposes above 278 °

Solubility (FNP 5): Freely soluble in water; very slightly soluble in ethanol

Specific rotation (FNP 5)	[α]25D: Between +145 ° and +151° (1% solution)
Chromatography	The retention time for the major peak in a liquid chromatogram of the sample corresponds to that for α-cyclodextrin in a chromatogram of reference α-cyclodextrin (available from Consortium für Elektrochemische Industrie GmbH, München, Germany or Wacker Biochem Group, Adrian, MI, USA) using the conditions described in the METHOD OF ASSAY.
PURITY	
Water (FNP 5)	Not more than 11% (Karl Fischer Method)
Residual complexant (1-decanol)	Not more than 20 mg/kg See description under TESTS
Reducing substances	Not more than 0.5% (as dextrose) See description under TESTS
Sulfated ash (FNP 5)	Not more than 0.1%
Lead (FNP 5)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in FNP 5, "Instrumental Methods."

TESTS

PURITY TESTS

Residual complexant (1-decanol)

Principle: After enzymatic digestion of the sample followed by solid-phase extraction, 1-decanol is determined by gas chromatography.

Buffer solution: Dissolve 606 mg Tris-buffer (Sigma-Aldrich, St. Louis, MO,USA) and 430 mg calcium sulfate (dihydrate) in 500 ml of water. Adjust pH with concentrated phosphoric acid to pH 6.5.

Internal standard (IS) solution: Add 50 mg 1-octanol (chromatography grade) to 250 ml tetrahydrofuran (THF).

Reference solution (25 mg/kg 1-decanol): Dissolve 75.0 mg 1-decanol (chromatography grade) in 100 ml IS solution. Transfer 200 :l of this stock solution into a 20-ml volumetric flask and fill with IS solution to the mark.

Sample solution: Dissolve 750 mg of sample and 50 mg of glucoamylase, EC 3.2.1.3, (e.g.,Gluczyme 8000, available from Wacker Chemie, Munich, Germany) in 7 ml of a 10 mM Tris-buffer (pH 6.5). Add 100 :l IS solution and 50 :l cyclodextrin glucosyltransferase preparation (500 U/ml) (Wacker Chemie, Munich, Germany). Close tightly, mix and incubate in a shaking water bath at 40° for 4 hours.
Condition an extraction column (Isolute C18 (10 ml) – ICT, Bad Homburg, Germany - or similar) by washing with methanol (2x10 ml) and water (4x10 ml). Pass the incubated solution slowly through the column and wash with water (2x10 ml). Gently pass nitrogen gas through the column to dry it (10 min). Apply 2.5 ml of THF to the column and let stand for 5 minutes. Then, elute the sample solution slowly.

Gas chromatography:
Column - Hewlett Packard HP-1 (25 m x 0.32 mm), 0.5:m FD
Carrier gas - helium (flow rate: 1 ml/min)
Detector - flame ionization

Temperatures - injection port: 265°; column: initial 60° (1 min isotherm); heating rate 20°/min; final 300° (7 min isotherm)
Injection volume: 1 µl
Measure the areas of the 1-decanol and 1-octanol peaks in the reference solution and sample solution.

Calculation: The concentration of 1-decanol (mg/kg) in the sample is:

$$C_{dec} = (C_{dec})_R \times (A_{dec}/A_{oct})_S / (A_{dec}/A_{oct})_R$$

where $(C_{dec})_R$ is the concentration of 1-decanol (mg/kg) of the reference solution; and $(A_{dec}/A_{oct})_S$ and $(A_{dec}/A_{oct})_R$ are the peak area ratios for 1-decanol to 1-octanol in the sample solution and reference solution, respectively.

Reducing substances

Note: Reducing substances are determined as dextrose. Dextrose levels are usually lower when determined by the following procedure in the presence of α-cyclodextrin compared to levels determined in its absence. Therefore, α-cyclodextrin reference standard is included in the procedure for the construction of the calibration curve.

Reagent solution: Weigh 10.0 g 3,5-dinitrosalicylic acid in a 1000-ml volumetric flask. Add 80 ml water and dissolve the 3,5-dinitrosalicylic acid by heating in a water bath. Prepare a solution of 16.0 g sodium hydroxide in 200 ml water and a solution of 300 g sodium potassium tartrate in 500 ml water. Transfer both solutions to the 1000-ml flask. Fill with water to the mark, shake the flask and let it stand for 24 hours. Filter (paper) the reagent solution prior to use if a precipitate appears.

Reference solution: Weigh accurately 1.0 g dextrose (anhydrous) in a 100-ml volumetric flask and fill with water to the mark.

Test solution: Weigh accurately 10.0 g of test sample into a 100-ml volumetric flask, add 80-90 ml water and dissolve the test sample (ultra-sonification bath, 15 minutes, 30°). Fill with water to the mark.

Calibration solutions: Weigh 1.0 g of reference α-cyclodextrin (available from Consortium für Elektrochemische Industrie GmbH, München, Germany or Wacker Biochem Group, Adrian, MI, USA) into each of eleven 10-ml volumetric flasks (numbered 0 to 10). Add 0, 0.1, 0.2, ..., 1.0 ml of reference solution to flasks nos. 0, 1, ... to 10, respectively. Fill all flasks with water to the mark.

Calibration curve: Assemble a set of eleven 10-ml volumetric flasks. Transfer 1 ml of each of the eleven calibration solutions into the flasks and add 1 ml of reagent solution to each flask. Heat each flask in the boiling water bath for 10 minutes. Cool rapidly to room temperature and fill with water to the mark. For each solution, measure the absorbance against water at 545 nm. Plot the data as absorbance vs dextrose concentration (mg/ml).

Analysis: Prepare a set of six 10-ml volumetric flasks (labeled a through f) and add 1 ml of the reagent solution to each. Transfer 1 ml of the test solution to flasks a, b, and c. Transfer 1 ml of the calibration solutions numbered 0, 3, and 6 to flasks d, e, and f. Thoroughly mix the contents of each flask and place in a boiling water bath for 10 minutes. Then, cool the flasks to room temperature, fill to the mark with water, and measure absorbance of the solutions against water at 545 nm.

Evaluation and calculation: The result is only valid if the absorbances of the solutions in flasks d-f do not deviate more than 5% from the calibration curve. Determine the reducing substance concentrations (mg/ml) of the solutions in

flasks a-c and calculate their mean, C_{RS}.
Then,

$$W_{RS} = 10\ C_{RS}$$

and

$$\% \text{ reducing substances} = 100 \times W_{RS}/0.01W_{TS}$$

where W_{RS} is the mean weight (mg) of reducing substance (as dextrose), determined from the absorbance readings, and W_{TS} is the weight (mg) of the test sample used to prepare the test solution.

METHOD OF ASSAY

Determine by liquid chromatography (FNP 5) using the following conditions:

Sample solution: Weigh accurately about 100 mg of test sample into a 10-ml volumetric flask and add 8 ml of deionized water. Dissolve the sample completely using an ultra-sonification bath (10-15 min) and dilute to the mark with purified deionized water. Filter through a 0.45-micrometer filter.

Reference solution: Weigh accurately about 100 mg of reference α-cyclodextrin into a 10-ml volumetric flask and add 8 ml of deionized water. Dissolve the sample completely using an ultra-sonification bath (10-15 min) and dilute to the mark with purified deionized water.

Chromatography: Liquid chromatograph equipped with a refractive index detector and an integrating recorder.
Column and packing: Nucleosil-100-NH2 (10 µm) (Machery & Nagel Co., Düren, Germany) or similar
- length: 250 mm
- diameter: 4 mm
- temperature: 40°

Mobile phase: acetonitrile/water (67/33, v/v)
Flow rate: 2.0 ml/min
Injection volume: 10 µl

Procedure: Inject the sample solution into the chromatograph, record the chromatogram, and measure the area of the α-cyclodextrin peak. Repeat for the reference solution. Calculate the percentage of α-cyclodextrin in the test sample as follows:

$$\% \text{ α-cyclodextrin (dry basis)} = 100 \times (A_S/A_R)(W_R/W_S)$$

where:

A_S and A_R are the areas of the peaks due to α-cyclodextrin for the sample solution and reference solution, respectively.
W_S and W_R are the weights (mg) of the test sample and reference α-cyclodextrin, respectively, after correcting for water content.

Annex 2

Analytical data of five batches of α-CD

Table 1 **Analytical data of five batches of α-CD**

Parameter[a]	Specifications	Batch No.				
		60P159[b]	60P160[b]	60P162[b]	60P163[b]	60P300[c]
Assay (%)	≥ 98	98.7	101.0	99.6	100.7	98.4
Loss on drying (%)	≤ 11	9.1	8.7	8.9	8.7	5.9
Reducing substances (%)	≤ 0.5	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*
Sulfated ash (%)	< 0.1	< 0.1*	< 0.1*	< 0.1*	< 0.1*	0.02
Volatile organics (ppm)	< 20	< 10	10.8	15	14	10
Heavy metals (ppm)	< 5	< 5*	< 5*	< 5*	< 5*	< 5*
Lead (ppm)	< 1	< 1*	< 1*	< 1*	< 1*	< 0.2*[f]
Optical density[d]	< 0.1	0.006	0.001	0.006	0.04	0.07
Specific rotation $[\alpha]_D^{20}$	+148 ±3	+150.0	+150.4	+150.5	+150.6	+149.0
Microorganisms (CFU/g)	<1000	< 40	< 40	< 40	< 40	< 10
Salmonella, E. coli (CFU in 10 g)	0	0	0	0	0	0

* Limit of detection
[a] as defined in "Specifications" (see Annex 1)
[b] Pilot scale
[c] Production scale
[d] 10% aqueous solution at 420 nm (1cm cuvette)
[e] 1% aqueous solution
[f] Analyses of three further lots of α-CD (60P301, 60P302, 60P303) also gave values below the limit of detection (0.2 ppm)

Annex 3

Critical control points and control standards of the production process

Table 1 Process controls for the manufacturing of alpha-cyclodextrin

Production step	In-Process control	Lower limit	Upper limit
Liquefied starch	DE-value	5.5	7.5
	Dry substance	31%	34%
	Weight	48000 lbs	52000 lbs
Enzyme addition	Weight	4 KU/kg starch	6 KU/kg starch
Complexing agent addition	Volume	95 g/kg starch	110 g/kg starch
Enzymatic reaction	Temperature	35° C	45° C
	pH	6.0	7.0
Complex separation	Temperature	35° C	45° C
	Bowl speed	Dependent on flow	Dependent on flow
	Scroll speed	Dependent on flow	Dependent on flow
Complex purification	Temperature	110° C	130° C
Complex separation	Temperature	45° C	75° C
	Bowl speed	Dependent on flow	Dependent on flow
	Scroll speed	Dependent on flow	Dependent on flow
Product stripping, evaporation	Temperature	100° C	130° C
	Solvent content		20 ppm
Crystallization	Temperature	15° C	40° C
Crystal filtration	Moisture		26%
Drying	Moisture		11%
	Temperature		90° C
Packaging	Weight	Dependent on size of container	Dependent on size of container

Table 2 Process controls for the manufacturing of alpha-cyclodextrin-glycosyltransferase

Production step	In-Process control	Lower limit	Upper limit
Fermentation	Temperature	25° C	30° C
	pH-value	6.9	7.1
	Dissolved oxygen	8%	21%
	The identity of the production strain is regularly checked with indicator plates		
Down-stream processing	Temperature	10° C	20° C
	pH-value	6	7
	Aerobic microroganisms		1000/ml

Annex 4

Protein and DNA content of a -CD

GENETIC ID

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Fairfield, iA 52557 USA
Phone: +1.641.472.9979
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Cover Notes to GMO Analysis Reports

Issued: **10/10/2001**
Order Num.: **78450**
Page 1

Wacker BioChem Corp

Attention: **Dr. Stefan Neumann**
Fax: 641- 969- 4929

These cover notes summarize the results for 1 GMO tests for samples received for analysis on 8/7/2001. Individual GMO Analytical Reports for each sample listed follow separately.

We thank you for the opportunity to serve you.

Dear Dr. Stefan Neumann, we are pleased to be able to tell you that we were able to remove inhibition and provide definite results. Please indicate if the sample ID information and customer information is as you want it. We also need to know where to send the invoice. Warm regards, Felicity

GID Sample Code / Customer Sample ID	Result(s)	Comments
010701 H02 60P300 Alfa CD	No recombinant E.coli DNA detected in the sample. No inhibition observed in the sample. (In the second advanced preparation) The recombinant E.Coli DNA was detectable in a dilution series down to 0.005ng/reaction. This corresponds, in a standard GMO test, to a limit of detection of 0.01% GMO.	

501 Dimick Drive
Fairfield, IA 52557 USA
Phone: +1.515.472.9979
www.genetic-id.com

GMO Analysis Report

Laboratory Analysis Performed For

Wacker BioChem Corp
1 Wacker Dr., Eddyville, IA, 52553 USA

Customer Sample Code: **60P300 Alfa CD**

Genetic ID Code: **010701 H02**

Date Order Received: **08/07/2001**

DateTested: **10/10/2001**

Sample Description: **Alfa cyclodextrin**

Gross Weight:

Method: ***PCR ANALYSIS***

Result: No recombinant E.coli DNA detected in the sample.

No inhibition observed in the sample. (in the second advanced preparation)

The recombinant E.Coli DNA was detectable in a dilution series down to 0.005ng/reaction. This corresponds, in a standard GMO test, to a limit of detection of 0.01% GMO.

Authorized By: Felicity Spaulding
Account Manager

Issued: **10/10/2001**

Disclaimer: This test is valid strictly for the sample submitted for analysis to the Genetic ID Laboratory. The test report shall not be construed as Genetic-ID Non-GMO certification of any product.

Dr. Friedrich Lottspeich MPI für Biochemie D-82152 Martinsried,Germany

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D-81379 München

7.7.02

Gel electrophoresis of Cavamax W6 Pharma

Method

Sample: Cavamax W6 Pharma, Batch Number 60P300

Stock solution: Cavamax W6 Pharma, Batch Number 60P300 was dissolved in water to a concentration of 100mg/mL water

Analysis date 3.7.2002

20µl of the stock solution was analysed in a 12% SDS polyacrylamide gel and silver stained

Results

No protein band could be detected. The detection limit of the method with the conditions applied is about 10 ng protein

Conclusion

In Cavamax W6 Pharma, Batch Number 60P300 no protein could be detected by SDS polyacrylamide gel electrophoresis. The detection limit of the method under the conditions used is 5 mg protein in 1 kg of Cavamax W6 Pharma.

Dr. Friedrich Lottspeich
7.7.2002

Dr. Friedrich Lottspeich MPI für Biochemie D-82152 Martinsried,Germany

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7.7.02

Gel electrophoresis of Cavamax W6 Pharma

Method

Sample: Cavamax W6 Pharma, Batch Number 60P301

Stock solution: Cavamax W6 Pharma, Batch Number 60P301 was dissolved in water to a concentration of 100mg/mL water

Analysis date 3.7.2002

20µl of the stock solution was analysed in a 12% SDS polyacrylamide gel and silver stained

Results

No protein band could be detected. The detection limit of the method with the conditions applied is about 10 ng protein

Conclusion

In Cavamax W6 Pharma, Batch Number 60P301 no protein could be detected by SDS polyacrylamide gel electrophoresis. The detection limit of the method under the conditions used is 5 mg protein in 1 kg of Cavamax W6 Pharma.

Dr. Friedrich Lottspeich
7.7.2002

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7.7.02

Gel electrophoresis of Cavamax W6 Pharma

Method

Sample: Cavamax W6 Pharma, Batch Number 60P302

Stock solution: Cavamax W6 Pharma, Batch Number 60P302 was dissolved in water to a concentration of 100mg/mL water

Analysis date 3.7.2002

20µl of the stock solution was analysed in a 12% SDS polyacrylamide gel and silver stained

Results

No protein band could be detected. The detection limit of the method with the conditions applied is about 10 ng protein

Conclusion

In Cavamax W6 Pharma, Batch Number 60P302 no protein could be detected by SDS polyacrylamide gel electrophoresis. The detection limit of the method under the conditions used is 5 mg protein in 1 kg of Cavamax W6 Pharma.

Dr. Friedrich Lottspeich
7.7.2002

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Proteinanalytik

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DURCHWAHL 85 78- 3964
TELEFAX (089) 85 78-28 02

Gel electrophoresis of Cavamax W6 Pharma

Method

Sample: Cavamax W6 Pharma, Batch Number 60P300

Stock solution: Cavamax W6 Pharma, Batch Number 60P300 was dissolved in water to a concentration of 100mg/mL water

Analysis date 6.6.2001

20µl of the stock solution was analysed in a 12% SDS polyacrylamide gel and silver stained

Results

No protein band could be detected. The detection limit of the method with the conditions applied is about 10 ng protein

Conclusion

In Cavamax W6 Pharma, Batch Number 60P300 no protein could be detected by SDS polyacrylamide gel electrophoresis. The detection limit of the method under the conditions used is 5 mg protein in 1 kg of Cavamax W6 Pharma.

Dr. Friedrich Lottspeich
7.6.2001

MAX-PLANCK-INSTITUT
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Proteinanalytik

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Amino acid analysis of Cavamax W6 Pharma

Method

Sample: Cavamax W6 Pharma, Batch Number 60P300

6 stock solutions were individually prepared and dissolved in water to a concentration of 80 mg/mL water

From each of the 6 stock solutions

a) 1 sample was prepared by taking 90µl of the stock solution and adding 10µl diaminopimelic acid (1mg/mL) as internal standard and directly analysed
b) 1 sample was prepared by taking 90µl of the stock solution and adding 10µl diaminopimelic acid (1mg/mL) as internal standard, hydrolysed with 6N HCl and analysed.

Analyses were performed on a Beckman 6300 amino acid analyser, using the conditions recommended by the manufacturer. The detection limit with the conditions used is for amino acids 20 pmol.

Results and conclusion

In the samples analysed no free amino acids could be detected. Therefore, direct amino acid analysis of Cavamax W6 Pharma, Batch Number 60P300 indicated that no free amino acids are present (detection limit: 20pmol/amino acid in 4 mg).

The analysis after hydrolysis is extremely difficult, since the hydrolysis procedure results in a bulky residue, which had to be extracted with small volumes of water and water/acetonitrile (50/50 v/v). Due to this handling procedure inevitably small amounts of contaminating amino acids are introduced to the sample. Almost exclusively the common contaminations like glycine serine, glutamic acid and aspartic acid were found. Considering the nature and variable amount of these amino acids found it can be concluded that the amino acids present are very probably simple contaminations introduced by the hydrolysis procedure and sample handling during extraction.

The complete absence of normal protein amino acids and considering the limit of detection of the method of 20 pmol/amino acid, proves that the maximal contamination of Cavamax W6 Pharma, Batch Number 60P300 with protein is below 10 mg protein per kg of Cavamax W6 Pharma, Batch Number 60P300

Dr. Friedrich Lottspeich
7.6.2001

Amino acid analyses of Cavamax W6 Pharma, Batch Number 60P300
without hydrolysis Raw data are given
injected 100 µl containing 7,2 mg Cavamax W6 Pharma, Batch Number 60P300

sample Nr.	1	2	3	4	5	6
Asx						
Thr						
Ser						
Glx						
Pro						
Gly						25,2
Ala						
Cys						
Val						
Met						
DAP	1277,3	1255,6	837,8 *	1303,8	1213,6	1272,7
Ile						
Leu						
Tyr						
Phe						
His						
Lys						
Arg						

*Missinjection

Amino acid analyses of Cavamax W6 Pharma, Batch Number 60P300
with hydrolysis Raw data are given
injected 100 µl containing 7,2 mg Cavamax W6 Pharma, Batch Number 60P300

sample Nr.	1	2	3	4	5	6
Asx			157,2			
Thr			68,5			
Ser	39,8	52,3	78,9	33,4		60,3
Glx	271,7	291,2	350,1	227	181,8	278,2
Pro						
Gly	325,1	559,4	319,3	216	179,9	362
Ala	154,3	175,6	146,4	73,7	63,6	149
Cys						
Val						
Met						
DAP	1009,6	1053,6	976,8	1083,8	1001,3	1007,8
Ile						
Leu	87,6	11,82	119,3	82	39,8	90,1
Tyr						
Phe						
His		9,3	15,4	15		18,8
Lys						
Arg						

DAP= Diaminopimelic acid (internal standard)

Annex 5

Projection of the α-CD intake by the dietary survey method

Methodology

The food consumption data used in the analyses of a -cyclodextrin intake (see attached Tables 1 through 12) are results of the 1994-96 USDA Continuing Survey of Food Intakes by Individuals (CSFII) and its Supplemental Children's Survey (CSFII 1998), as provided on CD-ROM (USDA, 2000). The CSFII 1994-96 was conducted between January 1994 and January 1997 with non-institutionalized individuals in the United States. In each of the three survey years, data were collected from a nationally representative sample of individuals of all ages. The CSFII 1998 was a survey of children ages 0 through 9 which was supplemental to the CSFII 1994-96. It used the same sample design as the CSFII 1994-96 and was intended to be merged with CSFII 1994-96 to increase the sample size for children. The merged surveys are designated as CSFII 1994-96, 1998. In the CSFII 1994-96, 1998, dietary intakes were collected through in-person interviews using 24-hour recalls on two nonconsecutive days approximately one week apart. A total of 21,662 individuals provided data for the first day; of those individuals, 20,607 provided data for a second day.

The population groups and number of survey respondents in each group are presented below:

Population Group	Number of Survey Respondents
Children 2 to 5 years	5,437
Children 6 to 12 years	2,089
Teenagers 13 to 19 years	1,212
Adults 20 years and older	9,221
Population 2 years and older	17,959
Breastfeeding children and pregnant and/or lactating females were not included in the analyses.	

The CSFII food codes were reviewed and all food codes representative of the proposed a-cyclodextrin use categories were identified. If a food code included one of the proposed use category foods as an ingredient, the CSFII Recipe Database Files and the CSFII "Food Code-to-Commodity Translation File" was used to identify the relevant proportion. The Food Code-to-Commodity Translation File translates each CSFII food code into Environmental Protection Agency (EPA) commodity percentages. The file was developed in a joint effort by the United States Department of Agriculture (USDA) and EPA to allow estimation of human exposures to pesticide residues and environmental contaminants through intakes of foods and beverages (EPA, 2000).

For the estimation of α-CD intake from chewing gum, which is not covered by CSFII, data of a Market Facts mail panel survey were used (for details see Table 13, foot-notes).

References:

Environmental Protection Agency. 2000. Food Commodity Intake Database (FCID) [CD-ROM], data and documentation. National Technical Information Service, Accession No. PB2000-500101.

U.S. Department of Agriculture, Agricultural Research Service. 2000. 1994-96, 1998 Continuing Survey of Food Intakes by Individuals [CD-ROM], data and documentation. National Technical Information Service, Accession No. PB2000-500027.

Table 1. Proposed Uses of a-Cyclodextrin

Food Category	Serving size	Maximum a-CD concentration in final food
1. Bakery products		
Breads and rolls yeast breads and rolls, also including hamburger and hotdog buns, yeast sweet rolls and coffee cakes	50 g	5%
Brownies	40 g	7%
Cakes (light weight) angel food, sponge cake	55 g	5%
Crackers (used as snacks) sweet and non-sweet crackers	30 g	10%
Grain-based bars breakfast bars, granola bars, rice cereal bars	40 g	7%
Coffee cakes, crumb cakes, muffins, biscuits, corn bread	55 g	5%
Refrigerated dough	-	5%
Dry baking mixes	-	5%
2. Beverages		
Dry mixes for beverages dry powder fruit-flavored beverage mixes	-	1%[1)]
Carbonated and noncarbonated sugarfree (diet) soft drinks	240 ml	1%
Fruit juices and juice drinks fruit juice, juice drinks, nectars, and fruit-milk drinks	240 ml	1%
Vegetable juice	240 ml	2%
Formula diets	240 ml	1%

meal replacements and nutritional supplements		
Soy and non-soy (imitation) milk	240 ml	2%
Instant coffee and tea	-	1%
Coffee whiteners powdered cream substitutes and coffee mixes containing whiteners	-	1%

3. Cereal and other grain products

Breakfast cereals, ready-to-eat		
<20 g/cup	15 g	2%
20-43 g/cup	30 g	9%
>=43 g/cup and biscuit-types	55 g	5%
Instant rice	140 g	2%[2)]
Pastas, including macaroni products and noodles	140 g	2%[3)]

4. Condiments

Condiments catsup, mustard	1 tsp - 1 tbsp	3%

5. Dairy products

Yogurt yogurt, yogurt beverages	225 g	2.5%
Dry mixes for pudding	-	1%
Dry mixes for milk-based beverages		2.5%
Frozen dairy desserts, reduced fat light ice creams, frozen yogurt, and related novelties	1/2 cup	2.5%

6. Fats and oils

Margarine, yellow-fat bread spreads (reduced- or fat-free)	1 tbsp	20%
Salad dressings and mayonnaise salad dressings, mayonnaise, sandwich spreads, mayonnaise-type dressings	15-30 g	5%

7. Snack Foods

Snacks chips, pretzels, popcorns, extruded snacks, grain-based snack mixes	30 g	1%

8. Soups

Canned soups	245 g	2%
Dry mixes for soups dry bouillon, instant soups	-	2%

9. Sugars and sweets

Hard candies, compressed candies, breath mints	2.5 - 15 g	15%

[1)] Assuming 20 g powder mix is used to prepare 240 ml beverage, the a-CD concentration in the powder mix is 12%.

[2)] Corresponding to 6.2% in dry rice (45 g dry rice yields 1 serving)

[3)] Corresponding to 5.1% in dry pasta (55 g dry pasta yields 1 serving)

Table 2. Estimated 2-Day Average Intake of a-Cyclodextrin by Consumers of Proposed Uses						
Population group and Food cateogry	Users		2-Day Average Intake per User			
			g per day		g per kgBW per day	
	Number	% of Population	Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old						
Bakery products	5200	96	3.1	5.6	0.18	0.34
Beverages	4798	88	3.2	6.1	0.20	0.38
Cereal/grain products	4791	88	2.7	5.2	0.16	0.32
Condiments	2453	45	0.3	0.7	0.02	0.04
Dairy products	1432	25	2.3	5.5	0.14	0.31
Fats and oils	2910	53	0.7	1.6	0.04	0.10
Snack foods	2852	53	0.2	0.5	0.01	0.03
Soups	1423	26	2.6	5.0	0.16	0.32
Sugars and sweets	487	9	1.5	3.2	0.09	0.17
All categories combined	5435	100	10.2	16.2	0.61	0.98
Children 6 to 12 years old						
Bakery products	2023	97	4.0	7.1	0.13	0.25
Beverages	1692	80	3.3	6.4	0.11	0.21
Cereal/grain products	1707	82	3.5	6.7	0.11	0.23
Condiments	1003	48	0.3	0.7	0.01	0.02
Dairy products	442	22	2.7	6.2	0.09	0.20
Fats and oils	1224	58	0.9	2.1	0.03	0.07
Snack foods	1206	57	0.3	0.6	0.01	0.02
Soups	451	22	3.2	6.8	0.10	0.22
Sugars and sweets	240	12	2.1	4.3	0.07	0.13
All categories combined	2089	100	11.8	18.7	0.38	0.65
Teenagers 13 to 19 years old						
Bakery products	1139	93	4.5	8.4	0.07	0.14
Beverages	896	74	4.2	8.9	0.07	0.14
Cereal/grain products	785	66	3.8	7.6	0.06	0.13
Condiments	595	49	0.5	1.0	0.01	0.02
Dairy products	164	13	3.1	6.2	0.05	0.09
Fats and oils	750	62	1.2	2.9	0.02	0.04
Snack foods	601	49	0.4	0.8	0.01	0.01
Soups	277	23	3.7	7.0	0.06	0.12
Sugars and sweets	88	6	2.7	5.0	0.05	0.11
All categories combined	1211	100	12.4	21.4	0.20	0.36

Table 2. Estimated 2-Day Average Intake of a-Cyclodextrin by Consumers of Proposed Uses						
Population group and Food cateogry	Users		2-Day Average Intake per User			
			g per day		g per kgBW per day	
	Number	% of Population	Mean	90th Percentile	Mean	90th Percentile
Adults 20 years and older						
Bakery products	8777	95	4.4	8.0	0.06	0.11
Beverages	6800	74	3.4	7.8	0.05	0.11
Cereal/grain products	5370	59	3.0	5.9	0.04	0.08
Condiments	2998	35	0.3	0.7	< 0.005	0.01
Dairy products	1675	18	3.0	6.2	0.04	0.09
Fats and oils	6625	72	1.4	3.1	0.02	0.04
Snack foods	3068	36	0.3	0.7	< 0.005	0.01
Soups	2524	28	3.5	7.4	0.05	0.11
Sugars and sweets	239	3	1.6	3.8	0.02	0.05
All categories combined	9189	100	11.3	20.2	0.16	0.28
Population 2 years and older						
Bakery products	17139	95	4.3	7.8	0.08	0.15
Beverages	14186	76	3.5	7.5	0.07	0.16
Cereal/grain products	12653	64	3.1	6.2	0.06	0.14
Condiments	7049	39	0.3	0.7	0.01	0.01
Dairy products	3713	18	2.9	6.1	0.06	0.12
Fats and oils	11509	68	1.3	2.9	0.02	0.05
Snack foods	7727	41	0.3	0.7	0.01	0.01
Soups	4675	27	3.5	7.3	0.06	0.14
Sugars and sweets	1054	5	1.9	4.1	0.05	0.11
All categories combined	17924	100	11.4	19.8	0.21	0.43

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent 2-Day average intakes per user generated using USDA sampling weights. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 3. Estimated Intake of a-Cyclodextrin from Proposed Uses in Bakery Products per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	3243	1.7	3.0	0.10	0.19
Lunch/brunch	5963	2.0	2.9	0.12	0.19
Dinner	2562	1.5	2.7	0.09	0.17
Supper	1650	1.7	2.9	0.10	0.19
Snack	3779	2.1	4.0	0.12	0.23
Unique eating occasion	17364	1.9	3.0	0.11	0.20
Children 6 to 12 years old					
Breakfast	1216	2.3	4.5	0.07	0.14
Lunch/brunch	2474	2.5	4.3	0.08	0.14
Dinner	1021	2.3	4.3	0.07	0.13
Supper	728	2.3	4.3	0.07	0.14
Snack	1158	2.7	5.2	0.09	0.16
Unique eating occasion	6679	2.4	4.3	0.08	0.14
Teenagers 13 to 19 years old					
Breakfast	628	3.0	5.4	0.05	0.09
Lunch/brunch	1138	3.1	5.2	0.05	0.09
Dinner	572	3.1	5.6	0.05	0.09
Supper	418	3.0	5.2	0.05	0.08
Snack	531	3.1	5.9	0.05	0.09
Unique eating occasion	3330	3.0	5.3	0.05	0.09
Adults 20 years and older					
Breakfast	7175	2.6	4.4	0.04	0.06
Lunch/brunch	8823	2.9	5.0	0.04	0.07
Dinner	4610	2.8	5.2	0.04	0.07
Supper	3677	2.8	5.1	0.04	0.07
Snack	3306	2.8	5.1	0.04	0.07
Unique eating occasion	27896	2.8	4.8	0.04	0.07
Population 2 years and older					
Breakfast	12262	2.6	4.4	0.04	0.08
Lunch/brunch	18398	2.8	4.8	0.05	0.10
Dinner	8765	2.7	5.0	0.05	0.09
Supper	6473	2.7	4.9	0.04	0.09
Snack	8774	2.8	5.2	0.06	0.11
Unique eating occasion	55269	2.7	4.7	0.05	0.09

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 4. Estimated Intake of a-Cyclodextrin from Proposed Uses in Beverages per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	3476	2.0	2.5	0.12	0.19
Lunch/brunch	3142	2.1	3.1	0.13	0.20
Dinner	1895	2.1	3.1	0.13	0.21
Supper	970	2.2	3.7	0.13	0.22
Snack	4676	2.0	2.6	0.12	0.20
Unique eating occasion	14628	2.1	3.1	0.13	0.21
Children 6 to 12 years old					
Breakfast	1146	2.3	3.7	0.07	0.12
Lunch/brunch	1022	2.5	3.8	0.08	0.14
Dinner	654	2.7	4.8	0.08	0.15
Supper	363	3.0	5.0	0.09	0.16
Snack	1118	2.5	4.7	0.08	0.14
Unique eating occasion	4395	2.6	4.8	0.08	0.14
Teenagers 13 to 19 years old					
Breakfast	559	3.2	5.0	0.05	0.09
Lunch/brunch	442	3.2	5.0	0.05	0.09
Dinner	288	3.5	6.3	0.06	0.11
Supper	145	3.9	7.8	0.06	0.11
Snack	580	3.7	7.2	0.06	0.11
Unique eating occasion	2080	3.6	6.2	0.06	0.10
Adults 20 years and older					
Breakfast	6092	1.8	3.7	0.03	0.05
Lunch/brunch	3307	2.9	5.0	0.04	0.08
Dinner	1996	2.8	5.3	0.04	0.08
Supper	1383	2.7	5.0	0.04	0.07
Snack	4756	2.5	4.8	0.03	0.07
Unique eating occasion	18272	2.5	5.0	0.03	0.07
Population 2 years and older					
Breakfast	11273	2.0	3.7	0.04	0.09
Lunch/brunch	7913	2.8	5.0	0.05	0.12
Dinner	4833	2.8	5.0	0.05	0.12
Supper	2861	2.8	5.0	0.05	0.12
Snack	11130	2.6	5.0	0.05	0.11
Unique eating occasion	39375	2.6	5.0	0.05	0.11

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 5. Estimated Intake of a-Cyclodextrin from Proposed Uses in Cereal and other Grain Products per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	5202	3.0	5.4	0.18	0.30
Lunch/brunch	1557	1.9	3.9	0.11	0.24
Dinner	1746	2.0	4.2	0.12	0.24
Supper	935	2.0	4.0	0.12	0.23
Snack	1027	2.1	3.9	0.13	0.23
Unique eating occasion	10537	2.5	4.6	0.15	0.27
Children 6 to 12 years old					
Breakfast	1867	4.1	6.7	0.13	0.23
Lunch/brunch	349	2.4	5.0	0.08	0.17
Dinner	604	3.0	5.6	0.10	0.20
Supper	323	2.7	5.6	0.09	0.18
Snack	303	3.4	6.1	0.11	0.19
Unique eating occasion	3458	3.5	6.3	0.11	0.22
Teenagers 13 to 19 years old					
Breakfast	614	5.2	8.1	0.09	0.14
Lunch/brunch	163	3.6	8.4	0.06	0.13
Dinner	269	3.9	7.7	0.06	0.13
Supper	157	3.4	6.3	0.05	0.10
Snack	117	4.4	8.6	0.08	0.14
Unique eating occasion	1325	4.4	7.9	0.07	0.13
Adults 20 years and older					
Breakfast	3852	4.1	6.8	0.06	0.09
Lunch/brunch	1249	3.0	6.1	0.04	0.09
Dinner	1951	3.5	7.0	0.05	0.10
Supper	1215	3.3	6.4	0.04	0.09
Snack	498	3.8	7.5	0.05	0.10
Unique eating occasion	8798	3.7	6.8	0.05	0.09
Population 2 years and older					
Breakfast	11535	4.1	6.8	0.09	0.18
Lunch/brunch	3318	2.9	5.8	0.06	0.12
Dinner	4570	3.3	6.5	0.06	0.13
Supper	2630	3.1	6.3	0.06	0.11
Snack	1945	3.6	6.7	0.08	0.16
Unique eating occasion	24118	3.6	6.5	0.07	0.15

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 6. Estimated Intake of a-Cyclodextrin from Proposed Uses in Condiments per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	170	0.3	0.5	0.02	0.03
Lunch/brunch	1596	0.4	0.9	0.02	0.05
Dinner	886	0.4	0.9	0.03	0.06
Supper	544	0.5	0.9	0.03	0.06
Snack	185	0.3	0.7	0.02	0.05
Unique eating occasion	3397	0.4	0.9	0.02	0.05
Children 6 to 12 years old					
Breakfast	46	0.4	0.9	0.01	0.02
Lunch/brunch	694	0.4	0.9	0.01	0.03
Dinner	358	0.6	1.4	0.02	0.04
Supper	234	0.5	1.2	0.02	0.03
Snack	92	0.3	0.5	0.01	0.02
Unique eating occasion	1436	0.5	0.9	0.01	0.03
Teenagers 13 to 19 years old					
Breakfast	38	0.6	1.8	0.01	0.02
Lunch/brunch	413	0.7	1.8	0.01	0.03
Dinner	174	0.7	1.4	0.01	0.02
Supper	150	0.6	1.4	0.01	0.02
Snack	60	0.6	0.9	0.01	0.02
Unique eating occasion	846	0.7	1.8	0.01	0.02
Adults 20 years and older					
Breakfast	171	0.5	0.9	0.01	0.01
Lunch/brunch	2052	0.4	0.9	0.01	0.01
Dinner	840	0.6	1.2	0.01	0.02
Supper	661	0.4	0.9	0.01	0.01
Snack	208	0.4	0.9	0.01	0.01
Unique eating occasion	3954	0.4	0.9	0.01	0.01
Population 2 years and older					
Breakfast	425	0.5	0.9	0.01	0.02
Lunch/brunch	4755	0.4	0.9	0.01	0.02
Dinner	2258	0.6	1.4	0.01	0.03
Supper	1589	0.5	0.9	0.01	0.02
Snack	545	0.4	0.9	0.01	0.02
Unique eating occasion	9633	0.5	0.9	0.01	0.02

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 7. Estimated Intake of a-Cyclodextrin from Proposed Uses in Dairy Products per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	419	4.0	7.8	0.24	0.46
Lunch/brunch	324	2.8	6.1	0.17	0.33
Dinner	202	2.7	6.1	0.17	0.33
Supper	82	2.5	5.7	0.15	0.32
Snack	1039	3.0	6.3	0.18	0.38
Unique eating occasion	2110	3.2	6.3	0.19	0.39
Children 6 to 12 years old					
Breakfast	121	5.2	9.4	0.17	0.31
Lunch/brunch	113	3.0	6.1	0.10	0.24
Dinner	62	3.2	6.3	0.10	0.17
Supper	29	3.1	7.5	0.10	0.21
Snack	270	4.3	7.8	0.14	0.26
Unique eating occasion	600	4.1	7.8	0.13	0.26
Teenagers 13 to 19 years old					
Breakfast	34	6.3	12.5	0.10	0.16
Lunch/brunch	40	5.2	8.1	0.09	0.15
Dinner	20	3.7	6.3	0.06	0.11
Supper	14	4.7	8.8	0.07	0.17
Snack	90	5.1	8.5	0.08	0.14
Unique eating occasion	199	5.2	8.8	0.08	0.15
Adults 20 years and older					
Breakfast	380	5.1	9.2	0.08	0.12
Lunch/brunch	450	4.2	6.3	0.06	0.10
Dinner	309	3.2	6.6	0.05	0.10
Supper	216	3.7	8.0	0.05	0.10
Snack	946	4.5	7.8	0.06	0.11
Unique eating occasion	2342	4.4	7.8	0.06	0.11
Population 2 years and older					
Breakfast	954	5.1	9.2	0.10	0.20
Lunch/brunch	927	4.0	6.3	0.08	0.16
Dinner	593	3.2	6.3	0.06	0.14
Supper	341	3.7	7.8	0.06	0.15
Snack	2345	4.3	7.8	0.09	0.17
Unique eating occasion	5251	4.3	7.7	0.08	0.17

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 8. Estimated Intake of a-Cyclodextrin from Proposed Uses in Fats and Oils per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	902	1.1	2.9	0.07	0.14
Lunch/brunch	1734	0.7	1.5	0.04	0.09
Dinner	1133	0.9	2.0	0.05	0.12
Supper	656	1.0	2.1	0.06	0.12
Snack	285	0.7	1.7	0.04	0.10
Unique eating occasion	4737	0.9	1.9	0.05	0.12
Children 6 to 12 years old					
Breakfast	318	1.5	2.9	0.05	0.11
Lunch/brunch	763	0.8	1.9	0.03	0.06
Dinner	506	1.3	2.9	0.04	0.08
Supper	288	1.3	2.9	0.04	0.10
Snack	124	0.9	1.9	0.03	0.07
Unique eating occasion	2013	1.1	2.8	0.04	0.08
Teenagers 13 to 19 years old					
Breakfast	139	1.6	2.9	0.03	0.05
Lunch/brunch	491	1.3	3.1	0.02	0.05
Dinner	332	1.7	3.9	0.03	0.06
Supper	190	1.4	2.9	0.02	0.05
Snack	99	1.4	3.3	0.02	0.05
Unique eating occasion	1260	1.5	3.1	0.02	0.05
Adults 20 years and older					
Breakfast	1766	1.7	2.9	0.02	0.05
Lunch/brunch	4851	1.2	2.8	0.02	0.04
Dinner	3356	1.8	3.5	0.02	0.05
Supper	2178	1.7	3.3	0.02	0.05
Snack	557	1.3	2.9	0.02	0.05
Unique eating occasion	12809	1.5	3.0	0.02	0.04
Population 2 years and older					
Breakfast	3125	1.6	2.9	0.03	0.06
Lunch/brunch	7839	1.1	2.8	0.02	0.04
Dinner	5327	1.7	3.3	0.03	0.06
Supper	3312	1.6	3.1	0.03	0.05
Snack	1065	1.2	2.9	0.02	0.05
Unique eating occasion	20819	1.4	2.9	0.02	0.05

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 9. Estimated Intake of a-Cyclodextrin from Proposed Uses in Snack Foods per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	21	0.3	0.4	0.02	0.03
Lunch/brunch	1306	0.2	0.4	0.01	0.03
Dinner	290	0.2	0.4	0.01	0.02
Supper	229	0.2	0.4	0.01	0.02
Snack	2449	0.3	0.6	0.02	0.03
Unique eating occasion	4338	0.3	0.5	0.02	0.03
Children 6 to 12 years old					
Breakfast	16	0.3	0.4	0.01	0.01
Lunch/brunch	640	0.3	0.5	0.01	0.02
Dinner	129	0.4	0.6	0.01	0.02
Supper	113	0.3	0.6	0.01	0.02
Snack	966	0.4	0.8	0.01	0.02
Unique eating occasion	1883	0.4	0.7	0.01	0.02
Teenagers 13 to 19 years old					
Breakfast	20	0.7	1.1	0.01	0.02
Lunch/brunch	285	0.4	0.9	0.01	0.02
Dinner	98	0.4	0.7	0.01	0.01
Supper	43	0.5	1.3	0.01	0.02
Snack	463	0.6	1.1	0.01	0.02
Unique eating occasion	918	0.5	1.1	0.01	0.02
Adults 20 years and older					
Breakfast	49	0.4	0.7	0.01	0.01
Lunch/brunch	1279	0.4	0.9	0.01	0.01
Dinner	385	0.4	0.8	0.01	0.01
Supper	361	0.4	0.7	< 0.005	0.01
Snack	2123	0.5	1.0	0.01	0.01
Unique eating occasion	4255	0.5	0.9	0.01	0.01
Population 2 years and older					
Breakfast	106	0.5	1.1	0.01	0.02
Lunch/brunch	3510	0.4	0.8	0.01	0.02
Dinner	902	0.4	0.8	0.01	0.01
Supper	746	0.4	0.7	0.01	0.01
Snack	6001	0.5	1.0	0.01	0.02
Unique eating occasion	11394	0.4	0.9	0.01	0.02

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 10. Estimated Intake of a-Cyclodextrin from Proposed Uses in Soups per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	74	4.4	8.2	0.29	0.52
Lunch/brunch	821	4.5	9.3	0.28	0.50
Dinner	533	3.5	8.5	0.21	0.47
Supper	233	3.8	7.5	0.22	0.43
Snack	148	4.3	9.9	0.25	0.47
Unique eating occasion	1832	4.1	9.3	0.25	0.49
Children 6 to 12 years old					
Breakfast	39	5.4	9.6	0.18	0.34
Lunch/brunch	160	6.5	11.9	0.21	0.41
Dinner	189	4.4	9.8	0.14	0.29
Supper	102	5.0	11.9	0.18	0.43
Snack	65	5.1	9.9	0.16	0.31
Unique eating occasion	561	5.2	9.9	0.17	0.35
Teenagers 13 to 19 years old					
Breakfast	20	7.3	11.9	0.13	0.21
Lunch/brunch	83	7.0	14.0	0.12	0.25
Dinner	120	6.1	12.2	0.10	0.20
Supper	56	5.4	9.9	0.08	0.18
Snack	52	6.0	10.1	0.10	0.17
Unique eating occasion	331	6.3	12.1	0.10	0.20
Adults 20 years and older					
Breakfast	90	6.7	12.2	0.10	0.19
Lunch/brunch	1287	6.0	10.1	0.09	0.17
Dinner	1008	5.3	10.1	0.08	0.17
Supper	590	5.2	11.2	0.07	0.16
Snack	173	4.3	9.8	0.06	0.14
Unique eating occasion	3174	5.5	10.3	0.08	0.17
Population 2 years and older					
Breakfast	223	6.4	10.6	0.13	0.24
Lunch/brunch	2351	5.9	10.1	0.11	0.23
Dinner	1850	5.2	10.1	0.09	0.19
Supper	981	5.1	10.8	0.09	0.20
Snack	438	4.7	9.9	0.09	0.22
Unique eating occasion	5898	5.5	10.1	0.10	0.21

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 11. Estimated Intake of a-Cyclodextrin from Proposed Uses in Sugars and Sweets per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	2	0.7	1.0	0.04	0.04
Lunch/brunch	29	2.4	3.2	0.14	0.22
Dinner	14	1.9	3.6	0.12	0.24
Supper	8	2.7	4.3	0.19	0.27
Snack	495	2.6	5.0	0.16	0.31
Unique eating occasion	562	2.6	4.5	0.16	0.31
Children 6 to 12 years old					
Breakfast	3	2.5	3.2	0.09	0.13
Lunch/brunch	32	3.7	8.5	0.13	0.28
Dinner	10	3.6	7.4	0.14	0.33
Supper	5	2.7	6.0	0.09	0.18
Snack	226	3.3	6.3	0.10	0.19
Unique eating occasion	285	3.3	7.5	0.11	0.23
Teenagers 13 to 19 years old					
Breakfast	0	---	---	---	---
Lunch/brunch	11	5.6	9.9	0.11	0.21
Dinner	2	2.3	3.9	0.04	0.07
Supper	2	2.4	4.0	0.05	0.08
Snack	83	4.7	7.8	0.08	0.15
Unique eating occasion	102	4.6	8.3	0.08	0.15
Adults 20 years and older					
Breakfast	4	4.7	7.5	0.06	0.12
Lunch/brunch	16	2.0	5.0	0.02	0.04
Dinner	8	5.1	9.0	0.07	0.10
Supper	17	3.0	7.5	0.03	0.08
Snack	227	2.4	5.1	0.03	0.07
Unique eating occasion	293	2.7	5.4	0.04	0.09
Population 2 years and older					
Breakfast	9	4.1	7.5	0.07	0.12
Lunch/brunch	88	3.2	8.5	0.08	0.22
Dinner	34	3.6	7.4	0.10	0.33
Supper	32	2.9	7.5	0.05	0.10
Snack	1031	3.0	6.3	0.08	0.16
Unique eating occasion	1242	3.1	7.2	0.08	0.17

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 12. Estimated Intake of a-Cyclodextrin from Proposed Uses in All Food Categories Combined per Eating Occasion

Population group and Eating occasion	Number of occasions	Intake per User			
		g per occasion		g per kgBW per occasion	
		Mean	90th Percentile	Mean	90th Percentile
Children 2 to 5 years old					
Breakfast	9105	3.4	6.2	0.20	0.38
Lunch/brunch	8801	3.2	6.2	0.19	0.37
Dinner	5399	2.8	5.7	0.17	0.35
Supper	2960	3.0	6.1	0.17	0.36
Snack	11691	2.2	4.5	0.13	0.27
Unique eating occasion	39009	2.9	5.6	0.17	0.34
Children 6 to 12 years old					
Breakfast	3305	4.3	7.7	0.14	0.25
Lunch/brunch	3240	3.8	7.1	0.12	0.24
Dinner	1990	3.9	7.9	0.12	0.25
Supper	1189	4.0	7.7	0.13	0.26
Snack	3580	2.7	5.9	0.09	0.18
Unique eating occasion	13616	3.6	7.2	0.12	0.24
Teenagers 13 to 19 years old					
Breakfast	1433	5.2	9.7	0.09	0.17
Lunch/brunch	1590	4.7	8.8	0.08	0.16
Dinner	1048	5.1	10.4	0.08	0.17
Supper	665	4.6	9.2	0.07	0.13
Snack	1718	3.4	7.5	0.06	0.12
Unique eating occasion	6610	4.5	9.2	0.07	0.15
Adults 20 years and older					
Breakfast	12583	4.0	7.8	0.06	0.11
Lunch/brunch	12014	4.6	9.1	0.06	0.13
Dinner	8077	4.7	9.9	0.07	0.14
Supper	5753	4.6	9.6	0.06	0.13
Snack	11046	2.7	5.7	0.04	0.08
Unique eating occasion	50931	4.0	8.3	0.06	0.11

Population 2 years and older					
Breakfast	26426	4.1	7.9	0.08	0.17
Lunch/brunch	25645	4.4	8.7	0.08	0.17
Dinner	16514	4.5	9.6	0.08	0.17
Supper	10567	4.4	9.2	0.08	0.16
Snack	28035	2.8	5.9	0.06	0.13
Unique eating occasion	110166	3.9	8.0	0.07	0.16

Data source: U.S. Department of Agriculture, 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII).
Notes: Estimates represent intakes per user per eating occasion generated using USDA sampling weights. Estimates of intake at "breakfast", "lunch/brunch", "dinner" and "supper" are based on all foods consumed at the respective meal (as identified by survey respondent) on each survey day. Estimates of intake per "snack" are based on each reported snacking occasion as specified by time of day and survey day. Estimates of intake per "unique eating occasion" are based on each reported intake of food as specified by time of day and survey day. Breastfeeding children, pregnant and/or lactating females, and individuals who provided only one 24-hour dietary recall were excluded from the analysis.

Table 13
Estimated Intake of α-CD by Consumers of Chewing Gum [a, b]

Population Group	Users		Mean Number of Pieces per User	Mean α-CD Intake per User [c]
	Number of Users	Percent of Survey Population		
Children: 2 to 5 years	77	39.1	2.1	0.63
Children: 6 to 12 years	122	45.7	2.9	0.87
Teenagers: 13 to 19 years	110	47.2	3.8	1.14
Adults: 20 years and older	522	29.8	2.9	0.87
Total population: 2 years and older	831	33.9	3.0	0.90

[a] Results based upon responses provided by 1044 households to a Market Facts mail panel survey conducted in 1995. The survey was not a probabilistic sample of individuals in the United States. Representation of regular and sugarless chewing gum use in U.S. households therefore is not assured. Intake estimates are based upon data from individuals who provided quantitative data in response to a question on use of sugarless chewing gum on the previous day.

[b] Proposed use of α-CD in regular and sugarless chewing gums is 10 g α-CD per 100 g gum.

[c] Weight of chewing gum assumed to be 3 g per piece (21 CFR § 101.12).

From:	Ricardo Carvajal
To:	McMahon, Carrie
Cc:	"Albert Bar"; Cora A. Seballos
Subject:	GRN 678 - Responses to questions from Evaluation Team
Date:	Wednesday, March 08, 2017 4:36:16 PM
Attachments:	GRN 678 Response to Evaluation Team 3-7-2017 (00305308).pdf

Dear Carrie:

Attached is the response to the questions from the Evaluation Team. Given the number of tables and annexes, we though it would be best to make the submission as a single PDF. We did not include Annex 4, which is the GRAS dossier for GRN 155, because I sent that to you this past Monday. Please let me know if you would like me to follow up with hard copies via FedEx, and if so, how many.

Thanks and best regards,
Ricardo

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Bioresco
Food Scientific and Regulatory Services

GRN 678

alpha-Cyclodextrin

Response to Evaluation Team Questions
of February 13, 2017

Prepared for:
Wacker Chemical Corp.
(formerly Wacker Biochem Corp.)

Prepared by:
Albert Bär Ph.D.

March 7, 2017

Content

Annex 1	Specifications of alpha-cyclodextrin as per Food Chemicals Codex, FCC 10 1S
Annex 2	Test methods applied for testing compliance of alpha-cyclodextrin with FCC 10 1S Monograph requirements
Annex 3	Certificates of analyses of 6 batches of alpha-CD produced at commercial scale
Annex 4	α-Cyclodextrin. Dossier prepared by Albert Bär PhD on behalf of Wacker Chemie GmbH, Munich, Germany, for the safety assessment of α-cyclodextrin, dated June 01, 2004.

1. INTRODUCTION

On November 1, 2016, Wacker Chemical Corporation ("Wacker" or "the Notifier") submitted a GRAS Notice for alpha-cyclodextrin. By letter dated November 28, 2016, the Food and Drug Administration (FDA) confirmed the filing of GRAS Notice No. GRN 000678.

The subject of that notice is alpha-cyclodextrin (hereinafter "alpha-CD") for use as an ingredient in processed and ultra-processed foods generally at levels of up to 3% (weight per weight) and in specified types of beverages at levels ranging from 1% to 2% (weight per volume).

On February 13, the FDA’s Evaluation Team asked several questions in relation to GRN 678. We here present the answers. The sequence and titles of the different sections of this document correspond to the Evaluation Team’s list of questions.

2. QUESTIONS RE PART 1 (SIGNED STATEMENTS AND CERTIFICATION)

The definition of "processed foods", as presented in footnote 2 of GRN 678, recites the definition proposed by Moubarac et al., 2014 (as referenced in GRN 678).

This very broad definition includes infant formulae, follow-on milks and other baby products. Although GRN 678 relies on this definition for estimating the daily intake of alpha-CD among adults and children 3 years of age and above, alpha-CD is not intended for use in infant formula as that term is defined in 21 CFR 106.3, nor in any foods for infants and young children 1 through 2 years of age.

3. QUESTIONS RE PART 2 (IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS AND PHYSICAL OR TECHNICAL EFFECT)

GRN 678 pertains to alpha-CD that complies with the description and purity criteria laid down in the Food Chemicals Codex 10, First Supplement (cf. Annex 1 for reference).

In GRN 678, the criteria for identity (optical rotation) and moisture content of alpha-CD were not recited because these two parameters were considered to not be directly relevant for the safety assessment of alpha-CD. The Notifier's alpha-CD complies with these two FCC criteria as shown in Table 1 and Table 2.

A complete comparison of the specifications applied by the Notifier for its alpha-CD and those laid down in FCC 10 1S is shown in Table 1.

The purity criteria for food-grade alpha-CD have been described in Section 2(3) of GRN 678. They correspond to those given in FCC 10 1S, except for lead for which a stricter limit value is applied by the Notifier.

The analytical methods that are applied by the Notifier to assure compliance of its alpha-CD with the identity and purity criteria laid down in FCC 10 1S are specified in Annex 2. USP rather than FCC methods are applied for "reducing substances", "residue on ignition", "specific rotation", lead and arsenic for the reasons described in Annex 2.

Analyses of six non-consecutive batches of alpha-CD produced at production scale are shown in Table 2. The data demonstrate that the Notifier's alpha-CD is fully compliant with the FCC 10 1S specifications for this substance.

4. QUESTIONS RE PART 3 (DIETARY EXPOSURE)

Table 1 shown on page 2 of GRN 678 presents the range of intended food uses and the corresponding maximum use levels of alpha-CD as first considered in GRN 155 and now revised in GRN 678. For processed and ultra-processed foods other than beverages a general maximum use level of 3% is now proposed.

As Table 1 of GRN 678 shows, alpha-CD will thus be used in a few types of processed or ultra-processed foods at a lower level (i.e. 3%) than proposed before in GRN 155 [e.g. 5% - 10% in bakery products (breads, crackers, rolls etc.)]. This reduction of certain use levels may explain why the estimated alpha-CD intake from foods among 3-5 year-old children is only 12% higher despite the wider conditions of use now proposed in GRN 678.

For beverages, the use levels remain the same in GRN 678 as in GRN 155, except for diet soft drinks for which the maximum use level is raised slightly from 1 to 1.05 %.

Since the range of alpha-CD uses proposed in GRN 678 is wider than that specified in GRN 155, the dietary exposure estimates for alpha-CD had to be revised.

As explained in Part 3, Section 3 of GRN 678, a different method than in GRN 155 was applied for estimating the alpha-CD intake from processed foods. For beverages, however, the intake data from GRN 155 could be used because the range of beverage applications remained unchanged. Yet, an adjustment had to be made for the intake of alpha-CD with soft drinks because the maximum level of use is now increased from 1 to 1.05% for this type of beverage.

The 2-day average intake of alpha-CD from beverages among children was described in Annex 5 (Table 2) of the dossier underlying GRN 155 and examined by the GRAS Panel at that time. Since the intended maximum level of alpha-CD use in beverages remained largely unchanged, these intake data could continue to be used.

The intake of alpha-CD from its combined use in processed and ultra-processed foods generally and in certain types of beverages (as specified in Table 1 of GRN 678) is presented in Part 3(5) of GRN 678 ("Intake of alpha-CD from its combined use in processed and ultra-processed foods and in certain types of beverages").

In Tables 3.1 - 3.3 of this present document, the newly estimated intakes of alpha-CD from processed and ultra-processed foods and beverages (as specified in Table 1 of GRN 678) are shown for the different age groups of consumers. Table 4 then summarizes these new intake estimates and compares them with the intake estimates in Annex 5 (Table 2) of the dossier that was examined by the Expert Panel in GRN 155 and that is now included with this response to the FDA as Annex 4 and thereby made publicly available.

This comparison indicates that the estimated alpha-CD intake of ≤ 5 year old children from all food and beverage sources combined is less than 50% higher with GRN 678 than it was with GRN 155 (Table 4). The lower use level of alpha-CD in bakery products (5 - 10% depending on type of food according to GRN 155 as opposed to 3% for any type bakery product according to GRN 678) and in ready-to-eat breakfast cereals (2 - 9% according to GRN 155 as opposed to 3% according to GRN 678) may explain why the percentage increase of the estimated daily alpha-CD intake is smaller in this age group than in the older age groups.

For 6 or 7 to 12 year old children, the estimated alpha-CD intake from all proposed uses combined is about 60% higher for GRN 678 than GRN 155[1]. For teenagers, this increase is even bigger (about 90%). The fact that processed and ultra-processed foods (all of which may contain up to 3% alpha-CD under GRN 678) account for about 70% of the total daily food intake may explain this difference (Baraldi et al., 2013; Moubarac et al., 2013a).

The 2-day average intake of alpha-CD from beverages among children has been described in Annex 5 (Table 2) of the dossier that was examined by the GRAS Expert Panel for GRN 155 (Annex 4). This data is now recited in Table 3.3 (providing the EDI for all age groups) of this present document.

[1] In the dietary survey on which GRN 155 was based, the population was grouped in 5 segments, ie. 2-5y, 6-12y, 13-19y and ≥ 20y olds (Environ, 2000). In contrast, the energy expenditure data that form the basis of the present alpha-D intakes are given for 3,5,7,9,12 and 18y old male and female subjects (Livingstone et al.,1992). Accordingly, the age groups are defined differently in GRN 155 and GRN 678 (see also foot-notes of Table 3.2 for basis of calculations).

In its list of questions, the FDA’s Evaluation Team draws the Notifier’s attention to seemingly contradictory statements regarding intake estimates for children. In addition to the explanation given above, it should be noted that the present calculations are based on data of total energy expenditure (except for beverage intake) rather than on dietary records that are known to have a bias towards underestimating energy intakes (Livingstone et al., 1992; Bandini et al., 1990). Thus, the intake estimates for alpha-CD presented in GRN 155 and GRN 678 differ to some extent also because of the different applied methods.

In its questions to Part 3 (dietary exposure), the Evaluation Team raises in its fourth bullet point the question about the intended conditions of alpha-CD use in beverages. Table 1 on page 2 of GRN 678 lists those use levels for both GRN 155 and GRN 678. As may be seen, the range of beverage applications and the use levels are identical, except for a small increase of the maximum use level in soft drinks from 1 to 1.05%.

In Part 2, Section 4, lines 7-12 of GRN 678 and its foot-note 10 it is re-iterated that the use levels of alpha-CD in beverages remain unchanged except for soft drinks (increase from 1 to 1.05%). With this increased maximum use level in soft drinks, the intake of alpha-CD from beverages raises in adults from 7.5 g/d in GRN 155 to 7.9 g/d in GRN 678 (see Table 3.3).

With respect to vegetable juice and soy milk, 2% is the correct maximum use level as confirmed in section (4) of GRN 678 (in the text and in foot-note 10). The alpha-CD intake from beverages in GRN 678 is based on Environ's intake study (Environ, 2000), in which a level of 2% alpha-CD was applied for vegetable juice and soy milk. However, Environ's figures of the total intake of alpha-CD from all types of beverages considered (and as determined by Environ) was increased by 5% in this document (Table 3.3) to reflect the increased use level of alpha-CD in soft drinks. This means that, for the purpose of intake estimation, a use level of 2.1% for vegetable juice and soy milk was also applied in the present estimation of the alpha-CD intake.

Shown in Table 3.1 is the estimated total intake by adult consumers of alpha-CD from all its intended uses, in foods and beverages combined at maximum levels of use.

5. QUESTIONS RE PART 4 (SELF-LIMITING LEVELS OF USE)

The statement made in GRN 678 under this title applies to both, the maximum levels of use specified in GRN 155 and GRN 678. In other words, there are no foodtechnological reasons that would preclude the use of alpha-CD at levels above those presented as maximum levels of use in GRN 678.

While there are certain self-limiting levels of use for alpha-CD because it may distort the flavor of a food, its texture or other organoleptic or foodtechnological property, exact limits cannot be specified and indeed depend upon the specific composition and characteristics of a food or beverage.

It follows that the maximum levels of use defined in GRN 678 are those that will limit the use of alpha-CD in foods. This is not unusual and there are other GRAS food ingredients for which the same applies [take other non-digestible carbo-hydrates such as isomaltodextrin (GRN 610) for an example].

6. QUESTION RE PART 6 (NARRATIVE)

The safety data on which GRN 155 was based were summarized for the first time in a dossier authored by Albert Bär PhD and dated June 01, 2004. This complete dossier that includes in its Annex 5 the data of an intake estimate by the dietary survey method (based on the then intended conditions of use) is included in this submission as Annex 4. Section 9.3 of that dossier summarizes the rationale that led to the conclusion that alpha-CD is safe under the then intended conditions of use leading to an intake of about 3.9 g alpha-CD per eating occasion and an aggregated estimated intake of 11.4 g/person/day.

The main facts that led to this conclusion were that (a) alpha-CD is not digested by the mammalian digestive enzymes, but (b) alpha-CD is completely fermented by the intestinal microbiota, like resistant starch or other fermentable dietary fibers, (c) only minute amounts of alpha-CD are absorbed unchanged (< 1%), (d) absorbed alpha-CD cannot be metabolized and is, therefore, excreted unchanged with the urine, (e) data from 13-week toxicity studies with rats and dogs provide no evidence for adverse reactions in the GI-tract, the kidneys, the liver or any other organs or tissues at alpha-CD intakes of up to 13 g/kg bw/d in rats and 10 g/kg bw/d in dogs, and (f) the intake of alpha-CD per eating occasion is well below the doses that were tolerated by adult volunteers without side-effects (10 g) or with minimal intestinal symptoms only (25 g).

Since the submission of GRN 0155 further studies with alpha-CD in animals and man have been published. The results of these studies are summarized in the following section, and demonstrate that the rationale that led to the conclusion of safety of alpha-CD in GRN 155 is still valid and fully applicable also to the newly proposed conditions of use that lead to a higher estimated daily intake of alpha-CD as described in GRN 678.

6.1 Studies in animals

No further standard toxicity tests with alpha-CD were published since submission of GRN 155 and none were performed to the Notifier's knowledge. The results of the toxicity studies relied on in GRN 155 are summarized in Table 5.

However, the potential beneficial effects of alpha-CD were examined in some animal studies. Although these studies are not directly relevant for the determination of safety of alpha-CD under its intended conditions of use and therefore were not referred to in GRN 678 (such as Artiss et al., 2006 and Wagner et al., 2008), they now are summarized below to provide a more comprehensive review of the current knowledge of the nutritional properties of this water soluble, non-digestible carbohydrate.

6.1.1 Studies in rodents

In a study with two groups of female LDL-receptor knock-out (LDL r-KO) mice (10 mice/group) a "Western" diet with or without 2.1% alpha-CD, this dose corresponding to 10% of the dietary fat content, was administered for 14 weeks. At sacrifice, there was no difference in food intake and body weight between treated mice and controls. However, plasma cholesterol and phospholipids were significantly lower in the treated animals. The fatty acid profile was improved (saturated and trans-fatty acids decreased, unsaturated fatty acidy increased). No deaths or adverse side-effects were reported. The alpha-CD intake was 0.945 g/kg bw/d (Wagner et al., 2008).

The effect of alpha-CD on weight gain, certain plasma parameters, liver lipid and fecal lipid was examined in four groups of 11-week old male Wistar rats receiving semi-purified low-fat (4% of diet) or high-fat (40% of diet) diets without and with alpha-CD (0.4% and 4% in low-and high-fat diet, respectively). The fat (soybean oil) was added mainly at the expense of starch, but there also were other minor compositional differences between low-fat and high-fat diets. There were no deaths reported and adverse effects due to the alpha-CD ingestion did not occur. The investigators concluded that alpha-CD prevented body weight gain, reduced serum TG and leptin levels and increased insulin sensitivity and fecal fat excretion under the conditions of this study (Artiss et al., 2006).

The effects of alpha-CD on nutrient digestibility, bile acid excretion, cecal microbiota and expression were examined in four groups of hamsters receiving alpha-CD (0 or 2%) and supplemental cholesterol (0 or 0.5%) for 28 days. There were no deaths or adverse effects reported. Alpha-CD reduced serum cholesterol in normocholesteremic but not hypercholesteremic hamsters. Triglycerides remained unaffected (Guevara, 2011).

In a study with apoE-knockout mice it was examined whether the admixture of alpha-CD to a Western high-fat diet would affect the development of aortic atherosclerotic lesions. In an initial pilot study, three groups of apoE-KO mice (n = 10/group) were fed a Western diet (WD) containing 21.2% fat (milk fat) and 34.1% sucrose (controls), the WD with 1.5% alpha-CD and the WD with 1.5% beta-CD for 11 weeks. Aortic lesion area was decreased significantly in mice fed WD-alpha CD, but not WD-beta CD, compared to controls (WD).

In the subsequent main study that included an additional comparison group receiving the WD with 1.5% inulin, and a group fed a low-fat diet (LFD), this specific effect of alpha-CD was reproduced. The lesion area of WDA- and LFD-fed apo-E-KO mice was similarly small. The dietary administration of alpha-CD did not affect body weight or food consumption. Plasma lipid levels were not changed significantly. Whether changes in the gut microbiome account for the observed beneficial effect of alpha-CD could not be determined with certainty (Sakurai et al., 2017). Studies with 2-hydroxypropyl-beta-CD revealed a similar effect on atherosclerosis in apo-E-KO mice that was attributed to "macrophage reprogramming" (Zimmer et al., 2016).

6.1.2 Studies in dogs

In a small cross-over study with 9 mixed-breed dogs, alpha-CD was administered at doses of 0, 3, 6 or 9 g alpha-CD twice per day after feeding a standard diet (2x 150 g per day). Each of the 3 periods lasted for 10 days, i.e. 6 days for adaptation followed by 4 days for feces collection. Daily food intake, fecal dry matter output and fecal scores did not differ between treatments. Body weights, condition scores and serum TG and cholesterol concentrations remained unaffected by the treatments. However, the ingestion of alpha-CD was associated with a small decrease in fat digestibility (Guevara et al., 2015).

6.2 Studies in humans

Clinical studies in humans with the purpose of examining the safety and tolerance of alpha-CD have not been performed and reported to our knowledge since submission of GRN 155.

However, several studies with alpha-CD were conducted in human volunteers in order to further investigate potential favorable effects of alpha-CD on blood lipids and glycemic control. An early study that remained unpublished (Diamantis & Bär, 2002) and the studies that have been published (in English) since submission of GRN 155 are summarized in Table 6.

Taken together these studies demonstrate that α-CD ingested at a high dose together with a starchy meal delays and dampens the glycemic and insulinemic response (Diamantis & Bär, 2002; Buckley et al., 2006; Gentilcore et al., 2001). This effect is most probably the consequence of the inhibition of pancreatic amylose by alpha-CD (Koukiekolo et al., 2001; Oudjeriouat et al., 2003). However, "gastrointestinal upsets" have been observed in some volunteers at such dose levels of alpha-CD and under the conditions of these three studies. Alpha-CD doses of 2 g ingested with a meal were usually (but with a few exceptions) well tolerated (Grunberger et al., 2007; Jarosz et al., 2013; Amar et al., 2016) but also remained without an effect on postprandial blood glucose levels (Jarosz et al., 2013).

The occurrence of intestinal side-effects (stool softening or even diarrhea), borborygmi, etc. are well known consequences of the ingestion of non-digestible, yet fermentable carbohydrates (polyols, soluble fermentable dietary fibers). Modulating factors are the gastric emptying time [depending on the ingestion of other foods (and their fact content)] and the individual sensitivity to such effects that are not considered adverse but that in fact have a self-limiting effect through the consumer’s food selection.

Potential favorable effects of alpha-CD on blood lipids have also been examined (reviewed by Gallaher & Plank, 2015). Such effects have been reported also for different other soluble dietary fibers (Surampudi et al., 2016).

6.3 Unpublished data

In Part 6, 3rd bullet point, the Evaluation Team inquired about published and unpublished data and information that the GRAS Panel considered.

The studies considered by the panel are published and are referenced in GRN 678. Unpublished information regarding the manufacturing process was included in supporting documentation for GRN 155, which was available to the panel. That documentation is included in this submission at Annex 4 and thus is publicly available.

7. QUESTIONS RE PART 7

The list of publications that was considered for GRN 678 is presented in Part 7 of the GRN. The Notifier is unaware of unpublished safety data that would be pertinent for the safety assessment of alpha-CD.

The citation of Park et al., (2012) now is completed with a reference to the FASEB's website (see Section 8, References).

In order to provide the complete picture of safety studies with alpha-CD on which GRN 155 was based, a table (Table 5) is now provided with the correct dose levels and citations.

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Table 1 **Comparison of specifications of alpha-cyclodextrin as laid down in FCC 10 1S and as applied by the Notifier**

Analysis	FCC limit	Notifier's limit
alpha-cyclodextrin	≥ 98%	≥ 98%
Reducing substances	≤ 0.5%	≤ 0.5%
Residue on ignition	≤ 0.1%	≤ 0.1%
Specific rotation	+145° - +151°	+145° - +151°
Water	≤ 11%	≤ 11%
Residual complexant	≤ 20 ppm	≤ 20 ppm
Lead	≤ 1 ppm	≤ 0.5 ppm
Total heavy metals	n.s.	≤ 5 ppm
Arsenic	n.s.	≤ 1.3 ppm
Total Microorganisms	n.s.	≤ 1000/g
E. coli	n.s.	0/10 g
Salmonella	n.s.	0/10 g

Abbreviations: n.s., not specified

Table 2 **Analyses of six non-consecutive batches of alpha-CD produced at commercial scale**

	Batch No				
	601091	**601084**	**601068**	**601055**	**601054**
α-Cyclodextrin content (%)	99	99	99	100	99
Residue on ignition (%)	0.1	0.0	0.0	0.1	0.0
Reducing substances (%)	<0.2	<0.2	<0.2	<0.2	<0.2
Heavy Metals (ppm)	<5	<5	<5	<5	<5
Lead (ppm)	<0.5	<0.5	<0.5	<0.5	<0.5
Arsenic (ppm)	<1.0	<1.0	<1.0	<1.0	<1.0
Residual 1-decanol (ppm)	<5	<5	<5	<5	<5
Loss on drying (%)	10.5	10.0	9.7	10.3	10.3
Microorganisms per g	<100	<100	<100	<100	<100
Salmonella/E. coli in 10g	0	0	0	0	0
Specific Rotation	+148°	+150°	+151°	+150°	+150°

Table 3.1 **Estimated intake by adult consumers of alpha-cyclodextrin from its combined use in processed and ultra-processed foods and in specified types of beverages at maximum levels of use (GRN 678)**

Source of alpha-CD	alpha-CD intake	
	Mean	**90th perc.**
Processed and ultra-processed foods (other than beverages)	16.7 g/d[1] (239 mg/kg bw/d)	29.4 g/d[1] (420 mg/kg bw/d)
Beverages (as specified in Table 1 of GRN 678)	3.7 g/d	7.9 g/d
Combined intake from all sources	20.4 g/d	37.3 g/d

[1] Assuming a body weight of 70 kg

Table 3.2 **Estimated average daily intake of alpha-CD from processed and ultra-processed foods (other than beverages) at maximum levels of use by children and teenagers (GRN 678) based on body weight and total daily energy expenditure data published by Livingstone et al., 1992**

Age	Children 3 - 5 y	Children 7 - 12 y	Teenagers 12 - 18 y
Body weight (group average)	17.2 kg[3]	33.5 kg[2]	57.6 kg[1]
Energy intake	78.8 kcal/kg bw/d[6]	64.0 kcal/kg bw/d[5]	49.4 kcal/kg bw/d[4]
Energy intake from food (80%)	63.0 kcal/kg bw/d	51.2 kcal/kg bw/d	39.5 kcal/kg bw/d
Intake of food (with average energy density 2 kcal/g)	31.5 g/kg bw/d	25.6 g/kg bw/d	19.8 g/kg bw/d
Intake of processed food (70% of all food)	22.1 g/kg bw/d	17.9 g/kg bw/d	13.8 g/kg bw/d
alpha-CD intake (3% in processed food)	662 mg/kg bw/d	538 mg/kg bw/d	415 mg/kg bw/d
Daily alpha-CD intake from processed and ultra-processed foods per person	11.4 g/d	18.0 g/d	23.9 g/d

[1] Average body weights of age groups 12, 15 and 18 year olds
[2] Average body weights of age groups 7, 9 and 12 year olds
[3] Average body weight of age groups 3, 4 and 5 year olds
[4] Average of total energy expenditure (TEE) of age groups 12, 15 and 18 year olds
[5] Average TEE of age groups 7, 9 and 12 year olds
[6] Average body weights of age groups 12, 15 and 18 year olds

Table 3.3 **Estimated daily intake by children, teenagers and adults of alpha-CD from processed and ultra-processed foods and specified beverages combined at maximum level of use**

Source of alpha-CD	Children 3 - 5 y	Children 7 - 12 y	Teenagers 12 - 18 y	Adults ≥ 20 y	
				Mean	90 perc.
Processed and ultra-processed foods (cf. Table 3.2)	11.4 g/d	18.0 g/d	23.9 g/d	16.7 g/d	29.4 g/d
Beverages (based on dietary survey data underlying GRN 155 but increased by 5% generally for beverages in order to adjust for the increase of the maximum use level in soft drinks from 1% to 1.05%)	3.4 g/d[1]	3.5 g/d[2]	4.4 g/d[3]	3.6 g/d	7.9 g/d
Intake from all sources	14.8 g/d	21.5 g/d	28.3 g/d	20.3 g/d	37.3 g/d[4]

[1] age 2 - 5 y for beverages (dietary survey data taken from dossier underlying GRN 155, Table 2)
[2] age 6 - 12 for beverages (dietary survey data taken from dossier underlying GRN 155, Table 2)
[3] age 13 - 19 for beverages (dietary survey data taken from dossier underlying GRN 155, Table 2)
[4] A 90th percentile consumer for both foods and beverages is likely overweight. Would intakes be calculated per kg body weight, the difference between mean and 90th percentile may be smaller.

Table 4 **Comparison between GRN 155 and GRN 678 of the total estimated daily intake of alpha-cyclodextrin from its combined uses in specified foods and specified types of beverages at maximum levels of use**

	GRN 155[1]		GRN 678[2]	
	Mean	90th perc.	Mean	90th perc.
Children (2-5 years) (3-5 years)	10.2 g/d	16.2 g/d	14.8 g/d[3]	n.d.
Children (6-12 years) (7-12 years)	11.8 g/d	18.7 g/d	21.5 g/d[3]	n.d.
Teenagers (13-19 years) (12-18 years)	12.4 g/d	21.4 g/d	28.3 g/d[3]	n.d.
Adults (≥ 20 years)	11.3 g/d	20.2 g/d	20.3 g/d[3]	37.3 g/d[3]

[1] 2-Day average intake by the dietary survey method (Environ, 2002).
[2] Calculated for foods from data on total energy expenditure (Livingstone et al., 1992)and for beverages from dietary survey data (Environ, 2002)
[3] Since certain beverages, such as soft drinks and energy drinks, are processed or ultra-processed foods (cf. footnote 2 of GRN 678), contribution to the total α-CD from all sources is taken into account twice. Hence these intake estimates from all sources are over-estimates.

n.d., not determined

Table 5 **Results of standard, oral toxicity studies with alpha-CD**

Type of study	Species (n)	Dose level (% of diet)	Results	NOAEL	References
Subchronic (4-wk) toxicity test	Wistar rats (5/sex/group)	0, 1, 5, 10, 15, α-CD; 5% β-CD	Persistent diarrhoea associated with reduced body weight gains and increased water consumption	5% (4 g/kg bw/d)	Lina & Bär, 2004a
Subchronic (13-wk) toxicity study	Wistar rats (20/sex/group)	0, 1.5, 5, 20% α-CD; 20% lactose	Slight cecal enlargement at 20% α-CD; increased urinary Ca excretion and decreased pH at 20% α-CD; increased fecal dry weight and nitrogen excretion and decreased fecal pH at 20% α-CD	20% (m: 12.6 g/kg bw/d; f: 13.9 g/kg bw/d)	Lina & Bär, 2004a
Subchronic (90-d) toxicity study	Beagle dogs (4/sex/group)	0, 5, 10, 20% α-CD	Slight cecal enlargement in the 10 and 20% dose groups; transient diarrhoea in a few dogs; decreased urinary pH in females of the 20% α-CD group	20% (m: 9.8 g/kg bw/d; f: 10.4 g/kg bw/d)	Lina & Bär, 2004b

Table 5 continued

Type of study	Species (n)	Dose level (% of diet)	Results	NOAEL	References
Embryotoxicity / teratogenicity study	Sprague-Dawley rats	0, 5, 10, 20% (day 6-16)	No differences between treated groups and controls except for an increased food consumption in the 10 and 20% α-CD group and sporadic increases of water consumption in all treated groups	20% (approx. 20 g/kg bw/d)	NTP, 1994a
Embryotoxicity / teratogenicity study	Swiss (CD-1) mice	0, 5, 10, 20% (day 6-16)	No differences between treated groups and controls except for an increase food consumption in the 20% α-CD group. Water intake was unaffected by the treatment	20% (approx. 49 g/kg bw/d)	NTP, 1994b
Embryotoxicity / teratogenicity study	Wistar rats (25f/group)	0, 1.5, 5, 10, 20% α-CD; 20% lactose (day 0-21)	No difference between treated groups and con-trols	20% (11 g/kg bw/d)	Waalkens-Berendsen & Bär 2004a
Embryotoxicity / teratogenicity study	New Zealand White rabbits (16f/group)	0, 5, 10, 20% α-CD; 20% lactose (day 0-29)	Transient mild diarrhoea in the 10 and 20% α-CD groups	20% (5.9-7.5 g/kg bw/d)	Waalkens-Berendsen et al., 2004

Abbreviations: m, male; f, female; bw, body weight; NOAEL, No Observed Adverse Effect Level

Table 6 **Tolerance of alpha-cyclodextrin in humans**

				Results			
Subjects, age, health condition	**Study design**	**n (total)**	**Treatments**	**Primary endpoints**	**Secondary endpoints; intestinal tolerance and adverse effects**	**Remarks**	**Reference**
Healthy adult males, fasted overnight		12	25 g α-CD single dose dissolved in 250 ml water; ingested together with 100 g fresh white bread.	Glycemic and insulinemic response: α-CD delays and reduces the glycemic and insulinemic response to the test meal.	Intestinal tolerance: 1 subject reported diarrhea, 3 reported abdominal discomfort		Diamantis & Bär, 2002
Healthy adult males (5) and females (5)	Double-blind, randomised, crossover; 48 hrs washout between treatments.	10	Boiled rice (50 starch) with 0, 2, 5 or 10 g α-CD added. Each subject received all 4 treatments.	Glycemic and insulinemic response at t 0, 15, 30, 45, 60, 90 and 120 min: α-CD reduces the glycemic response in a dose-related manner. Effect is significant at 5 and 10 g α-CD.	Intestinal tolerance "GI-upsets" reported by 1/10 subjects at low-dose, 2/10 at mid-dose and 3/10 at high-dose α-CD intake.	Greater satiety at high dose of α-CD Lower palatability at high dose of α-CD.	Buckley et al., 2006
Overweight (not obese), nondiabetic, adult males (14) and females (27)	Randomized, (28 compliant) controlled, double-blind crossover.	41 (28 compliant)	6 g α-CD/d (2 g after each fat containing meal) for 1 month.	Body weight, body fat, blood lipids, insulin, leptin, adiponectin unchanged. Significant decrease of total and LDL-Chol decreased in hypercholesteremic participants. Adiponectin unchanged.	Intestinal tolerance: No GI effects reported		Comerford et al. 2011

Healthy older adults (age 68 - 76 y) (7 males, 3 females)	Double-blind, randomized.	10	Ingestion of 100 g sucrose (in 300 ml drink) with or without 10 g α-CD.	Gastric emptying, blood glucose, serum insulin: Gastric emptying slowed by α-CD; blood glucose and insulin slightly decreased at 60 and 90 and 120 min, resp.	Intestinal tolerance: 3/10 subjects reported loose stools 5 hrs after α-CD ingestion. Symptoms resolved within 7 hrs after completion of study. [Note: 3/13 recruited subjects dropped out after experiencing diarrhea on completion of the first visit (treatment not specified)].		Gentilcore et al., 2011
Obese type-2 diabetics (BMI ≥ 30, ≥ 30 y old)	Double-blind parallel design with randomized allocation of subjects to α-CD group (at start n = 33 in treated group and n = 34 in control group). Drop-outs n = 13 in treated group and n = 7 in control group, all due to insufficient compliance with		Ingestion of 2 1-g tablets with α-CD or placebo (not specified) with each fat containing meal (≥ 20 g fat), i.e. ≤ 6 g α-CD/d.	Blood sampling before and after 1, 2 and 3 months of study: glucose, creatinine, ALAT, Chol tot., HDL-Chol, LDL-Chol, TG, CRP, fructosamine: (a) Not significant decrease of Chol tot in treated group and increase in control group; difference between group	Secondary endpoints: Serum 25-OH-vit.D, leptin, insulin, adiponectin, HbA1c at start and end of study. None of the participants reported adverse side effects.		Grunberger et al., 2007

	protocol.			is significant. (b) Significant increase of adiponectin relative to placebo group (interpreted as beneficial effect).			
Health adults (18 - 65 y old)	Double-blind, placebo controlled cross-over study (n = 34)		Ingestion of 2 g α-CD or cellulose (placebo) following a high-fat breakfast	Fasting and postprandial (1 - 3 h) glucose, cholesterol (LDL, HDL, total), TG No significant treatment related effects at each point in time. However, significant time by treatment interaction indicating a TG lowering effect of α-CD in hyper-lipidemic but not normolipidemic subjects	No adverse GI effects reported		Jarosz et al., 2013; Fletcher, 2013
Overweight and obese subjects	Randomized controlled crossover study with three 7-day treatment periods	21	(1) α-CD 30g/d (2) water-soluble modified cellulose (6 g/d) (3) isocaloric placebo drink Two servings/ day (breakfast, dinner)	Fecal 4-day fat excretion does not differ between α-CD and comparison (cellulose) treatment periods	None reported	Study is pub-lished as abstract only.	Park, 2012

			for 4 conse-cutive days				
Healthy subjects (18 - 75 y old)	Double-blind, placebo-controlled, cross-over study (n = 75)		3 x 2 pills/ day with main meals for 14 weeks Verum: 1 g α-CD + excipients Placebo: Dialcium phosphate and cellulose + excipients	Blood lipids, and blood glucose parameters: Modest reduction in small LDL-particles. Other parameters unaffected by α-CD treatment.	Standard clinical chemical parameters, lipophilic vitamins, RBC, WBC: no adverse effects due to α-CD treatment. Minor gastro-intestinal symptoms in 8% of subjects during α-CD treatment and in 3% during placebo treatments (difference not statistically significant).		Amar et al., 2016

Annex 1

Specifications of alpha-cyclodextrin as per Food Chemicals Codex, FCC 10 1S

alpha-Cyclodextrin

alpha-Cyclodextrin

Published in: FCC 10 1S
First Published: FCC 6, Second Supplement
Last Revised: FCC 6, Second Supplement

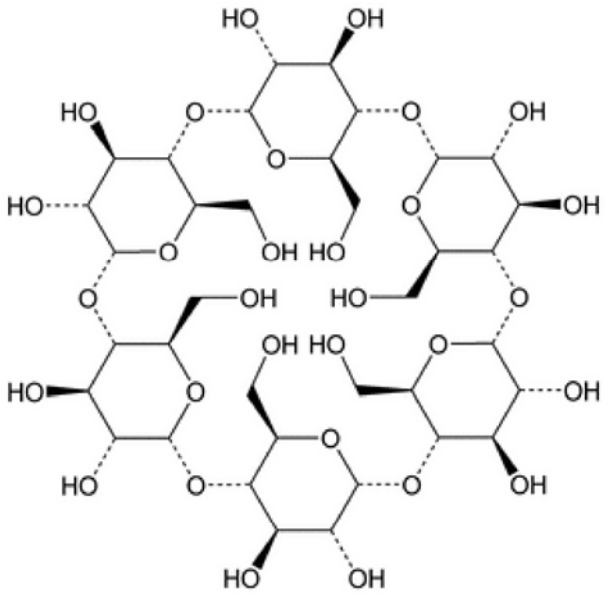
α-Schardinger dextrin

α-Dextrin

Cyclohexaamylose

Cyclomaltohexose

α-Cycloamylose



$(C_6H_{10}O_5)_6$
Formula wt: 972.85
INS: 457
CAS: [10016-20-3]
UNII: Z1LH97KTRM [alfadex]

DESCRIPTION

Alpha-Cyclodextrin occurs as a virtually odorless, white or almost white crystalline solid. It is a non-reducing cyclic saccharide consisting of six α-(1→4)-linked D-glucopyranosyl units produced by the action of cyclodextrin glucosyltransferase (CGTase, EC 2.4.1.19) on

hydrolyzed starch. Recovery and purification of alpha-cyclodextrin may be carried out using one of the following procedures: precipitation of a complex of alpha-cyclodextrin with 1-decanol, dissolution in water at elevated temperature and re-precipitation, steam-stripping of the complexant, and crystallization of alpha-cyclodextrin from the solution; or chromatography with ion-exchange or gel filtration followed by crystallization of alpha-cyclodextrin from the purified mother liquor; or membrane separation methods such as ultra-filtration and reverse osmosis. It is freely soluble in water and very slightly soluble in ethanol.

Function: Carrier; encapsulating agent; stabilizer

Packaging and storage: Store in tight containers in a dry place.

IDENTIFICATION

- **PROCEDURE**

Acceptance criteria: The retention time of the major peak in the chromatogram of the *Sample solution* is the same as that of the *Standard solution* in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (67:33, v/v)

Standard solution: 10 mg/mL USP Alpha-Cyclodextrin RS[NOTE— Ultra-sonication for 10–15 min may be necessary to aid in complete dissolution.]

Sample solution: 10 mg/mL filtered through a 0.45-µm filter [NOTE— Ultra-sonication for 10–15 min may be necessary to aid in complete dissolution.]

Chromatographic system, *Appendix IIA*

Mode: High-performance liquid chromatography

Detector: Refractive index

Column: 25-cm × 4-mm, packed with a monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support (10 µm particle diameter)[1]

Column temperature: 40°

Flow rate: 2.0 mL/min

Injection volume: 10 µL

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. Calculate the percentage of alpha-cyclodextrin in the portion of the sample taken by the equation:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100\%$$

r_U = peak response for alpha-cyclodextrin from the *Sample solution*
r_S = peak response for alpha-cyclodextrin from the *Standard solution*
C_U = concentration of the sample in the *Sample solution* (mg/mL)
C_S = concentration of alpha-cyclodextrin in the *Standard solution* (mg/mL)

Acceptance criteria: NLT 98%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **LEAD,** ***Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I, Appendix IIIB***

  Acceptance criteria: NMT 1 mg/kg

Organic Impurities

- **REDUCING SUBSTANCES (AS DEXTROSE)** [NOTE— Dextrose levels are usually lower when determined by the following procedure in the presence of alpha-cyclodextrin, compared to levels determined in its absence. An alpha-cyclodextrin reference standard is therefore utilized in this procedure for the calibration.]

  Reagent solution: Weigh 10.0 g of 3,5-dinitrosalicylic acid in a 1000-mL volumetric flask. Add 80 mL of water, and dissolve the 3,5-dinitrosalicylic acid by heating in a water bath. Prepare a solution of 16.0 g sodium hydroxide in 200 mL of water and a solution of 300 g sodium potassium tartrate in 500 mL of water. Transfer both solutions to the 1000-mL flask. Dilute with water to volume, shake the flask, and let it stand for 24 h. Filter (paper) the reagent solution prior to use if a precipitate appears.

  Standard stock solution: 10 mg/mL dextrose (on the anhydrous basis)

  Standard solutions: 0 to 1.0 mg/mL dextrose prepared as follows: weigh 1.0 g of alpha-cyclodextrin standard[2] into each of eleven 10-mL volumetric flasks (numbered 0 to 10). Add 0, 0.1, 0.2, ..., 1.0 mL of *Standard solution* to flasks nos. 0, 1, ... to 10, respectively. Dilute all flasks with water to volume.

  Sample solutions: 100 mg/mL [NOTE— Ultra-sonication for 10–15 min at 30° may be necessary to aid in complete dissolution.]

  Calibration curve: Assemble a set of eleven 10-mL volumetric flasks. Transfer 1 mL of each of the eleven *Standard solutions* into the flasks, and add 1 mL of *Reagent solution* to each flask. Heat each flask in the boiling water bath for 10 min. Cool rapidly to room temperature, and dilute all flasks with water to volume. For each solution, measure the absorbance against water at 545 nm. Generate a standard curve by plotting absorbance vs. the concentration, in mg/mL, of dextrose in the *Standard solutions*.

  Analysis: Prepare a set of six 10-mL volumetric flasks (labeled a through f), and add 1 mL of *Reagent solution* to each. Transfer 1 mL of the *Sample solution* to flasks "a," "b," and "c". Transfer 1 mL of the *Standard solutions* numbered 0, 3, and 6 to flasks "d," "e," and "f". Thoroughly mix the contents of each flask, and place in a boiling water bath for 10 min. Then, cool the flasks to room temperature, fill to the mark with water, and measure absorbance of the solutions against water at 545 nm. The result is only valid if the absorbances of the solutions in flasks "d," "e," and "f" do not deviate more than 5% from the corresponding absorbances from the *Calibration curve.* Calculate the percentage reducing substance (as dextrose) in the sample taken using the following equation:

$$\text{Reducing substances} = C_{RS}/C_U \times 100\%$$

C_{RS} = average of the reducing substance concentrations (as dextrose), in mg/mL, from flasks "a," "b," and "c," calculated using the *Calibration curve*

C_U = concentration of the sample in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5% (as dextrose)

- **RESIDUAL COMPLEXANT (1-DECANOL)**

Tris buffer solution: Dissolve 606 mg of tris (hydroxymethyl) aminomethane and 430 mg of calcium sulfate dihydrate in 500 mL of water. Adjust the pH to 6.5 with phosphoric acid.

Internal standard solution: Add 50 mg of 1-octanol to 250 mL of tetrahydrofuran.

Standard stock solution: 750 µg/mL 1-decanol in *Internal standard solution*

Standard solution: 7.5 µg/mL 1-decanol in *Internal standard solution:* diluted from *Standard stock solution*

Sample solution: Dissolve 750 mg of sample and 50 mg of glucoamylase[3] (EC 3.2.1.3) in 7 mL of *Tris buffer solution.* Add 100 µL of *Internal standard solution* and 50 µL of cyclodextrin glucosyltransferase preparation (500 U/mL).[4] Close tightly, mix, and incubate in a shaking water bath at 40° for 4 h.

Condition a C18 solid-phase extraction column[5] by washing with methanol (2 × 10 mL) and water (4 × 10 mL). Quantitatively transfer the digested sample solution to the conditioned column, and slowly pass it through the column. Wash the column with water (2 × 10 mL). Gently pass nitrogen through the column to dry it (10 min). Apply 2.5 mL of tetrahydrofuran to the column, let stand for 5 min, and collect the eluate. Use the eluate as the *Sample solution*.

Chromatographic system, *Appendix IIA*

Mode: Gas chromatography

Detector: Flame ionization

Column: 25-m × 0.32-mm capillary column coated with a 0.5-µm layer of dimethylpolysiloxane gum[6]

Column temperature: 60° for 1 min, 20°/min to 300°, 300° for 7 min

Injection port temperature: 265°

Carrier gas: Helium

Flow rate: 1 mL/min

Injection syringe: Heated, gas-tight

Injection volume: 1 µL

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the concentration (mg/kg) of 1-decanol in the sample taken using the following formula:

$$\text{Result} = (R_U/R_S) \times C_S \times (2.5/S) \times 1000$$

R_U = internal standard ratio (1-decanol peak response/1-octanol response) from the *Sample solution*

R_S = internal standard ratio (1-decanol peak response/1-octanol response) from the *Standard solution*

C_S = concentration of 1-decanol in the *Standard solution* (µg/mL)

2.5 = dilution factor for the *Sample solution* from the mL of tetrahydrofuran applied to the solid-phase extraction column
S = mg of sample taken to prepare the *Sample solution*
1000 = µg/mg to mg/kg conversion factor

Acceptance criteria: NMT 20 mg/kg

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE DETERMINATION, *Appendix IIB***

  Acceptance criteria: Decomposes above 278°

- **RESIDUE ON IGNITION (SULFATED ASH), *Appendix IIC***

  Sample: 1 to 2 g

  Acceptance criteria: NMT 0.1%

- **OPTICAL (SPECIFIC) ROTATION, *Appendix IIB***

  Sample solution: 10 mg/mL

  Acceptance criteria: $[\alpha]_D^{25}$ between +145° and +151°

- **WATER, *Water Determination, Method I, Appendix IIB***

  Acceptance criteria: NMT 11%

---

[1] Nucleosil 100 NH2 (Macherey-Nagel Co, Düren, Germany), or equivalent.

[2] Available from Consortium für Elektrochemische Industrie GmbH (München, Germany), or Wacker Biochem Group (Adrian, MI, USA).

[3] Gluczyme 8000 (Wacker Chemie, Munich, Germany).

[4] Available from Wacker Chimie (Munich, Germany).

[5] Isolute C18, 10 mL (ICT, Bad Homburg, Germany), or equivalent.

[6] HP-1 (Agilent Technologies), or equivalent.

Please check for your question in the FAQs (http://www.usp.org/frequently-asked-questions/food-chemicals-codex-fcc) before contacting USP.

Topic/Question	Contact	Expert Committee
Monographs	Kenny Xie (mailto:KYX@usp.org) Scientific Liaison (240)221-2052	FI2015 Food Ingredients 2015

Annex 2

Test methods applied for testing compliance of alpha-cyclodextrin with FCC 10 1S Monograph requirements

WACKER

JOHANNA GOODWIN
F-S-Q/EDY

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To Whom It Concerns

☐ As discussed
☐ Thank you
☐ To be kept on file

Requested action:

☒ For your information
☐ For review and comment
☐ Take appropriate action
☐ Contact me
☐ Please return

1 March 2017

Cavamax® W6 Alpha Cyclodextrin FCC Compliance

Wacker Specifications and test methods for Cavamax® W6 Food Alpha Cyclodextrin are equivalent to FCC10 Monograph requirements.

Analysis	FCC Limit	Method used	Justification
Cyclodextrin content	NLT 98%	HPLC	Based on current USP: uses a C-18 column, MeOH / water mobile phase; former USP used same column and mobile phase as current FCC.
Reducing substances	NMT 0.5%	USP	USP more widely accepted.
Residue on ignition	NMT 0.1%	USP	USP method is dried at a lower temperature and is performed without amm. carbonate, compared to FCC method. Both are dried to constant weight and thus considered equivalent.
Specific Rotation	+145°-+151°	USP	Minor difference at ↑ 5°C.
Water	NMT 11%	Halogen Dryer	Loss on Drying via Halogen Dryer validated in house as suitable alternative.
Residual complexant (1-decanol)	NMT 20ppm	GC	Parameters in Monograph differ than in house method. Preparation is the same. See equivalence report
Lead	NMT 1ppm	ICP-MS	USP <730> is preferred to AAS for its ability to detect multiple analytes concurrently. ICP-MS is also used by USDA/FSIS/OPHS CLG-TM3.01 and EPA 3050/6020.
Arsenic	--	ICP-MS	USP <730> is preferred to AAS for its ability to detect multiple analytes concurrently. ICP-MS is also used by USDA/FSIS/OPHS CLG-TM3.01 and EPA 3050/6020.
Total Microorganisms	--	Photometric Test	AOAC PTM 071203, Validated equivalent to USP
E.coli	--	Photometric Test	AOAC PTM 101101, Validated equivalent to USP
Salmonella	--	Photometric Test	Validated equivalent to USP.

Testing conducted shows methods for analysis in the FCC monograph give results equivalent to the methods utilized by Wacker final product analysis.

Cyclodextrin Purity
All results of analysis are stated as % cyclodextrin content (dry basis).

Table 1. Purity results

Sample	USP method	FCC method
601054	99.4	99.6
601089	98.6	99.5
601090	98.1	99.4
601091	99.3	100.5
601092	99.5	100.3

Reducing Substances
All results of analysis are stated as % reducing sugars.

Table 2. Reducing Substances results

Sample	USP method	FCC method
601054	0.0043	0.0008
601082	0.0028	0.0028
601083	0.0107	0.0039
601084	0.0097	0.0024
601091	0.0052	0.0013

Specific Rotation
The results obtained by the FCC method at 25°C are consistent with the results via USP method at 20°C.

Table 3. Specific Rotation results

Sample	USP method	FCC method
601082	151.0°	150.9°
601083	150.7°	150.6°
601084	150.6°	150.4°
601091	147.7°	148.0°
601092	149.8°	149.6°

Water
The results obtained by the FCC method compare with Loss on Drying by Halogen Dryer. All results of analysis are stated as %.

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Table 4. Water results

Sample	Halogen Dryer	FCC method
601054	10.4	10.1
601082	10.3	10.1
601083	10.4	10.1
601084	10.3	10.1
601091	10.0	9.7

Residual Complexant

The results obtained by the FCC method compare with current methods. The detection limit for the method is 5ppm, all samples fall below this limit.

Table 5. Residual Complexant (1-Decanol) results

Sample	Wacker Method	FCC method
601054	1.77	0.85
601082	0.80	0.00
601083	0.80	0.36
601084	1.78	0.00
601091	1.15	0.72

Annex 3

Certificates of analyses of 6 batches of alpha-CD produced at commercial scale

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Date of delivery

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CAVAMAX® W6 Food

date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601054	0,000	19.05.2015	18.05.2018

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,0	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,3	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	150	145	151

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date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601055	0,000	21.05.2015	20.05.2018

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	100	98	-
Residue on ignition	USP/NF	%	0,1	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,3	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	150	145	151

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date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601068	0,000	15.06.2015	14.06.2018

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,0	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 2,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	9,7	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	151	145	151

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date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601084	0,000	08.07.2016	08.07.2019

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,0	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,0	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	150	145	151

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CAVAMAX® W6 Food

date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601091	0,000	22.07.2016	22.07.2019

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,1	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,5	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	148	145	151

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From: Ricardo Carvajal
To: McMahon, Carrie
Cc: "Albert Bar"; Cora A. Seballos
Subject: RE: GRN 678 - Responses to questions from Evaluation Team
Date: Thursday, March 09, 2017 9:39:40 AM
Attachments: Annex 3 - Batch analyses 3-9-17 (00305465).pdf

Dear Carrie:

As part of Annex 3, we inadvertently submitted an inspection certificate for a batch analysis (for batch 601068) that erroneously reports an arsenic value of <2 (as opposed to <1). That certificate was issued on 02/03/17. Wacker had investigated the issue and confirmed that that the reported value of <2 was attributable to a reporting error. The correct value is <1 and is reflected in a corrected inspection certificate, which was issued on 03/06/2017 and is included in the attached version of Annex 3. If you would prefer that I resubmit the entire PDF that I submitted yesterday with the attached version of Annex 3, please let me know. My apologies for any inconvenience.

Best regards,
Ricardo

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Admitted in the District of Columbia
www.hpm.com
Visit the HPM blog at: www.fdalawblog.net

From: Ricardo Carvajal
Sent: Wednesday, March 08, 2017 4:35 PM
To: 'McMahon, Carrie'
Cc: 'Albert Bar'; Cora A. Seballos
Subject: GRN 678 - Responses to questions from Evaluation Team

Dear Carrie:

Attached is the response to the questions from the Evaluation Team. Given the number of tables and annexes, we though it would be best to make the submission as a single PDF. We did not include Annex 4, which is the GRAS dossier for GRN 155, because I sent that to you this past Monday. Please let me know if you would like me to follow up with hard copies via FedEx, and if so, how many.

Thanks and best regards,
Ricardo
Ricardo Carvajal, J.D., M.S.

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Annex 3

Certificates of analyses of 6 batches of alpha-CD produced at commercial scale

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Date of requisition

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Customer No. Fax

CAVAMAX® W6 Food

date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601091	0,000	22.07.2016	22.07.2019

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,1	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,5	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	148	145	151

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Customer No. Fax

CAVAMAX® W6 Food

date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601084	0,000	08.07.2016	08.07.2019

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,0	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,0	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	150	145	151

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Inspection certificate EN 10204 - 3.1

Date of delivery

Delivery note

Requisition No.

Date of requisition

Customer material

Order No.

Customer No. Fax

CAVAMAX® W6 Food

date of issue: 03/06/2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601068	0 kg	06/15/2015	06/2018

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0.0	-	0.1
Reducing substances	USP	%	< 0.2	-	0.5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0.5	-	0.5
Arsenic	TITRATION	ppm	< 1.0	-	1.3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	9.7	-	11.0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	151	145	151

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Date of requisition

Order No.
/ 000000 /

Customer No. Fax

CAVAMAX® W6 Food

date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	601055	0,000	21.05.2015	20.05.2018

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	100	98	-
Residue on ignition	USP/NF	%	0,1	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
Residual complexant (1-decanol)	GC	ppm	< 5	-	20
Loss on drying	Halogen Dryer	%	10,3	-	11,0
Microorganisms per g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	< 100	-	1000
Salmonella/E. coli in 10 g	MICROBIOLOGICAL PHOTOMETRIC TEST	-	0	-	0
Specific rotation	FCC	°	150	145	151

Wacker Chemical Corporation
Johanna Goodwin, Quality Assurance Manager, Biosolutions-Eddyville
Eddyville, IA 52553-7010
Tel: 641-969-3005 / Fax: 641-969-4929

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WACKER

Inspection certificate EN 10204 - 3.1

Date of delivery

Delivery note

Requisition No.
/

Date of requisition

Order No.
/ 000000 /

Customer No. Fax

CAVAMAX® W6 Food

date of issue: 02.03.2017

Material	Batch	NET	Date of manufacture	Best use before end
60071344	**601054**	**0,000**	**19.05.2015**	**18.05.2018**

Technical data	Test method/Inspection condition	Unit	Measured value	Lower limit	Upper limit
Cyclodextrin content	USP/NF	%	99	98	-
Residue on ignition	USP/NF	%	0,0	-	0,1
Reducing substances	USP	%	< 0,2	-	0,5
Heavy metals	USP/NF	ppm	< 5	-	5
Lead	USP/NF	ppm	< 0,5	-	0,5
Arsenic	TITRATION	ppm	< 1,0	-	1,3
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This certificate was issued by machine and is valid without a signature.

From:	Ricardo Carvajal
To:	McMahon, Carrie
Cc:	Albert Bar
Subject:	GRN 678 - additional information on intestinal tolerance
Date:	Friday, April 07, 2017 12:40:17 PM
Attachments:	Wacker Dossier Alpha-CD_Supplementary Information_010417.pdf

Dear Ms. McMahon:

Attached is the additional information regarding intestinal tolerance that we mentioned in our call of March 30.

Best regards,
Ricardo

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Bioresco
Food Scientific and Regulatory Services

GRN 678

alpha-Cyclodextrin

Supplementary Information to
Response to Evaluation Team Questions
of February 13, 2017

Prepared for:
Wacker Chemical Corp.
(formerly Wacker Biochem Corp.)

Prepared by:
Albert Bär Ph.D.

April 01, 2017

Content

Table 1 Estimated daily intake of three resistant dextrins

1. INTRODUCTION

The intake of alpha-CD from its concomitant use in all processed and ultra-processed foods at the maximum use level of 3% (w/w) results in an estimated daily intake of alpha-CD of 20.3 and 37.3 g/d for the mean and 90th percentile consumer, respectively (cf. Part 2, Section 3(5) of GRN 678).

The effect on fecal fat excretion of dietary alpha-CD and, for comparison, a water-soluble modified cellulose was examined in a randomized, controlled cross-over study with 31 healthy volunteers who consumed each of these two glucose polymers for a period of 7 days. The daily dose of 30 g of each test substance was ingested in two portions, i.e. 15 g at breakfast and 15 g at dinner. Intestinal side-effects were not reported by the authors of this study that at this time is published in the form of an abstract only (Park et al., 2012).

2. INTESTINAL TOLERANCE OF RESISTANT DEXTRINS IN COMPARISON

Since alpha-CD is a water-soluble, non-digestible glucose polymer, its intestinal tolerance is expected to correspond to that of other non-digestible, yet fermentable glucose polymers, such as isomaltodextrin and resistant dextrin that have been the subject of GRAS Notices 610 and 436, respectively. The estimated daily intakes of these two resistant dextrins (as considered in GRN 610 and GRN 436) and of alpha-CD (as presented in GRN 678) are shown in Table 1.

Resistent dextrin was found to be well tolerated when given at doses of 30 or 45 g/d (Pasman et al., 2006). At higher doses, i.e. 60 and 80 g/d, flatulence was observed but not diarrhea (Van den Heuvel et al., 2004). In another study, resistant dextrin ("Nutriose FB") was administered in step-wise increasing daily doses from 20 g/d to 100 g/d over a period of 20 days followed by a 5-day period with ingestion at this top dose level. The daily dose was ingested in six equal portions, i.e. 16.7 g resistant dextrin was ingested at each eating occasion. Except for flatulence (in 2/10 subjects) and slight abdominal pain in 1/10 subjects at intakes above 50 g/d, i.e. about 8.3 g per eating occasion, no side-effects were reported. However, during the initial adaptation period, two subjects noted diarrhea for one day (Vermorel et al., 2004).

Such intestinal side-effects are well known to occur also after the ingestion of sugar alcohols (polyols) such as sorbitol that are incompletely absorbed but fermented by the intestinal microbiota.

The observation of "gastrointestinal upsets" in 3/10 subjects and loose stool in 3/10 subjects ingesting single doses of 10 g alpha-CD (consumed without a preceding adaptation period) is consistent with this overall picture of minor intestinal symptoms that may occur after intake of low-digestible or non-digestible carbohydrates in sensitive individuals.

Therefore, it also is not surprising that "GI upsets" and loose stools were observed in 3/10 subjects in each of the two human studies with administration of a single 10-g alpha-CD dose (Buckley et al., 2006; Gentilcore et al., 2011). These observations are not necessarily conflicting with the apparent absence of side-effects in the study by Park et al., 2014 because other factors such as differences in the composition of the food with which the low digestible dextrin is ingested and thus the gastric emptying time can also play a role.

For a specific resistant dextrin, Fibersol-2, the maximum single oral dose that does not cause diarrhea was determined in 10 healthy adult subjects. The test substance was ingested in aqueous solution after breakfast. The composition of the (presumably Japanese) breakfast was not described. The maximum single dose that did not cause diarrhea was 1 g/kg bw/d under the conditions of this study. However, mild transient symptoms such as "gargling sounds" and flatus were observed at all dose levels tested (0.7 - 1.1 g/kg bw) (Kishimoto et al., 2013).

In a study on effects of a resistant dextrin, Fibersol-2, on colonic transit time, 15 g of this product dissolved in water were consumed together with breakfast. Side effects that could be attributed to this treatment were not reported (Ruiz et al., 2016).

Considering the structural (glucose polymer) and physiological (non-digestibility yet colonic fermentability) of alpha-cyclodextrin and resistant dextrins, this data indicate that alpha-cyclodextrin, even if used at the maximum proposed level (3%) in all processed and ultra-processed foods, does not present a risk of intestinal side effects that are adverse to human health. Minor side-effects, such as flatulence that may occur under certain circumstances and/or in specifically sensitive individuals, are the same as may occur after the consumption of other non-digestible polymeric carbohydrates or incompletely absorbed polyols.

3. CONCLUSION

Alpha-CD belongs to the group of low- or non-digestible polymeric carbohydrates and, more specifically, to the group of glucose polymers which encompasses products such as resistant (malto-) dextrins and polydextrose. While there are some differences in the physiochemical properties and the degree of polymerization between such products (cf. Table 1 of GRN 436), they all have a certain water-binding capacity and they all are fermented partly or completely by the colonic microbiota.

Observations of intestinal symptoms, such as flatulence, soft stool or even diarrhea that are observed after the ingestion of excessive single doses are a result of these properties. The fact that such side-effects disappear on continued exposure or indeed do not even appear if doses are increased step-wise ("adaptation") demonstrates that the microbial fermentation plays an important role in the handling of ingested non-digestible carbohydrates. However, other factors such as the gastric emptying time and an individual sensitivity to gastrointestinal effects play also a role.

The difference between estimated total daily intakes of resistant dextrin, isomaltodextrin and alpha-CD from all intended uses combined is comparatively small. The data including intestinal tolerance data that have led to the General Recognition of Safety of resistant dextrin and isomaltodextrin are therefore a sufficient basis for extending that status also to alpha-CD for which in fact a more comprehensive toxicological data set is available.

4. REFERENCES

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Table 1 Estimated daily intake of three resistant dextrins

Non-digestible dextrin	GRN	Estimated intake from all sources combined			
		g/person/d		g/kg bw/d	
		Mean	90th perc.	Mean	90th perc.
Resistant dextrin	436	17.4	32.6	0.3	0.6
Isomaltodextrin	610	16.3	32.7	0.265	0.552
Alpha-cyclodextrin	678	20.4[1]	37.3[1]	0.292[1]	0.533[1]
	155	11.3[2]	20.2[2]	0.160[2]	0.280[2]

Notes:

Depending upon the food, the purpose of use of a non-digestible dextrin, cost considerations etc., one or the other dextrin or perhaps mixtures thereof will be used. A cumulative use of these dextrins at maximum levels of use is not expected and indeed has explicitly not been a consideration in GRN 610.

The expression of the intake of dietary fibers in terms of body weight, i.e. mg/kg bw/d, is of limited value because of their non-digestibility and non-absorption.

[1] Adults (≥ 20 years) (cf. Table 3.1 of GRN 678 for data)
[2] Adults (≥ 20 years) (cf. Table 5 of GRN 155)