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June 25, 2004

BY MESSENGER

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration (DBG&R)
5100 Paint Branch Parkway (HFS-255)
College Park, Maryland 20740-3835

Re: Notice of a GRAS Exemption for α-Cyclodextrin

Dear Sir\Madam:

Pursuant to the proposed rule outlined at 62 Fed. Reg. 18939 (April 17, 1997), Wacker Chemical Corporation hereby submits notification that the use of its α-cyclodextrin product is exempt 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) through scientific procedures.

To facilitate your review, this notification is submitted in the format suggested under proposed 21 C.F.R. § 170.36 (c) (see 62 Fed. Reg. at 18961). Four copies of the GRAS Exemption Claim and Additional Information documents, including one for the U.S.D.A. Food Safety Inspection Service, are enclosed. Also enclosed is an electronic copy (Microsoft Word) of the documents.

Sincerely,

(b)(6)

Diane B. McColl

DBM/lmr
Enclosure

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RECEIVED
Jun 28, 04
OFFICE OF
FOOD ADDITIVE SAFETY

GRAS EXEMPTION CLAIM

We hereby claim that the use of alpha-cyclodextrin (α-CD) in foods is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because we have determined that such use of α-CD is generally recognized as safe (GRAS).

(1) Name and address of the notifier:

Mr. Christian Gimber
Wacker Chemical Corporation
3301 Sutton Road
Adrian, Michigan 49221-9397
517-264-8793
517-264-8795 (fax)

(2) Common or usual name of the substance that is the subject of the GRAS exemption claim:

Alpha-cyclodextrin, α-cyclodextrin, α-CD, alpha-dextrin, α-dextrin

(3) Applicable conditions of use of the notified substance:

(a) Foods in which α-CD is to be used:

α-CD may be used for increasing the dietary fiber content of solid, semi-liquid and liquid foods alike. Because of its ability to form complexes with certain organic molecules, α-CD may also fulfill certain food-technological functions, e.g. as a flavor adjuvant; a coloring adjunct; or a carrier, stabilizer, or solubilizer of certain vitamins (*i.e.*, retinol acetate, vitamins K_1 and C, riboflavin, and β-carotene) and fatty acids.

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(b) Maximum levels of use of α-CD in foods:

Use Level	Food Category
5%	Breads, rolls, cakes, baking mixes and refrigerated doughs; dressings and mayonnaise
7%	Brownies and bars
10%	Crackers (sweet and non-sweet); chewing gum
1%	Snack foods; pudding mixes (dry); diet soft drinks; beverage mixes; fruit juices; instant coffees and teas; coffee whiteners (dry); formula diets; meal replacements; nutritional supplements
2%	Vegetable juices; soy milk and non-soy (imitation) milk; instant rice, pasta and noodles (prepared); canned and dry soups (prepared)
2-9%*	Ready-to-eat breakfast cereals
3%	Condiments
2.5%	Yogurt; milk beverage mixes; frozen dairy desserts
20%	Reduced fat spreads
15%	Hard candy

* Use level differs with serving size (<20 g/cup, 2%; 20-43 g/cup, 9%;>43 g/cup, 5%).

(c) Purposes for which α-CD is used:

Fiber supplementation; carrier or stabilizer for flavors (flavor adjuvant); carrier, stabilizer or solubilizer for colors, vitamins and fatty acids; improve "mouthfeel" in beverages.

(d) Description of the population expected to consume α-CD:

Individuals consuming at least one of the food categories described above.

(4) Basis for the GRAS determination:

The basis of the GRAS determination is through scientific procedures.

(5) Review and Copying Statement:

The data and information that are the basis for Wacker Chemical Corporation's GRAS determination are available for the Food and Drug Administration's (FDA's) review and copying at reasonable times at the offices of the notifier, or will be sent to FDA upon request.

(b)(6)

Christian Gimber
Wacker Chemical Corporation
3301 Sutton Road
Adrian, Michigan 49221-9397
517-264-8793
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ADDITIONAL INFORMATION

(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

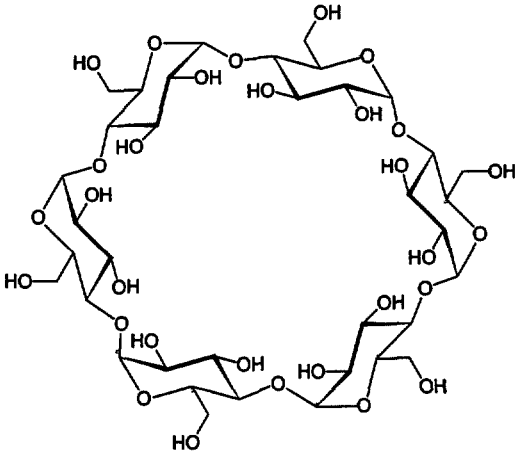
10016-20-3

(c) Empirical formula

$(C_6H_{10}O_5)_6$

(d) Structural formula

α-CD is the cyclic polymer of six α-1,4-linked glycopyranosyl units



(e) Method of manufacture of α-CD

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 $\geq$ 98.0%.

(f) 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.

(g) Any content of potential human toxicants

The potential impurities of α-CD are residues of the CGTase 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 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 by the Food and Drug Administration (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 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).

(h) 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.

(2) Information on any self-limiting levels of use

None.

(3) Detailed summary of the basis for the notifier's determination that a particular use of the notified substance is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because such use is GRAS

A substantial body of evidence supports the safety of α-CD as a food ingredient. The data demonstrate that α-CD is not digested by the mammalian digestive enzymes, but completely fermented by the intestinal microbiota, like resistant starch or other fermentable dietary fibers (Marshall and Miwa, 1981; Kondo et al., 1990; McCleary et al, 2004; Antenucci and Palmer, 1984). A bolus dose of 25 g α -CD produced no increase in blood glucose or insulin in humans, which is consistent with the low digestibility seen in animals (Diamantis and Bär, 2002; Diamantis et al, 2004). Only minute amounts of α-cyclodextrin are absorbed unchanged (< 1%) and absorbed α-CD is excreted unchanged in the urine (Diamantis & Bär, 2002; Gaebert and Korff, 2004; Diamantis et al., 2004; van Ommen & de Bie, 1995 as cited in WHO, 2002; van Ommen et al., 2004). Data from 13-week toxicity studies in the rat (Lina, 1992 as cited in WHO, 2002; Lina & Bär, 2004a) and in the dog (Til & van Nesselrooij, 1993 as cited in WHO, 2002; Lina & Bär, 2004b) 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. Studies in mice, rats and rabbits show that α-CD is not fetotoxic, embryotoxic or teratogenic (Price *et al.*, 1996; NTP, 1994a, 1994b; Waalkens-Berendsen and Smits van Prooije, 1992 as cited in WHO, 2002; Waalkens-Berendsen et al., 2004; Waalkens-Berendsen and Bär, 2004). α-CD is not mutagenic in standard Ames tests and did not produce any chromosomal damage or damage of the mitotic apparatus in an *in vivo* mouse bone marrow micronucleus test (Blijleven, 1991; Immel, 1991 both as cited in WHO, 2002 and Waalkens-Berendsen and Bär, 2004). The gastrointestinal tolerance of α-CD was examined in adult volunteers, who consumed α-CD without any side-effects (10 g) or only minimal intestinal symptoms (25 g) (Diamantis and Bär, 2002; Diamantis et al., 2004).

Recently, a specially-convened panel of independent experts (Expert Panel), qualified by scientific training and experience to evaluate the safety of food ingredients, independently and collectively critically evaluated the published and unpublished scientific information and data deemed appropriate or pertinent to the safety of α-CD under the conditions of intended use in foods. The Expert Panel also evaluated data and information concerning the method of manufacture, the chemical and physical properties of the product, the product specifications and analytical data, and the specific conditions of intended use of α-CD in foods and

dietary supplements. The Panel unanimously concluded that under the conditions of intended use in foods, Wacker's α-CD meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practice, is "generally recognized as safe" (GRAS) based on scientific procedures. A copy of the Expert Panel's confidential GRAS opinion is provided in Appendix 1.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated α-CD in June 2001 for technological uses in food that would result in a combined Estimated Daily Intake (EDI) of 1.7 and 3.0 g/day for the mean and 90th percentile consumer, respectively. On basis of the available safety studies on α-CD and studies on the related β- and γ-CD, JECFA allocated an Acceptable Daily Intake (ADI) "not specified[1]" to α-CD (WHO, 2002). 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, was recently considered by JECFA at its 63rd meeting. JECFA again granted an ADI "not specified" to α-CD (WHO, 2004).

In Australia and New Zealand, α-CD is currently under review as a novel food. A draft assessment report was published by the Food Standards Australia New Zealand (FSANZ) on May 26, 2004. The EDI for the average Australian consumer was estimated at 17g/d. Based on its evaluation of the available safety data and other pertinent information, FSANZ recommends approval of the use of α-CD as a novel food with no specified limits of use (FSANZ, 2004).

Products consisting of a mixture of α-, β-, and γ-cyclodextrins (with 5-70% α-cyclodextrin) have been sold in Japan for food use in amounts of more than 100 metric tons/year for several years.

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[1] 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).

(4) Probable Consumption of the Substance

Estimated daily intakes (EDIs), averaged over 2 days, were calculated from the different proposed uses of α-CD in food, excluding chewing gum, using the dietary survey approach which relies on food consumption data from the 1994-96 and 1998 Continuing Surveys of Food Intakes by Individuals. The mean and 90th percentile intake (of users of all age groups combined) of α-CD from all of the intended uses in food (except chewing gum) is estimated to be 11.4 and 19.8 g/person/day (approximately 0.21 and 0.43 g/kg bw/day), respectively. On a kilogram body weight basis, young children (ages 2 to 5 years) are estimated to consume the highest amount of α-CD with a mean and 90th percentile intake of 0.61 and 0.98 g/kg bw/day, respectively. The intake of α-CD in chewing gum was calculated using a separate survey on chewing gum use in the United States, from which it was determined that additional amounts of α-CD may be ingested with chewing gum (0.9 g/person/day) (Environ, 2002).

(5) Basis for concluding, in light of the data and information described above, that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

Based on a critical review of the scientific evidence, including, e.g., physical and chemical identity information, manufacturing process, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, an independent panel of scientific experts (Expert Panel), 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 product is generally recognized as safe (GRAS) through scientific procedures. See Appendix 1 for confidential Expert Panel GRAS opinion.

The Expert Panel's conclusion is consistent with the findings of the Joint FAO/WHO Expert Committee on Food Additives (WHO, 2002, 2004).

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Appendix 1

EXPERT PANEL OPINION ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF α-CYCLODEXTRIN FOR USE IN FOODS

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Panel), qualified by their scientific training and relevant national and international experience in evaluating the safety of food and food ingredients[1], was specially convened by Wacker Chemie GmbH (Wacker) to conduct a comprehensive review of the pertinent data and information to determine whether α-cyclodextrin would be "generally recognized as safe" (GRAS) based on scientific procedures, under the conditions of its intended use in foods.

A thorough search of the published scientific literature relating to the safety of α-cyclodextrin was conducted through May 2004 by Bioresco Bioresearch Management and Consulting Ltd. The Panel, independently and collectively, critically evaluated a comprehensive package of the publicly available scientific information and data compiled from such literature and other published sources by Bioresco, as well as any other data and information, published or unpublished, deemed appropriate or pertinent to the safety of α-cyclodextrin. In addition, the Panel evaluated data and information concerning the method of manufacture, the chemical and physical properties of the product, the product specifications and analytical data, and the conditions of intended use in foods and dietary supplements.

Following independent, critical evaluation of all such data and information deemed necessary, the Panel met on December 16, 2000, conferred by telephone thereafter, and unanimously concluded that under the conditions of intended use in foods, Wacker's α-cyclodextrin meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practice, is "generally recognized as safe" (GRAS) based on scientific procedures. Provided below is a summary of the basis for the Panel's conclusion.

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1 Curricula vitae evidencing the expertise of the Panel members are available upon request.

Introduction

Cyclodextrins are cyclic α-(1-4)-linked maltooligosaccharides consisting of 6, 7, and 8 glucose units for α-, β-, and γ-cyclodextrin, respectively. 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 (1903-1911) on microbial food spoilage, *Bacillus macerans*, a heat-resistant, α-cyclodextrin-producing microorganism was isolated (Schardinger, 1903; French, 1957). Since then, many other bacteria have been identified that produce cyclodextrins from starch by means of extracellular cyclodextrin glucosyltransferases (CGTases).

α-Cyclodextrin (CAS No. 10016-20-3), also known as cyclohexa-amylose, cyclomalto-hexose or α-dextrin, has an empirical formula of $(C_6H_{10}O_5)_6$ with a molecular weight of 973 Daltons. It is a cyclic polymer of six α-1.4-linked glycopyranosyl units and is shaped like a hollow truncated cone or torus. Because the hydrogen atoms of the glucose moieties are facing the inner side of the torus, while the hydroxyl groups are located on the outer side, α-cyclodextrin has a hydrophobic cavity and, at the same time, a hydrophilic outer surface making it freely water soluble (13.0 g/100 ml at 25°C). The hydrophobic cavity enables α-cyclodextrin to form inclusion complexes with certain lipophilic substances which fit in its cavity entirely or with part of their molecular structure. This formation of reversible inclusion complexes is the basis for certain applications of cyclodextrins in dry food preparations (excess water would in most cases result in a dissociation of the complex), cosmetics, and pharmaceutical preparations (Hedges et al., 1995; Nagatomo, 1985; Loftsson and Masson, 2001).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated α-cyclodextrin in June 2001 for technological uses in food that would result in a combined Estimated Daily Intake (EDI) of 1.7 and 3.0 g/day for the mean and 90th percentile consumer, respectively. On basis of the available safety studies on α-cyclodextrin and studies on the related β- and γ-cyclodextrin, JECFA allocated an Acceptable Daily Intake (ADI) "not specified" (WHO, 2002) to α-cyclodextrin.

In Australia and New Zealand, α-cyclodextrin is currently under review as a novel food. A draft assessment report was published by the Food Standards Australia New Zealand (FSANZ) on May 26, 2004. The EDI for the average Australian consumer was estimated at 17g/d. Based on its evaluation of the available safety data and other pertinent information, FSANZ recommends approval of the use of α-cyclodextrin as a novel food with no specified limits of use (FSANZ, 2004).

Products consisting of a mixture of α-, β-, and γ-cyclodextrins (with 5-70% α-cyclodextrin) have been sold in Japan for food use in amounts of more than 100 metric tons/year for several years.

Manufacturing Process

α-, β-, and γ-cyclodextrins are formed by the action of CGTases, which are amylolytic enzymes that are produced naturally by different strains of *Bacilli* and other organisms (*e.g.*, *Klebsiella pneumoniae*, *K. oxytoca*, *Micrococcus* sp., *Thermoanaerobacter* sp., and *Brevibacterium* sp.) (Bikbulatova *et al.*, 2000; Tonkova, 1998; Sicard and Saniez, 1987; Schmid, 1989; Bender, 1977). Typically, mixtures of α-, β-, and γ-cyclodextrins are formed by the action of CGTases on amylose; however, different CGTases produce α-, β-, and γ-cyclodextrins in different proportions (Yim *et al.*, 1997). The CGTase from *K. oxytoca* produces mainly α-cyclodextrin and therefore is referred to as "α-CGTase".

The α-CGTase used by Wacker for the production of α-cyclodextrin is obtained from a recombinant strain of *Escherichia coli* K12. 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 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 host for the α-CGTase expression and secretion vector is an *E coli* K12 strain. The *E. coli* K12 strain belongs to risk group 1 in the classification of human etiologic agents (NIH, 2002), and is used in the production of Chymosin A, which has been affirmed as GRAS for use in foods by the U.S. Food and Drug Administration (FDA) (21 C.F.R. § 184.1685(a)(2); 55 Fed. Reg. 10932 (Mar. 23, 1990)). *E. coli* K12 also has been recommended as a safe host organism by the EU Commission (EU Commission, 1997).

The α-CGTase preparation used by Wacker for the production of α-cyclodextrin 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. 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).

For the production of α-cyclodextrin, Wacker applies a solvent process in which 1-decanol is used as a selective complexant for removing α-cyclodextrin from the reaction mixture and enhancing the yield of the process. Briefly, liquefied starch solution (food grade) is treated in a closed stainless steel vessel with α-CGTase

000022

(described above) at appropriate pH and temperature conditions. 1-Decanol (purity >98%; 21CFR§172.864; FEMA Nr. 2365) 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%.

Specifications

Analyses of 5 (3 non-consecutive) batches of α-cyclodextrin showed that the product meets appropriate food grade specifications:

Assay	≥98%
Loss on drying	≤11%
Reducing substances	≤0.5%
Sulfated ash	<0.1%
Volatile organics	<20 ppm
Heavy metals	<5 ppm
Lead	<1 ppm
Optical density (10% aqueous solution at 420 nm)	<0.1
Specific rotation $[\alpha]_D^{20}$	+148±3
Microorganisms	<1000 CFU/g
Salmonella, E. coli	0 CFU/10 g

In order to examine the efficacy of the purification process, three batches of α-cyclodextrin were analyzed for protein. No protein could be detected with gel electrophoresis (limit of detection 5 ppm) and amino acid analysis (limit of detective 10 ppm) (Lottspeich, 2002). In addition, α-cyclodextrin was analyzed for the presence of DNA from the recombinant source organism (*E.coli* K12) of the α-CGTase using a quantitative PCR method. DNA was not detected in the one batch sample tested (limit of detection: 0.005 ng/reaction) (Genetic ID Laboratory, 2001).

Uses in food and estimated daily intake

α-Cyclodextrin is not digested to absorbable glucose in the small intestine to any significant extent, but it is fermented by the colonic microflora like many other

low-digestible carbohydrates. Therefore, α-cyclodextrin, has the nutritional properties of a fermentable dietary fiber, similar to so-called "resistant starch". In animal studies, α-cyclodextrin was found to increase fecal bulk and to lead to stool softening if fed at high dietary concentrations. In humans, α-cyclodextrin attenuated the glycemic response to co-ingested starch, an effect that has been observed also with other dietary fibers such as β-glucan or guar gum (Diamantis & Bär, 2002; Diamantis et al., 2004). With regard to these physiological functions, α-cyclodextrin has the properties of a dietary fiber.

Because of its high stability under food processing conditions and its low viscosity in aqueous solutions, α-cyclodextrin may be used for increasing the dietary fiber content of solid, semi-liquid and liquid foods alike.

Because of its ability to form complexes with certain organic molecules, α-cyclodextrin may also fulfill certain food-technological functions, e.g. as a flavor adjuvant, coloring adjunct; or carrier, stabilizer, and solubilizer of certain vitamins (*i.e.*, retinol acetate, vitamins K_1 and C, riboflavin, and β-carotene) and fatty acids.

The conditions of intended use of α-cyclodextrin in foods are as follows:

Food category	**Maximum use level**
<u>Bakery products</u>	
Breads, rolls, cakes, baking mixes and refrigerated doughs	5%
Brownies and bars	7%
Crackers (sweet and non-sweet)	10%
<u>Beverages</u>	
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%
<u>Cereals and other grain products</u>	
RTE breakfast cereals	2-9%[a)]
Instant rice, pasta and noodles	2% (prepared)
<u>Condiments</u>	3%

000024

Dairy products	
Yogurt, milk beverage mixes and frozen dairy desserts	2.5%
Pudding mixes	1% (dry)
Fats and oils	
Reduced fat spreads	20%
Dressings and mayonnaise	5%
Snack foods	1%
Canned and dry soups	2% (prepared)
Hard candy	15%
Chewing gum	10%

[a)] Use level differs with serving size (<20 g/cup, 2%; 20-43 g/cup, 9%;≥43 g/cup, 5%).

Estimated daily intakes (EDIs), averaged over 2 days, were calculated from the different proposed uses of α-cyclodextrin in food, excluding chewing gum, using the dietary survey approach which relies on food consumption data from the 1994-96 and 1998 Continuing Surveys of Food Intakes by Individuals. The mean and 90th percentile intake (of users of all age groups combined) of α-cyclodextrin from all of the intended uses in food (except chewing gum) is estimated to be 11.4 and 19.8 g/person/day (approximately 0.21 and 0.43 g/kg bw/day), respectively. On a kilogram body weight basis, young children (ages 2 to 5 years) are estimated to consume the highest amount of α-cyclodextrin with a mean and 90th percentile intake of 0.61 and 0.98 g/kg bw/day, respectively. The intake of α-cyclodextrin in chewing gum was calculated using a separate survey on chewing gum use in the United States, from which it was determined that additional amounts of α-cyclodextrin may be ingested with chewing gum (0.9 g/person/day) (Environ, 2002).

Absorption, disposition, metabolism and excretion (ADME) Studies

The results of four experiments conducted in rats provide a coherent picture of the absorption, disposition, metabolism and excretion of α-cyclodextrin (van Ommen & de Bie, 1995 as cited in WHO, 2002; van Ommen et al., 2004).

Like the results of *in vitro* experiments with salivary and pancreatic amylase (Marshall and Miwa, 1981; Kondo et al., 1990; McCleary, 2002), it was found that ingested α-cyclodextrin is resistant to the action of the digestive enzymes and is

000025

not hydrolyzed to a significant extent in the small intestine. Because of its relatively high molecular volume and its hydrophilic surface, only a very small fraction of ingested α-cyclodextrin is absorbed. Since intravenously administered α-cyclodextrin (50 mg/kg bw) 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. With orally administered ^{14}C-α-cyclodextrin (200-1000 mg/kg bw), not more than about 1-2% of the administered dose was recovered from the urine produced by the rats during the first 8 hours following dosing (van Ommen & de Bie, 1995, as cited in WHO 2002; van Ommen et al., 2004). All the non-digestible and not absorbed α-cyclodextrin reaches the colon where the α-cyclodextrin ring is readily opened by microbial enzymes. The resulting linear malto-oligosaccharides are further hydrolyzed and fermented *via* well established metabolic pathways (Antenucci and Palmer, 1984). These results are in keeping with those of previous metabolism studies of cyclodextrins in rats (Andersen *et al.*, 1963; Antlsperger & Schmid, 1996).

In humans, no significant increase of blood glucose and insulin levels was noted after the intake of a bolus dose of 25 g α-cyclodextrin, which supports the low digestibility of α-cyclodextrin seen in animals. However, the absorption of α -cyclodextrin appears to be even lower in humans than in rats as only about 0.01% of the administered dose was recovered from the urine which was excreted during the 3-hour period following ingestion of this dose (Diamantis & Bär, 2002; Gaebert, 2004; Diamantis et al., 2004).

Safety Studies

Acute studies

α -Cyclodextrin has a very low acute toxicity as shown by a study in mice in which the oral administration of α-cyclodextrin at a dose of 10 g/kg bw did not result in any mortality or other signs of intoxication (Immel, 1991, as cited in WHO, 2002). LD_{50} tests in rats and mice with parenteral (i.v., i.p.) administration of α-cyclodextrin gave LD_{50} values of 500-1000 mg/kg bw) (Frank *et al.*, 1976; Riebeek, 1990a,b).

Short-term studies

In an early, limited study, groups of mature male mice were given α- or β-cyclodextrin by gavage for 15 days at a dose of 0 or 60 mg/kgbw/d. Growth of the animals, relative liver weights and fat content of the livers did not differ between the groups (Miyazaki et al., 1979).

000026

Short-term toxicity studies conducted according to OECD Guidelines and following the rules of Good Laboratory Practice include a 28-day study and a 13-week study in rats and a 13-week study in dogs.

In a 28-day range-finding study, groups of Wistar rats (5/sex/group) were fed diets containing 0, 1, 5, 10, or 15% α-cyclodextrin or 5% β-cyclodextrin for 28 days. All rats survived to the end of the study. Transient diarrhea was reported during the first week of treatment in the 5% β-cyclodextrin groups. In rats fed 15% α-cyclodextrin, diarrhea started on day 6 and persisted throughout the study. Body weights of this group were below controls [males: -26% ($p<0.01$); females: -7% (not statistically significant)]. Food intake was reduced in males fed 15% α-cyclodextrin; whereas water intake was increased in males and females. Some hematological and clinical chemistry parameter changes were reported but these were not dose related and/or occurred in one sex only, with the exception that plasma alkaline phosphatase was increased significantly in rats (both sexes) fed 15% α-cyclodextrin. In addition, plasma aspartate aminotransferase and alanine aminotransferase levels tended to be slightly elevated in rats fed 5% (statistically significant in females only). Absolute and relative liver weights were significantly decreased in males fed 15% α-cyclodextrin and a similar trend was observed in females (statistically significant for relative but not absolute liver weight). Cecum weights (full and empty) were slightly increased in rats fed 5% α- or β-cyclodextrin and, more markedly, in the 15% α-cyclodextrin group. A few other organ weight changes observed in males but not females of the 15% α-cyclodextrin group were attributed to the lower body weights of this dose group. No gross abnormalities were detected at necropsy that could be attributed to α-cyclodextrin treatment. Microscopic examination of the main organs (*i.e.*, 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 fed 15% α-cyclodextrin, but no signs of inflammation or degenerative changes of the intestinal mucosa were observed. A depletion of glycogen in periportal hepatocytes in males fed 15% α-cyclodextrin was attributed to their impaired nutritional state. The decreased liver weight reported in this group was probably another manifestation of this condition. The increased water consumption in animals of the 15% α-cyclodextrin group may have been related to the loss of fluids with the diarrhea. In the absence of morphological changes in the kidneys, it is not expected that this was an adverse renal effect, particularly since no renal effects were reported in the following 13-week study conducted at higher dietary concentrations (Lina & Bruyntjes, 1987, as cited in WHO, 2002; Lina & Bär, 2004a).

In a 13-week subchronic toxicity study, groups of Wistar rats (20/sex/group) were fed diets containing 0, 1.5, 5, or 20% α-cyclodextrin for 13 weeks. A comparison group was fed a diet containing 20% lactose. Three additional groups (10

000027

rats/sex/group) were fed 0 or 20% α-cyclodextrin or 20% lactose for 13 weeks and were then maintained on basal diet for a 4-week recovery period. All animals, with the exception of 1 female fed 5% α-cyclodextrin (terminated due to a prolapsed vagina), survived until their scheduled termination. Soft stools were reported during the first few weeks in most rats (particularly males) fed 20% α-cyclodextrin or lactose. No other treatment-related signs were reported. Mean body weights were slightly reduced in males, but not females, fed 20% α-cyclodextrin, reaching statistical significance at the end of the study. Males fed 20% lactose exhibited reduced body weights throughout the study. The reduction of body weights, however, did not exceed 10% at any time (day 91: -6.1 and -7.8% in the 20% α-cyclodextrin and lactose groups, respectively). Food intakes were increased by 6.7 and 5.6% in males and females fed 20% α-cyclodextrin, respectively (the difference compared to controls was significant on a few occasions). Otherwise, food intakes were not different from controls. Food conversion efficiency calculated during the first 4 weeks was approximately 10-15% below control values in males fed 20% α-cyclodextrin or lactose, but did not reach statistical significance. In females, no differences in food conversion efficiency were reported. A slight increase of water consumption was noted for a few days during the first week of treatment in rats fed 20% α-cyclodextrin and males fed 20% lactose. Hematological and clinical chemistry parameters as well as semi-quantitative urinalyses did not reveal adverse changes that could be attributed to the α-cyclodextrin treatment. During the urine concentration test (day 87/88), similar urine volumes were produced among the test groups. In fresh urine samples collected from rats that 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-cyclodextrin 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. Females of the lactose group also exhibited increased fecal weights (not significant) and increased nitrogen excretion (significant). Corresponding, yet somewhat more pronounced changes of these fecal parameters were observed in rats fed 20% α-cyclodextrin. After 13 weeks, the absolute and relative cecal weights (full and empty) were increased in males and females fed 20% lactose and, to a greater extent, 20% α-cyclodextrin. The rats fed 20% lactose also exhibited increased relative weights of the spleen and liver (females), and the testes, adrenals and brain (males). For the spleen ($p<0.01$), testes (not significant) and adrenals (not significant), a slight increase was noted also in males fed 20% α-cyclodextrin. 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 attributable to the α-cyclodextrin treatment. It was concluded that the ingestion of α-cyclodextrin for 13 weeks at dietary concentrations of up to 20% was well tolerated and did not produce any signs of toxicity. The slightly decreased body weights and feed

000028

conversion efficiency in males fed 20% α-cyclodextrin were not considered to be adverse effects due to the fact that α-cyclodextrin is metabolically available only indirectly after fermentation by the intestinal microflora resulting in lower physiological energy values than that of the starch for which it was substituted in the test diets. The NOAEL for α-cyclodextrin in this study was 20%, equivalent to 10 g/kg bw/d, i.e., the highest dose level tested (Lina, 1992 as cited in WHO, 2002; Lina & Bär, 2004a).

In a 13-week subchronic toxicity study in non-rodent species, groups of Beagle dogs (4/sex/group) were fed diets containing 0, 5, 10, or 20% α-cyclodextrin for 90 days. 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 concentrations of α-cyclodextrin but was slightly less pronounced in females than in males. Body weights did not differ significantly between the groups but the weight gain of high-dose females was somewhat reduced from week 4 onwards. Food intake was slightly increased, particularly in high-dose males. Correspondingly, the calculated food efficiency was slightly, yet not significantly, decreased in this group. No treatment-related differences were observed with respect to opthalmoscopic examinations, hematological parameters, clinical chemistry analyses, and semi-quantitative urinalyses. Only urinary pH was slightly below control values in high-dose males (not significant) and high-dose females ($p<0.05$). No abnormalities were reported at necropsy that were attributable to treatment. Organ weight data revealed some cecal enlargement in dogs fed 10 or 20% α-cyclodextrin, reaching statistical significance in males only. On microscopic examination, no treatment-related effects were observed in any of the various organs and tissues. Transient diarrhea, slight cecal enlargement and slightly increased acidity of the urine were the only effects attributable to α-cyclodextrin treatment. The intensity of these physiological changes was much less than that commonly observed in dogs fed low digestible carbohydrates. It was concluded that the 20% dietary dose level, equivalent to an intake of 5 g/kg bw/d, was a NOAEL in this study (Til & van Nesselrooij, 1993 as cited in WHO, 2002; Lina & Bär, 2004b).

Teratogenicity studies

Groups of pregnant Swiss Albino mice (number per group not stated) were fed a diet containing 0, 5, 10, or 20% α-cyclodextrin (equal to 0, 14, 23, and 49 g/kg bw/day, respectively) during gestation days 6 to 16. On day 17, the dams were killed and the fetuses were removed and examined. Maternal relative food consumption was increased on days 16-17 in high-dose dams. No α-cyclodextrin-related effects on maternal food intake were noted at the other concentrations. Maternal relative water consumption was unaffected by treatment. The percent resorptions/litter, average number of live fetuses/litter, average fetal body

000029

weight/litter, and sex ratio/litter did not differ from control values at all concentrations. The incidence of external visceral or skeletal malformations showed no treatment-related effects. The incidence of variations was similarly unaffected by the α-cyclodextrin treatment. Under the conditions of this test, α-cyclodextrin at 20% in the diet was not teratogenic (Price et al., 1996; NTP, 1994b).

Groups of pregnant Sprague-Dawley rats (number per group not stated) were fed a diet containing 0, 5, 10, or 20% α-cyclodextrin (approximately 0, 4,2, 9.0, or 20 g/kg bw/day, respectively) during gestation days 6 to 16. On day 20, the dams were killed and the fetuses were removed and examined. α-Cyclodextrin ingestion caused a dose-dependent increase in maternal relative food consumption during the dosing period (days 6-16) which remained elevated in the 2 highest dose groups until day 18 and then returned to normal on days 18-20. Maternal water consumption during the dosing period showed no distinct dose-related effects; however, sporadic, individually significant increases in water consumption were reported in all treatment groups. Maternal water consumption remained elevated on days 16-18 at the 2 highest dose levels. Despite treatment-related increases in maternal food consumption, average maternal body weight or body weight gain was not affected by α-cyclodextrin ingestion. 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 concentrations. The incidence of external visceral or skeletal malformations showed no treatment-related effects. The incidence of variations was similarly unaffected by the treatment. Under the conditions of this test, α-cyclodextrin at 20% in the diet was not teratogenic (Price *et al.*, 1996; NTP, 1994a).

Groups of pregnant Wistar rats (25/group) were fed a diet containing 0, 1.5, 5, 10, or 20% α-cyclodextrin during gestation days 0 to 21. A separate group was fed a diet containing 20% lactose. The dams were killed on day 21 and fetuses were removed and examined. Generally, α-cyclodextrin was well tolerated and no deaths or abortions occurred in any group. Diarrhea did not occur and soft stool was observed only in 1 high-dose dam on day 11. Maternal weight gains and food consumption were similar in all groups during gestation, except for a slightly increased food intake in high-dose dams from days 6-21 and decreased food intake in dams fed 10% α-cyclodextrin from days 16-21. Water intake was increased from day 16-21 in the high-dose group and from day 0-21 in the lactose group. Reproductive performance was not affected by α-cyclodextrin ingestion. Necropsy of the dams did not reveal gross changes attributable to treatment. Examination of the fetuses for external, visceral and skeletal malformations and anomalies did not reveal any fetotoxic, embryotoxic or teratogenic effects. A significantly increased incidence of renal pelvic cavitation, a visceral variation, was reported in rats fed 20% α-cyclodextrin (33% of fetuses affected) and 20%

000030

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 groups (including lactose group) excluding rats fed 20% α-cyclodextrin. Under the conditions of this study, α-cyclodextrin at 20% in the diet (equivalent to about 10 g/kg bw/d) was not fetotoxic, embryotoxic or teratogenic (was not fetotoxic, embryotoxic or teratogenic (Waalkens-Berendsen and Smits van Prooije, 1992 as cited in WHO, 2002; Waalkens-Berendsen et al., 2004)

Groups of pregnant New Zealand White rabbits (16/group) were fed diets containing 0, 5, 10, or 20% α-cyclodextrin during gestation days 0 to 29. A separate group was fed a diet containing 20% lactose. Dams were killed on day 29 and fetuses were removed and examined. With the exception of mild transient diarrhea in 1 high-dose rabbit, the treatments were well tolerated. Reduced food intake in high-dose dams during the first week resulted in reduced weight gains during week 1 and 2. After week 2, no differences in weight gain were reported and terminal body weights were similar in all groups. No signs of maternal toxicity were noted at any concentration. Reproductive performance was not affected by α-cyclodextrin ingestion. 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 lactose group, the incidence of placental cysts was higher than in controls. Visceral and skeletal examination of fetuses did not reveal any malformations, anomalies or variations that were attributable to α-cyclodextrin ingestion. In the lactose group, the incidence of unossified front phalanges and the total incidence of skeletal retardations were significantly reduced. Under the conditions of this study, a-cyclodextrin at 20% in the diet (equivalent to about 6 g/kg bw/d) was not fetotoxic, embryotoxic or teratogenic (Waalkens-Berendsen and Smits van Prooije, 1992 as cited in WHO, 2002; Waalkens-Berendsen et al., 2004).

Genotoxicity studies

α-Cyclodextrin is not mutagenic in standard Ames tests and did not produce any chromosomal damage or damage of the mitotic apparatus in an *in vivo* mouse bone marrow micronucleus test (Blijleven, 1991; Immel, 1991 both as cited in WHO, 2002).

000031

Studies in humans

In an early study with α-cyclodextrin of unspecified purity, doses of 50 g/day given to two type-2 diabetic subjects were reported to produce nausea in one subject (von Hoesslin and Pringsheim, 1923). In another study, cyclodextrin (consisting mainly of α-, with some β- and γ-cyclodextrin) was consumed at doses of 50-100 g/day by diabetic patients. Some subjects reported nausea and, occasionally, diarrhea. Ingestion of the cyclodextrin preparation did not lead to an elevation of urinary glucose excretion as was seen after the ingestion of bread (von Hoesslin and Pringsheim, 1927).

More recently, the gastrointestinal tolerance of α-cyclodextrin 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-cyclodextrin dissolved in 250 ml water was administered to subjects who had fasted overnight. One subject reported diarrhea and 3 other subjects reported abdominal discomfort. However, these effects were rated as "mild" and did not prevent the subjects from further participation in the study. The ingestion of 10 g α-cyclodextrin dissolved in 250 ml water with 100 g fresh white bread was not associated with any intestinal side effects in any of the subjects (Diamantis and Bär, 2002; Diamantis et al., 2004).

Other Safety Related Aspects

Interaction with the absorption of nutrients

Considering the similar functionality of β-cyclodextrin and α-cyclodextrin as complex-forming agents, it would appear that the conclusion of non-interference with vitamin absorption of β-cyclodextrin (Bellringer *et al.*, 1995; WHO, 1996; Toyoda *et al.*, 1997) applies equally to α-cyclodextrin. Furthermore, the possibility of impaired vitamin absorption due to ingestion of α-cyclodextrin can also be dismissed based on the following:

1. The formation of inclusion complexes with cyclodextrins is reversible (Connors, 1995; Stella *et al.*, 1999);
2. A number of studies have shown that complexes of fat-soluble vitamins or other lipophilic compounds with β- or γ-cyclodextrin are bioavailable and actually may have a higher bioavailability than those that are not in complexed form (Szejtli and Bolla, 1980; Pitha, 1981; Szejtli *et al.*, 1983; Horiuchi *et al.*, 1988; Uekama *et al.*, 1988; Bardos *et al.*, 1989); and
3. α-Cyclodextrin 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).

000032

For these reasons, an impaired bioavailability of vitamins is not expected from the use of α-cyclodextrin in food. In addition, since α-cyclodextrin has a low viscosity and a chemical structure which lacks anionic and cationic groups, it also is not expected to impair the small intestinal absorption of minerals.

Effects on cell membranes

In comparative studies on the *in vitro* effects of different cyclodextrins on cell membranes (*e.g.*, erythrocytes), α-cyclodextrin was significantly better tolerated than β-cyclodextrin, but not as well as γ-cyclodextrin (Szejtli *et al.*,1986; Ohtani *et al.*, 1989). At concentrations of ≥1 mM, α-cyclodextrin was able to extract phospholipids from cell membranes but this was not associated with morphological changes or death of the cells (Ohtani *et al.*, 1989). In comparison to the low absorption of α-cyclodextrin in humans (0.01%) or rats (1%), the cyclodextrin concentrations applied in these in-vitro studies are very high. Therefore, the in-vitro effects on cell membranes have no relevance for the safety of ingested α-cyclodextrin in animals or man.

Cyclodextrin-mediated changes of intestinal permeability

Interaction of certain cyclodextrins with components of cell membranes (cholesterol, phospholipids) changes their fluidity and permeability (Irie *et al.*, 1982). The observation of a higher permeation through an intestinal cell culture model (Caco-2) cell monolayers of 2 peptides in the presence of α-cyclodextrin lead to the hypothesis that α-cyclodextrin might temporarily open tight junctions (Haeberlin *et al.*, 1996). However, experiments with other markers (mannitol, sulfanilic acid) which do not form complexes with α-cyclodextrin do not support this hypothesis (Nakanishi *et al.*, 1990; 1992; Ono *et al.*, 2001).

Discussion

In vitro studies with salivary and pancreatic amylase demonstrate that α-cyclodextrin is not digested. α-Cyclodextrin shares this indigestibility with β-cyclodextrin, and distinguishes it fundamentally from γ-cyclodextrin which is readily digested by pancreatic amylase. In conventional rats, unabsorbed α-cyclodextrin is completely fermented by the intestinal microflora. 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-cyclodextrin is very low (1%). Systemically administered ^{14}C-α-cyclodextrin was excreted unchanged with the urine and was not metabolized to any significant extent.

000033

Being non-digestible by the enzymes of the human alimentary tract, α-cyclodextrin complies with the most important criterion of a dietary fiber. α-Cyclodextrin also exhibits some of the physiological benefits of a dietary fiber as shown by fecal bulking and cholesterol-lowering effects in rats and a blunting effect on postprandial blood glucose levels in man.

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

Assuming that α-cyclodextrin 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-cyclodextrin intake is estimated to be approximately 11.4 g/person/day for the consumers ("users") of such foods (90th percentile consumer: 19.8 g/person/day). Use of α-cyclodextrin in chewing gum will contribute about 0.9 g/person/day. The consumption of α-cyclodextrin is spread evenly over the main meals and between-meals eating occasions during the day. The intake per eating occasion is estimated at 3.9 and 8.0 g for the mean and 90th percentile consumer, respectively. These levels are below those that are tolerated without intestinal symptoms (10 g single dose) or mild symptoms only (25 g single dose). Children of age 2-5 years exhibit the highest estimated intake (relative to body weight) with values of 10.2 and 16.2 g/day and 0.61 and 0.98 g/kg bw/day for the mean and 90th percentile users. This exposure is at least 10 times lower than the NOAEL in rats and dogs (90-day studies).

The toxicity of α-cyclodextrin was examined in standard *in vitro* and *in vivo* toxicity tests. Ames tests and a micronucleus test demonstrate that α-cyclodextrin is not genotoxic. In acute toxicity tests with parenteral administration, the LD_{50} of α-cyclodextrin varied between 500-1000 mg/kg bw (depending upon species and route of administration). In two 13-week oral toxicity tests, rats and dogs received α-cyclodextrin with the diet at concentrations 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-cyclodextrin. No reactions to the treatment were observed on histopathological examination of tissues and organs. It was concluded that α-cyclodextrin ingested at dietary levels of up to 20%, corresponding to about 13 g/kg bw/day (rats) and 10 g/kg bw/day (dogs), was tolerated without any adverse effects. Four embryotoxicity/teratogenicity studies in mice, rats and rabbits with oral administration of α-cyclodextrin at dietary levels of up to 20% (approximately 49, 11-20, and 5.9-7.5 g/kg bw/day for mice, rats, and rabbits, respectively) also did not reveal any treatment-related, adverse effects.

000034

Given the lack of genotoxicity and toxicity in subchronic studies, and considering the metabolism of α-cyclodextrin, long term oral toxicity/carcinogenicity studies are not required for this substance.

A human tolerance study showed that a single bolus dose of 25 g α-cyclodextrin may result in "mild" gastrointestinal effects (*i.e.*, nausea, abdominal discomfort). A single bolus dose of 10 g α-cyclodextrin produced no such effects. Since these effects are transient and not cumulative, the maximum tolerated daily dose is a multiple of the maximum tolerated bolus dose.

In conclusion, there is a substantial body of evidence supporting the safety of α-cyclodextrin as a food ingredient. The data demonstrate that α-cyclodextrin 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-cyclodextrin are absorbed unchanged (< 1%). Absorbed α-cyclodextrin 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-cyclodextrin intakes of up to 13 g/kg bw/d in rats and 10 g/kg bw/d in dogs. The intended use of α-cyclodextrin in foods would result in an aggregated estimated intake of 11.4 g/person/day. The EDI calculations also indicate that the intake of α-cyclodextrin per eating occasion will be about 3.9 g, an amount well below the doses that were tolerated by adult volunteers without side-effects (10 g) or minimal intestinal symptoms only (25 g). On this basis, it can be concluded that α-cyclodextrin would be safe for its intended uses in food.

000035

Conclusion

Based on our critical review of the scientific evidence, we conclude that, under the conditions of its intended use in foods, Wacker Chemie GmbH's α-cyclodextrin, meeting appropriate food grade specifications and manufactured in accordance with good manufacturing practices, is generally recognized as safe (GRAS), through scientific procedures.

By:

(b)(6)

James W. Anderson, M.D — 6/25/04 Date

Joseph F. Borzelleca, Ph.D. — Date

Dennis T. Gordon, Ph.D. — Date

000036

Conclusion

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By:

James W. Anderson, M.D	Date
(b)(6)	23 June 2004
Joseph F. Borzelleca, Ph.D.	Date
Dennis T. Gordon, Ph.D.	Date

000037

Conclusion

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By:

James W. Anderson, M.D	Date
Joseph F. Borzelleca, Ph.D.	Date
(b)(6)	June 23, 2004
Dennis T. Gordon, Ph.D.	Date

000038

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000044

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