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May 19, 2000

Office of Premarket Approval (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C St. SW  
Washington, DC 20204

Subject: Notice of a GRAS exemption for gamma cyclodextrin

Dear Sir or Madame:

Pursuant to the proposed rule outlined at 62 Fed. Reg. 18939 (April 17, 1997), Wacker Biochem Corp. hereby submits notification that a particular use of a substance (gamma-cyclodextrin,  $\gamma$ -CD) 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). For ease of review by FDA, this notification is submitted in the format suggested under proposed 21 C.F.R. § 170.36(c) (62 Fed. Reg. at 18961). Also enclosed is an electronic copy (Microsoft Word 97) of the claim (GRAS Notification Claim.doc) and the additional information (GRAS Additional Info.doc).

Sincerely,

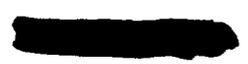


David B. Clissold

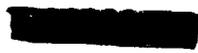
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## GRAS NOTIFICATION CLAIM

We hereby claim that the use of gamma-cyclodextrin ( $\gamma$ -CD) for use as a stabilizer, emulsifier, carrier, and formulation aid 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  $\gamma$ -CD is generally recognized as safe (GRAS).

**(1) Name and address of the notifier:**

Dr. Gerhard Schmid, President  
Wacker Biochem Corp.  
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:**

Gamma cyclodextrin;  $\gamma$ -cyclodextrin; cyclooctaamylose; cyeomaltocctaose

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

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

Bread spreads, frozen dairy desserts, ready to eat dairy desserts, desserts prepared from dry mixes, fruit fillings, cheese and cream fillings, chewing gum, dietary supplements (see Appendix 1).

**(b) Levels of use in such foods:**

Up to 20% in bread spreads, 1-5% in all other foods listed above (see Appendix A).

**(c) Purposes for which the substance is used:**

Stabilizer, emulsifier, carrier, formulation aid

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

Individuals consuming at least one of the food categories described above (see Appendix A).

000004

**GRAS NOTIFICATION CLAIM**

Page 2

**(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 the notifier's GRAS determination will be sent to FDA upon request.



David B. Clissold  
Attorney for Wacker Biochem Corp.

Please address correspondence to:

Dr. Gerhard Schmid, President  
Wacker Biochem Corp.  
3301 Sutton Road  
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Appendix A

000005

## Appendix A

### Applications of $\gamma$ -CD and Maximum Levels of Use

Food	Use level
Carrier for flavors, sweeteners and colors	< 1%
Dry mixes for beverages	< 1%
Dry mixes for soups	< 1%
Dry mixes for dressings, gravies and sauces	< 1%
Dry mixes for puddings, gelatins and fillings	< 1%
Instant coffee and instant tea	< 1%
Coffee whiteners	< 1%
Compressed candies	< 1%
Chewing gum	< 1%
Breakfast cereals (ready-to-eat)	< 1%
Savory snacks and crackers	< 1%
Spices and Seasonings	< 1%
Carrier for vitamins	
For use in dry food mixes and dietary supplements	< 90% <sup>1</sup>
Carrier for PUFAs <sup>2</sup>	
For use in dry food mixes and dietary supplements	< 80% <sup>1</sup>
Flavor modifier	
Soya milk	< 2%
Stabilizer	
Bread spreads (fat-reduced)	< 20%
Frozen dairy desserts	< 3%
Baked goods (excl. bread, but incl. dough and baking mixes)	< 2%
Bread	< 1%
Fruit-based fillings	< 3%
Fat-based fillings	< 5%
Processed cheese	< 3%
Dairy deserts (ready-to-eat and prepared from dry mixes)	< 3%

<sup>1</sup> % (By weight) of  $\gamma$ -CD relative to the nutrient for which  $\gamma$ -CD is used as carrier.

<sup>2</sup> Polyunsaturated fatty acids.

**Additional Information**

000007

## ADDITIONAL INFORMATION

(1) **Identity of the notified substance**

(a) **Chemical name**

Gamma cyclodextrin;  $\gamma$ -cyclodextrin; cyclooctaamylose; cyclomaltoctaoase;  $\gamma$ -CD

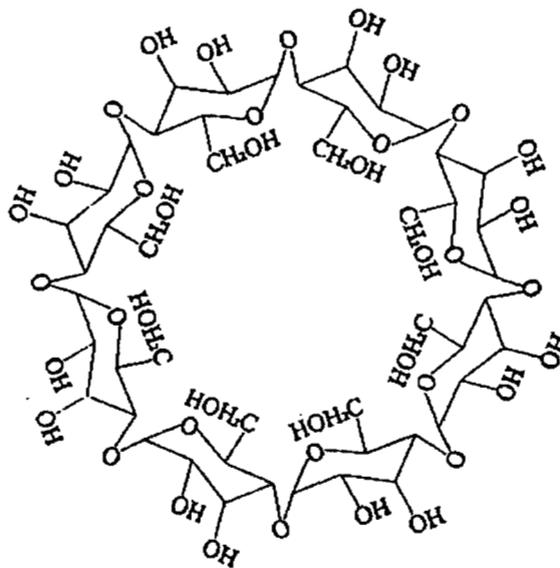
(b) **Chemical Abstracts Service (CAS) Registry Number**

17465-86-0

(c) **Empirical formula**

$(C_6H_{10}O_5)_8$

(d) **Structural formula**



(e) **Method of manufacture (excluding any trade secrets)**

$\alpha$ -,  $\beta$ - and  $\gamma$ -Cyclodextrin (CD) 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  $\alpha$ -1,4-linkage, and

transfers it to the C-4 position of the non-reducing end to produce  $\alpha$ -,  $\beta$ - or  $\gamma$ -CD (Schmid, 1989). Typically, mixtures of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD are formed by the action of CGTases on starch, with the  $\beta$ -form being predominant for thermodynamic reasons. Different CGTases produce  $\alpha$ -,  $\beta$ -, and  $\gamma$ -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).

The product being considered here, is produced using CGTase from a genetically modified strain of *Escherichia coli* K12 and applying the solvent process for separation of the obtained  $\gamma$ -CD.

In the first step of  $\gamma$ -CD production, food-grade, liquefied starch is treated with CGTase under controlled pH and temperature conditions. Cyclohexadecen-1-one (CHDC) or another appropriate complexant is added to precipitate formed  $\gamma$ -CD. The complex is removed and purified. The complexant is separated from  $\gamma$ -CD by extraction with n-decane, a component of “odorless” light petroleum hydrocarbons which may be used in some food processes (21 CFR § 172.884). According to the specifications of  $\gamma$ -CD, total residue levels of volatile organic compounds (i.e., CHDC and n-decane) will not exceed 20 ppm (FAO, 1998).  $\gamma$ -CD is obtained by crystallization as a white powder with a purity of  $\geq 98\%$  (on dry matter basis).

#### (f) **Characteristic properties**

$\gamma$ -cyclodextrin is a non-reducing cyclic saccharide consisting of eight  $\alpha$ -(1,4) linked D-glycopyranosyl units. It is a virtually odorless, white or almost white, crystalline solid. It is freely soluble in water, and very slightly soluble in ethanol.

Cyclodextrins are cyclic  $\alpha$ -(1-4) -linked maltooligosaccharides.  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin consist of 6, 7, and 8 glucose units, 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 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.

Due to the steric arrangement of the glucose units, the inner side of the torus-like cyclodextrin molecules is less polar than the outer side. This enables cyclodextrins to form inclusion complexes with various organic compounds. This property forms the basis for numerous applications of cyclodextrins in foods, as well as pharmaceutical and cosmetic products (Szeitli, 1982; Nagatomo, 1985; Vaution et al., 1987; Pszczola, 1988; Allegre & Deratani 1994; Thompson, 1997). In foods, cyclodextrins can protect volatile compounds from evaporation, and chemically sensitive products from oxidation or photodegradation. Cyclodextrins also can stabilize emulsions and foams, mask certain undesirable tastes and odors, provide bulk, and improve texture (Hedges et al., 1995). 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. With its larger ring size,  $\gamma$ -cyclodextrin ( $\gamma$ -CD) has wider applications in this respect than  $\alpha$ - or  $\beta$ -CD.

**(g) Any content of potential human toxicants**

The CGTase is obtained from a genetically modified strain of *Escherichia coli* K12. *E. coli* K12 is a nonpathogenic and nontoxic host organism which has been used for the production of other food ingredients such as chymosin and which is recognized as safe (FDA, 1990). The present strain expresses a CGTase gene of a *Bacillus* strain of the firmus/lentus group, an ubiquitous group of aerobic, gram-positive, alkalophilic, non-pathogenic microorganisms. For constructing this strain, a vector was used which is derived from pBr322, a widely used vector which is considered to be safe. The obtained CGTase preparation was non-mutagenic in Ames tests using *S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100, with and without metabolic activation (S9-mix) (van Delft, 1997, cited in WHO, 1999).  $\gamma$ -CD does not contain any CGTase activity because the enzyme is inactivated by heat and is removed completely during the  $\gamma$ -CD production process. Any nonproteinaceous, hydrophilic or lipophilic by-products present in the CGTase preparation would also be removed by the applied purification steps.

CHDC (CAS 3100-36-5) which may be applied in the  $\gamma$ -CD production as a complexant, is a colorless, waxy, solid product which is practically insoluble in water. CHDC occurs naturally in civet, the secretion from the civet cat (Ding and Fu, 1986; Prigge et al., 1989). Civet is GRAS under 21CFR §182.50. The safety of CHDC has been examined in a number of studies. CHDC is not genotoxic in

Ames tests and the in vivo micronucleus test (Wilmer, 1986, cited in WHO, 1999; Willems, 1986, cited in WHO, 1999). Its LD<sub>50</sub> (p.o.) exceeds 4.6g/kg bw in mice and 10g/kg bw in rats (Spanjers, 1986, cited in WHO, 1999; Dickhaus & Heisler, 1985, cited in WHO, 1999). Subchronic (28-day) oral toxicity tests in mice and rats revealed NOELs of 300 and 45-50 mg/kg bw/d, respectively (Lina, 1992, cited in WHO, 1999; Lina et al., 1986, cited in WHO, 1999). At the estimated daily intake (EDI) of  $\gamma$ -CD (4 g/person/day, for calculation see below), the CHDC intake would be less than 1  $\mu$ g/kg bw/d.

According to the specifications of  $\gamma$ -CD, total residue levels of volatile organic compounds (i.e., CHDC and n-decane) will not exceed 20 ppm (FAO, 1998).

#### (h) Specifications for food-grade material

From FAO, 1998. See also Appendix 1 (draft Food Chemicals Codex specification for  $\gamma$ -cyclodextrin) Requirements and Tests.

### CHARACTERISTICS

#### IDENTIFICATION

**Solubility:** Freely soluble in water; very slightly soluble in ethanol

**Specific rotation:**  $[\alpha]_D^{25}$ : Between +174° and +180° (1% solution)

**Reaction with iodine:** To 0.2 g of the sample in a test-tube add 2 ml of 0.1 N iodine solution. Heat the mixture in a water bath and allow to cool at room temperature. A clear brown solution is formed.

**Chromatography:** The retention time for the major peak in the liquid chromatogram of the sample solution corresponds to that for  $\gamma$ -cyclodextrin in the chromatogram of the standard solutions prepared as directed in the Method of Assay.

#### PURITY

**Water:** Not more than 11% (Karl Fischer Method)

**Volatile Organic Compounds:** Not more than 20 mg/kg. See description under TESTS.

**Reducing Substances:** Not more than 0.5% (as glucose)

**Sulfated ash:** Not more than 0.1%

**Lead:** Not more than 1 mg/kg. Reflux about 5 g of the sample, accurately weighed, with 30 ml nitric acid for 1 h. Remove the reflux condenser and attach a condenser to the flask. Continue to heat and collect the distilled nitric acid. Allow the residue to cool, add 20 ml of water and again allow to cool. Add 2 ml of orthophosphoric acid, dilute to 100 ml and determine the lead content of the solution by *atomic absorption spectroscopy*.

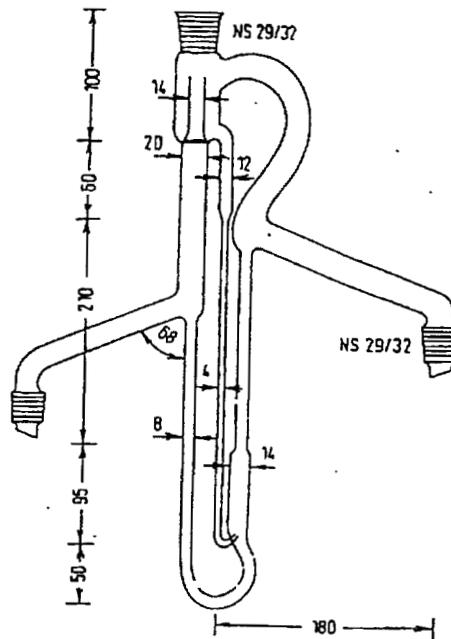
## TESTS

### PURITY TESTS

**Volatile organic compounds:** See also Appendix 1 (Draft Food Chemicals Codex specification, Volatile Organic Compounds).

Dissolve 50 g of the sample in about 700-ml distilled water in a 1-liter round bottom flask and add a magnetic stirrer. Attach the flask to the lower part of a Bleidner apparatus (see Figure 1) and connect a 100-ml round bottom flask containing about 70 ml hexane and a few boiling stones to the other side of the apparatus. Fill the Bleidner apparatus with equal amounts of water and hexane and place a reflux condenser on the top. Heat both flasks with heating mantels to boiling. Stir the 1-liter flask well by the magnetic stirrer. Keep the content of the two flasks boiling for 8 h. After cooling, remove the 100-ml flask, transfer the content to a 100 ml volumetric flask, and fill to mark with hexane.

Figure 1



Analyze the hexane solution by *gas chromatography* using the following conditions:

Column

length: 30 m

diameter: 0.32 mm

stationary phase: 95% dimethyl, 5% diphenyl polysiloxane, 0.25  $\mu$ m

Injector: 280°

Temperature: 70° (4 min) – 250°, 10°/min

Carrier

gas: nitrogen

flow: 70 ml/min

Detection: FID, 280°

Calculate the area(s) under the peak for each volatile organic compound and convert it to mg/kg  $\gamma$ -cyclodextrin using the response factor of 8-cyclohexadecen-1-one. The response factor is determined from a calibration curve using 8-cyclohexadecen-1-one concentrations of 0.1-6 mg/100 ml hexane.

**Method of Assay:** See also Appendix 1 ((Draft Food Chemicals Codex specification, Assay).

Principle:  $\gamma$ -cyclodextrin is identified by *liquid chromatography* and quantified by comparison to reference standards containing standard cyclodextrins.

Preparation of Sample Solution: Weigh accurately about 100 mg of sample into a 10-ml volumetric flask and add about 8 ml of purified deionized water. Bring to complete dissolution by using an ultrasonic bath for 10-15 min. After cooling to room temperature, dilute to mark with purified deionized water.

Preparation of Standard Solution: Use reference  $\gamma$ -cyclodextrin (available from Consortium für Elektrochemische Industrie GmbH, München, Germany or Wacker Biochem Group, Adrian, MI, USA). Prepare a solution of the reference material as described for the sample solution.

Apparatus: Liquid chromatograph maintained at a constant temperature of 40° and equipped with a refractive index detector.

#### Conditions

##### Column

length: 25 cm

diameter: 4 mm

packing: Nucleosil-10 NH<sub>2</sub> (Machery & Nagel Inc.) or equivalent

particle size: 10  $\mu$ m

pore size: 100 Å

Solvent: Acetonitrile/Water: (67:33)

Flow rate: 2 ml/min

Injection volume: 9  $\mu$ l

Procedure: From five analytical runs of each solution the mean values of the peak areas of the reference and the sample are calculated.

#### Calculation

Calculate the content of  $\gamma$ -cyclodextrin in the sample using the formula:

$$C = \frac{A_S \times m_R \times (100 - w_R) \times 100}{A_R \times m_S \times (100 - w_S)} \%$$

where

C = percentage of  $\gamma$ -cyclodextrin in the sample  
A<sub>S</sub> = Mean value of peak areas of sample solution

$A_R$	=	Mean value of peak area of reference solution
$m_S$	=	Amount of sample in sample solution (mg)
$m_R$	=	Amount of reference material in reference solution (mg)
$w_S$	=	Water content of sample (%)
$w_R$	=	Water content of reference material (%)

**(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) Biological studies**

*i) Absorption, disposition, metabolism and excretion (ADME)*

In vitro studies showed that human salivary amylase as well as human and porcine pancreatic amylase are unable to hydrolyse  $\alpha$ -CD and  $\beta$ -CD to any measurable extent. On the other hand, they hydrolyse  $\gamma$ -CD readily, yielding mainly maltose, some maltotriose and smaller amounts of glucose (Abdullah et al., 1966; Marshall & Miwa, 1981; Kondo et al., 1990).

A series of four ADME studies with  $^{14}\text{C}$ - $\gamma$ -CD in conventional and germ-free rats demonstrated that the metabolism of  $\gamma$ -CD resembles closely that of starch and linear dextrans (de Bie et al., 1998). Ingested  $\gamma$ -CD is rapidly and essentially completely digested by salivary and pancreatic amylase to products which also result from the digestion of starch and linear dextrans. Glucose is the only absorbed product of this digestive process (Jones et al., 1987). The absorption by passive diffusion of intact  $^{14}\text{C}$ - $\gamma$ -CD is very low (< 0.02%) (de Bie et al., 1998).

Intravenously administered  $^{14}\text{C}$ - $\gamma$ -CD was rapidly eliminated from the circulation in rats ( $t_{1/2}$ , 15-20 min). About 90% of the administered  $\gamma$ -CD was excreted in urine within 24 hours. The remaining 10% of the dose was probably excreted into the gastrointestinal tract with bile and saliva. In addition, some  $^{14}\text{C}$ - $\gamma$ -CD may have been degraded by plasma and tissue amylases and  $\alpha$ -glucosidases. Rapid urinary excretion of intravenously administered  $\gamma$ -CD was also observed in rabbits and one dog (Matsuda et al., 1985).

**(b) Toxicological studies***i) Acute Studies*

The acute toxicity of  $\gamma$ -CD was examined in mice and rats receiving single doses of the test substance by gavage or by subcutaneous, intraperitoneal, or intravenous injection. The design and results of these studies are summarized in Table 1. On oral administration, no deaths occurred at  $\gamma$ -CD levels of up to 16g/kg bw in mice and 8g/kg bw in rats (Matsuda et al., 1983; Immel, 1991, cited in WHO, 1999). Intraperitoneal and subcutaneous administration of  $\gamma$ -CD did also not produce any immediate or delayed adverse effects (Matsuda et al., 1983; Riebeek et al., 1990c, cited in WHO, 1999). However, when  $\gamma$ -CD was administered intravenously to mice (5, 7.5, or 10 g/kg bw) and rats (2.5, 3.75 or 5 g/kg bw), some dose-related signs of toxicity, such as piloerection and sluggishness, were observed within one hour to a few days after treatment in all dose groups. In both mice and rats, some deaths occurred within a few days (1/10, 4/10 and 5/10 mice, and 0/10, 4/10 and 6/10 rats in the low-, mid-, and high-dose group, respectively). The surviving animals recovered and appeared healthy at the end of the 14-day observation period. Macroscopic examination of the animals did not reveal any treatment-related gross alterations (Riebeek et al., 1990b, cited in WHO, 1999)

*ii) Subchronic studies with oral administration of  $\gamma$ -CD*

Subchronic oral toxicity studies with  $\gamma$ -CD were conducted in rats and dogs. The design of the studies and the obtained No-Observed-Adverse-Effect-Levels (NOAEL) are presented in Table 2.

In a two-week toxicity study, six groups of 5 young male Wistar rats received diets to which 0, 5, 10, 15, or 20%  $\gamma$ -CD, or 20% lactose were added at the expense of starch. The ingestion of  $\gamma$ -CD was generally well tolerated. Body weights tended to be slightly below controls in all treated groups (including the lactose group), but no dose-related trend was apparent. Feces were somewhat softer in all treated groups (more in the lactose group). Diarrhea was not observed. Plasma alkaline phosphatase (AP) was increased in the 20%  $\gamma$ -CD group but plasma aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) did not differ between treated groups and the controls. The liver and kidney weights did not differ between groups, but cecum weights (full and empty) were increased slightly in the 10, 15 and 20%  $\gamma$ -CD groups and, more pronounced, in the 20% lactose group. No gross abnormalities were detected at necropsy that could be attributed to the  $\gamma$ -CD treatment (Lina & Bär, 1998).

In a subchronic (13-week) oral toxicity study, groups of 20 male and 20 female Wistar rats received diets with 0, 1.5, 5 or 20%  $\gamma$ -CD. A comparison group received a diet with 20% lactose.  $\gamma$ -CD and lactose were added at the expense of starch. Three satellite groups (10/sex/group) received the control diet, a diet with 20%  $\gamma$ -CD, or a diet with 20% lactose. After a treatment period of 13 weeks, the animals of the main groups were killed. The rats of the satellite groups continued the treatment for another 4 weeks (recovery period). Due to a feeding error the 20%  $\gamma$ -CD group was terminated, and a corresponding study consisting of a control and a 20%  $\gamma$ -CD group was added. There were no deaths during the study. Somewhat softer stools were observed during a few days in some animals of the 5 and 20%  $\gamma$ -CD groups, and the lactose group. Otherwise, no signs of treatment-related reactions were seen. Mean body weights were slightly, yet significantly reduced in males, but not females, of the 20%  $\gamma$ -CD and lactose groups. The hematological and clinicochemical parameters as well as the semiquantitative urine analyses did not reveal changes that could be attributed to the  $\gamma$ -CD treatment. Urinary pH was decreased and calcium increased in males and females of the lactose group. The ingestion of  $\gamma$ -CD did not influence these parameters. During a urine concentration test performed on days 87/88, similar urine volumes were produced in all treatment groups. At the end of the treatment period, the absolute and relative weights of the full and empty cecum were increased in males and females of the 20% lactose group and, to a lesser extent, 20%  $\gamma$ -CD group. At the end of the recovery period, there was no difference of cecum weights between the 20%  $\gamma$ -CD group and the control animals, but a slight increase was still evident in the 20% lactose group. Relative adrenal weight was increased in the 20%  $\gamma$ -CD group (males) and the lactose group. There was a very slight increase in relative liver weight in males of the 20%  $\gamma$ -cyclodextrin group as well as in the females of the lactose control group. There were no organ weight changes at the end of the recovery period. There were no gross pathological changes attributable to treatment with  $\gamma$ -CD. Histopathological examination did not reveal any abnormalities that could be attributed to the treatment. The effects observed after feeding of  $\gamma$ -CD in the diet at concentrations up to 20% appear to be largely related to the presence of fermentable carbohydrate in the large intestine. Similar, yet generally more pronounced effects were observed when the diet contained 20% lactose. It was concluded that the ingestion of  $\gamma$ -CD for 13 weeks at dietary levels of up to 20% (corresponding to intakes of 11.4 and 12.7 g/kg bw/d for male and female rats, respectively) was well tolerated and did not produce any signs of toxicity (Lina & Bär, 1998).

In a 3-month subchronic toxicity study, groups of Beagle dogs received diets with 0, 5, 10 or 20%  $\gamma$ -CD (4/sex/group). The test substance was added to the diet at

the expense of starch. All dogs remained in good health during the study. Transient diarrhea occurred in all  $\gamma$ -CD groups. The incidence and severity of diarrhea was related to the dietary levels of  $\gamma$ -CD. It was somewhat higher in females than in males. No treatment-related differences were observed with respect to ophthalmoscopic observations, hematological parameters, clinicochemical analyses of the plasma, and semiquantitative urine analyses. Only the urinary pH was slightly below control levels in males of the 20% dose group. No abnormalities were seen at necropsy that could be attributed to the treatment. The organ weight data revealed some cecal enlargement in the 10 and 20% dose group. Relative ovary weights were significantly increased in the 10 and 20% groups, but this was probably a result of an unusually low ovary weight in the controls. An increase of relative liver weights in males of the 10 and 20% dose groups also was considered to lack toxicological relevance because it was not associated with changes in plasma liver enzyme levels or histopathological alterations. On microscopic examination, treatment-related effects were not observed in any of the various organs and tissues. Transient diarrhea, slight cecal enlargement and a slightly increased acidity of the urine were the only effects that could be attributed to the  $\gamma$ -CD treatment. The intensity of these physiological changes was much less than is observed commonly in response to the ingestion of low digestible carbohydrates. It was concluded that the intake of  $\gamma$ -CD at dietary levels of up to 20% (corresponding to intakes of approximately 7.7 and 8.3 g/kg bw/d in male and female dogs, respectively) was tolerated without any toxic effects (Til & Bär, 1998).

*iii) Chronic study with oral administration of  $\gamma$ -CD*

In a 1-year chronic toxicity study, groups of 20 male and 20 female Wistar rats received diets to which 0, 5, 10 or 20%  $\gamma$ -CD were added at the expense of pregelatinized potato starch. General condition and behavior, mortality, food intake, body weights, ophthalmoscopic observations, blood cell parameters, plasma and urinary electrolytes, and the results of semiquantitative urinalysis did not differ between controls and treated groups. No toxicological relevance was attributed to small increases in the high-dose groups of plasma AP (significant in week 14), ALAT (significant in males in weeks 14 and 26), and ornithine carbamoyl transferase (OCT) [(significant in males (week 14) and females (weeks 14 and 26))] because they were not associated with any histopathological changes of the liver, and because they were small, not progressive with duration of the treatment, and not consistent among individual animals (i.e., different animals exhibited increases at different points in time). Moreover, AP and ALAT were not increased in studies with intravenous administration also to rats of  $\gamma$ -CD (OCT was not measured) (Donaubauer et al., 1998). Thus, it is concluded that the

increases seen in the 1-year oral toxicity study were not a direct result of  $\gamma$ -CD exposure.

Organ weights were not affected by the  $\gamma$ -CD treatment, except for the cecum which was slightly enlarged in the males of the high-dose group (increase in females was not statistically significant), and the testes which were slightly heavier in the high-dose groups (increase was significant for relative but not for absolute organ weight). Macroscopic and histopathological examination of the organs and tissues did not reveal any abnormalities that could be related to  $\gamma$ -CD treatment. It was concluded that the 20% dose level represents the NOAEL.  $\gamma$ -CD intake at this level was 8.7 and 10.8 g/kg bw/d for male and female rats, respectively (Lina 1999).

A comparison of the results of the 2-week, 3-month and 1-year oral toxicity studies in rats demonstrates that, at the 20%  $\gamma$ -CD dose level, slight cecal enlargement was the only consistent treatment-related effect. Plasma AP and ALAT levels were transiently increased in the 1-year study but no such increases were seen in the 90-day oral toxicity study or in the studies with intravenous administration of  $\gamma$ -CD. Plasma triglyceride and phospholipid levels were slightly decreased in males of the high-dose group of the 1-year study but not in the corresponding group of the 90-day study. Plasma creatinine concentrations were transiently increased in females (but not males) of the 1-year study but not in males or females of the 90-day study. Relative weights of the testes were increased in the 10 and 20%  $\gamma$ -CD groups of the 1-year study and the 20% lactose group of the 90-day study but not in the 20%  $\gamma$ -CD group of the 90-day study (Lina, 1999; Lina & Bär, 1998). Histopathological changes attributable to the ingestion of  $\gamma$ -CD, were not observed in any study (Lina & Bär, 1998; Lina, 1999).

iv) *Subchronic studies with intravenous administration of  $\gamma$ -CD*

Two subchronic toxicity studies with daily intravenous administration of  $\gamma$ -CD were performed in rats. The design of these studies and the resulting No-Observed-Effect-Levels (NOEL) are summarized in Table 3.

In a 1-month toxicity study,  $\gamma$ -CD was administered intravenously in single daily doses of 0 (controls), 200, 630, or 2000 mg/kg bw to groups of Wistar rats 15 rats/sex/group, for 30 consecutive days. To each group, a satellite group (5 rats/sex/group) was attached which received the same treatment followed by a 4-week recovery period without treatment. There were no mortalities during the course of the study. Body weights were slightly reduced in male rats of the mid- and high-dose groups during the first half of the treatment period. Hemoglobin

and hematocrit values were decreased in male rats of the mid-dose group and male and female rats of the high-dose group. The reticulocyte counts were significantly elevated in male and female animals of the high-dose group. A statistically significant, dose-dependent reduction in the thrombocyte count was noted in females of the mid- and high-dose groups. These changes were reversed after the recovery period. Biochemical measurements revealed elevated creatinine and urea levels in the serum of male and female animals of the high-dose group at the end of the treatment period. No changes were seen at the end of the recovery period. Urinalysis revealed an increased incidence of hemoglobin for male and female rats of the high-dose group. This observation was confirmed by the presence of erythrocytes in the sediment of all animals of this group. An increased number of markedly granulated, irregularly colored epithelia also were observed in the urine of these animals. These changes were reversed at the end of the recovery period. On post-mortem examination the majority of animals of the high-dose group was found to have relatively light-colored and, in some cases, irregularly colored kidneys. A statistically significant, dose-related increase of the relative spleen weight was noted in males of the mid- and high-dose group, and in females of all groups. The relative weights of lungs and adrenals were increased in males and females of the high-dose group. Increases of other relative organ weights were limited to one sex (livers in females of the mid- and high-dose groups, kidneys in females of the high-dose group). None of these organ weight changes were seen at the end of the recovery period, except for the spleen and kidney which remained somewhat increased in females of the high-dose group. Histopathological examination revealed resorptive vacuolation of renal epithelial cells (proximal convoluted tubules) and extensive pulmonary histiocytosis (massive accumulation of alveolar macrophages) in all animals of the high-dose group and a few animals of the mid-dose group. At the end of the recovery period, some residual lung and kidney changes were observed in a few animals of the high-dose group. It was concluded that the low-dose level (200 mg/kg bw) was the NOEL (Donaubauer et al., 1998).

In a 3-month intravenous toxicity study,  $\gamma$ -CD was administered in daily doses of 0, 60, 120, or 600 mg/kg bw to groups of Wistar rats (15/sex/group) for 90 days. To each group, a satellite group (5/sex/group) was attached which received the same treatment followed by a 5-week recovery period without treatment. There were no mortalities or signs of toxicity during the study. Body weights were decreased in males of the high-dose group on days 7 and 10, but not thereafter. A dose-related decrease in food consumption was observed during the first few days of the study. Hematological measurements revealed significantly lower erythrocyte counts, hemoglobin, and hematocrit values in females of the high-dose group. The reticulocyte counts of these animals were significantly increased and

the thrombocyte counts tended to be decreased. Hematological parameters did not differ between treated groups and controls at the end of the recovery period, except for increased thrombocyte counts in females of the high-dose group. Biochemical analyses revealed a statistically significant decrease of plasma bilirubin in animals of the high-dose group. These changes were reversed at the end of the recovery period. Post-mortem macroscopic examinations revealed an increased incidence of enlarged iliacal lymph nodes in males and females of the high-dose group. This effect is likely to be related to inflammation at the injection site. The relative weights of lungs, liver and spleen were increased in male rats of the high-dose group. The relative weights of heart, lungs, kidneys, spleen, and adrenals were increased in females of the high-dose group. All changes were reversed at the end of the recovery period. Histopathological examination revealed simple hyperplasia and hyperplastic foci of the mucosa of the urinary bladder, as well as pulmonary histiocytosis (foam-cell macrophage aggregates) of males and females in the high-dose group. Slight resorptive vacuolation was found in the epithelia of the renal tubuli of a few males and females of the high-dose group. At the end of the recovery period, no changes were detected in the bladder and kidneys of the treated rats. The reversibility of the bladder changes indicates that the hyperplasia was not pre-neoplastic. In the lungs, some residual changes were still seen in a few animals, but fibrosis was not detected in any instance. It was concluded that the NOEL was 120 mg/kg bw (Donaubauer et al., 1998).

The effects observed in these two intravenous toxicity studies are not unique for  $\gamma$ -CD but also have been seen with other cyclodextrins (Coussement et al., 1990). The changes of hematological parameters in association with increased urinary hemoglobin and an increased relative weight of the spleen could result from membrane effects of the infused cyclodextrin which may decrease the half-life of erythrocytes and thrombocytes. Similar hematological changes were reported from a 3-month rat toxicity study with daily intravenous administration of 400 mg 2-hydroxypropyl- $\beta$ -CD /kg bw (Coussement et al., 1990). In vitro, hydroxypropyl- $\beta$ -CD has a slightly higher hemolytic effect than  $\gamma$ -CD (Rajewski et al., 1995).

Resorptive vacuolation of renal tubular cells results from the uptake of urinary cyclodextrin by the epithelial cells via pinocytosis followed by fusion of the pinocytotic vesicles with lysosomes. This effect has been seen in studies with acute or subchronic parenteral administration of different cyclodextrins as well as linear carbohydrates such as inulin, dextran or sucrose (Coussement et al., 1990; Frank et al., 1976; Hiasa et al., 1981; Fillastre et al., 1967; Kief & Engelbart, 1966)

**(c) Embryotoxicity/teratogenicity studies**

In an embryotoxicity/teratogenicity study in rats,  $\gamma$ -CD was fed at dietary concentrations of 0, 1.5, 5, 10 or 20% to groups of 25 presumed pregnant female Wistar rats from day 0 to 21 of gestation. A comparison group received a diet with 20% lactose. The additions to the diet of  $\gamma$ -CD and lactose were made at the expense of starch. There were no deaths during the study. Maternal bodyweight gains were similar in all groups. On necropsy of the dams, there were no adverse effects which could be related to treatment. The reproductive performance was similar for all groups. Examination of the fetuses did not reveal any treatment-related increase in gross, skeletal or visceral abnormalities. Under the conditions of this assay,  $\gamma$ -CD showed no evidence of maternal toxicity, embryotoxicity, fetotoxicity and teratogenicity at dietary concentrations of up to 20% corresponding to an intake of 11 g/kg bw/d (Waalkens-Berendsen et al., 1998b).

In an embryotoxicity/teratogenicity study in rabbits,  $\gamma$ -CD was administered to groups of sixteen, artificially inseminated New Zealand White rabbits at dietary concentrations of 0, 5, 10, or 20% from day 0-29 of gestation. A comparison group received a diet containing 20% lactose.  $\gamma$ -CD and lactose were added to the diets at the expense of starch. Transient mild diarrhea occurred in 2 and 3 rabbits of the 10 and 20%  $\gamma$ -CD groups, respectively, during the first few days of the treatment. However, terminal body weights were similar in all groups. There were no deaths during the study. No signs of maternal toxicity were observed, and reproductive performance was similar in all groups. On necropsy of the does, there were no adverse effects which could be related to the  $\gamma$ -CD treatment. Visceral and skeletal examinations of the fetuses did not reveal any treatment-related malformations, anomalies or variations. The decrease in the incidence of hemorrhagic fluid in the 5 and 20% dose groups was considered unlikely to be treatment-related. Under the conditions of the study,  $\gamma$ -CD had no adverse effect on reproductive performance and was not embryotoxic, fetotoxic or teratogenic at dietary concentrations of up to 20% corresponding to an intake of 5-7 g/kg bw/day (Waalkens-Berendsen et al., 1998a).

**(d) Special studies on genotoxicity**

The type and experimental condition of genotoxicity studies with  $\gamma$ -CD are summarized in Table 4. The uniformly negative results demonstrate that  $\gamma$ -CD is not mutagenic, not clastogenic, and does not produce any chromosomal damage or damage of the mitotic apparatus (Blijleven, 1991; de Vogel & van Delft, 1996; Immel, 1991)

**(e) Irritation/sensitization studies**

In a skin irritation/sensitization test in guinea pigs, a 3%  $\gamma$ -CD solution was injected intradermally with Freund's Complete Adjuvans (FCA) (controls: water with or without FCA). Topical application of  $\gamma$ -CD in Vaseline after one and three weeks did not provoke signs of irritation or cutaneous delayed hypersensitivity (Prinsen, 1992).

In an acute eye irritation test, dry  $\gamma$ -CD was applied in the conjunctival cul-de-sac of 3 rabbits. Except for a transient, very slight redness of the conjunctiva, no reaction to the treatment was seen. It was concluded that  $\gamma$ -CD is not irritating or corrosive to the eye (Prinsen, 1990).

**(f) Tolerance in humans**

The gastrointestinal tolerance of  $\gamma$ -CD was examined in a double-blind, placebo-controlled, cross-over study in 24 healthy human volunteers. Single doses of 8 g maltodextrin (control) or 8 g  $\gamma$ -CD were consumed with 100 g yogurt as a mid-morning snack. Administration of a single dose of  $\gamma$ -CD was considered sufficient for examining gastrointestinal tolerance because signs of intolerance typically appear within a short period (0.5 - 6 hours) after ingestion of low digestible carbohydrates and because repeated ingestion leads to an adaptation of the colonic microflora and thus to a higher tolerance. The incidence of subjective gastrointestinal side effects as well as the number and consistency of feces passed was not significantly different between control and test treatment during the 8-hour post-treatment observation period. Flatulence which is the earliest and most frequent side-effect accompanying the ingestion of low digestible

carbohydrates, was noted by two subjects after ingestion of maltodextrin and  $\gamma$ -CD (Koutsou et al., 1999).

**(g) Other safety related aspects**

*i) Interaction with the absorption of nutrients*

Since  $\gamma$ -CD can form inclusion complexes with fat-soluble vitamins and polyunsaturated fatty acids (PUFAs), it needs to be considered whether the use of this substance could impair the bioavailability of these nutrients. The effects of ingested  $\gamma$ -CD on the absorption of essential nutrients are not to be expected for the following reasons.

First, it must be noted that the formation of inclusion complexes is reversible (Connors, 1995). It follows from this that in the presence of other food components, or stomach and intestinal contents, complexed vitamins will be replaced by other organic compounds which have a higher affinity to the cyclodextrin cavity, or are present at higher concentrations.

Second, the rapid and complete digestion of  $\gamma$ -CD by salivary and pancreatic amylase will result in a disappearance of  $\gamma$ -CD from the digesta.

Third, it has been shown that complexation with cyclodextrins actually increases the bioavailability of fat-soluble vitamins or other lipophilic compounds probably because the cyclodextrin complexes have a higher water-solubility (Szejtli et al., 1983; Bárdos et al., 1989).

Fourth, it has been shown in a one-year oral toxicity study with  $\beta$ -CD in dogs that the ingestion of  $\beta$ -CD at dietary concentrations of up to 5% did not influence the plasma levels of vitamins A, D, and E and the liver concentrations of vitamin A and B (D was not measured in the liver) (Bellringer et al., 1995).

#### ii) *Effects on cell membranes*

Cyclodextrins can induce hemolysis of erythrocytes in vitro, presumably due to a cyclodextrin-mediated extraction of cholesterol and other lipids from the erythrocyte membrane (Irie et al., 1982). On incubation of erythrocytes with increasing concentrations of cyclodextrins in isotonic buffer for 30 minutes, hemolysis was initiated at 3 mM  $\beta$ -CD, 6 mM  $\alpha$ -CD, and 16 mM  $\gamma$ -CD. The higher tolerance of  $\gamma$ -CD was confirmed in other studies in which  $\gamma$ -CD concentrations of between 15 to >30 mM were required for the induction of hemolysis (Leroy-Lechat et al., 1994; Okada et al., 1988; Yoshida et al., 1988). At concentrations of  $\geq 1$  mM,  $\beta$ -CD leads within 30 minutes to a significant release of cholesterol and protein from erythrocyte membranes. On the other hand, more than 20 mM  $\gamma$ -CD was required to induce a similar effect (Ohtani et al., 1989). Observations from other in vitro studies using different models confirm that membrane effects are lowest with  $\gamma$ -CD and highest with  $\beta$ -CD (Leroy-Lechat et al., 1994; Bar & Ulitzur, 1994).

However, for the assessment of the safety of ingested  $\gamma$ -CD, the results of these in vitro studies have no relevance since only a very small fraction of ingested  $\gamma$ -CD is absorbed unchanged (<0.02%) and since plasma  $\gamma$ -CD concentrations will therefore be by several orders of magnitude below levels that were associated with membrane effects (de Bie et al., 1998). Direct evidence for an absence of membrane effects of ingested  $\gamma$ -CD is provided by the oral toxicity studies in

which  $\gamma$ -CD was administered to rats at doses of up to 20% in the diet (Lina & Bär, 1998; Lina, 1999).

#### (4) Probable Consumption of the Substance

The ability to form complexes with a wide variety of organic molecules coupled with a relatively high water-solubility makes  $\gamma$ -CD a versatile food ingredient.

It may be used as a carrier and stabilizer for flavors, typically in a ratio of about 5-20 parts of flavor with 100 parts of  $\gamma$ -CD (Thoss et al., 1993). By forming inclusion compounds,  $\gamma$ -CD also can stabilize certain sensitive colors (e.g., lycopene, anthocyanin), fat-soluble vitamins, and polyunsaturated fatty acids (PUFAs) (Tamura et al., 1997; Linssen et al., 1991).  $\gamma$ -CD-stabilized preparations of vitamins and PUFAs are useful for the formulation of dietary supplements and meal replacements (formula diets) in powder form.

$\gamma$ -CD can stabilize emulsions of fats and oils. This property is useful for the preparation of bread spreads in which the typical use levels of  $\gamma$ -CD would not exceed 20%. In frozen dairy desserts,  $\gamma$ -CD improves the melting behavior at a concentration of less than 3%. In ready-to-eat dairy desserts, or in desserts prepared from dry mixes with the admixture of milk (e.g., chocolate mousse),  $\gamma$ -CD stabilizes the fat/water emulsion and the foam, also at levels up to 3%.

The addition of about 1-2%  $\gamma$ -CD to dough increases the volume of baked products (Mulderink, 1986, cited in Linssen et al., 1991). In no-fat or low-fat doughs, an admixture of 1%  $\gamma$ -CD is sufficient to achieve this effect.

Depending upon the food system,  $\gamma$ -CD can improve the retention of water (e.g., in fruit fillings) or fat (e.g., in cheese and cream fillings). In fruit fillings, not more than 3%  $\gamma$ -CD is required for achieving the intended effect. In fat fillings, up to 5%  $\gamma$ -CD may be required for preventing the so-called "oiling-out".

The estimated daily intake (EDI) of  $\gamma$ -CD from the above described uses in food has been calculated using food consumption data from the 1989-91 Continuing Survey of Food Intakes by Individuals (CSFII) and assuming that each food (or food component) which may contain  $\gamma$ -CD, indeed contained it at the highest, technologically feasible concentration (Amann et al., 1998). The one- and three-day intakes of  $\gamma$ -CD were calculated on both per-capita and per-user bases. "Users" were defined as individuals consuming a product of at least one of the food categories concerned on at least one occasion. Intakes from chewing gum and dietary supplements (vitamin and PUFA preparations) were estimated separately since their consumption data are not included in the CSFII database.

The mean one-day intake of  $\gamma$ -CD from all intended food uses combined (except chewing gum, dietary supplements and meal replacements) was estimated at 4.1 g/person/day for users. The so-called "heavy user", i.e., the 90th percentile user, was estimated to ingest about 8.8 g/person/day  $\gamma$ -CD on the same basis. For the 3-day average intake, values of 4.0 and 7.5 g/person/day  $\gamma$ -CD were obtained for the mean and 90th percentile user, respectively. On a per kg body weight basis, the highest  $\gamma$ -CD intakes are expected for children of age 2-6 (0.2 and 0.4 g/kg bw for the mean and 90th percentile users, respectively).

The intake of  $\gamma$ -CD with chewing gum was calculated from a separate survey on chewing gum use in the U.S. It was found that  $\gamma$ -CD intake from chewing gum is small (0.07 g  $\gamma$ -CD/person/per day) (Amann et al., 1998).

The average (50th percentile) user of vitamin and mineral supplements consumes daily about 28 mg fat-soluble vitamins (mainly vitamin E) (Moss et al., 1989; Subar & Block, 1990). This amount requires about 0.25 g  $\gamma$ -CD for stabilization.

The highest intake of  $\gamma$ -CD would result from its use in meal replacements (in powder form) in which it may be used to stabilize added PUFAs. A portion of a formula diet which is designed for replacing one daily meal, should contain at least 1g PUFA. If  $\gamma$ -CD was used for its stabilization, a  $\gamma$ -CD intake of about 4 g/meal would result. Replacement of all daily meals requires a PUFA intake of at least 4.5 g/day. For the stabilization of this amount, about 18 g  $\gamma$ -CD would be needed. However, replacement of all food by formula diet is practiced only by a small number of consumers for limited periods of time (e.g., during stringent weight-loss programs under medical supervision). The intake of 18 g  $\gamma$ -CD/day represents, therefore, an extreme case.

**(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.**

The information in this notification was reviewed by an independent panel of experts:

I.C. Munro, Ph.D., CanTox, Inc.

P.M. Newberne, D.V.M., Ph.D., Dept. of Pathology, Boston University School of Medicine

V.R. Young, Ph.D., Laboratory of Human Nutrition, School of Science, MIT.

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, Drs. Munro, Newberne, and Young concluded that Wacker Biochem's  $\gamma$ -CD product is, through scientific procedures, generally recognized as safe (GRAS) under the conditions of its intended use in foods. The Expert Panel's review has been accepted for publication by Food and Chemical Toxicology ("Safety assessment of  $\gamma$ -Cyclodextrin," Munro, I.C., Newberne, P.M., and Young, V.R.). The Expert Panel's conclusion is consistent with the findings of the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1998).

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Table 1

Results of acute toxicity studies with  $\gamma$ -CD

Species	Sex	n/sex/ group	Route	LD <sub>50</sub> mg/ kg bw	Reference
Mouse	M, F	10	p.o.	>16000	Matsuda et al., 1983
Mouse	M, F	10	s.c.	> 4000	Matsuda et al., 1983
Mouse	M, F	10	i.v.	> 4000	Matsuda et al., 1983
Mouse	M, F	5	i.v.	~10000	Riebeek, 1990a <sup>1</sup>
Mouse	M, F	15	p.o.	>15000	Immel, 1991 <sup>1</sup>
Rat	M, F	10	p.o.	> 8000	Matsuda et al., 1983
Rat	M, F	10	s.c.	> 2400	Matsuda et al., 1983
Rat	M, F	10	i.v.	> 2400	Matsuda et al., 1983
Rat	M, F	5	i.v.	> 3750	Riebeek, 1990b <sup>1</sup>
Rat	M	3	i.p.	> 4600	Riebeek, 1990c <sup>1</sup>

<sup>1</sup> Cited in WHO, 1999

Table 2

Toxicity studies with oral administration of  $\gamma$ -CD

Type of study	Species (n)	Dose levels (% of diet)	NOAEL	Reference
Subacute (2-week) toxicity test	Wistar rats (5m/group)	0, 5, 10, 15, 20% $\gamma$ -CD; 20% lactose	20%	Lina & Bär, 1998
Subchronic (13-week) toxicity study	Wistar rats (20/sex/group)	0, 1.5, 5, 20% $\gamma$ -CD; 20% lactose	20% (m: 11.4 g/kg bw/d; f: 12.7 g/kg bw/d)	Lina & Bär, 1998
Subchronic (90-day) toxicity study	Beagle dogs (4/sex/group)	0, 5, 10, 20% $\gamma$ -CD	20% (m: 7.7 g/kg bw/d; f: 8.3 g/kg bw/d)	Til & Bär, 1998
Chronic (1-year) toxicity study	Wistar rats (20/sex/group)	0, 5, 10, 20% $\gamma$ -CD	20% (m: 8.7 g/kg bw/d; f: 10.8 g/kg bw/d)	Lina & Bär, 1998
Embryotoxicity/teratogenicity study	Wistar rats (25f/group)	0, 1.5, 5, 10, 15, 20% $\gamma$ -CD; 20% lactose	20% (11 g/kg bw/d)	Waalkens-Berendsen et al., 1998b
Embryotoxicity/teratogenicity study	New Zealand White rabbits (16f/group)	0, 5, 10, 20% $\gamma$ -CD; 20% lactose	20% (5-7 g/kg bw/d)	Waalkens-Berendsen et al., 1998a

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

**Table 3**

**Toxicity studies with intravenous administration of  $\gamma$ -CD**

Type of study	Species	Dose levels (mg/kg bw)	NOEL	Reference
Subchronic (1-month) toxicity test	Rats (5/sex/group)	0, 200, 630, 2000	200 mg/kg bw	Donaubauer et al., 1998
Subchronic (3-month) toxicity test	Rats (15/sex/group)	0, 60, 120, 600	120 mg/kg bw	Donaubauer et al., 1998

Abbreviations: bw, body weight; NOEL, No-Observed-Effect-Level

**Table 4**

**Results of genotoxicity studies with  $\gamma$ -CD**

Test	Test system	Concentration	Result	Reference
Ames Test <sup>1</sup>	S. typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100	0, 0.002 0.02, 0.2, 2 or 20 mg/plate	Negative	Blijleven, 1991
Mouse micronucleous test	Mouse bone marrow	15g/ kg bw	Negative	Immel, 1991
In vitro chromosome aberation test <sup>1</sup>	Human lymphocytes	1250, 2500, 5000 $\mu$ g/ml	Negative	de Vogel & van Delft, 1996

<sup>1</sup> With and without metabolic activation (rat liver S9 fraction)



## Appendix 1

This Appendix contains the "Requirements" and "Tests" sections of the specification for  $\gamma$ -cyclodextrin that will be submitted to the Food Chemicals Codex. Methods referenced are in the Food Chemicals Codex, 4<sup>th</sup> Ed., unless otherwise indicated.

### REQUIREMENTS

#### Identification

**Reaction with iodine:** To 0.2 g of the sample in a test-tube add 2 mL of 0.1 N iodine solution. Heat the mixture in a water bath and allow to cool at room temperature. A clear brown solution is formed.

**Chromatography:** The retention time for the major peak in the liquid chromatogram of the *Assay Preparation* corresponds to that in the chromatogram of the *Standard Preparation* obtained as directed in the *Assay*.

**Assay:** Not less than 98% of  $\gamma$ -cyclodextrin ( $C_6H_{10}O_5$ )<sub>8</sub>, calculated on the anhydrous basis.

**Heavy metals (as Pb):** Not more than 5 mg/kg.

#### Microbial Limits:

**Aerobic Plate Count:** Not more than 1000 CFU per g.

**Coliforms:** Negative in 25 g.

**Salmonella:** Negative in 25 g.

**Reducing Sugars:** Not more than 0.5%.

**Residue on Ignition:** Not more than 0.1%.

**Specific rotation:**  $[\alpha]_D^{25}$ : Between +174° and +180° (1% solution).

**Volatile Organic Compounds:** Not more than 20 mg/kg.

**Water:** Not more than 11%.

### TESTS

#### Assay

**Principle**  $\gamma$ -cyclodextrin is identified by *liquid chromatography* and quantified by comparison to reference standards containing standard cyclodextrins.

**Mobile Phase** Prepare a filtered and degassed mixture of acetonitrile and water (67:33).

**Standard Preparation** Use reference  $\gamma$ -cyclodextrin (available from Consortium für Elektrochemische Industrie GmbH, München, Germany or Wacker Biochem Group, Adrian, MI, USA). Prepare a solution of the reference material as described for the *Assay Preparation*.

**Assay Preparation** Weigh accurately about 100 mg of sample into a 10-mL volumetric flask and add about 8 mL of purified deionized water. Bring to complete dissolution by using an ultrasonic bath for 10-15 min. After cooling to room temperature, dilute to mark with purified deionized water.

**Chromatographic System** Use a liquid chromatograph maintained at a constant temperature of 40° and equipped with a refractive index detector, a 4 mm  $\times$  25 cm column packed with Nucleosil-10 NH<sub>2</sub> (Machery & Nagel Inc.) or equivalent (particle size: 10  $\mu$ m, pore size: 100 Å). The flow rate is 2 mL/min.

**Procedure** The injection volume is 9  $\mu$ l. From five analytical runs of each solution the mean values of the peak areas of the reference and the sample are calculated. Calculate the content of  $\gamma$ -cyclodextrin in the sample using the formula:

$$C = \frac{A_S \times m_R \times (100 - w_R) \times 100}{A_R \times m_S \times (100 - w_S)} \%$$

where C = percentage of  $\gamma$ -cyclodextrin in the sample, A<sub>S</sub> = mean value of peak areas of sample solution, A<sub>R</sub> = mean value of peak area of reference solution, m<sub>S</sub> = amount of sample in sample solution (mg), m<sub>R</sub> = amount of reference material in reference solution (mg), w<sub>S</sub> = water content of sample (%), and w<sub>R</sub> = water content of reference material (%).

**Heavy Metals** A solution of 4 g in 25 mL of water meets the requirements of the Heavy Metals Test (Method I), Appendix IIIB, using 20  $\mu$ g of lead ion (Pb) in the control (*Solution A*).

#### **Microbial Limits:**

**Aerobic Plate Count** Proceed as directed in chapter 3 of the *FDA Bacteriological Analytical Manual*, 8<sup>th</sup> Edition, 1995

**Coliforms** Proceed as directed in chapter 4 of the *FDA Bacteriological Analytical Manual*, 8<sup>th</sup> Edition, 1995

**Salmonella** Proceed as directed in chapter 5 of the *FDA Bacteriological Analytical Manual*, 8<sup>th</sup> Edition, 1995

**Reducing Sugars** Proceed as directed under Reducing Sugars Assay, Appendix X.

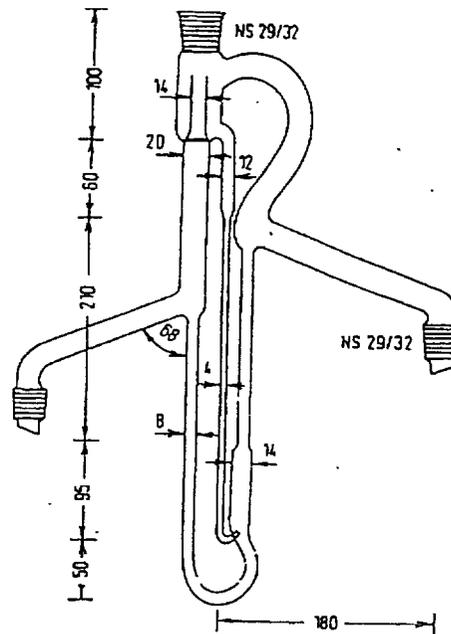
**Residue on Ignition** Ignite 10 g as directed in the General Method, Appendix II C.

**Specific Rotation** Proceed as directed under Optical (Specific) Rotation, Appendix II B. Determine in a solution containing 1 g of a previously dried sample in 100 mL water at 25°.

### **Volatile Organic Compounds**

*Procedure* Dissolve 50 g of the sample in about 700 mL distilled water in a 1-liter round bottom flask and add a magnetic stirrer. Attach the flask to the lower part of a Bleidner apparatus (see Figure 1) and connect a 100 mL round bottom flask containing about 70 mL hexane and a few boiling stones to the other side of the apparatus. Fill the Bleidner apparatus with equal amounts of water and hexane and place a reflux condenser on the top. Heat both flasks with heating mantels to boiling. Stir the 1-liter flask well by the magnetic stirrer. Keep the content of the two flasks boiling for 8 h. After cooling, remove the 100-mL flask, transfer the content to a 100 mL volumetric flask, and fill to mark with hexane.

Figure 1



**Chromatographic System** Analyze the hexane solution by gas chromatography using a 0.32 mm  $\times$  30 m column with a 0.25  $\mu$ m 95% dimethyl, 5% diphenyl polysiloxane stationary phase. The injector temperature is 280°. Use nitrogen at a flow rate of 70 mL/min as the carrier gas. The column temperature is set at 70° (4 min), then increased to 250° at a rate of 10°/min. Detection is FID, 280°

**Determination** Calculate the area(s) under the peak for each volatile organic compound and convert it to mg/kg  $\gamma$ -cyclodextrin using the response factor of 8-cyclohexadecen-1-one. The response factor is determined from a calibration curve using 8-cyclohexadecen-1-one concentrations of 0.1-6 mg/100 mL hexane.

**Water** Determine as directed under *Water Determination* by the *Karl Fischer Titrimetric Method*, Appendix II B.

**Packaging and Storage** Store in well-closed containers.

SUBMISSION END

000043

000043



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May 24, 2000

## BY HAND DELIVERY

Linda S. Kahl  
 Center for Food Safety and Applied Nutrition (JFS-206)  
 Food and Drug Administration  
 200 C Street S.W.  
 Washington, D.C. 20204

Re: GRAS Exemption Notification for Gamma-Cyclodextrin

Dear Linda:

As discussed yesterday, we wish to confirm the availability for inspection of the supporting data for the GRAS exemption notification for gamma-cyclodextrin, submitted by David Clissold on May 19, 2000 on behalf of Wacker Biochem Corporation. Therefore, please amend the "Review and Copying Statement" in the GRAS notice as follows:

The data and information that are the basis for Wacker Biochem Corporation's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at the office of Hyman, Phelps and McNamara, P.C., 700 Thirteenth Street N.W., Suite 1200, Washington, D.C. 20005, or will be sent to FDA upon request.

We trust that the above amendment clarifies the availability of Wacker's supporting data and information.

000044

Linda S. Kahl  
May 24, 2000  
Page 2

HYMAN, PHELPS & MCNAMARA, P.C.

If you have any further questions regarding the pending GRAS notification for gamma-cyclodextrin, please do not hesitate to contact me or my associate, David Clissold.

Sincerely,

Diane B. McColl  
Counsel to Wacker Biochem Corporation

DBM/dmb

cc: Gerhard Schmid  
President, Wacker Biochem Corporation

000045

AM



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August 31, 2000

BY FACSIMILE/CONFIRMATION COPY BY MAIL

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Dr. Linda S. Kahl  
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Washington, D.C. 20201

2000 SEP 19 P 12:00

Re: Lead (Pb) Specification in Notice of GRAS Exemption for  $\gamma$ -Cyclodextrin

Dear Drs. Laumbach and Kahl:

Thank you very much for finding time in your busy schedules to speak to Albert Bär, Ph.D. and me last week. As discussed, Wacker Biochem. Corp. (Wacker) wishes to clarify the specification for lead (Pb) in Appendix 1 of the above referenced Notice of GRAS Exemption (GRAS Notice).

Appendix 1 of Wacker's GRAS Notice was a draft of a specification for  $\gamma$ -Cyclodextrin that Wacker plans to submit to the Food Chemicals Codex-4<sup>th</sup> Edition (FCC-IV). Appendix 1 showed a requirement for "Heavy metals (as Pb)" as "Not more than 5 mg/kg" (page 000039) using "Heavy Metals Test (Method I), Appendix IIIB" of the FCC-IV (page 000040). Wacker agrees to alter this requirement and test method in the

000048

Dr. Andrew D. Laumbach  
Dr. Linda S. Kahl  
August 31, 2000  
Page 2

HYMAN, PHELPS & MCNAMARA, P.C.

will be submitted to FCC-IV. The limit for lead (Pb) that will be submitted to FCC-IV will be "not more than 1 mg/kg" as determined by the Flame Atomic Absorption Spectrophotometric Method (Method I) under the Lead Limit Test, Appendix IIIB of the FCC-IV.

The specification for lead (Pb) in Wacker's GRAS Notice (not more than 1 mg/kg) is not affected by this clarification regarding Appendix 1.

Thank you again for speaking with us regarding this issue.

Sincerely

David B. Clissold

DBC/dmb

cc: Dr. Gerhard Schmid, President  
Wacker Biochem Corp.

Albert Bär, Ph.D.  
Bioresco Ltd.

000049