Bureau Nieuwe Voedingsmiddelen
Novel Foods Unit

α-Cyclodextrine

α-Cyclodextrin

Tweede beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten

Second opinion regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients

aan/to:

de Minister van Volksgezondheid, Welzijn en Sport
the Minister of Health, Welfare and Sport

Nr. 2005-07BNV, Den Haag, 24 november 2005
No. 2005-07BNV, The Hague, 24 November 2005

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Beoordeling

Inleiding

Aan de orde is een tweede beoordeling volgens de Europese Verordening 258/97, over het gebruik van α-cyclodextrine als een nieuw voedselingrediënt. De aanvraag is ingediend door de firma Bioresco namens de producent, Wacker Chemie GmbH (Duitsland). α-Cyclodextrine is een cyclisch oligosaccharide, een ringvormige verbinding bestaande uit zes suikereenheden. Deze stof is geen gewoon bestanddeel van bekende voedingsmiddelen, maar wordt in de natuur door micro-organismen gevormd uit zetmeel. De industriële productie van α-cyclodextrine vindt plaats door een enzymatische bewerking van zetmeel, gevolgd door enkele zuiveringsstappen. Vanwege bijzondere fysisch-chemische eigenschappen zijn cyclodextrines al eerder gebruikt in geneesmiddelen en cosmetica en als additief in voedingsmiddelen. De in dit dossier voorgestelde toepassing betreft het gebruik van grotere hoeveelheden α-cyclodextrine als ingrediënt in een breed assortiment voedingmiddelen. Voor deze nieuwe toepassing is in de EU een veiligheidsbeoordeling als nieuw voedingsmiddel vereist. In het kader van de desbetreffende toelatingsprocedure is deze tweede beoordeling uitgevoerd door het Bureau Nieuwe Voedingsmiddelen van het College ter Beoordeling van Geneesmiddelen. Het bureau heeft hiervoor de Commissie Veiligheidsbeoordeling Nieuwe Voedingsmiddelen geraadpleegd, hierna genoemd 'de commissie VNV'.

Eerste beoordeling

De eerste beoordeling van de aanvraag voor markttoelating is verricht in België door de Hoge Gezondheidsraad (*Conseil Supérieur d'Hygeniène: CSH*). De CSH concludeert dat er geen bezwaar is tegen toelating van α-cyclodextrine als nieuw ingrediënt, mits er geen substantiële toename zal plaatsvinden van de consumptie van voedingsmiddelen met dit ingrediënt.

Bevindingen van de commissie VNV

De commissie VNV heeft geen bezwaar tegen de toelating van α-cyclodextrine als nieuw voedselingrediënt, maar maakt zelf enkele kritische opmerkingen bij het dossier. Zij baseert haar oordeel op de informatie in het dossier (zie de samenvatting in bijlage A) en de eerste beoordeling van de CSH (bijlage B). Tevens heeft zij gebruik gemaakt van informatie van de *Joint FAO/WHO Expert Committee on Food Additives* (JECFA) [1] [2].

α-Cyclodextrine wordt gevormd door de inwerking van het bacteriële enzym *cyclodextrin glycosyltransferase* op zetmeel van levensmiddelenkwaliteit. Een gen dat

[1] Evaluation of certain food additives. WHO Technical Report Series #928, p. 16-20. (http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf)

[2] WHO food additives series: 48. Safety evaluation of certain food additives and contaminants. Alpha-cyclodextrin. (http://www.inchem.org/documents/jecfa/jecmono/v48je10.htm)

codeert voor het genoemde enzym is daartoe overgezet van de bacterie *Klebsiella oxytoca* M5a1 naar de bacterie *Escherichia coli* K12. Het enzympreparaat wordt door ultrafiltratie geïsoleerd uit het cultuurmedium van deze recombinante bacteriestam. Dit enzym produceert uit zetmeel vooral α-cyclodextrine, maar ook β- en γ-cyclodextrines kunnen worden gevormd, die respectievelijk uit zeven of acht glucose-eenheden bestaan. Na de enzymatische bewerking wordt α-cyclodextrine geïsoleerd door toevoeging van de stof 1-decanol, waarmee het specifiek een onoplosbaar complex vormt. Bij de daaropvolgende zuiveringsstappen wordt het 1-decanol verwijderd door stoomdestillatie en wordt α-cyclodextrine met een zuiverheid van meer dan 98% verkregen door kristallisatie. De CSH bespreekt in haar beoordeling het gebruikte micro-organisme en mogelijke verontreinigingen in het eindproduct, afkomstig van de toevoeging van het enzympreparaat en van de zuiveringsstap met 1-decanol. De CSH concludeert dat het gebruikte micro-organisme veilig is en accepteert de voorgestelde specificatie van het eindproduct, inclusief de mogelijke aanwezigheid van ten hoogste 20 mg per kg 1-decanol in het eindproduct. Ook de JECFA heeft hier bij haar beoordeling rekening mee gehouden. De commissie VNV heeft geen bezwaren op basis van de beschrijving van het productieproces en de specificaties van het eindproduct.

De aanvrager gaat uit van toepassing van α-cyclodextrine in een breed assortiment voedingsmiddelen. In het dossier is een lijst opgenomen met voorgestelde gehaltes α-cyclodextrine in verschillende typen voedingsmiddelen (tabel 4 van het dossier). De aanvrager gebruikt deze gegevens in combinatie met voedselconsumptiegegevens uit de Verenigde Staten om een schatting te maken van de verwachte inname van α-cyclodextrine voor verschillende leeftijdsgroepen (tabel 5 en annex 5 van het dossier). Daarbij is aangenomen dat het voorgestelde maximale gehalte α-cyclodextrine aanwezig is in alle voedingsmiddelen die dit ingrediënt kunnen bevatten. Voor alle leeftijdsgroepen samen komt deze berekening uit op een gemiddelde inname van 10,7 gram α-cyclodextrine per dag (18,8 gram per dag voor het 90 percentiel). De eerste beoordeling van de CSH noemt overigens iets hogere getallen, omdat zij ook rekening houdt met de mogelijke inname van 0,6 tot 0,9 gram α-cyclodextrine per dag via kauwgom. Per kilogram lichaamsgewicht is de geschatte inname het hoogst voor kinderen van twee tot vijf jaar oud: gemiddeld 0,6 gram per kilogram per dag (0,96 gram per kilogram per dag voor het 90 percentiel). De commissie VNV vindt het teleurstellend dat Europese voedselconsumptiegegevens in het dossier ontbreken.

Op basis van verschillende onderzoeksgegevens stelt de aanvrager dat α-cyclodextrine in het maagdarmkanaal niet door het lichaam zelf wordt verteerd en nauwelijks wordt geabsorbeerd. Wel wordt het afgebroken door micro-organismen in de darm. De CSH staat in haar eerste beoordeling ook stil bij de mogelijkheid van interacties van α-cyclodextrine met vetoplosbare stoffen en de denkbare gevolgen hiervan voor absorptie van bepaalde voedingsstoffen. Door zijn chemische structuur als een ring met een hydrofobe binnenzijde, kan α-cyclodextrine vetoplosbare moleculen reversibel binden. De CSH noemt in haar beoordeling dat zij hierover aanvullende informatie heeft gevraagd aan de aanvrager. De aanvrager zou vervolgens de informatie in hoofdstuk 9.3 van het dossier verder hebben onderbouwd. De commissie VNV heeft geen inzage in de aanvullende gegevens, maar merkt op dat ook de JECFA op basis van beschikbare gegevens voor β-cyclodextrine meent dat een effect van consumptie van α-cyclodextrine

op de absorptie van vetoplosbare voedingsstoffen niet waarschijnlijk is. Hoewel de commissie VNV geen concrete aanwijzingen heeft om hieraan te twijfelen, beveelt zij aan om deze veronderstelling ook experimenteel te bevestigen.

De eerste beoordeling van de CSH bevat een korte samenvatting van het in het dossier beschreven toxicologisch onderzoek aan α-cyclodextrine. Ook is een uitgebreide toxicologische beoordeling van α-cyclodextrine uitgevoerd door de JECFA. De bevindingen van de JECFA zijn ook van toepassing op de beoordeling van dit dossier. Een eerste JECFA-beoordeling had betrekking op het gebruik van α-cyclodextrine als voedseladditief voor een aantal gespecificeerde toepassingen. Bij die gelegenheid kende de JECFA de status "*ADI not specified*" toe (ADI = *Acceptable Daily Intake*). Dit betekent dat de JECFA op basis van de beschikbare informatie over de eigenschappen en het voorgestelde gebruik van deze stof geen gezondheidsrisico voorziet[3]. Daarbij werd uitgegaan van een verwachte inname van gemiddeld 1,7 gram α-cyclodextrine per dag (3 gram per dag voor de 90 percentiel). Bij een volgende gelegenheid verklaarde de JECFA de status "*ADI not specified*" ook van toepassing op het gebruik van α-cyclodextrine als ingrediënt. Bij die beoordeling werd uitgegaan van een totale inname van 65 gram α-cyclodextrine per dag, al tekende men hierbij aan dat de werkelijke inname waarschijnlijk aanzienlijk lager zal zijn. Dit laatste blijkt ook uit de eerder genoemde schattingen voor de inname van α-cyclodextrine in het dossier. Verder blijkt uit de gegevens in het dossier dat bij sommige proefpersonen milde gastro-intestinale klachten optraden bij een inname van 25 gram α-cyclodextrine in één keer. Volgens de aanvrager worden dergelijke effecten ook waargenomen voor andere minder goed verteerbare koolhydraten, wat ook door de JECFA wordt bevestigd. De commissie VNV onderschrijft de conclusies van de JECFA.

Conclusie

Samenvattend is de commissie VNV het eens met de conclusie dat α-cyclodextrine veilig kan worden gebruikt voor de toepassingen die in het dossier zijn beschreven. Het betreurt de commissie wel dat geen Europese voedselconsumptiegegevens zijn gebruikt voor de schatting van de inname van α-cyclodextrine. Ook beveelt de commissie aan om experimenteel te bevestigen dat consumptie van α-cyclodextrine geen effect heeft op de opname van vetoplosbare nutriënten.

[3] Principles for the safety assessment of food additives and contaminants in food. (http://www.inchem.org/documents/ehc/ehc/ehc70.htm)

English courtesy translation

Introduction

The subject in question is a so-called second opinion, in accordance with European Regulation 258/97, regarding the use of α-cyclodextrin as a novel food ingredient. The application was submitted by the company Bioresco, on behalf of the producer, Wacker Chemie GmbH (Germany). α-Cyclodextrin is a cyclic oligosaccharide, consisting of six sugar units. It is not a normal constituent of known foods, but occurs in nature as a microbial metabolite of starch. The industrial production of α-cyclodextrin starts with an enzymatic treatment of food-grade starch. A number of purification steps complete the process. Because of their special physico-chemical properties, cyclodextrins have been used previously in pharmaceuticals and cosmetics, and as food additives. This dossier describes the use of larger quantities of α-cyclodextrin as an ingredient in a broad range of foods. This new application requires a safety assessment as a novel food in the EU. In the subsequent assessment procedure, this second opinion is issued by the Novel Foods Unit of the Medicines Evaluation Board Agency, after consultation of the Committee on the Safety Assessment of Novel Foods (VNV Committee).

Initial assessment

The initial assessment of the application for market authorization was carried out by the Belgian food assessment body, the *Conseil Supérieur d'Hygeniène* (CSH). The CSH concludes that there is no objection to the authorisation of α-cyclodextrin as a novel food ingredient, provided that there is no substantial increase in the consumption of foods containing α-cyclodextrin.

Findings of the VNV Committee

The VNV Committee does not object to authorisation of α-cyclodextrin as a novel food ingredient. However, it does have some critical comments regarding this dossier. The committee bases its view on the information in the dossier (see annex A for the summary) and the report of the first assessment by the CSH (see annex B). In addition, the committee used information from the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [1][2].

α-Cyclodextrin is produced by the action of the bacterial enzyme *cyclodextrin glycosyltransferase* on food-grade starch. For this purpose, a gene encoding the enzyme was transferred from the bacterium *Klebsiella oxytoca* M5a1 to the bacterium *Escherichia coli* K12. The enzyme preparation is isolated from the culture medium of this recombinant bacterial strain by ultrafiltration. Using starch as a substrate, this enzyme predominantly produces α-cyclodextrin. β-Cyclodextrin and γ-cyclodextrin can also be formed, consisting respectively of seven or eight sugar units. After this reaction 1-decanol is added, specifically

[1] Evaluation of certain food additives. WHO Technical Report Series #928, p. 16-20. (http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf)

[2] Evaluation of certain food additives. WHO Technical Report Series #928, p. 16-20. (http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf)

forming an insoluble complex with α-cyclodextrin. In the following purification steps, 1-decanol is removed by steam distillation. Crystallisation results in a final product of over 98% purity. In the initial assessment, the CSH discusses the micro-organism used and possible impurities in the final product, originating from the enzyme preparation or the purification step using 1-decanol. The CSH concludes that the used micro-organism is safe. Furthermore, it has no objection to the proposed specification of the final product, including the possible presence of up to 20 mg per kg 1-decanol, a fact that the JECFA has also taken into account. The VNV Committee has no objections regarding the described production process and the specification of the final product.

The applicant proposes the use of α-cyclodextrin in a broad range of foods. The dossier contains a list of proposed levels of α-cyclodextrin in different types of foods (table 4 in the dossier). The applicant uses these figures in combination with food survey data from the United States to estimate the expected intake of α-cyclodextrin by individuals in different age groups (described in table 5 and annex 5 of the dossier). This calculation assumes that α-cyclodextrin is present at the proposed maximum level in all the foods that can contain this ingredient. The mean intake for all age groups combined was estimated to be 10.7 grams of α-cyclodextrin per day (18.8 grams per day for the 90th percentile). The initial assessment by the CSH mentions slightly higher values, taking into account the possible consumption of an additional 0.6 to 0.9 grams of α-cyclodextrin from chewing gum. Expressed per kg body weight, the highest mean intake estimate is 0.6 g/kg/day for 2-5 year old children (0.96 g/kg/day for the 90th percentile). The VNV Committee is disappointed by the lack of European food survey data in the dossier.

The applicant states that experimental evidence shows that α-cyclodextrin is not digested by the human body itself, nor is it absorbed from the gut in significant amounts. However, α-cyclodextrin is fermented by micro-organisms in the gut. In the initial assessment, the CSH also considers the possibility of interactions of α-cyclodextrin with lipophilic substances, and the possible consequences for the absorption of certain nutrients. The chemical structure of α-cyclodextrin as a ring-like molecule with a hydrophobic interior, allows the reversible binding of lipophilic substances. The CSH mentions having requested additional information from the applicant regarding this issue. According to the initial assessment report, the applicant has subsequently backed up the information in chapter 9.3 of the dossier. The VNV Committee has not seen this additional information, but points out that the JECFA has stated that, based on analogy with results for β-cyclodextrin, it is unlikely that consumption of α-cyclodextrin will affect the absorption of lipophilic nutrients. The VNV Committee has no evidence to the contrary, but nevertheless recommends that this assumption should be confirmed experimentally.

In its initial assessment, the CSH briefly summarises the toxicological studies described in the dossier. An extensive toxicological assessment of α-cyclodextrin has also been performed by the JECFA. The observations by the JECFA are also relevant for the assessment of this dossier. In its first assessment, the JECFA considered the use of α-cyclodextrin as a food additive for specified purposes. On that occasion, the JECFA has allocated an ADI “not specified” (ADI = Acceptable Daily Intake). This means that, on the basis of available data on the properties of this substance and its proposed use, the JECFA

sees no hazard to health[3]. In this assessment, a mean intake of 1.7 grams of α-cyclodextrin per day was estimated (3 grams per day for the 90th percentile). On a following occasion, the JECFA also allocated an ADI "not specified" for the use of α-cyclodextrin as a food ingredient. During that assessment, a estimated total intake of 65 grams of α-cyclodextrin per day was taken into consideration. The JECFA nevertheless expected the true intake to be significantly lower. Estimates in this novel food dossier for consumption of α-cyclodextrin confirm this expectation. The dossier also describes mild gastrointestinal complaints in some subjects following a bolus intake of 25 grams of α-cyclodextrin. According to both the applicant and the JECFA, similar effects were also observed for other carbohydrates of low digestibility. The VNV Committee supports the conclusions of the JECFA.

Conclusion

In summary, the VNV Committee concurs with the conclusion that α-cyclodextrin can safely be used as a food ingredient for the application described in the dossier. However, the VNV Committee was disappointed that no European food survey data were used to estimate the daily intake of α-cyclodextrin. Furthermore, the VNV Committee recommends that it is experimentally confirmed that consumption of α-cyclodextrin will not affect the absorption of lipophilic nutrients.

[3] Principles for the safety assessment of food additives and contaminants in food. (http://www.inchem.org/documents/ehc/ehc/ehc70.htm)

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Bioresco
Food Scientific and Regulatory Services

ALPHA-CYCLODEXTRIN

Application submitted on behalf of Wacker Chemic GmbH, Germany, for evaluation pursuant to Regulation (EC) No 258/97 on novel foods and novel food ingredients

Executive Summary

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Date: October 8, 2004

INTRODUCTION

Alpha-cyclodextrin (α-CD) is a cyclic α-(1→4)-linked maltooligosaccharide consisting of six glucose molecules. It occurs in nature as an extracellular oligosaccharide, which is produced by certain microorganisms, such as Bacillus macerans, from starch. On a commercial scale, α-CD is produced from liquefied starch using a bacterial cyclodextrin glucosyl-transferase (CGTase), an enzyme of the α-amylase family.

α-CD is not digested by the human digestive enzymes. However, it is fermented completely by the intestinal microbiota. Hence, α-CD is, in physiological terms, a soluble, fermentable dietary fiber, comparable to – for example – fructooligo-saccharides or resistant dextrins. Accordingly, α-CD is intended to be used in foods primarily for its nutritional properties as a dietary fiber.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated α-CD at its 63rd meeting (June 2004) as a nutritive substance (dietary fiber) with an aggregate daily intake from all uses of 11.4 and 19.8 g/d for the mean and 90th percentile consumer, respectively. An ADI "not specified" was allocated. Specifications are published in the FAO Compendium of Food Additive Specifications (Addendum 9). A toxicological monograph, which was prepared for α-CD at the 57th meeting of JECFA, has been published (WHO Food Additive Series 48). Publications of all pivotal safety studies have appeared recently [Regulatory Toxicology and Pharmacology, Vol. 39 (Suppl. 1), 2004].

In Australia/NZ, α-CD is currently under review as a novel food. The assessment report by Food Standards Australia New Zeeland (FSANZ) recommends approval of α-CD as a novel food.

In the US, a GRAS-Notice has been submitted to the Food and Drug Administration (GRN No. 155).

In Japan, α-CD is already consumed for many years with mixed cyclodextrin products that contain 5-70% α-CD and are used in food production.

MANUFACTURING PROCESS

α-CD is produced by the action of α-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 (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. 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 formed α-CD is continuously 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. The product has a purity of >98%. 1-Decanol is present at levels of <20ppm. Protein from the enzyme

preparation and DNA from the source microorganism of the enzyme could not be detected in α-CD.

INTENDED USE AND ESTIMATED DAILY INTAKE

Being non-digestible by the enzymes of the human alimentary tract, α-CD complies with the most essential element of the definition of dietary fiber. α-CD also exhibits some of the physiological benefits of dietary fiber as shown by fecal bulking, a slight cholesterol lowering effect in rats and a blunting effect on postprandial blood glucose levels in man. α-CD is, therefore, useful for the fiber enrichment of foods.

Being soluble and stable under all temperature and pH conditions typically encountered in food production, α-CD may be added as a dietary fiber to solid, semi-liquid and liquid foods. Typical use levels vary between about 1% (most types of beverages), 2% (cereal products, pasta etc.), 5% (certain baked products) and 7-10% (cereal bars, crackers). Since α-CD may distort the flavor of foods if added in excessive amounts, there is a self-limiting effect to its use.

Assuming that α-CD is incorporated at maximum feasible levels in all foods in which it may fulfill a useful nutritional function, the average α-CD intake is estimated to be approximately 10.7 g/person/day for the consumers ("users") of such foods (90^{th} percentile consumer: 18.8 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 4.0 and 7.8 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 as are typically associated with an excessive intake of low digestible carbohydrates (25 g single dose).

SAFETY STUDIES

The toxicity of α-CD was examined in standard in-vitro and in-vivo toxicity test. 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). 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.

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. 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)]. Protein and DNA derived from the source organism of the CGTase preparation could not be detected in α-CD.

1-Decanol, which is used as complexant for the precipitation of α-CD, has been used as a flavor for many years. 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 sings of maternal toxicity and there was no evidence of teratogenic effects. The pre- and post-implantation losses were less increased with 1-decanol than with ethanol. Considering that in humans the 1-decanol intake from α-CD would be less than 4 µg/kg bw/d, this effect, observed at a 5 x 10^5 times higher dose, has no practical relevance.

The interaction between α-CD and certain lipophilic molecules to form inclusion complexes has no relevance for the absorption of vitamins or other nutritive substances because the complexed "guest molecules" are easily released in the presence of water and/or the chyme. In addition, α-CD is not known to form complexes with retinol, vitamin D and vitamin E, probably because the size of its central cavity is too small. An impairment by α-CD of mineral absorption also is not to be expected because the α-CD molecule lacks anionic groups.

In conclusion, there is a substantial body of evidence to support the safety of α-CD under the conditions of its intended use as a food ingredient (dietary fiber).

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Rapport d'évaluation initiale du Conseil Supérieur d'Hygiène concernant une demande d'autorisation d'alpha-cyclodextrine (Wacker Chemie GmbH) au titre du Règlement (CE) n°258/97 relatif aux nouveaux aliments et aux nouveaux ingrédients alimentaires

(CSH 8088 – Emis et approuvé par le Groupe de Travail « Alimentation, Nutrition, y compris Sécurité alimentaire » le 29 juin 2005)

Initial assessment report from the Public Health Board concerning an application for the authorisation of alpha-cyclodextrin (Wacker Chemie GmbH) under Regulation (EC) No 258/97 on novel foods and novel food ingredients

(CSH 8088 – Issued and approved by the Working Group on Food, Nutrition and Food Safety on 29 June 2005)

1. INTRODUCTION

1. The application was submitted in Belgium by Bioresco Ltd., Basel, Switzerland on behalf of Wacker Chemie GmbH, Munich, Germany.

2. Alpha-cyclodextrin is a cyclical malto-oligosaccharide α -(1→4) comprising 6 glucose molecules. It occurs naturally in the form of extracellular oligosaccharide produced by microorganisms such as *Bacillus macerans*.

3. The substance is produced on a commercial scale by a cyclodextrin-glucosyltransferase of bacterial origin, using liquefied starch as the substrate. The enzyme is derived from a recombinant strain of Escherichia coli K-12. The gene is derived from a non-pathogenic, non-toxicogenic strain of *Klebsiella oxytoca*.

4. The applicant has placed the product in Class 1.2 "Pure chemicals or simple mixtures from non-GM sources, the source of the NF has no history of food use in the Community. " This means that structured schemes I, II, III, IX, XI, XII and XIII must be presented (cf. Commission Recommendation 97/618/EC).

2. SPECIFICATION OF THE NOVEL FOOD : STRUCTURED SCHEME I

α-cyclodextrin (α-CD) is a cyclical polymer comprising 6 α -(1→4) linked glycopyranosyl units. It is also known under the name of cyclohexa-amylose and cyclomalto-hexaose. Its molecular weight is 973 daltons.

α-CD is water-soluble, has a melting point of around 250°C and crystallises with 6 mol water. It is stable in the pH conditions of foodstuffs but hydrolyses in highly acidic environments. The rate of hydrolysis is slower than in linear oligosaccharides.

α-CD has no reactive functional groups nor a reductive final position. It does not react with the other ingredients and does not turn brown in colour as occurs with the Maillard reaction.

It is thermostable in the conditions applied during the technological processes used for foodstuffs. It undergoes enzymatic hydrolysis with microbial α-amylases, but not with human α-amylases (saliva and pancreas).

On account of its specific structure (a hollow truncated cone with a hydrophobic cavity and a hydrophilic external wall), it possesses complexing properties, forming inclusion complexes with some organic molecules. Its applications in food, pharmaceuticals and cosmetics are based on this property. The applicant states that this is not the basis of the application.
Cyclodextrins (CD) occur naturally and are produced by several microorganisms, including *Bacillus macerans*. There are no known data concerning the intake of CD in food.
Studies on *Klebsiella oxytoca* show that cyclodextrins play a role in the intake of starch as a source of C; starch is transformed outside the cell into CD by CGTase (cyclodextrin glucosyltransferase), then the CD are taken up by the cell thanks to a special transport system. Inside the cell, the ring is open and the malto-oligosaccharides formed are hydrolysed and catabolised.

Impurities may come from a different source.
The raw material, liquefied starch, produces malto-oligosaccharides, maltose and glucose.
The α-CGTase preparation is obtained by filtering through a 0.2 µm filter and has a molecular weight of approximately 20 000 daltons. Impurities from the fermentation medium occur. The high molecular mass fraction consists mainly of the enzyme and fragments of lysed *E. coli* cells.
At the time of preparation, components with a high molecular mass are separated from the α-CD during a precipitation stage using a complexing agent. The residual protein is denatured by heating when subsequently purified.
Protein determination by PAGE has shown that the residual protein content is less than 5 mg protein per kg of α-CD. These results have been confirmed by amino acid determination following acid hydrolysis.
The absence of DNA from a recombinant source of CGTase has been determined by a quantitative PCR method. The detection limit is 0.005 ng per reaction, which is equivalent to a limit of 0.01 % GMO in a standard GMO test.
The low molecular mass fraction comprises nutrients from the fermentation medium: amino acids, peptides, glucose, minerals, vitamin B1 as well as small amounts of tetracycline, IPTG and sorbic acid. These impurities are removed at the first and second stages of precipitation.
The complexing agent, 1-decanol, is removed at the second stage of precipitation by steam distillation. Analysis has shown that the residual content is less than 15 ppm.

The following **specifications** apply:
Purity: at least 98 % α-CD on an anhydrous base
Ash content: not exceeding 0.1 %
Reducing sugars: not exceeding 0.5 %
Heavy metals (such as lead): not exceeding 5 ppm
Arsenic: not exceeding 3 ppm
Lead: not exceeding 0.5 ppm
Volatile organic substances (1-decanol): not exceeding 20 ppm
Microbiological aspects are examined in point XII.

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The CSH raised the following questions in December 2004:

1. The CSH wished to have results demonstrating the absence of tetracycline and IPTG residues. Both products are evidently used during the fermentation process.
2. The CSH also wished to have information on *E. coli* residues originating from the fermentation medium.

Applicant's replies and the CSH's opinion:

1. **Tetracycline and IPTG residues**

   The applicant pointed out that methods had been developed in response to this question.
   Analyses had been carried out on 3 batches of α-CD. The detection limit of the methods developed was:
   0.5 ppm for tetracycline
   1 ppm for IPTG
   The two components could not be detected in the three samples examined. HPLC methods were used.
   The applicant pointed out that it was logical that residues of the two production aids could not be detected.
   Ultrafiltration was applied for purification, as well as double precipitation of the α-CD.

   The applicant had answered the question and shown that no tetracycline or IPTG residues could be detected.

2. **E. coli residues**

   The applicant refers to the dossier which provides evidence that the strain used is safe; residues of the organism are therefore irrelevant from the point of view of safety.
   With regard to the purity of α-CD, it also emerges from the dossier that no *E. coli* residues can be found.
   The applicant refers once more to the complex purification process as an explanation for these findings.

3. **Conclusion**

   The applicant has answered both questions and has shown that:
   - no tetracycline or IPTG residues can be found
   - no *E. coli* residues can be detected.

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3. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NF: STRUCTURED SCHEME II

Plan of the procedure

- solution of liquefied starch
- addition of CGTase and 1-decanol
- enzyme reaction at 40°C
- isolation of α-CD/decanol complex
- purification of the complex by dissolving in water and reprecipitation
- removal of 1-decanol in a stripping column
- concentration of the α-CD solution
- crystallisation of α-CD
- filtration of crystals
- drying
- packing

Products used during the procedure:

- liquefied starch: raw material
- NaOH: processing aid
- CGTase preparation: enzyme
- water: solvent
- 1-decanol: complexing agent

Production of the enzyme CGTase

Klebsiella oxytoca M5a1 secretes an effective CGTase. The coding gene for this enzyme was isolated, characterised, cloned in pHE3, isolated again and imported into the expression vector pJF118EH. *Escherichia coli* K12 was used as the host organism.

During enzyme production, the recombined organism is grown on a standard medium of glucose, mineral salts, casein hydrolysate as a source of N, micro-nutrients, vitamin B1 and yeast extract.

The enzyme is extracted by the usual methods:

- removal of cells and particles by centrifugation,
- cooling of the supernatant and two-stage filtration up to a pore diameter of 0.2 µm,
- concentration by cross-flow ultrafiltration on membranes with a 20 000 dalton cut-off,
- addition of 5g/l sorbic acid.

The usual quality control checks are carried out during fermentation.

Safety of microorganisms

Klebsiella oxytoca is a gram-negative, optionally anaerobic organism, belonging to the Enterobacteria family. It is found in faecal microbiota but also in water and the rhizosphere of rice (fixation of N). There are pathogenic strains, but *K. oxytoca* is in Risk Group 1 or group of organisms not associated with disease in healthy adult humans, in accordance with the NIH Guidelines for Research Involving Recombinant DNA Molecules (2002).

The strain used as a donor for the CGTase gene is *K. Oxytoca* M5a1. This strain is described as being well characterised. The UK Advisory Committee on Genetic Modification states that the strain has a history of safe use and negligible ability to survive in man.

The vector used is derived from vector pJF118EH, which is considered to be safe (EU Commission, COM (95) 640 final, 1997).

A strain of *E. coli* K12 is used as the host organism for the expression and secretion of CGTase. This strain belongs to Risk Group 1, according to the NIH, 2002. This organism is used to produce chymosin and is considered safe (Fed. Reg. 1990 and EU Commission, COM (95) 640 final, 1997).

Comment: The CSH has focused particular attention on the safety of the microorganism and the vector used. The CSH notes that these points do not pose a problem.

Safety of the CGTase preparation

With regard to the safety of the CGTase preparation, a number of toxicity tests were carried out:

- Ames in vitro genotoxicity test
- in vitro chromosomal aberration test
- subchronic toxicity in rats

The applicant concluded that there was no problem. If all the organic substances present in the enzyme preparation were present in the α-CD, exposure would then be at least 1000 times less than the NOAEL. Obviously, only a part of these substances is found in the finished product on account of the purification applied.

Legal status of the enzyme preparation

The applicant pointed out that the enzyme is removed completely from the finished product after the production process. Tests on protein and amino acid residues had been carried out in order to do this. The applicant pointed out that the enzyme preparation is not an additive or an adjuvant.

Comment: The CSH agreed with this proposal.

Safety of the complexing agent: 1-decanol

1-decanol (decyl alcohol) is used as a complexing agent in the production process. It is a colourless liquid with a melting point of 7°C and boiling point of 220-235°C. It is not very water-soluble and has a floral, fruity, waxy odour. 1-decanol is used as an ingredient in flavourings.

There are no data available concerning absorption, distribution, metabolism and excretion of 1-decanol. It is generally accepted that it is resorbed as primary aliphatic alcohol and oxidises into aldehyde. It is then transformed into the corresponding acid and metabolised by β-oxidation in the citric acid cycle.

The toxicity of 1-decanol has been examined in a number of studies:

- gene mutation test on *B. subtilis* H17 and M45 with 17 µg per plate
  negative result
- acute oral toxicity in rats: two studies
  LD50 : >5 and 12.8 g/kg of body weight
  In mice: LD50: 6,5 g/kg of body weight
- embryotoxicity: teratogenicity in rats
  exposure through inhalation of 100mg/m^3; 6 hours/day; for 19 days
  no sign of toxicity
- embryotoxicity/teratogenicity in rats of a series of primary alcohols, including 1-decanol
  oral administration of 400 mg per day for 15 days
  no sign of toxicity observed for decanol (though for methanol and ethanol).

Comment: The CSH agrees with the applicant's proposal, given the low toxicity of decanol and the low quantities present in α-CD.

Legal status of 1-decanol

1-decanol has been evaluated by the JECFA as a "flavouring substance" and there are no problems regarding its safety. Daily intake is estimated at 28 µg per person per day. The EU has included 1-decanol in the list of "flavouring substances" under FL No 02.024. In the USA, 1-decanol has GRAS status (Fema No 2365). It is included in the list of fatty alcohols (21 CFR & 172.864) for use in the synthesis of food additives and other substances permitted in food and in the list of synthetic flavouring substances 21 CFR & 172. 515).

Comment : It is a processing aid.

4. HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NF: STRUCTURED SCHEME III

The applicant points out that the NF is derived from starch and not from an organism and that no other information is therefore required.

Comment: The CSH agrees with this point of view.

5. ANTICIPATED INTAKE / EXTENT OF USE OF THE NF: STRUCTURED SCHEME IX

The average and 90 percentile, calculated on the basis of all foods likely to contain α-CD (except chewing gum), is equal to 0.21g/kg per day ; and 0.43 g/kg per day (11.4 and 19.8 g/day), respectively.
For children from 2 to 5 years, the average and 90 percentile is equal to 0.61 and 0.98 g/kg per day, respectively.
The concentration of α-CD in chewing gums is 10%; intake by this route (3 chewing gums) would be 0.6-0.9 g/day.

Comparison of data supplied with toxicological data and conclusion:
cf. point 8 (= Structured scheme XIII)

6. NUTRITIONAL INFORMATION ON THE NF: STRUCTURED SCHEME XI

BIOLOGICAL STUDIES

In vitro digestibility studies
Not digestible by human and pig salivary and pancreatic amylases (as opposed to gamma-cyclodextrin)

Bioavailability studies in animals: analogy to fermentable fibres
(From ^{14}C-alpha-cyclodextrin (α-CD) versus ^{14}C-potato starch).
- period of from 3.5 -9.5 hours for expiration of CO2 following oral intake of 1.5 g (sign of fermentability); confirmed following the administration of 200 and 1000 mg per kg of body weight in rats; 1% of the 14c is excreted unaltered in the urine between 0 and 4 hours after administration. Fermentation via bacterial amylases and cyclodextrinases
- intravenous injection (50 mg/kg): half-life of 88 minutes; excretion of 80% within 8 hours, mainly in the urine.

Digestibility in man: confirmation of fermentability
90% of the oral dose is recovered in the ileal effluent of ileostomised patients.
Administration of 10, 25 or 50 g of α-CD +/-50g starch in healthy or diabetic volunteers/ No rise in glycaemia and urinary glucose with α-CD alone (0.01% of the dose of α-CD consumed excreted in urine).

Interaction with the absorption of other substances/nutrients
- Lyophilic substances (NB: α-CD: vehicle for the administration of Ibuprofen)
Formation of reversible complexes with lipophilic substances (not with retinol, vitamins D and E ("size")).
- Minerals: No harmful effect anticipated on the absorption of minerals ("fermentable fibres" effect)
- Dietary carbohydrates: cf. "digestibility" experiments: Delay and reduction of the glycaemic response of starch in the presence of α-CD.

7. MICROBIOLOGICAL INFORMATION ON THE NF: STRUCTURED SCHEME XII

The applicant pointed out that no micro-organisms were involved in the α-CD production process.

Comment: microorganisms are used in CGTase production and are therefore used indirectly in the process.
Structured scheme XII must therefore be included.

The application contained data relating to the analysis of 5 batches in respect of the microorganisms present. This showed that microbial specifications were largely met
- microorganisms: < 1000 CFU/g
- *Salmonella*: 0 CFU/10g
- *E. coli*: 0 CFU/10g

Comment: The CSH agrees with the data concerning microbial purity.

8. TOXICOLOGICAL INFORMATION ON THE NF: STRUCTURED SCHEME XIII

TOXICOLOGICAL STUDIES

Acute: LD50 in Sprague-Dawley rats : 1g/kg after intravenous (iv) administration (renal toxicity: osmotic nephrosis) and between 0.75 and 1g/kg by intraperitoneal (ip) injection; between 500 and 750 mg/kg in mice.

Short and medium-term oral administration:
- No effect following administration of 60 mg/mouse (15 days)
- Increase in caecum weight and decrease in body weight and liver weight in rats receiving a 15% dose of α-CD for 28 days. No histopathological changes were detected; this was confirmed in a 13-week study of intake at a dose of 20% (corresponds to 12.6 and 13.9 g/kg of weight for male and female rats).
- No relevant effect (except for a decrease in the pH of urine) in dogs which had received 5 to 20% α-CD.

No reference to studies analysing the long-term carcinogenic and reproductive effects

Embryogenicity / teratogenicity
No effect in in vivo studies (administration of 5 to 20% to pregnant Albino mice). 20% is equivalent to 49.3 g/kg per day. Studies carried out on female rats (same doses) show no embryogenicity; idem in female rabbits. NOAEL in rats: 11g /kg per day; in rabbits: 7.5g/kg per day.

Mutagenicity
Ames test and micro-nucleus tests negative.

Toxicity tests on rabbit skin and mucous membranes
No skin irritation/corrosion lesions (0.5g; analyses within 72 hours of application);
Ocular instillation of 0.062g α-CD powder: ocular irritation 1 hour after instillation; lesions disappeared after 14 days (conclusion: irritant but not corrosive agent). Instillation of solution (14% maximum): conjunctival swelling, which disappeared within 24 h (non irritant, non corrosive in solution).

Effects on cell integrity and membrane permeability
Haemolysis in the presence of 6 mM of α-CD.
Intestinal permeabilisation (paracellular penetration of ^{14}C-mannitol): no effect with 0.1 and 1% α-CD; in some there was an effect when increased to 5%; in others there was no effect at 7.3%; at 5% there was no effect on transepithelial resistance on Caco-2.
α-CD has no effect (as opposed to β and γ CD) on the absorption of sulphanilic acid and the release of cholesterol.

Immunological effects
Coupling of α-CD to albumin did not have an immunogenic effect (oral tolerance test in mice; proliferation of splenocytes in mice).

Studies in human volunteers
2 NIDDM patients + 50 g/day α-CD = nausea in one person 10 min after ingestion.
Healthy volunteers
+ 50 or 100 g/day = nausea and occasional diarrhoea in "a few" people
+ 25 g (bolus) : diarrhoea in 1/12 ;
+ 10g with white bread: no intestinal effects
Dossier page 143: analogy to fructans (fructo-oligosaccharide (FOS) inulin) in terms of gastro-intestinal tolerance. But there are individual variations in tolerance when a large dose of fructans is administered.

Comparison of the data supplied (cf. point 5/structured scheme IX) **with toxicological data and conclusion:**
It is concluded from in vivo studies that, according to the available data, α-CD has no effect at doses 10 to 100 times higher than those which would be consumed at the 90 percentile. The safety factor of 100 is therefore not applicable in all cases.
With regard to the potential contaminant (1-decanol), on the basis of the maximum concentration which might be found in the finished product and the average food intake, intake would not exceed 4 μg/kg per day; according to the report, "1 decanol is metabolised rapidly by the intestinal mucosa ... and pre- and post-implantation problems are observed in gestating rats at doses $5x10^5$ times higher".
The safety factor (100) is therefore respected, in the light of the data submitted by the applicant.

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In February 2005, the CSH raised questions concerning:
1. the interaction of α-cyclodextrin with lipophilic compounds
2. possible secondary effects in "sensitive" patients
3. potential toxicity of 1-decanol at the doses likely to be consumed

The company's replies and the CSH's opinion:
1. Interaction of α-cyclodextrin with lipophilic compounds:

Since α-cyclodextrin has a tendency to form complexes with lipophilic substances, the question was raised as to whether the presence of this compound in food was likely to modify the bioavailability of medicinal products or nutrients consumed at the same time.

The company's attitude was that it was the responsibility of pharmaceutical companies and not agri-food companies to investigate interactions between foodstuffs and medicinal products, pointing out that the intake of food was a "normal situation", while the intake of medicinal products was an "exceptional situation" and that any possible interactions must be mentioned in the leaflet.

The company answered the question, however, backing up the data presented in Section 9.3 of the dossier. They maintain that:
- the complexes formed between α-cyclodextrin and lipophilic substances are reversible and easily separated when solubilised.

- Adding α-cyclodextrin *per se* as a "dietary fibre" to the foodstuff does not have the same effects on bioavailability as the "voluntary" preparation of complexed forms for the purpose of increasing the solubility of lipophilic medicinal products.
- The α-cyclodextrin "cavity" is such that only molecules of low molecular weight can enter it (volatile substances added as additives, for example, but the effect is reversible, as stated earlier).

Opinion of the CSH:
Although being unable to agree with the first part of the company's answer, since the agri-food industry does indeed have overall responsibility in this matter, including the risk of potential toxicity, the answers are reassuring and backed up by conclusive data.

1. Potential secondary effects in "sensitive" patients

The company stated that no study had been carried on persons suffering from gastro-oesophageal reflux.
They put forward the following argument: α-cyclodextrin may be similar to certain viscous soluble fibres, which tend to have a positive effect on this aspect. However, they admit that the potential effect of α-cyclodextrin on reflux should be similar to that of other non-digestible oligosaccharides found in food.

Opinion of the CSH: following research, the CSH in fact considers that, in view of the potential intake, any intolerance in particularly sensitive individuals will not be greater than that caused by "natural" oligosaccharides found in food, and the novel food does not therefore differ substantially from numerous foods which already contain non-digestible oligosaccharides.

3. Potential toxicity of 1-decanol at the doses likely to be consumed

The applicant points out that:
1. the potential ingested dose of 1-decanol is less than 300 micrograms per day, which is much "lower than the toxic dose in *in vivo* toxicity studies".
2. 1-decanol is largely metabolised by b-oxidation in the enterocyte, which greatly reduces the risk of it entering the general circulation.

Opinion of the CSH: the CSH regrets that the applicant did not carry out a comparison/extrapolation of the doses with figures, the majority of studies being obtained from information obtained in vitro. The safety factor (100) for in vivo toxicity has, however, been established in the studies available to us.

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9. GENERAL CONCLUSION

On the basis of the toxicological dossier, it is possible to accept the addition of alpha-cyclodextrin as recommended, provided that there is not a substantial increase in the consumption of foods likely to contain alpha-cyclodextrin, which might lead to an increase in the daily intake.