

## Initial Assessment

### $\gamma$ -Cyclodextrin

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**Novel Food Classification:** 1.1.

#### Introduction

An application for the authorisation of  $\gamma$ -Cyclodextrin as a novel food ingredient was submitted by Wacker Chemie GmbH, and accepted by the Food Safety Authority of Ireland (FSAI) on February 26<sup>th</sup>, 2010.

$\gamma$ -Cyclodextrin and an enzyme used in its production do not have a history of food use in the EU which means the novel ingredient falls within the scope of novel food Regulation EC No. 258/97. The application for authorisation of  $\gamma$ -Cyclodextrin as a novel food ingredient was prepared pursuant to Commission Recommendation 97/618/EC concerning the scientific aspects and the presentation of information in support of an application to market novel foods and novel food ingredients in the EU. The applicant considers  $\gamma$ -Cyclodextrin to fall within the novel food category of “foods and food ingredients with a new or intentionally modified primary molecular structure”, as set out in *Article 1.2.(c)* of the novel food Regulation. In order to assess wholesomeness,  $\gamma$ -Cyclodextrin was considered to fall into class 1.2. by the applicant; “Pure chemicals or simple mixtures from non-GM sources with no history of food use in the community”, (Commission Recommendation 97/618/EC). However, following discussions with the applicant, it was clarified that  $\gamma$ -Cyclodextrin is produced from starch which is derived from non-GM maize. Non-GM maize (corn) has a history of consumption within the Community and thus the novel ingredient is placed in class 1.1.

$\gamma$ -Cyclodextrin is produced from food-grade starch by the amylolytic enzyme cyclodextrin glucanotransferase (CGTase), which is produced naturally by certain bacteria. The novel ingredient is intended for use in an unspecified range of foods as a source of glucose with a moderate glycaemic and insulinemic impact.

In 2001, a novel food application was submitted to the Italian novel food Competent Authority for the use of  $\gamma$ -Cyclodextrin as a stabilisation agent. While the safety assessment identified no major safety concerns, the Italian Authority concluded that in light of the purpose of its intended use, it should be considered under additives rather than novel food legislation, a conclusion supported by other Member States. As this application relates to the use of  $\gamma$ -Cyclodextrin as a source of slow release glucose in foodstuffs, the Food Safety Authority of Ireland (FSAI) is satisfied that the novel food Regulation is the appropriate mode of authorisation.

The information addressing the safety of  $\gamma$ -Cyclodextrin is set out using the schemes set out in Commission Recommendation 97/618/EC.

## **I. Specification of the novel food**

Starch is made up of amylose (20-30%) and amylopectin (70-80%). Amylose is a linear polymer of D-Glucose units while amylopectin is similar but with regular branching. Cyclodextrins are enzymatically created cyclic molecules with a polar exterior allowing them to be water soluble, and a mildly hydrophobic cavity which forms weak complexes with certain organic compounds in dry, oily or low water content foods. The most common cyclodextrins are  $\alpha$ -,  $\beta$ - and  $\gamma$ - which have six, seven and eight member rings respectively. The applicant provides the specifications of  $\gamma$ -Cyclodextrin that were adopted by JECFA in 1999.

## **II. Effect of the production process applied to the novel food**

$\gamma$ -Cyclodextrin is produced from liquefied food-grade starch by an enzymatic process, the precise mechanism of which is not yet fully understood. The process described uses a complexing agent, 8-cyclohexadecen-1-one (CHDC) to purify  $\gamma$ -Cyclodextrin from the mix of cyclodextrins liberated from the starch matrix. The complexant is then separated from the precipitated complex by extraction with n-decane to yield high purity  $\gamma$ -cyclodextrin. The purification of  $\gamma$ -Cyclodextrin involves a series of precipitation and centrifugation steps that result in a white crystalline powder with a water content of approximately 10%.

The cyclodextrin glucanotransferase (CGTase) enzyme used to produce the cyclodextrins from starch is derived from a recombinant *E. coli* K12 incorporating the CGTase gene from a soil bacterium of the *Bacillus firmus/lentus* group. This use of a recombinant protein in an enzymatic process is similar to the use of recombinant chymosin in cheese production and does not incur GM labelling rules (Regulation EC No 1829/2003) since the CGTase is considered a processing aid and is not present in the final product (Directive 2000/13/EC).

## **III. History of the organism used as the source of the novel food**

The application dossier could be considered slightly confusing with regard to the use of the term “source organism”. The source of the liquefied starch, which is the actual source of the  $\gamma$ -Cyclodextrin, was not specified in the original application dossier, though it was subsequently clarified by the applicant as non-GM maize, which has a long history of safe use in food production. The section on “History of the organism used as the source of the NF” includes information on *E. coli* K12, which is best placed in the section dealing with the production process and does not raise any concern since CGTase is considered a processing aid not present in the final product.

## **IV. – VIII.**

The  $\gamma$ -Cyclodextrin is produced from liquefied starch which is derived from non-GM maize. PCR analysis demonstrates that DNA from the recombinant (GM) bacteria (*E.*

*coli* K12) which produced the CGTase enzyme is not detectable in the final product that is >98% pure  $\gamma$ -Cyclodextrin.

### **IX. Anticipated intake/extent of use of the novel food**

$\gamma$ -Cyclodextrin is metabolised relatively slowly resulting in a gradual release of glucose into the system. The applicant explores a number of possible uses of  $\gamma$ -Cyclodextrin in a variety of food types, but for reasons of cost, texture and taste concludes that its ultimate use will likely be limited to specialty foods for diabetics or pre-diabetics, endurance athletes or those on calorie-controlled diets. The  $\gamma$ -Cyclodextrin levels proposed for foods range between 20% in spreads, 10% in nutrition bars and 5-8% in beverages and formula diets. The theoretical “worst case scenario” applied by the applicant envisages a formula diet with  $\gamma$ -Cyclodextrin as the sole source of carbohydrate, or 40% of the energy, providing a patient with 1,800 kcal/day. This would result in an intake of 180g of  $\gamma$ -Cyclodextrin in a day (3 g/kg bw/day) which is approximately one third of the reported NOAEL of approximately 10 g/kg bw/day established in a one-year chronic toxicity study in rats. The applicant assumes that, in reality,  $\gamma$ -Cyclodextrin will only make up a third of the daily carbohydrate intake (1g/kg bw/day) resulting in a safety margin of approximately 10 when compared with the NOAEL in rats of approximately 10 g/kg bw/day. This along with the limited exposure anticipated by the applicant suggests that there are no apparent health risks involved with the consumption of  $\gamma$ -Cyclodextrin at the proposed levels.

### **X. Information from previous human exposure to the novel food or its source**

The source of  $\gamma$ -Cyclodextrin in this application, as clarified by the applicant, is non-GM maize which has a long history of safe use in the EU. While  $\gamma$ -Cyclodextrin has a relatively short history of use in countries like the USA, Australia and New Zealand, it does not have a history of consumption within the EU and the applicant therefore has not provided specific information under this heading.

### **XI. Nutritional information on the novel food**

$\gamma$ -Cyclodextrin is processed by the body in the same way as starch or linear dextrans, and complete digestibility has been demonstrated in human and animal studies.  $\gamma$ -Cyclodextrin is digested by salivary and pancreatic amylase to glucose, and has the same physiological energy value as glucose, maltose or starch. Some concerns had arisen with respect to the ability of  $\gamma$ -Cyclodextrin to complex with certain organic compounds which could impair the bioavailability of lipophilic essential nutrients (e.g. fat-soluble vitamins) or drugs. However, the dynamic, reversible nature of complex formation, along with the ready digestibility of  $\gamma$ -Cyclodextrin by pancreatic amylase means that any impact resulting from complex formation would be transient, with little or no effect on bioavailability. In fact, bioavailability of fat soluble vitamins may even be enhanced by complex formation, possibly due to greater water-solubility of the  $\gamma$ -Cyclodextrin mediated complex.

### **XII. Microbiological information on the novel food**

Food grade starch with a suitable microbiological profile is used as the source of  $\gamma$ -Cyclodextrin, while high temperatures used in the production process would make it difficult for microorganisms to survive.

### **XIII. Toxicological information on the novel food**

A range of acute, short and longer term toxicity studies in rats were considered by the applicant. While the majority of these studies related to the oral administration of  $\gamma$ -Cyclodextrin, some involved intravenous applications which are of limited relevance for a food safety assessment. A 90 day toxicity study in dog and a developmental toxicity study in rabbit, along with *in vitro* and *in vivo* genotoxicity studies make up the remaining toxicity studies provided.

#### ***Absorption, Distribution, Metabolism and Excretion (ADME)***

Ingested  $\gamma$ -Cyclodextrin is readily hydrolysed by salivary and pancreatic amylase to glucose, which is absorbed from the intestinal tract to enter the normal metabolic pathway. Absorption of intact  $\gamma$ -Cyclodextrin is minimal at an estimated 0.02%.

#### ***Toxicity***

Data provided by the applicant demonstrates that  $\gamma$ -Cyclodextrin is of low acute toxicity, with no evidence of toxicity at doses as high as 16 g/kg bw/day in mice, and 8 g/kg bw/day in rats. Only a few mild physiological effects, typical of the administration of high molecular weight carbohydrates, were evident in thirteen week oral toxicity studies in rats and dogs. In these studies,  $\gamma$ -cyclodextrin ingested at dietary levels of up to 20%, corresponding to about 12 g/kg bw/day (rats) was tolerated without any adverse effects. Transient changes in some clinical chemistry are not considered to be of toxicological relevance. A NOAEL of 8.7 and 10.8 g/kg bw/day for male and female rats, respectively, was the result of a one year chronic toxicity study in which rats were fed diets containing up to 20%  $\gamma$ -cyclodextrin. A diet containing up to 10%  $\gamma$ -cyclodextrin as part of a two year carcinogenicity study in rats did not produce any non-neoplastic or neoplastic histopathological changes that could be attributed to  $\gamma$ -cyclodextrin. Genotoxicity due to  $\gamma$ -cyclodextrin was not evident from the Ames test, a chromosome aberration test or an *in vivo* micronucleus test. Oral administration of  $\gamma$ -cyclodextrin at levels of up to 20% in embryotoxicity/teratogenicity studies in rats and rabbits did not identify any treatment-related adverse effects. The endpoint of fertility (reproductive toxicity) was not specifically assessed, but no effects were reported on the gonads in repeat dose toxicity studies. Significant adverse effects were also not evident from a 90-day oral toxicity study in dog using  $\gamma$ -cyclodextrin levels of up to 8 g/kg bw/day.

Studies on the irritation and sensitising potential of  $\gamma$ -cyclodextrin on the skin, eyes and muscle of animals proved negative, but in any case are of limited value for the safety assessment of a food.

#### ***Human studies***

Two human studies on  $\gamma$ -cyclodextrin ingestion were cited. Single doses of 50 g  $\gamma$ -cyclodextrin, or rapidly digested maltodextrin were administered to 32 healthy adult volunteers in a double-blind, randomized cross-over study. Subjective gastrointestinal tolerance symptoms were recorded on a visual analog rating scale for two 24-hour

periods following ingestion of the test products. Information on stool frequency and consistency was also collected. The results demonstrated that the two treatments were both well tolerated, with treatment-related effects consisting of mild nausea, cramping, flatulence and distension being seen after both treatments, albeit slightly increased after  $\gamma$ -cyclodextrin. Breath hydrogen excretion was not significantly elevated over an 8 hour postprandial period, suggesting that  $\gamma$ -cyclodextrin is primarily hydrolysed by digestive amylases in the small intestine, rather than being a substrate for microbial fermentation. Similar results had been obtained in an earlier double-blind, randomized cross-over study comparing the tolerance of 24 volunteers to 8 g of orally administered  $\gamma$ -cyclodextrin or maltodextrin. The incidence of mild symptoms, as described for the higher dose study above, was similar after both carbohydrates involving five of the 24 volunteers.

### ***Toxicity of CGTase and impurities 8-cyclohexadecen-1-one (CHDC) and n-decane***

The CGTase used in the production of  $\gamma$ -cyclodextrin is inactivated and removed during the process, as demonstrated by the absence of protein bands in silver stained SDS-PAGE gels. However, the applicant provides data from studies to examine the safety of the crude enzyme preparation and to detect any toxic by-products that may result from its production. A 13-week subchronic oral toxicity test was conducted with CGTase from *E. coli* K12 expressing the CGTase gene of *Klebsiella oxytoca*. The highest dose level tested in this study (260 mg total organic solids/kg bw/day) showed no adverse effect.

In a 28-day oral toxicity study with CHDC, a slightly changed staining pattern of hepatocytes was noted in rats of the high-dose group (0.75% CHDC in the diet). The NOAEL in this study was 45 mg/kg bw/day.

In a repeated dose toxicity/reproduction study with n-decane, the highest dose level tested of 1,000 mg/kg bw/day was found to be a NOAEL. Data on the closely related n-nonane can also be used in the safety assessment of n-decane. In 90-day oral toxicity studies in female F344 rats and male C57BL/6 mice, the lowest dose level tested of 0.1 g/kg bw/day was determined to be the NOAEL in both species. Using an uncertainty factor of 1,000, a Reference Dose (RfD) of 0.1 mg/kg bw/day was derived from this study.

### **Conclusions**

In a “worst case scenario”, a formula diet including  $\gamma$ -cyclodextrin as the sole carbohydrate would provide an estimated intake of 3 g/kg bw/day, approximately one third of the NOAEL value derived from toxicity studies in rats. The applicant concludes that  $\gamma$ -cyclodextrin would make up less than a third of the daily carbohydrate consumed, resulting in an average intake of 1 g/kg bw/day, leaving a safety margin greater than 10 which is sufficient for such a carbohydrate.

The metabolism of  $\gamma$ -cyclodextrin is similar to that of other digestible carbohydrates in the diet, which when considered alongside the extensive toxicological data from experimental animals leads to the conclusion that  $\gamma$ -cyclodextrin will be safe and well tolerated. The results of the human volunteer studies suggest that some minor nausea or gastrointestinal discomfort may occur at the average daily intake of 1 g/kg bw/day. However, these symptoms are expected to be the same for any carbohydrate with characteristics similar to  $\gamma$ -cyclodextrin and are likely to be self-limiting.

The NOAEL for CHCD, a potential impurity in  $\gamma$ -cyclodextrin was found to be 45 mg/kg bw/day in a 28 day study in rats. CHDC residues can be present in  $\gamma$ -cyclodextrin at less than 2 ppm (2 mg/kg), and at an estimated intake of 60 g/person/day, the CHDC intake would be less than 2  $\mu$ g/kg bw/d. The safety margin between this maximum intake and the NOAEL in rats (28-day study) is approximately 25,000, which is considered adequate. With an estimated  $\gamma$ -cyclodextrin intake of 60 g/person/day, the intake of n-decane, considering a proposed oral Reference Dose (RfD) of 0.1 mg/kg bw/d, would be less than 3  $\mu$ g n-decane/kg bw/d. This would give a safety margin of at least 30 which does not raise any toxicological concerns.

### **Recommendation**

The FSAI is satisfied that the use of  $\gamma$ -Cyclodextrin as a novel food ingredient is not likely to pose a significant health risk to consumers, and that it meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation EC (No) 258/97.