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Safety assessment of γ-cyclodextrin

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Abstract

Gamma-cyclodextrin (γ-CD) is a cyclic α-(1,4)-linked oligosaccharide consisting of eight glucose molecules. Like other cyclodextrins, γ-CD can form inclusion complexes with a variety of organic molecules because the inner side of the torus-like molecule is less polar than the outer side. In foods, γ-CD may be used as a carrier for flavors, vitamins, polyunsaturated fatty acids, and other ingredients. It also has useful properties as a stabilizer in different food systems. The daily intake from all its intended uses in food at highest feasible concentrations has been estimated at 4.1 g/person/day for consumers of γ-CD containing foods. The present review summarizes the safety data of γ-CD. The toxicity studies consist of standard genotoxicity tests, subchronic rat studies with oral and intravenous administration of γ-CD for up to 3 months, a subchronic (3-month) toxicity study in dogs, a (1-year) oral toxicity study in rats, and embryotoxicity/teratogenicity studies in rats and rabbits. In the studies with oral administration, γ-CD was given at dietary concentrations of up to 20%. All these studies demonstrated that γ-CD is well tolerated and elicits no toxicological effects. Metabolic studies in rats showed that γ-CD is rapidly and essentially completely digested by salivary and pancreatic amylase. Therefore, the metabolism of γ-CD closely resembles that of starch and linear dextrins. A human study with ingestion of single doses of 8 g γ-CD or 8 g maltodextrin did not reveal a difference in gastrointestinal tolerance of these two products. An interaction of ingested γ-CD with the absorption of fat-soluble vitamins or other lipophilic nutrients is not to be expected because the formation of inclusion complexes is a reversible process, γ-CD is readily digested in the small intestine, and studies with β-CD, a non-digestible cyclodextrin, have shown that the bioavailability of vitamins (A, D, and E) is not impaired. On basis of these studies it is concluded that γ-CD is generally recognized as safe (GRAS) for its intended uses in food.

1. Introduction

Cyclodextrins are cyclic α-(1,4)-linked maltooligosaccharides. α-, β-, and γ-cyclodextrin consist of 6, 7, and 8 glucose units, respectively (Fig. 1). 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 to 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 (Allegre and

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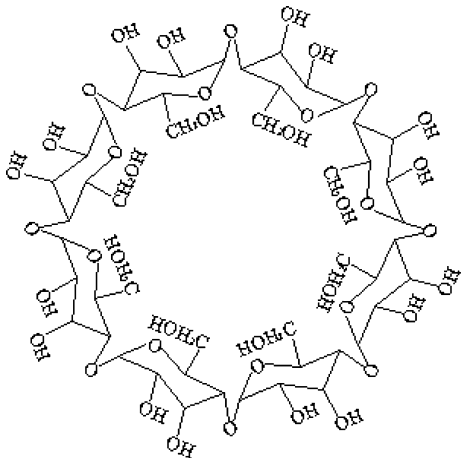

Fig. 1. Chemical structure of γ-cyclodextrin.

Deratani, 1994; Nagatomo, 1985; Pszczola, 1988; Szejtli, 1982; Thompson, 1997; Vaution et al., 1987). 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, γ-cyclodextrin (γ-CD) has wider applications in this respect than α- or β-CD.

The present report summarizes the results of biological and toxicological studies of γ-CD, and the authors' assessment of the safety of γ-CD under the conditions of its intended use in food.

2. Manufacturing process

α-, β-, and γ-Cyclodextrin 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 and Saniez, 1987; Schmid, 1989, 1991; Starnes, 1990; Tonkova, 1998). CGTases degrade starch by a cyclization reaction. There is evidence that the enzyme recognizes the 6, 7 or 8 glucose units from the non-reducing end of an amylose molecule, attacks the adjacent α-1,4-linkage, and transfers it to the C-4 position of the non-reducing end to produce α-, β- or γ-CD (Schmid, 1989). Typically, mixtures of α-, β-, and γ-CD are formed by the action of CGTases on starch, with the β-form being predominant for thermodynamic reasons. Different CGTases produce α-, β-, and γ-CD in different proportions during the initial phase of the reaction. The ratio of the formed cyclodextrins is also influenced by other conditions such as the reaction time, temperature, and presence of ethanol (Goel and 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 and 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 γ-CD.

In the first step of γ-CD production, food-grade, liquified starch is treated with CGTase under controlled pH and temperature conditions. Cyclohexadecen-1-one (CHDC) or another appropriate complexant is added to precipitate formed γ-CD. The complex is removed and purified. The complexant is separated from γ-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 γ-CD, total residue levels of volatile organic compounds (i.e., CHDC and *n*-decane) will not exceed 20 ppm (FAO, 1998). γ-CD is obtained by crystallization as a white powder with a purity of ⩾98% (on dry matter basis).

The CGTase was obtained from a genetically modified strain of *E. coli* K12. *E. coli* K12 is a non-pathogenic and non-toxigenic 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 *Salmonella 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). γ-CD does not contain any CGTase activity because the enzyme is inactivated by heat and is removed completely during the γ-CD production process. Any non-proteinaceous, 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 γ-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). In the United States, civet is GRAS under

21CFR § 182.50.[1] 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_{50} (p.o.) exceeds 4.6 g/kg bw in mice and 10 g/kg bw in rats (Spanjers, 1986, cited in WHO, 1999; Dickhaus and 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/day, respectively (Lina, 1992, cited in WHO, 1999; Lina et al., 1986, cited in WHO, 1999). At the estimated daily intake (EDI) of γ-CD (4 g/person/day, for calculation see below), the CHDC intake would be less than 1 μg/kg bw/day.

3. Uses in food and estimated daily intake

The ability to form complexes with a wide variety of organic molecules coupled with a relatively high-water solubility makes γ-CD a versatile food ingredient.[1]

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 γ-CD (Thoss et al., 1993). By forming inclusion compounds, γ-CD also can stabilize certain sensitive colors (e.g., lycopene and anthocyanin), fat-soluble vitamins, and polyunsaturated fatty acids (PUFAs) (Linssen et al., 1991; Tamura et al., 1997). γ-CD-stabilized preparations of vitamins and PUFAs are useful for the formulation of dietary supplements and meal replacements (formula diets) in powder form.

γ-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 γ-CD would not exceed 20%. In frozen dairy desserts, γ-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), γ-CD stabilizes the fat/water emulsion and the foam, also at levels up to 3%.

The addition of about 1–2% γ-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% γ-CD is sufficient to achieve this effect.

Depending upon the food system, γ-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% γ-CD is required for achieving the intended effect. In fat fillings, up to 5% γ-CD may be required for preventing the so-called "oiling-out."

The estimated daily intake (EDI) of γ-CD from the above described uses in food has been calculated using food consumption data from the 1989 to 1991 Continuing Survey of Food Intakes by Individuals (CSFII) and assuming that each food (or food component) which may contain γ-CD, indeed contained it at the highest, technologically feasible concentration (Amann et al., 1998). The 1- and 3-day intakes of γ-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 data base.

The mean 1-day intake of γ-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 γ-CD on the same basis. For the 3-day average intake, values of 4.0 and 7.5 g/person/day γ-CD were obtained for the mean and 90th percentile user, respectively. On a per kilogram body weight basis, the highest γ-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 γ-CD with chewing gum was calculated from a separate survey on chewing gum use in the US. It was found that γ-CD intake from chewing gum is small (0.07 g γ-CD/person/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 and Block, 1990). This amount requires about 0.25 g γ-CD for stabilization.

The highest intake of γ-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 1 g PUFA. If γ-CD was used for its stabilization, a γ-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 γ-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 γ-CD/day represents, therefore, an extreme case.

[1] In the US, food ingredients include both food additives approved by the US Food and Drug Administration (FDA) and substances that are generally recognized as safe (GRAS). General recognition of safety requires both technical evidence of safety and common knowledge of the substance throughout the scientific community knowledgeable about the safety of ingredients added to food (21 CFR 170.30).

4. Biological studies

4.1. 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 hydrolyze α-CD and β-CD to any measurable extent. On the other hand, they hydrolyze γ-CD readily, yielding mainly maltose, some maltotriose, and smaller amounts of glucose (Abdullah et al., 1966; Kondo et al., 1990; Marshall and Miwa, 1981).

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

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

5. Toxicological studies

5.1. Acute studies

The acute toxicity of γ-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 γ-CD levels of up to 16 g/kg bw in mice and 8 g/kg bw in rats (Matsuda et al., 1983; Immel, 1991, cited in WHO, 1999). Intraperitoneal and subcutaneous administration of γ-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 γ-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 1 h 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 (Riebek et al., 1990b; cited in WHO, 1999).

5.2. Subchronic studies with oral administration of γ-CD

Subchronic oral toxicity studies with γ-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 2-week toxicity study, six groups of 5 young male Wistar rats received diets to which 0, 5, 10, 15, or 20% γ-CD, or 20% lactose were added at the expense of starch. The ingestion of γ-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% γ-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

Table 1
Results of acute toxicity studies with γ-CD

Species	Sex	*n*/sex/group	Route	LD_{50} (mg/kg bw)	Reference
Mouse	M,F	10	p.o.	>16,000	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.	~10,000	Riebeek (1990a)[a]
Mouse	M,F	15	p.o.	>15,000	Immel (1991)[a]
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)[a]
Rat	M	3	i.p.	>4600	Riebeek (1990c)[a]

[a] Cited in WHO, 1999.

Table 2
Toxicity studies with oral administration of γ-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% γ-CD; 20% lactose	20%	Lina and Bär (1998)
Subchronic (13-week) toxicity study	Wistar rats (20/sex/group)	0, 1.5, 5, 20% γ-CD; 20% lactose	20% (m: 11.4 g/kg bw/day; f: 12.7 g/kg bw/day)	Lina and Bär (1998)
Subchronic (90-day) toxicity study	Beagle dogs (4/sex/group)	0, 5, 10, 20% γ-CD	20% (m: 7.7 g/kg bw/day; f: 8.3 g/kg bw/day)	Til and Bär (1998)
Chronic (1-year) toxicity study	Wistar rats (20/sex/group)	0, 5, 10, 20% γ-CD	20% (m: 8.7 g/kg bw/day; f: 10.8 g/kg bw/day)	Lina and Bär (1998)
Embryotoxicity/ teratogenicity study	Wistar rats (25f/group)	0, 1.5, 5, 10, 15, and 20% γ-CD; 20% lactose	20% (11 g/kg bw/day)	Waalkens-Berendsen et al. (1998b)
Embryotoxicity/ teratogenicity study	New Zealand White rabbits (16f/group)	0, 5, 10, 20% γ-CD; 20% lactose	20% (5–7 g/kg bw/day)	Waalkens-Berendsen et al. (1998a)

Abbreviations: m, male; f, female; bw, body weight; NOAEL, no-observed-adverse-effect-level.

and empty) were increased slightly in the 10, 15, and 20% γ-CD groups and, more pronounced, in the 20% lactose group. No gross abnormalities were detected at necropsy that could be attributed to the γ-CD treatment (Lina and 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% γ-CD. A comparison group received a diet with 20% lactose. γ-CD and lactose were added at the expense of starch. Three satellite groups (10/sex/group) received the control diet, a diet with 20% γ-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% γ-CD group was terminated, and a corresponding study consisting of a control and a 20% γ-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% γ-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% γ-CD and lactose groups. The hematological and clinico-chemical parameters as well as the semiquantitative urine analyses did not reveal changes that could be attributed to the γ-CD treatment. Urinary pH was decreased and calcium increased in males and females of the lactose group. The ingestion of γ-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% γ-CD group. At the end of the recovery period, there was no difference of cecum weights between the 20% γ-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% γ-CD group (males) and the lactose group. There was a very slight increase in relative liver weight in males of the 20% γ-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 γ-CD. Histopathological examination did not reveal any abnormalities that could be attributed to the treatment. The effects observed after feeding of γ-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 γ-CD for 13 weeks at dietary levels of up to 20% (corresponding to intakes of 11.4 and 12.7 g/kg bw/day for male and female rats, respectively) was well tolerated and did not produce any signs of toxicity (Lina and Bär, 1998).

In a 3-month subchronic toxicity study, groups of Beagle dogs received diets with 0, 5, 10 or 20% γ-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 γ-CD groups. The incidence and severity of diarrhea was related to the dietary levels of γ-CD. It was somewhat higher in females than in males. No treatment-related differences were observed with respect to ophthalmoscopic observations, hematological parameters, clinico-chemical 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 necrospy 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 γ-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 γ-CD at dietary levels of up to 20% (corresponding to intakes of approximately 7.7 and 8.3 g/kg bw/day in male and female dogs, respectively) was tolerated without any toxic effects (Til and Bär, 1998).

5.3. Chronic study with oral administration of γ-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% γ-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 γ-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 γ-CD exposure.

Organ weights were not affected by the γ-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 γ-CD treatment. It was concluded that the 20% dose level represents the NOAEL. γ-CD intake at this level was 8.7 and 10.8 g/kg bw/day 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% γ-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 γ-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% γ-CD groups of the 1-year study and the 20% lactose group of the 90-day study but not in the 20% γ-CD group of the 90-day study (Lina, 1999; Lina and Bär, 1998). Histopathological changes attributable to the ingestion of γ-CD were not observed in any study (Lina, 1999; Lina and Bär, 1998).

5.4. Subchronic studies with intravenous administration of γ-CD

Two subchronic toxicity studies with daily intravenous administration of γ-CD were performed in rats. The design of these studies and the resulting NOELs are summarized in Table 3.

In a 1-month toxicity study, γ-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-

Table 3
Toxicity studies with intravenous administration of γ-CD

Type of study	Species (mg/kg bw)	Dose levels	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.

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 was 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 and 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 reabsorptive 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, γ-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 γ-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-β-CD/kg bw (Coussement et al., 1990). In vitro, hydroxypropyl-β-CD has a slightly higher hemolytic effect than γ-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; Fillastre et al., 1967; Frank et al., 1976; Hiasa et al., 1981; Kief and Engelbart, 1966).

5.5. Embryotoxicity/teratogenicity studies

In an embryotoxicity/teratogenicity study in rats, γ-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 γ-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, γ-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/day (Waalkens-Berendsen et al., 1998b).

In an embryotoxicity/teratogenicity study in rabbits, γ-CD was administered to groups of 16, artificially inseminated New Zealand White rabbits at dietary concentrations of 0, 5, 10, or 20% from day 0 to 29 of gestation. A comparison group received a diet containing 20% lactose. γ-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% γ-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 γ-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, γ-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).

5.6. Special studies on genotoxicity

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

5.7. Irritation/sensitization studies

In a skin irritation/sensitization test in guinea pigs, a 3% γ-CD solution was injected intradermally with Freund's complete adjuvans (FCA) (controls: water with or without FCA). Topical application of γ-CD in vaseline after 1 and 3 weeks did not provoke signs of irritation or cutaneous delayed hypersensitivity (Prinsen, 1992).

In an acute eye irritation test, dry γ-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 γ-CD is not irritating or corrosive to the eye (Prinsen, 1990).

5.8. Tolerance in humans

The gastrointestinal tolerance of γ-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 γ-CD were consumed with 100 g yogurt as a mid-morning snack. Administration of a single dose of γ-CD was considered sufficient for examining gastrointestinal tolerance because signs of intolerance typically appear within a short period (0.5–6 h) 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-h 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 γ-CD (Koutsou et al., 1999).

Table 4
Results of genotoxicity studies with γ-CD

Test	Test system	Concentration	Result	Reference
Ames test[a]	*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 micro-nucleus test	Mouse bone marrow	15 g/kg bw	Negative	Immel (1991)
In vitro chromosome aberration test[a]	Human lymphocytes	1250, 2500, 5000 μg/ml	Negative	De Vogel and van Delft (1996)

[a] With and without metabolic activation (rat liver S9 fraction).

6. Other safety-related aspects

6.1. Interaction with the absorption of nutrients

Since γ-CD can form inclusion complexes with fat-soluble vitamins and PUFAs, it needs to be considered whether the use of this substance could impair the bioavailibility of these nutrients. The authors concluded that effects of ingested γ-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 γ-CD by salivary and pancreatic amylase will result in a disappearance of γ-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 (Bárdos et al., 1989; Szejtli et al., 1983).

Fourth, it has been shown in a 1-year oral toxicity study with β-CD in dogs that the ingestion of β-CD at dietary concentrations of up to 5% did not influence the plasma levels of vitamins A, D, and E and the liver concentrations of vitamin A and E (D was not measured in the liver) (Bellringer et al., 1995; Toyoda et al., 1997).

6.2. 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 cyclodextrines in isotonic buffer for 30 min, hemolysis was initiated at 3 mM β-CD, 6 mM α-CD, and 16 mM γ-CD. The higher tolerance of γ-CD was confirmed in other studies in which γ-CD concentrations of between 15 and >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 ⩾1 mM, β-CD leads within 30 min to a significant release of cholesterol and protein from erythrocyte membranes. On the other hand, more than 20 mM γ-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 γ-CD and highest with β-CD (Bar and Ulitzur, 1994; Leroy-Lechat et al., 1994).

However, for the assessment of the safety of ingested γ-CD, the results of these in vitro studies have no relevance since only a very small fraction of ingested γ-CD is absorbed unchanged (<0.02%) and since plasma γ-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 γ-CD is provided by the oral toxicity studies in which γ-CD was administered to rats at doses of up to 20% in the diet (Lina, 1999; Lina and Bär, 1998).

7. Discussion

There is a substantial body of evidence to support the safety of γ-CD as a food ingredient.

Data from in vitro and in vivo studies demonstrate that γ-CD is digested and metabolized like starch or linear dextrins. Only minute amounts of γ-CD may be absorbed unchanged (<0.02%).

The toxicity of γ-CD was examined in standard in vitro and in vivo toxicity tests. Ames tests, a chromosome aberration test, and a micronucleus test demonstrate that γ-CD is not genotoxic. In acute oral toxicity tests, γ-CD was well tolerated without signs of toxicity even at the highest doses tested (16 g/kg bw/day p.o. in mice). In two 13-week oral toxicity tests, rats and dogs received γ-CD with the diet at dietary levels of up to 20%. A few mild, intestinal effects (minimal cecal enlargement and transient stool softening) suggested that a small fraction of the high-γ-CD dose may not have been digested completely or, alternatively, that γ-CD had a slight inhibitory effect on the digestion of dietary starch by pancreatic amylase. Otherwise, no reactions to the treatment were observed. It was concluded that γ-CD ingested at dietary levels of up to 20%, corresponding to about 12 g/kg bw/day (rats) and 8 g/kg bw/day (dogs), was tolerated without any adverse effects. Embryotoxicity/teratogenicity studies in rats and rabbits with oral administration of γ-CD at dietary levels of up to 20% also did not reveal any treatment-related, adverse effects.

In a 1-year rat toxicity study, γ-CD was administered at dietary levels of up to 20%. γ-CD intake at this level was 8.7 and 10.8 g/kg bw/day in male and female rats, respectively. There were no adverse effects in response to the treatment. A few transient increases of plasma enzyme levels were considered not to be related to the γ-CD treatment. A slight increase of the cecum weight in the 20% dose group was the only change that could be attributed to the ingestion of γ-CD. However, cecal enlargement is a well-known consequence of the ingestion of low-digestible carbohydrates in rats which is not considered to have any relevance for human safety (WHO, 1987).

Two rat studies with daily intravenous administration of γ-CD for periods of 1 and 3 months, demonstrate that systemically administered γ-CD is well tolerated at doses which are more than three orders of magnitude higher than those which could possibly result from the ingestion of γ-CD.

Given the lack of genotoxicity and considering the metabolism of γ-CD which corresponds to that of starch, long-term oral toxicity/carcinogenicity studies are not required for this substance.

A human tolerance study showed that a single dose of 8 g γ-CD did not result in more gastrointestinal side-effects than the ingestion of 8 g maltodextrin. Considering the lack of toxicity of γ-CD in extensive animal studies and the metabolic similarity between γ-CD and starch, further and more extensive tolerance testing in humans, while useful, is not essential.

Adverse effects of ingested γ-CD on the absorption of certain nutrients (fat-soluble vitamins, PUFAs) are not to be expected because complex formation of γ-CD with these nutrients is reversible, and γ-CD is digested rapidly by pancreatic amylase. A study on the effect of β-CD on the absorption of fat-soluble vitamins in dogs did also not provide any evidence for an inhibitory effect of cyclodextrin on vitamin absorption.

8. Conclusion

Based on a critical evaluation of the forgoing data and information, the authors conclude that γ-CD is generally recognized as safe (GRAS) under the above described conditions of intended use in foods. Disregarding the use of γ-CD in formula diets, the combined uses result in an estimated daily intake of 4.1 and 8.8 g/day for the mean and 90% percentile consumer, respectively. The use in formula diets for complete meal replacement may result in a γ-CD intake of up to 18 g/person/day.

This conclusion of safety is in keeping with the result of the safety assessment of γ-CD by the Joint FAO/WHO Expert Committee on Food Additives which has allocated an ADI "not specified" to this substance (WHO, 1999, 2000).

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