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July 27, 2006

REC'D JUL 31 2006

Via Federal Express

Dr. Laura M. Tarantino
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: Submission of GRAS Notification for Erythritol (from *Trichosporonoides megachiliensis*)

Dear Dr. Tarantino:

Pursuant to proposed 21 C.F.R. § 170.36(c) and on behalf of our client, Mitsubishi-Kagaku Foods Corporation of Tokyo, Japan, we hereby notify the agency of our determination on the basis of scientific procedures that erythritol is generally recognized as safe (GRAS) when used in a variety of foods. As with all GRAS substances, the described uses of erythritol are exempt from the premarket clearance requirement applicable to food additives under section 409 of the Food, Drug, and Cosmetic Act.

We trust you will find the enclosed notification acceptable. Should any questions arise during the review process, please do not hesitate to contact us, preferably by telephone, so that we may respond as quickly as possible.

Sincerely,

David R. Joy

cc: Ms. Yukino Nagai
Mr. Takahiro Abe

Encl: GRAS Notification in triplicate

Erythritol GRAS Notification

RECD JUL 31 2006

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1. Claim regarding GRAS status

Mitsubishi Kagaku-Foods Corporation hereby notifies the agency through its attorneys of its determination that erythritol produced through fermentation of *Trichosporonoides megachiliensis* is generally recognized as safe (GRAS) when added to food as described below.

As such, the covered uses of erythritol are exempt from the premarket clearance requirements of the Federal Food, Drug, and Cosmetic Act.

i. Name and address of the notifier

Ms. Yukino Nagai
Mitsubishi Kagaku-Foods Corporation
3-9, Ginza 1-chome, Chuo-ku
Tokyo 104-0061 Japan
phone: 011 813 3563 1696

ii. Common or usual name of the notified substance

The common or usual name of the notified substance is:

Erythritol

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iii. Conditions of use

The conditions of use proposed by Mitsubishi are identical to those reported to FDA in GRAS Notification (GRN) 76. A table reproducing these levels (copied from FDA's response letter to GRN 76) is presented below for convenient reference.

Food	Level of use
Reduced- and low-calorie carbonated and non-carbonated beverages; Dairy drinks (chocolate and flavored milks)	3.5 percent
Frozen dairy desserts (regular ice cream, soft serve, sorbet); Puddings (instant, phosphate set); Yogurt (regular and frozen)	10 percent
Bakery fillings (fruit, custard, cream, pudding); Cakes and cookies (regular and dietetic)	15 percent
Fat-based cream used in modified fat/calorie cookies, cakes and pastries; Chewing gum; Soft Candies (non-chocolate, plain chocolate, chocolate coated)	60 percent

Hard candies (including pressed candy, mints, and cough drops)	99 percent
Sugar substitutes (carrier)	100 percent

The purpose of the present submission is to assert that erythritol produced using the microorganism, *Trichosporonoides megachiliensis,* is GRAS. GRN 76 was presented as encompassing erythritol produced using a different microorganism, *Moniliella pollinis.*

iv. Basis for the GRAS determination

The GRAS determination for erythritol is based upon scientific procedures.

v. Statement of availability of data and information

The data and information that are the basis for Mitsubishi's GRAS determination are available for review and copying by FDA at the offices of Keller and Heckman, LLP, 1001 G Street, N.W., Washington, D.C. 20001. These documents will be sent to FDA upon request.

We further note that most information forming the basis for Mitsubishi's GRAS determination has already been submitted to FDA in the form of GRAS Affirmation Petition No. 7G0422, which is hereby incorporated by reference.

July 27, 2006

David R. Joy
Attorney for the Notifier
Keller and Heckman, LLP
1001 G Street, N.W.
Washington, D.C. 20001

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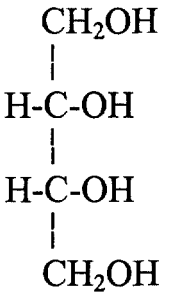
2. Detailed Information About the Identity of the Notified Substance

i. Name: erythritol

ii. CAS Registry Number: 149-32-6

iii. Synonyms: 1,2,3,4-butanetetrol
meso-erythritol
tetrahydroxybutane

iv. Chemical Structure:



v. Method of manufacture:

Erythritol is manufactured by the pure culture fermentation using safe and suitable microorganisms that act on carbohydrate-based media. Upon completion of the fermentation process, the microorganisms are heat-killed and separated from the fermentation broth. Other impurities are also removed to produce a finished substance of not less than 99.5% purity.

As mentioned above, GRAS Notification No. 76 covers erythritol manufactured using *Moniliella pollinis*. Mitsubishi's manufacturing process for erythritol is based on the fermentative conversion of glucose to erythritol catalyzed by a microorganism named *Trichosporonoides megachiliensis* (previously named *Aureobasidium sp.*). The fermentation step is followed by separation and purification from the fermented broth.

For the fermentation process, the strain inoculum preparation is transferred under aseptic conditions into a sterile medium containing glucose syrup and corn steep liquor. All ingredients used are of food grade quality or food compatible. The fermentation temperature is maintained within a specified range. When the glucose is completely consumed, the fermentation broth is heated to kill the culture organisms.

Purification processes are kept at temperatures suitable to ensure sterility. Dead cells are separated from the fermentation broth by filtration. The supernatant is first passed through ion-exchange resins to remove salts, impurities, and colorants. The supernatant is then passed through activated charcoal. The purified solution is further purified using ultrafiltration (molecular weight cut-off: 6 kD) and concentrated under reduced pressure and then crystallized by cooling. The slurry is centrifuged and the solid washed with deionized water. The wet product is air-dried and packed. The resulting erythritol is at least 99.5% pure by high-performance liquid chromatography (HPLC) analysis.

vi. Specifications:

Specifications for erythritol are included in the 5th Edition of the *Food Chemicals Codex*. Copies of these specifications are presented in Appendix 1 for convenient reference. Mitsubishi's erythritol meets all requirements of the current *Food Chemicals Codex* monograph. We note that both microorganisms, *Trichosporonoides megachiliensis* and *Moniliella pollinis*, are mentioned in the *Food Chemicals Codex* monograph for erythritol.

Included as Appendix 2 is a summary analytical report confirming that five batches satisfy the purity specifications of the FCC.

The Joint (FAO/WHO) Expert Committee on Food Additives (JECFA) has developed similar purity specifications for erythritol, which also cite the use of both microorganisms. (Ref. 1).

3. Information on any self-limiting levels of use

Not applicable.

4. Summary of Basis for Notifier's GRAS Determination

This Notification relies upon data and information previously submitted to FDA to support its evaluations of erythritol in response to GRAS Notification No. 76 and the GRAS Affirmation Petition filed jointly by Mitsubishi, Cerestar, and Nikken (GRASP No. 7G0422).

Mitsubishi Chemical Corporation, Cerestar Holding B.V., and Nikken Chemicals Co., Ltd., jointly submitted GRASP No. 7G0422, which claimed GRAS status for erythritol when used in a variety of foods including sugar substitutes, hard and soft candies, chewing gum, and beverages. The data and information contained in GRASP No. 7G0422 was developed jointly by the three submitters and pertained to erythritol produced using either microorganism, *Trichosporonoides megachiliensis* or *Moniliella pollinis*.

Rather than converting the pending GRASP into a GRAS Notification, Cerestar's GRAS Notification, GRN 76, incorporated the GRASP by reference and claimed expanded uses for

erythritol as GRAS. These expanded uses were supported by a supplemental statement made by a GRAS panel of qualified experts and a revised dietary exposure estimate.

Because GRN 76 referred only to the use of a single microorganism, Mitsubishi considers it appropriate to notify the agency that erythritol produced using *Trichosporonoides megachiliensis* is also GRAS, under the same conditions of use reviewed in GRN 76.

Erythritol has been studied extensively and evaluated numerous times by food safety experts. An extensive review article on the safety of erythritol was published in *Food and Chemical Toxicology* in 1998 (Ref. 2). In 1996, an entire issue of *Regulatory Toxicology and Pharmacology* was devoted to studies demonstrating the safety of erythritol (Ref. 3). The reported studies include acute, subchronic, and chronic oral toxicity studies in rats, mice, and dogs. They also include teratogenicity, reproduction, genotoxicity, and metabolic studies, as well as human tolerance studies.

Erythritol occurs endogenously and naturally in the diet in foods such as mushrooms, watermelons pears, grapes, wine, beer, soy sauce, and cheese, at levels up to 0.13%. The safety of erythritol is further supported by its chemical structure, *i.e.*, erythritol is positioned in the homologous series of sugar alcohols, between glycerol and xylitol, a series that also includes other common food ingredients such as sorbitol and mannitol. As is typical of sugar alcohols, ingested erythritol is significantly excreted unchanged in the urine and partially undergoes microbial fermentation to volatile fatty acids in the large intestine.

In 1999, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated erythritol and established an acceptable daily intake (ADI) 'not specified' (Appendix 3). JECFA's ADI 'not specified' is applied to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health.

In 2003, the Scientific Committee on Food (SCF) of the European Union issued an opinion on erythritol, concluding that its use as a food additive is acceptable and that a numerical ADI was not needed (Appendix 4). The SCF specifically acknowledged the use of *Trichosporonoides megachiliensis* and referred to this method of production as the Mitsubishi/Nikken method. The SCF considered rare reports, cited below, of allergic reactions or allergic-like reactions linked to erythritol. This information was also included in GRASP No. 7G0422.[1]

On July 5, 2006, the European Union adopted a set of amendments to its food additive legislation authorizing the addition of erythritol to foods generally in accordance with good manufacturing practice. More specifically, two pieces of EU food additive legislation were amended, the Sweeteners Directive (94/35/EC) and the so-called Miscellaneous Additives

[1] Supplemental Submission of January 17, 2001, from Diane McColl to Rosalie Angeles with updated GRAS panel statement.

Directive (95/2/EC). Under the Sweeteners Directive, erythritol is now authorized at *quantum satis* levels of use in a variety of foods, identical to the manner in which the other polyol sweeteners are authorized. Under the Miscellaneous Additives Directive, erythritol may be added to foods generally, except beverages, unprocessed foods, and certain other exceptions (but including liquers and including frozen, unprocessed fish, crustaceans, molluscs, and cephalopods) at *quantum satis* levels for purposes other than sweetening (Ref. 4).

In 1997, FDA amended the health claim regulation regarding noncariogenic carbohydrate sweeteners to include erythritol in response to a petition filed jointly by Mitsubishi, Cerestar, and Nikken (Ref. 5). FDA's authorization of a health claim regarding erythritol indicates that the petitioners demonstrated to FDA's satisfaction that the substance is safe and lawful under the applicable food safety provisions of the Federal Food, Drug, and Cosmetic Act, as mandated by 21 C.F.R. § 101.14(b)(3)(ii).

In 2001, FDA responded favorably to GRAS Notification No. 76 in which Cerestar asserted that erythritol is GRAS under the same conditions of use covered by the present submission (Appendix 5).

Erythritol has been used since 1990 in Japan as a component of candies, sugar substitutes, chocolates, soft drinks, chewing gum, jellies, jams, and yogurt.

Both microorganisms, *Trichosporonoides megachiliensis* and *Moniliella pollinis*, have been reviewed and found acceptable by JECFA, the SCF and the Committee on Food Chemicals Codex. The fermentation broth containing erythritol is separated from the organisms and subjected to purification treatment similar to those for the carbohydrate sweeteners and sugar alcohols, *e.g.*, ion-exchange resin, activated charcoal, and crystallization. The final product is a material containing not less than 99.5% erythritol.

The safety of *Trichosporonoides megachiliensis,* was demonstrated in an acute oral toxicity study in which an erythritol fermentation broth containing the organism was administered to rats. No deaths or abnormalities attributable to the test substance were observed. A minimum lethal dosage of the fermentation broth in rats therefore exceeds the 5000 mg/kg b.w. dose in this study (Ref. 6).

i. Intake Estimate

As indicated above, Mitsubishi claims GRAS status for erythritol under the same conditions of use reported in GRN 76. In its response letter to GRN 76, the agency indicated that its own calculations of the estimated daily intake for erythritol under the reported conditions of use are 13 g/person/day at the mean and 30 g/person/day at the 90th percentile. Because of competition in the market from other sugar alcohols, it is unlikely that this maximum theoretical EDI for erythritol will be achieved. It is clear, however, that this would represent a safe level of exposure. In the human clinical studies, no significant gastrointestinal effects were found at daily doses up to 1 g/kg body weight, or 60 g/person/day for an adult (Ref. 7).

ii. Basis for concluding there is consensus among qualified experts

The safety of erythritol has been confirmed by a GRAS panel consisting of Drs. William Berndt, Joseph Borzelleca, Gary Flamm, and Ian Munro (Ref. 8). The GRAS panel subsequently reviewed expanded levels of use, as reported in GRAS Notification No. 76. Qualified experts within JECFA and the European Union's Scientific Committee on Food independently confirmed the safety of erythritol when added to foods generally as a sweetener, and did not consider it necessary to establish a numerical ADI.

Appendices

Appendix 1: Food Chemicals Codex monograph for erythritol

Appendix 2: Batch Analyses for 5 batches of Mitsubishi's erythritol

Appendix 3: WHO (2000). Erythritol. *Safety evaluation of certain food additives and contaminants.* WHO Technical Report Series: 44, pp 15-70. ICPS International Programme on Chemical Safety in Cooperation with the Joint FAO/WHO Expert Committee on Food Additives (JECFA), World Health organization, Geneva.

Appendix 4: SCF (2003), SCF/CS/ADD/EDUL/215 Final, 24 March 2003, Opinion of the Scientific Committee on Food on Erythritol

Appendix 5: Rulis (2001), Agency Response Letter to GRAS Notice No. GRN 000076 (erythritol)

References

Reference 1: 53rd JECFA (1999), FNP 52 Add 7. (JECFA purity specifications for erythritol, citing use of both microorganisms)

Reference 2: Munro, Berndt, et al. Erythritol : An interpretive Summary of Biochemical, Metabolic, Toxicological and Clinical Data. *Food and Chemical Toxicology* 36 (1998) 1139-1174.

Reference 3: *Regulatory Toxicology and Pharmacology*, Volume 24, Number 2, October, 1996, Part 2 of 2 Parts.

Reference 4: Directive 2006/52/EC of the European Parliament and of the Council of 5 July 2006 amending Directive 95/2/EC on food additives other than colours and sweeteners and Directive 94/35/EC on sweeteners for use in foodstuffs. Official Journal of the European Union, L204/10, 26 July 2006.

Reference 5: 62 Fed. Reg. 63653, Dec. 2, 1997 (amendment to health claim regulation for noncariogenic sweeteners).

Reference 6: Mitsubishi Kasei Corporation (1990). Acute toxicity study on erythritol broth in rats. Internal Report. Included in GRASP No. G70422 as Appendix VII-6.

Reference 7: Tetzloff, Dauchy, *et al.* Tolerance to Subchronic, High-Dose Ingestion of Erythritol in Human Volunteers, *Reg. Tox. and Pharmacology* **24** S286-S295 (1996)

Reference 8: Berndt, Borzelleca, Flamm, and Munro, Erythritol: A Review of Biological and Toxicological Studies, *Reg. Tox. and Pharmacology* **24** S191-S197 (1996)

Reference 9: Hino, Kasai, *et al.* (2000). A case of allergic urticaria caused by erythritol. *J Dermatol* 27: 163-165.

Reference 10: Yunginger, Jones, *et al.* (2001) Allergic reactions after ingestion of erythritol-containing foods and beverages. *J Allergy Clin Immunol* 108: 650.

Reference 11: GRAS Affirmation Petition No. 7G0422 (FDA Docket No. 97G-0063).

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Appendix 1 - Food Chemicals Codex monograph for erythritol

Appendix 1

Food Chemicals Codex monograph for erythritol

Appendix 2 - Batch Analyses for 5 batches of Mitsubishi's erythritol

Appendix 2

Batch Analyses for 5 batches of Mitsubishi's erythritol

Appendix-2

Results of product analysis of Erythritol

11-Jul-06

		Lot No.				
FCC 5th Ed. Requirements		0606004	0606005	0606006	0606007	0606008
Identification	-	pass	pass	pass	pass	pass
Assay	from 99.5% to 100.5%	99.9	100.0	100.0	100.0	100.0
Lead	Not more than 1mg/kg	pass	pass	pass	pass	pass
Loss on drying	Not more than 0.2%	0.037%	0.044%	0.057%	0.070%	0.073%
Reducing Sugars (as glucose)	Not more than 0.3%	pass	pass	pass	pass	pass
Residue on ignition	Not more than 0.1%	0.020%	0.017%	0.003%	0.003%	0.015%
Ribitol and Glycerol	Not more than 0.1%	0.01%	0.01%	0.01%	0.01%	0.01%

Appendix 3 - JECFA Toxicological Monograph for Erythritol

Appendix 3

JECFA Toxicological Monograph for Erythritol

Pages 0000021 - 000050 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Appendix 4 - Opinion of the Scientific Committee on Food (SCF) on Erythritol

Appendix 4

Opinion of the Scientific Committee on Food (SCF) on Erythritol

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees II; scientific co-operation and networks

Scientific Committee on Food

SCF/CS/ADD/EDUL/215 Final
24 March 2003

Opinion of the Scientific Committee on Food

on

Erythritol

(opinion expressed on 5 March 2003)

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Opinion on Erythritol

Terms of reference

To evaluate the safety of erythritol to be used as a food additive and to confirm its non-laxative effect as claimed by the petitioners. Additionally, to confirm the energy value of erythritol (0 to 0.2 kcal/g) as claimed by the petitioners.

Background

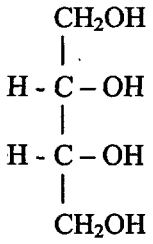
Erythritol is a four-carbon sugar alcohol (polyol) that has sweetness approximately 60-80% that of sucrose. It occurs naturally in minor amounts in some fruits (watermelon, pear and grape (Shindou et al., 1989). It also occurs in mushrooms, fermented foods (wine, sake, beer, soy sauce) (Shindou et al., 1988; Mitsubishi Chemical Corporation 1991) and cheese (Shindou and Sihizuka, 1996).

Erythritol has been used as a food ingredient in Japan since 1990 (Cerestar Holding B.V., Mitsubishi Chemical Corporation and Nikken Chemicals Co., Ltd, 1999). It has been approved in USA since 2001 (U.S. FDA, 2001).

Erythritol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on its fifty-third meeting and assigned an ADI "not specified" (JECFA, 1999a).

Technical data

The systematic name for erythritol is 1,2,3,4-Butanetetrol. It has the following chemical identity:

EINECS-number:	205-737-3
C.A.S.-number:	149-32-6
Chemical formula.	$C_4H_{10}O_4$
Structural formula:	
Formula weight:	122.12
Description:	White, odourless, non-hygroscopic, heat-stable crystals. It has a sweetness approximately 60-80% that of sucrose.
Solubility:	Freely soluble in water, slightly soluble in ethanol, insoluble in diethyl ether.
Melting range:	Between 119° and 123°

Erythritol which is intended for use as a low calorie sweetener is produced by two different manufacturing processes, the Misubishi/Nikken method (M/N-method) and the Cerestar method (C-method) from wheat or corn starch by enzymatic hydrolysis yielding glucose. This is fermented by yeast-like fungi such as *Trichosporonoides megachiliensis* (M/N-method) or *Moniliella pollinis* (C-method). The fermentation broth is heated to kill the production organism and dead cells are removed by filtering. Once erythritol is separated from the fermentation broth, it is purified by ion exchange resin, activated charcoal, ultrafiltration and crystallisation. The final crystalline product is more than 99% pure (Cerestar Holding B.V., Mitsubishi Chemical Corporation and Nikken Chemicals Co., Ltd, 1999).

The purity criteria proposed by the applicants are in good agreement with analytical results obtained from different batches representing both manufacturing processes. It is noted that the proposed level of lead is twice as much as in the JECFA purity criteria (JECFA, 1999b).

Predicted exposure

The mean intake per person of erythritol estimated by the petitioners from natural occurrence in foods like wine, cheese, fruits, chocolate and mushrooms varies between countries, being e.g. 24 mg/day in USA and 105 mg/day in Japan. In comparison, the figure of 47 mg/day is a Danish estimate (DVFA, 2001).

The main food applications and maximum use levels of erythritol proposed by the petitioners include:

Food category	Maximum level of erythritol in product % by weight
Table top substitutes	99.9
Low-calorie beverages including sport drinks	3.5
Sugar-free chewing gum	60
Reduced calorie and sugar-free chocolate	50
Candies, not sugar-free: soft (fudges)	40
hard	50
Fondants and creams	60
Lozenges	99
Bakery (pastry) products (cookies biscuits, cakes pastries with cream filling/topping), not sugar-free	7-60

The calculations of estimated mean daily intake of erythritol using Danish disappearance and consumption data (DVFA, 2001) and the proposed main food applications and maximum use levels of erythritol in those are 18 to 27 g/day depending on sex and age, being the highest for 7 to 10 year old boys. When expressed as relative intake estimates these figures are for women 0.4 g/kg bw assuming an average body weight of 64 kg, for men 0.4 g/kg bw assuming an average body weight of 80 kg, for girls 1.3 g/kg bw with a reference body weight of 27.3 kg, and for boys 1.5 g/kg bw with a reference body weight of 27.8 kg. These are considered as "worst case " calculations of the average consumption for adults and children as

they are based on the assumption that the erythritol intake derives from products where erythritol has replaced sugar.

In comparison, the U.S. Food and Drug Administration's own calculations of estimated daily intake of erythritol under conditions of use proposed by Cerestar, are 13 g/day at the mean and 30 g/day at the 90th percentile (U.S. FDA, 2001).

The petitioners provided lower estimates of intake based on several assumptions regarding the percentage of sugar-fee products to be consumed. The estimates were 9.6 g/day and 11.2 g/day at the 90th percentile for the total population and for teenagers respectively.

Microbiological evaluation

The application from the petitioners involves the use of two fermentation processes for the production of erythritol each using a different fungus. *Trichosporonoides megachiliensis* Inglis & Sigler 1992, which is used in one of the processes was described from isolates associated with alfalfa leafcutter bees (Inglis & Sigler 1992) and the strain used in the process was isolated from soil in a sugar cane plant and selected following UV and NTG (nitrosomethyl guanidine) treatment. *Moniliella pollinis* (Hennebert & Verachtert) de Hoog & Gueho 1984, which is used in the other process, is the type strain of the organism and was isolated from pollen (Dooms *et al.* 1971; de Hoog & Gueho 1984).

Both *M.pollinis* and *T.megachiliensis* are osmophilic fungi and together with related species can contaminate high sugar foods such as syrups, jams and honey. Some *Moniliella* species are found in low pH foods (pickles, sauces) and both *Moniliella* spp. and *Trichosporonoides* spp. have been isolated from foods high in fat (e.g. margarine, ghee) (Samson & van Reenen-Hoekstra 1988).

Both fungi are able to grow at 35-37°C. A search of the literature, major fungus culture collection and taxonomic databases failed to reveal any documented evidence of *M.pollinis* or *T. megachiliensis* being pathogenic to humans. Related species have been isolated from clinical material although they appear to be rare occurrences (Kockova-Kratochvilova *et al.* 1987; McKenzie *et al.* 1984).

There is no indication in the literature that *T.megachiliensis* or *M.pollinis* produce toxic metabolites or antimicrobial compounds. However, both fungi have been reported to produce pigmented metabolites in culture (Dooms *et al.*, 1971; Inglis & Sigler 1992) although the petitioners have provided information indicating that colour changes in the fermentation broth are minor and can be explained by variation in broth components and the effect of heat treatment.

The fermentation broth produced by both fungi has been tested for toxicogenic activity. The isolate of *T.megachiliensis* was tested by feeding the fermentation broth to rats in doses up to 2000 mg dry weight per kg body weight with no indications of toxicity (Kashima Laboratory Mitsubishi Chemical Safety Institute Ltd., 1995). A repeated-dose (4-week) oral toxicity study of *M.pollinis* fermentation broth in rats demonstrated that dietary levels up to 2.5% (the highest dose tested) corresponding to an overall intake of 2.1 g/kg bw/day did not induce any treatment related changes (Lina, 2002).

According to the petitioners, use of the erythritol product will not expose consumers to the producing organisms, which will be destroyed by heat treatment of the fermentation broth and removed by filtration and purification. The petitioners provided end product microbiological specifications for their products together with some information indicating consistency between batches. There should be no microorganisms of public health significance if good manufacturing/hygienic practice and Hazard Analysis Critical Control Point principles are applied throughout the manufacturing processes.

No information was found in the literature concerning microbiological contamination problems associated with erythritol and the water content of the erythritol product (<0.2%) is too low to support microbial growth.

Absorption, distribution, excretion and biotransformation

In animals and humans, depending on doses, 60 to more than 90 % of ingested erythritol is rapidly absorbed from the small intestine and excreted unchanged in the urine (Bornet et al. 1996a, b; Dean et al., 1996; Hiele et al., 1993; Ishikawa et al., 1996; Lina et al., 1996; Nakayama, 1990a, b; Noda, 1994; Noda and Oku, 1990, 1992; Noda et al., 1988; Noda et al., 1996; Oku and Noda 1992a, b; Tetzlof et al., 1996 Til et al., 1996; van Ommen et al., 1990, 1996).

Consumption of erythritol with foods appears to delay absorption (Bornet et al. 1996b). Absorbed erythritol is rapidly distributed throughout the body in animals and humans, with peak plasma, serum and/or blood concentrations generally occurring within 1 hour of ingestion (Bornet et al., 1996a, b; Ishikawa et al., 1996; Nakayama, 1990a b, c, d; Noda et al., 1994, 1996). In humans, plasma erythritol levels have been reported to reach a maximum of about 3 to 25 mmol/l within 30 to 120 min following ingestion of 0.3 to 1 g erythritol/kg bw (Noda et al., 1994; Bornet et al., 1996a, b). Bile concentrations of erythritol have been reported to be proportional to plasma erythritol concentrations (Westendorf and Czok, 1983). Erythritol has been reported to transfer across the human placenta (Jansson et al., 1993) and to pass slowly from the plasma to cerebrospinal fluid and brain of sheep (Dziegielewska et al., 1979). No metabolite of erythritol has been observed in rats (Noda and Oku, 1992; Noda et al., 1996; van Ommen et al., 1996) or in humans (Hiele et al., 1993; Noda et al., 1994) indicating that erythritol is not metabolised to a significant extent in the body. Unabsorbed erythritol does however undergo microbial fermentation in the colon to volatile short-chain fatty acids (Noda and Oku, 1990, 1992) or is excreted in the faeces. In the rat at high doses, a higher proportion of the ingested dose undergoes fermentation as the dosage is increased (Noda and Oku, 1992; Oku and Noda 1990a) and as a result of pre-adaptation to erythritol in the diet (Oku and Noda 1990a). In dogs less than 2% of ingested erythritol is excreted unchanged via faeces (Dean et al., 1996; Noda et al., 1996). Similar results have been reported in rats (Lina et al., 1996; Noda and Oku, 1990; Noda et al., 1996; Oku and Noda, 1990a, b; Til et al., 1996; Van Ommen et al., 1996). The biliary excretion of erythritol has been estimated in dogs at 1% (Lewis et al., 1982).

Animal studies

Acute toxicity

When tested for acute toxicity, erythritol is essentially non-toxic e.g. after oral administration LD_{50} in dogs is greater than 5g/kg bw (Ozeki et al., 1988), LD_{50} in rats is 13.1 g/kg bw for

males and 13.5 g/kg bw for females (Yamamoto et al. 1987) or greater than 18 g/kg bw (Beck et al., 1938). In these studies, the effects recorded in animals that died were those commonly associated with the dosing of large volumes of hypertonic solutions.

Sub-acute and sub-chronic toxicity

Increased thirst, increase in caecal and/or kidney weights, and transient diarrhoea in the 10% groups and occasionally in 5% groups were the most apparent adverse effects reported (the effects known to occur in rodents fed polyols) in two 28-day studies in rats (Oku and Noda, 1990a; Til and Modderman, 1996) and in a 28-day study in the rat (Shibata et al., 1991), conducted to further evaluate the findings of increased blood urea nitrogen (BUN) observed in some sub-chronic studies (Kamata, 1990a, b; Yamamoto et al., 1989; see below). In another 28-day toxicity study in rats, specifically designed to assess the potential effect of erythritol on the renal function, and in which the highest dose of erythritol was 5% in the diet, the only relevant and statistically significantly altered clinical parameter was an increased water intake in the high-dose group of the sham treated and nephrectomised rats compared to the controls (Kanai et al., 1992). This effect was considered to be evidence of a diuretic effect of erythritol.

In sub-chronic rodent studies (Kamata, 1990a; Til et al., 1991, 1992, 1996; Yamamoto et al., 1989) the following effects were seen in some or all studies: soft stool and/or diarrhoea and reduced body weight in groups exposed to high oral doses of erythritol (20% in the diet or ≥4g/kg bw by gavage), an increased feed intake, increased water intake, changes in some clinico-biochemical parameters (e.g. increased alkaline phosphatase (ALP), decreased γ-glutamyltransferase (GGT), increased BUN, decreased plasma protein and chlorine, decreased sodium, decreased calcium and increased potassium), increased urination/urine volume, changes in several urinary parameters (e.g. increased excretion of sodium, potassium, calcium, chlorine, protein, GGT and *N*-acetyl glucosaminidase (NAG)), changes in organ weights such as increased weights of caecum, kidneys and adrenal glands and decreased thymus weight. Furthermore, a slight dilatation of renal tubules was reported in the high-dose group (8 g/kg bw/day) in one study (Yamamoto et al. 1989). All these effects were considered of non–specific nature being a result of physiological responses to the diuretic and osmotic actions of high doses of erythritol.

Treatment of dogs with erythritol in high doses (5 g/kg bw: Yamaguchi, 1990 and Kamata, 1990b; Dean and Jackson, 1992; Dean et al., 1996: 10% in the diet equivalent to 3.8 g/kg bw) in sub-chronic studies was associated with increased water intake and increased urination/urine volume irrespective of duration of the study and the route of administration (Yamaguchi, 1990: 13 weeks by gavage; Kamata, 1990b: 6 months intravenously; Dean and Jackson, 1992; Dean et al., 1996: 1 year in the diet). Furthermore, some renal changes were recorded in the 13-week study (Yamaguchi, 1990): eosinophilic degeneration, slight dilatation and pycnosis of tubules in 2 of 5 males in the high dose group (5g/kg bw) for each finding and epithelial desquamation, hydropic degeneration, slight necrosis of tubules in one of 5 males in the high dose group. In the 6-month study (Kamata, 1990b), a dose related increase in BUN was recorded but it was considered of no toxicological significance as (i) a renal functional test *(PSP clearance test)* did not reveal any evidence of renal function impairment, (ii) there were no histopathological changes in the kidneys, and (iii) there was no concomitant increase in creatinine concentrations to indicate the presence of renal damage. The results of the 1-year study indicated that ingestion up to 10% erythritol in the diet (equivalent of 3.8

g/kg bw/day) did not cause any overt signs of toxicity (Dean and Jackson, 1992; Dean et al., 1996).

Chronic toxicity and carcinogenicity

One chronic (78-weeks) study in rats with dietary levels of 0, 1, 3, or 10% erythritol (equal to 0.46, 1.4 and 5 g/kg bw/day for males and 0, 0.54, 1.7 and 5.7 g/kg bw/day for females) (Til and van Nesserooij, 1994) and another 2-year chronic toxicity/carcinogenicity study in rats with dietary levels of erythritol of 0, 2, 5, or 10% (equal to 0, 0.9, 22, and 4.6 g/kg bw/day for males and 0, 1.0, 2.6, and 5.4 g/kg bw/day) (Lina et al., 1994; 1996) demonstrated that erythritol did not affect survival and had no carcinogenic effect.

The spectrum of effects recorded in the 78-week toxicity study (Til and van Nesserooij, 1994) included soft faeces in the high-dose group during the two first weeks only, slight decrease in mean body weights of both sexes in the 10% erythritol group (the difference with the controls being statistically significant in males on days: 14, 21, 28, 77, 168 and from day 308 onwards), increase in water intake with increasing dietary levels of erythritol in both sexes (the differences with the controls being generally statistically significant in the 10% group and in the 3% and 1% groups on several occasions during the study), increased plasma alkaline phosphatase activity (ALP) (the difference with the controls reaching a statistical significance in week 13 for females and in weeks 26 and 78 for males in the 10% group), higher urine volume in the 10% group (the difference with the controls being statistically significant only in weeks 12 and 25), increased urinary calcium excretion over 16-hour period in both sexes in the 10% group (the difference with the controls being statistically significant at nearly all stages throughout the study[1] [2]), statistically significantly increased absolute and relative weights of the full caecum in the 3% and 10% groups and of the empty caecum in the 10% group, statistically significantly increased relative kidney weights, and absolute and relative spleen weights, and absolute thyroid weights in females in the 3% group.

The effects observed in a 2-year rat study with erythritol (Lina *et al.* 1994, 1996) were: reduced body weights (the difference with the controls being statistically significant for males in the 2% group at termination, in the 5% group from week 8 to the termination and in the 10% group from week 3 to the termination, and in females in the 10% group during most weeks after week 10), increased feed intake in both sexes in the 10% group (the difference being statistically significant on several occasions), increased water intake (the difference being statistically significant on most time points for males in the 10% group and occasionally for males in the 5% and for females in the 10% groups), a few statistical differences at various sampling times in haematological parameters and of blood chemistry parameters, consistent but not statistically significantly increase in plasma ALP of both sexes in the 10% group, increased the 24-hr urine production (the difference being statistically significant in the 5% group in week 26 and in the 10% group in weeks 26, 42, 50 and 78), decreased urine osmolarity in the 10% group (the difference being statistically significant in weeks 26, 42 and 50), changes in urinary pH (statistically significant decrease in the 5% group in week 26 and in the 10% group in weeks 26, 42, 50, 78, and statistically significant increase in the 5% and 10% groups at termination), increased urinary excretion of protein (the difference being

[1] Statistically significantly increased urinary calcium excretion/16hrs in the high-dose group was recorded on day 87 for both sexes, on day 179 for males only, on day 543 for females only.

[2] Increased calcium excretion could be due to increased absorption from the gut, a phenomenon reported with certain other low molecular weight organic compounds, which are fermented in the colon (poorly absorbed and poorly metabolised carbohydrates).

statistically significant in the 10% group in weeks 42 and 78), of low molecular weight protein (LMP) (the difference being statistically significant in the 10% group in weeks 42, 50 and 78), of enzymes (the difference being statistically significant for GGT in the 2% group in week 50, in the 5% group in weeks 26 and 50 and in the 10% group in weeks 26, 42, 50 and 78, and for NAG in the 5% group in weeks 26 and in the 10% group in weeks 26, 42, 50 and 78), and of electrolytes (the difference being statistically significant in the 10% group for sodium in weeks 26, 50, 78, for potassium in week 78, for calcium on all occasions measured, for phosphate in weeks 26, 42, 50 and 78 and in the 5% group for phosphate in week 26, for citrate on all occasions except for week 26), increased absolute and relative weights of full and empty caecum of both sexes in the 10% group (the difference being statistically significant at all time points i.e. weeks 52, 78 and 102) and in the 5% group (in weeks 72 and 102), increased relative kidney weights in the 10% group (the difference being statistically significant for males in weeks 52 and 78 and for females at termination), increased relative liver weights of males in the 10% group (the difference being statistically significant in week 78 and 102), increased weights of adrenals in the 10% group (the difference being statistically significant for females in the absolute and relative weights at termination and for males in the relative weights in week 78), increased relative weight of spleen of males in the 10% group (the difference being statistically significant in week 78). Histopathological findings revealed statistically increased nephrocalcinosis in the 5 and 10% groups in week 78, and statistically significantly increased pelvic nephrocalcinosis (pelvic epithelial mineralisation) in females of the 2%, 5%, and 10% groups).

Genotoxicity

Mutagenic potential of erythritol was investigated in two Ames' tests (Blijleven, 1990; Kawamura et al., 1996) and in an *in vitro* chromosome aberration test in Chinese hamster fibroblasts (Kawamura et al., 1996; Nakatsuru et al., 1988).

In the first Ames' test (Blijleven, 1990) erythritol, both in the absence and in the presence of an exogenous source of metabolic activation (Aroclor 1254-induced rat liver S9) showed no evidence of mutagenic activity at concentrations of up to 30.0 mg/plate when tested in five histidine-requiring *Salmonella typhimurium* strains (TA1535, TA1537, TA1538, TA98, TA100). Also in the second Ames' test (Kawamura et al., 1996) erythritol, both in the absence and in the presence of an exogenous source of metabolic activation, showed no evidence of mutagenic activity following incubation with histidine-requiring *Salmonella typhimurium* strains (TA98, TA100, TA1537) or with *Escherichia coli* strain WP2 *uvrA* at doses up to 5000 µg/plate. In strain TA1535, in the presence of S9, many of the erythritol treated groups showed a greater number of revertant colonies than the controls. These increases, however, failed to show a dose-response relationship, and tended to show considerable variation in number. As a result, the increased revertant numbers observed in TA1535 were not considered evidence of mutagenic activity.

In an *in vitro* chromosome aberration assay in Chinese hamster fibroblast cells, erythritol at concentrations up to 10.0 mM, both in the absence and in the presence of an exogenous source of metabolic activation, failed to produce a significant increase in the incidence of abnormal cells, polyploid cells, total chromosomal aberrations, break or exchange types aberrations, thus demonstrated no genotoxic activity (Kawamura et al., 1996; Nakatsuru et al., 1998).

Reproductive and developmental toxicity
In a peroral exposure by gavage of mice (doses 0, 1, 2, 4, or 8g/kg bw/day) the effects recorded in maternal animals of both sexes were sporadic diarrhoea and increased water intake at 4 and 8 g/kg bw/day, and dilatation of renal tubules in 2 of 24 males at 8g/kg bw/day. The only statistically significant differences in reproductive parameters, compared to the controls, were a lower implantation rate in the 4 g/kg bw group, and decreased a male: female ratio in the 8 g/kg bw/day group but there was no clear dose-response pattern and both parameters were within the normal historical range (Tateishi, 1989).

In the second study (Tateishi et al., 1992) an intravenous administration of erythritol to mice in doses of 0, 1, 1.73, or 3 g/kg bw/day had no effect on conventional reproductive performance parameters or on foetal development. The effects recorded in maternal animals were limited to the high-dose group and included the death of two males and one female, statistically significantly increased water intake in the female mice before mating, and a dilatation of the renal tubules and a dilatation of the Bowman's capsule in one male and a dilatation of the renal tubules in two females from the high-dose group.

In a 2-generation study in rats (Smits-van Prooije et al., 1996a; Waalkens-Berendsten et al., 1996), groups of 24 animals of both sexes were fed diets containing 0, 2.5, 5, or 10% erythritol for approximately 10 weeks before mating and during the gestation for two consecutive generations (F_0 and F_1) with one litter per generation. Diarrhoea was recorded only in the high-dose group of F_0 and F_1 generations during the first few days of treatment. Body weight of F_0 generation in the high-dose group was below those of controls (the difference being statistically significant for males only). However, the feed intake of F_0 generation in the high-dose group (both sexes) was statistically significantly reduced during the first week only. Thereafter the feed intake of both sexes was statistically significantly higher than in controls with the exception for females in weeks 2 and 3 of gestation and lactation. F_1-males and females exhibited the reduced body weight, the difference from controls being statistically significant for males during week 0-8 and 17-18, and for females during weeks 0-4. However, the rates of body weight gain of males and females of this dose group were not different from those of the controls. Erythritol did not affect reproductive performance of the parental rats (F_0 and F_1). There were no effects on the development of offspring. Histological examination did not reveal any abnormalities.

Results of the three studies indicated that erythritol even at high doses had no adverse effects on fertility or on the developing foetus.

Erythritol was administered intravenously to pregnant mice (mated with untreated males) in doses 0, 1, 2, or 4 g/kg bw/day on days 6-15 of gestation (Ota et al., 1990). Administration of erythritol at 4 g/kg bw/day to dams from F_0-generation was associated with maternal hypoactivity and staggering gait, the death of two dams, periodically decreased feed intake and increased water intake, slightly higher (but not statistically significantly) incidence of fetuses with external abnormalities and a statistically significantly higher incidence of foetuses with skeletal abnormalities. Erythritol in doses up to 4 g/kg bw/day had no effect on body weights of pups (F_1) during lactation and after weaning, or on developmental parameters, behaviour or reproductive performance of the F_1 generation.

When erythritol was administered to pregnant rats from day 0 to day 21 at dietary levels of 0, 2.5, 5, or 10% the only effects recorded were a statistically significantly reduced maternal body weight and body weight gain in week 2 of gestation in the high-dose group, and a statistically significantly higher number of postimplantation losses and higher placental weight in the low-dose group compared to the controls (Smits-van Prooije, 1993; Smits-van Prooije et al., 1996b).

Mated female rabbits received 0, 1, 2.24, or 5 g/kg bw/day of erythritol intravenously on days 6 to18 of gestation (Hashima Laboratory, 1989; Shimizu et al., 1996). Maternal effects such as polyuria, auricular oedema and lethargy were observed in the high-dose group. Furthermore, water intake was statistically significantly higher than that in the controls from day 7 to 13 of gestation in the low- and mid-dose groups and from day 7 to 17 of gestation in the high-dose group. No effects were observed in the reproductive performance of the dams or on foetal development at any treatment level.

Human studies

Potential influence of erythritol on carbohydrate metabolism

In 5 male healthy volunteers, a single oral dose of erythritol (0.3g/kg bw as 20% aqueous solution) had no significant effect on serum glucose or insulin concentrations. Furthermore, ingestion of erythritol was not associated with any changes in serum cholesterol, triglycerides, free fatty acids, sodium, potassium or chloride levels (Noda et al., 1994). Urinary volume, osmotic pressure, concentration of sodium, potassium and chloride were not significantly different when compared to those after ingestion of the same dose of glucose. Approximately 90% of the ingested dose was excreted in the urine within 48 hours. Furthermore, plasma glucose and insulin levels were not affected in 3 male and 3 female healthy volunteers after a single oral dose of erythritol of 1g/kg bw dissolved in 250 ml of water (Bornet et al., 1996a), or in 12 male and 12 female healthy volunteers after single administration of 0.4 or 0.8 g/kg bw erythritol in form of a midmorning snack (Bornet et al. 1996b).

In 5 noninsulin-dependent diabetic patients (sex not stated), the results of a single dose study (20 g/person in 100 ml aqueous solution) indicated no significant effects of erythritol on carbohydrate metabolism (Ishikawa et al., 1992, 1996). Furthermore, a two-week daily administration of erythritol (20 g/person/day in solution throughout the day with the usual diet) to 11 noninsulin-dependent outpatients (3 males and 8 females) had no effect on blood glucose control (Ishikawa et al. 1996; Miyashita et al. 1993). These limited studies indicate that erythritol does not adversely affect carbohydrate metabolism.

Gastrointestinal tolerance

In studies investigating the laxative effect of erythritol in healthy volunteers after single oral doses up to 78 g (Bornet et al., 1996a) in aqueous solution given on an empty stomach the diarrhoea was not observed at 30 g/person corresponding to 0.46 g/kg bw (Umeki, 1992) and 0.47 g/kg bw (Takahashi, 1992a) in 6/6 and 8/8 males, respectively and to 0.57 g/kg bw in 4/4 females (Takahashi, 1992a). The minimum dose of erythritol causing laxation in these studies ranged from 0.6 to 0.7 g/kg bw. A single administration of erythritol incorporated into a jelly ingested by 14 males and 24 females 2 to 3 hours after a meal did not cause laxation at doses below 0.70 g/kg bw (Oku and Okazaki, 1996). Female subjects seemed to show a greater tolerance than males to the laxative effect of erythritol. Other reported symptoms associated

with diarrhoea were abdominal pain, nausea, intestinal rumbling and/or increased intestinal movements/cramps/spasms, flatulence and thirst. In all studies, the gastrointestinal symptoms showed recovery within 24 hrs after dosing.

In the studies with single daily doses of erythritol mentioned above (Umeki, 1992; Takahashi, 1992 a; Oku and Okazaki, 1996) sucrose and sorbitol were used as reference compounds for gastrointestinal tolerance. Sucrose in tested doses of 0.92 (Umeki, 1992), 1.0 (Takahashi, 1992a) and 1.2 g/kg bw (Oku and Okazaki, 1996), depending on the study, had no laxative effect. Sorbitol in aqueous solution caused laxation in all 6 male subject in a dose of 0.15 g/kg (Umeki, 1992) and in 3 of 8 male subjects at 0.16 g/kg bw (Takahashi, 1992a) while 0.19 g/kg bw had no effect in all 4 females (Takahashi, 1992a). When incorporated into a jelly, sorbitol doses of less than 0.25 g/kg bw caused laxation in 43% (6/14) of the males but not in females (0/24) (Oku and Okazaki, 1996). These results indicate that the laxative effect of erythritol is weaker than that of sorbitol.

When the effect of a repeated dosing was investigated in 8 male and 2 female healthy volunteers, ingestion of erythritol in two daily doses of 20 g in aqueous solution 2-3 hours after breakfast or lunch during 5 consecutive days (corresponding to 40 g/day equivalent to 0.64 and 0.74 g/kg bw in men and women respectively) caused gastrointestinal pain and diarrhoea in one man but not in other subjects (Takahashi, 1992b). In contrast, no signs of gastrointestinal intolerance were recorded in 12 male healthy volunteers when erythritol was given during 7 consecutive days in total daily doses of 0.3 g/kg bw on day 1, of 0.6 g/kg bw on day 2 and of 1 g/kg bw thereafter divided in five portions ingested with food or beverages (Tetzloff et al., 1996). However, when 1 g/kg bw was administered as a single dose in 250 ml water after an overnight fast to 3 male and 3 female healthy volunteers diarrhoea was observed in 2 out of 6 healthy subjects (sex not stated), while the other subjects had abdominal spasms, discomfort and flatulence (Bornet et al., 1996a).

Potential allergenicity of erythritol

Erythritol is a simple sugar alcohol and not known to undergo covalent binding to proteins. It is not a reactive compound and it is not metabolised to reactive metabolites (Bornet et al., 1992; Hiele et al., 1993; Noda et al., 1994). Therefore, it is unlikely that erythritol should cause allergic reactions when consumed with foods. Furthermore, it occurs naturally in some foods, which are not known to be common allergens. Additionally, no reactions, which would indicate any allergic sensitivity in humans, were reported in the above mentioned human studies with erythritol.

When the allergic potential of erythritol was investigated in the heterologous passive cutaneous anaphylaxis reaction in rats, erythritol elicited no hypersensitivity reactions or increases in IgE antibody production (Kawauchi et al., 1989a). Also in guinea pigs several antigenicity tests with erythritol (active systemic anaphylaxis test, homologous passive cutaneous test, active cutaneous anaphylaxis - delayed type hypersensitivity test and passive haemagglutination test) were negative, indicating no immunoreactive or sensitising effect of the compound (Kawauchi et al., 1989b).

There are three well-described cases of severe allergic or allergic-like adverse reactions after erythritol ingestion (Hino et al., 2000; Yunginger et al., 2001). All three patients had a history

of reactions after eating or drinking food with added erythritol: two women developed generalised urticaria and a man generalised urticaria or hypotension. Erythritol itself and not a contaminant seems to be responsible for the reaction; the pathophysiologic mechanisms (e.g. IgE or non IgE mediated) underlying these reactions remain obscure. According to the same authors (Yunginger et al., 2001) the estimated prevalence of adverse reactions to erythritol containing products is less than 1 per million people.

Energy value of erythritol

The caloric value of erythritol, as declared by the petitioners, is 0 - 0.2 kcal/g. The determination of the energy value was performed using a factorial method - a recognised approach for the determination of the energy values of fermentable non-digested carbohydrates (Livesey, 1992).

Some human studies (Noda et al., 1988, 1994; Ishikawa et al., 1992) indicated that 90% or more of erythritol was absorbed and not metabolised following doses of up to 20 g/day. Animal studies suggested a fractional absorption in the small intestine in the same range as described in humans (Nakayama, 1990a; Noda et al., 1996; Oku and Noda, 1990a). Metabolism of erythritol may occur to a very small extent, but no metabolite of this sugar alcohol has been observed in animals (Noda et al., 1996). The study of metabolism of erythritol in humans also suggested a very low rate of metabolism (if any) (Hiele et al., 1993). Erythritol that is not absorbed from the small intestine passes into the large intestine where it can be fermented by colonic microorganisms into short-chain fatty acids (SCFAs) (Noda and Oku, 1992). The conversion of erythritol to SCFAs and gases in the human colon is associated with a loss of about 25% of caloric value. Furthermore, the loss of caloric value of about 25% (from 14 to 30%) due to production of bacterial mass in colon should be added. Other minor losses such as heat production and faecal excretion are difficult to quantify. In all animal studies some faecal erythritol was noted. In studies with adapted rats where erythritol was quantified, faecal excretion of erythritol was usually between 1 and 5% of intake (Lina et al., 1994; Til et al., 1991). In vitro studies in humans (Barry et al., 1992; Hiele et al., 1993) suggested that erythritol is not fermented by human colonic micro-flora to the same extent as it is in rats, but no precise figure can be established.

On the basis of available information concerning the various components in the factorial equation for erythritol the caloric value for erythritol is confirmed to be less than 0.9 kJ/g; or less than 0.2 kcal/g, in humans given oral doses lower than 25g/day or 0.34 g/kg bw.

Comments

The level for lead proposed by the petitioners is twice as much as in the JECFA purity criteria (JECFA, 1999). It seems more appropriate to base the safety of erythritol as a food additive not on the lead level proposed by the petitioners, but on the JECFA criterion for lead (not more than 0.5 mg/kg) considering that erythritol may be used in relatively high quantities in certain foods based on the proposed application and anticipated maximum levels of erythritol in food products.

The effects of erythritol in animals have been investigated in a large number of studies (Dean and Jackson, 1992; Dean et al., 1996; Kamata, 1990a, b; Kanai et al., 1992; Lina et al., 1994,

1996; Oku and Noda, 1990a; Shibata et al., 1991; Smits-van Prooije et al., 1996a, b; Smits-van Prooije, 1993; Tateishi, 1989; Til and Modderman, 1996; Til and van Nesselroijl, 1994; Til et al., 1991, 1992, 1996; Waalkens-Berendsten et al., 1996; Yamaguchi, 1990; Yamamoto et al. 1989). They included transient occurrence of loose stool/diarrhoea, decreased body weight gain, increased water consumption, increased urine volume, increased urinary excretion of electrolytes (particularly sodium, potassium and calcium) and urinary enzymes (e.g. NAG, GGT), increased serum ALP and BUN, increased absolute or relative caecal weights, increased absolute or relative kidney weights and histopathological changes in kidneys such as a dilatation of renal tubules, calcium deposits in kidneys/pelvic nephrocalcinosis. These effects were recorded in rats and dogs when high doses of erythritol were used i.e. ≥ 5% in the diet, ≥ 2.5 g/kg bw/day by gavage or ≥ 2.2 g/kg bw/day intravenously.

Other findings reported in sub-chronic and chronic studies mentioned above were minor isolated changes in haematological parameters or in blood chemistry, urinanalysis and organ weights. These changes were either of small magnitude, or did not show a dose-response effect, or were not consistent across sex, time and study and therefore were considered to have no relationship to erythritol treatment.

The decreased body weight /body weight gain recorded in some of the sub-chronic and chronic rodent studies and in a two generation reproduction study is most likely attributable to the reduced caloric value of erythritol, as the feed intake was either comparable or slightly increased in the groups treated with high doses of erythritol when compared to the controls. In a short-term study, however, diarrhoea may also have played a role in decreasing the body weight.

The loose stools/diarrhoea in rats and effects on caecal weights in rats and mice treated orally with erythritol could be due to the loading of the large intestine of unabsorbed erythritol at high doses. The fact that the caecal enlargement was not observed in rats receiving erythritol intravenously supports this explanation.

The increased absolute and or /relative kidney weights reported in one of the short-term studies and in the sub-chronic and chronic rodent studies by the oral or intravenous routes of exposure and in one sub-chronic dietary dog study could be the result of the increased urine output recorded in these studies. A possible explanation might be that increased urine output increased the workload of the kidneys, increasing functional tissue activity and therefore increasing the kidney weight. This explanation seemed likely, as no increase in kidney weight was recorded in rats treated intravenously with erythritol for 6 months and allowed a 4-week recovery period, and based on the reported observation that diuresis resulting from a high-dose exposure of rodents to carbohydrates was accompanied by increased absolute and or relative kidney weights (Ogino et al., 1994). Furthermore, the slight dilatation of the renal tubules could be attributable to exposure to the substance with osmotic/diuretic activity.

Other effects such as increased total excretion of electrolytes and of urinary enzymes could also be attributable to the osmotic/diuretic activity of erythritol. In addition, these findings were not consistent from study to study, and were not correlated with any indication of renal disease.

The pelvic nephrocalcinosis recorded in the 2-year rat study was most likely associated with the recorded increase in calcium excretion. It has to be noted that pelvic nephrocalcinosis in that study was not limited to the 10% erythritol treatment but was also recorded for the 10% mannitol group. Furthermore, nephrocalcinosis has also been recorded in other rat studies with poorly absorbed / poorly metabolised carbohydrates (Bär, 1985) and it has been suggested to be associated with the increased calcium excretion as a result of increased calcium absorption due to the osmotic loading of the gastrointestinal tract (Bär, 1985).

The reported increases in serum ALP in rat studies could most likely be attributed to the osmotic activity of erythritol, which reached the large intestine after ingestion at high doses in a similar way as it was demonstrated with other osmotically active carbohydrates (Bär et al. 1995; Moser *et al.* 1980; Schaafsma and Visser 1980; Woutersen 1987).

The finding of an increased BUN recorded in some studies was further elucidated in a 28-day rat study and demonstrated to be associated with loss of electrolytes, particularly sodium, resulting from the diuretic action of erythritol.

Although no gene mutation test in mammalian cells was performed with erythritol the available negative results of Ames' tests and in *in vitro* chromosome aberration test in Chinese hamster fibroblasts were considered sufficient for evaluation of possible mutagenic activity of the compound considering that other polyols were not mutagenic.

The results of human studies on gastrointestinal tolerance indicated that the NOEL for gastrointestinal symptoms was between 0.5 to 1.0 g/kg bw. However, when erythritol was administered in water a dose of 0.5 g/kg bw was the lowest NOEL for diarrhoeic effect. The finding of a relatively higher incidence of gastrointestinal effects with acute bolus dosing in solutions and on an empty stomach may reflect greater passage of the osmotically active erythritol into the large intestine as a result of reduced absorption. The estimation of a daily intake of erythritol based on Danish disappearance and consumption data and under conditions of use proposed by petitioners indicates that the laxative threshold may be exceeded especially by young consumers and through ingestion of erythritol in beverages.

Conclusions

There are extensive animal studies and some human trials on erythritol. The Committee concluded that the effects seen in the animal studies were attributable to physiological and adaptive responses to the rapid absorption and excretion of erythritol and to the osmotic activity of unabsorbed erythritol and its fermentation products in the gut. The intestinal effects are common to all the polyols.

Erythritol does have a laxative effect, but at higher doses than other polyols. The NOEL for the laxative effect of erythritol in humans is around 0.5 g/kg bw for a single dose.

In accordance with the Committee's earlier opinion on other polyols it is considered inappropriate to establish a numerical ADI for erythritol.

The Committee considers that the use of erythritol as a food additive is acceptable. However, as with other polyols, this should not be interpreted as meaning the acceptance of unlimited

use in all foods at any technological level, because the laxative effect should be borne in mind.

The Committee confirms the caloric value of erythritol to be less than 0.9 kJ/g (or less than 0.2 kcal/g) for daily intakes not exceeding 25 g/day.

The Committee recommends that the limit for lead in the specifications should be not higher than 0.5 mg/kg.

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Appendix
List of short-term, sub-chronic, chronic, reproductive and developmental toxicity studies and no-effect and effect levels

Study	Reference	NOEL	LOEL
28-day toxicity study in rats	Oku and Noda, 1990a	Not established	5% in the diet
28-day toxicity study in rats	Til and Modderman, 1996	Not established	5% in the diet
Sub-chronic (13-week) feeding study with erythritol in mice	Til *et al.*, 1996	5% in the diet	10% in the diet
Sub-chronic (13-week) feeding study with erythritol in rats	Til *et al.*, 1996	5% in the diet	10% in the diet
A 13-week oral subacute toxicity study of NIK-242 with four week recovery period in rats. (gavage)	Yamamoto *et al.*, 1989	2 g/kg bw	4 g/kg bw
A 6 months intravenous chronic toxicity study of NIK-242 in rats with 1 month recovery period	Kamata, 1990a	1 g/kg bw	1.73 g/kg bw
A 13-week oral subacute toxicity study of NIK-242 in dogs with 4-week recovery period. (gavage)	Yamaguchi, 1990	1.25 g/kg bw	2.5 g/kg bw
A 6 months intravenous chronic toxicity study of NIK-242 in Beagle dogs with 1 month recovery period	Kamata, 1990b	Not established	1 g/kg bw
Chronic (1-year) oral toxicity study of erythritol in dogs	Dean *et al.*, 1996; Dean and Jackson, 1992	5% in the diet (corresponding to 1.7 g/kg bw)	10% in the diet (corresponding to 3.8 g/kg bw) *(the highest dose used)*
Chronic (78-week) oral toxicity study with erythritol in rats	Til and van Nesselrooij, 1994	3% in the diet (corresponding to 1.5 g/kg bw)	10% in the diet (corresponding to 5.4 g/kg bw) *(the highest dose used)*

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Chronic (2-year) oral toxicity and carcinogenicity study with erythritol in rats	Lina *et al.*,1996, Lina *et al.*, 1994	2% in the diet (corresponding to 1 g/kg bw)	5% in the diet (corresponding to 2.4 g/kg bw)
Oral reproduction study of erythritol (NIK-242) with mice prior to and in the early stage of pregnancy (gavage)	Tateishi, 1989	General toxicity: 2 g/kg bw Reproductive performance and foetal development: 8 g/kg bw	General toxicity: 4 g/kg bw Reproductive performance and foetal development: No LOEL as the highest dose was NOEL
Fertility study of NIK-242 in ICR strain mice (intravenous dosing)	Tateishi *et al.*, 1992	General toxicity: 1.73 g/kg bw Reproductive performance and foetal development: 3 g/kg bw	General toxicity: 3 g/kg bw Reproductive performance and foetal development: No LOEL as the highest dose was NOEL
Dietary two-generation reproduction study with erythritol in rats	Smits-van Prooije *et al.*, 1996a, Waalkens-Berendsten *et al.*, 1996	10% in the diet (corresponding to 7.6 g/kg bw	No LOEL as the highest dose was NOEL
Teratology study of NIK-242 in mice (intravenous dosing)	Ota *et al.*, 1990	Maternal and developmental toxicity: 2 g/kg bw	Maternal and developmental toxicity: 4 g/kg bw *(the highest dose used)*
Oral embryotoxicity/ teratogenicity study with erythritol in rats	Smits-van Prooije, 1993, Smits-van Prooije *et al.*, 1996b	Maternal toxicity: 5% in the diet (corresponding to 3.4 g/kg bw) Developmental toxicity: 10% in the diet (corresponding to 6.7 g/kg bw)	Maternal toxicity: 10% in the diet (corresponding to 6.7 g/kg bw) Developmental toxicity: No LOEL as the highest dose was NOEL
Teratology study of erythritol in rabbits (intravenous dosing)	Shimizu *et al.*, 1996, Hashima Laboratory, 1989	Maternal toxicity: 2.24 g/kg bw Reproductive performance and foetal development: 5 g/kg bw	Maternal toxicity: 5 g/kg bw *(the highest dose used)* Reproductive performance and foetal development: No LOEL as the highest dose was NOEL

Appendix 5 - FDA Response Letter to GRAS Notice No. GRN 000076 (erythritol)

Appendix 5

FDA Response Letter to GRAS Notice No. GRN 000076 (erythritol)

U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
September 11, 2001

Agency Response Letter
GRAS Notice No. GRN 000076

Diane B. McColl
Hyman, Phelps, & McNamara, P.C.
700 13th St. NW
Washington, DC 20005-5929

Re: GRAS Notice No. GRN 000076

Dear Ms. McColl:

The Food and Drug Administration (FDA) is responding to the notice, dated April 30, 2001, that you submitted on behalf of Cerestar Holding, B. V. (Cerestar) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). FDA received the notice on April 30, 2001 and designated it as GRAS Notice No. GRN 000076.

The subject of the notice is erythritol. The notice informs FDA of the view of Cerestar that erythritol is GRAS, through scientific procedures, for use as a flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer in a variety of foods as described in Table 1 (below). Based on these conditions of use, Cerestar informs FDA that the estimated daily intake (EDI) of erythritol would be one gram per person per day (g/p/d) at the mean and 4 g/p/d at the 90th percentile. (1)

Erythritol is a naturally occurring four-carbon sugar alcohol. Its chemical name is 1,2,3,4-butanetetrol and its Chemical Abstracts Service Registry Number (CAS Reg. No.) is 149-32-6. It has a sweetness of about 60-80 percent that of sucrose. Erythritol is manufactured using the fermentative conversion of glucose to erythritol by a non-toxicogenic and non-pathogenic organism, *Moniliella pollinis*. The fermented broth is heated to kill the microorganisms, crystallized, washed, redissolved and purified using an ion exchange resin. The erythritol solution is purified further by ultrafiltration and recrystallization. The resulting erythritol is at least 99.5 percent pure and complies with the specifications for erythritol set forth in the Food Chemicals Codex, 4th edition Second Supplement (2000).(2)

Table 1
Conditions of Use Proposed by Cerestar

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Food	Level of use

Reduced- and low-calorie carbonated and non-carbonated beverages; Dairy drinks (chocolate and flavored milks)	3.5 percent
Frozen dairy desserts (regular ice cream, soft serve, sorbet); Puddings (instant, phosphate set); Yogurt (regular and frozen)	10 percent
Bakery fillings (fruit, custard, cream, pudding); Cakes and cookies (regular and dietetic)	15 percent
Fat-based cream used in modified fat/calorie cookies, cakes and pastries; Chewing gum; Soft Candies (non-chocolate, plain chocolate, chocolate coated)	60 percent
Hard candies (including pressed candy, mints, and cough drops)	99 percent
Sugar substitutes (carrier)	100 percent

In its notice, Cerestar describes the deliberations of a panel of individuals (Cerestar's GRAS panel) who evaluated the data and information that are the basis for its GRAS determination. Cerestar considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. Cerestar's notice includes two reviews published by members of its GRAS panel (the 1996 panel report and the 1998 interpretive review) and an unpublished report authored by its GRAS panel (the May 2000 panel report).

In the 1996 panel report, Cerestar's GRAS panel concludes that the use of erythritol in certain foods (sugar substitutes, hard candies, soft candies, reduced- and low-calorie beverages, fat-based cream for use in cookies, cakes, and pastries, dietetic cookies, wafers and chewing gum) is GRAS based on scientific procedures. In the 1998 interpretive review, Cerestar's GRAS panel provides a comprehensive review of the data and information, already summarized in the 1996 panel report, in anticipation of a review by JECFA (the Joint Food and Agriculture Organization/World Health Organization's (FAO/WHO) Expert Committee on Food Additives).[3]

The published reviews included in Cerestar's GRAS notice describe studies in rats, dogs, and humans (including diabetics). From these studies, Cerestar's GRAS panel concludes that most ingested erythritol is rapidly absorbed via the small intestine. Cerestar's GRAS panel also concludes that this absorbed erythritol is excreted unchanged in the urine 24 hours after a single oral dose. Cerestar's GRAS panel concludes that any unabsorbed erythritol undergoes microbial fermentation to volatile fatty acids in the colon. Cerestar's GRAS panel further concludes that erythritol is well-tolerated by humans and produces no meaningful gastrointestinal or renal effects when ingested with food and beverages at levels providing up to one gram per kilogram body weight per day (g/kg bw/day), corresponding to a daily intake of 60 g/day (i.e., for a 60 kg adult).

The published reviews included in Cerestar's GRAS notice also describe acute, subchronic, chronic, carcinogenicity, reproductive toxicity, teratogenicity, and mutagenicity studies conducted with erythritol. From these toxicological studies, Cerestar's GRAS panel concludes that erythritol is without carcinogenic and teratogenic potential, and does not exhibit mutagenic or clastogenic activity *in vitro*. Cerestar's GRAS panel reports that no reproductive or developmental toxicological effects were observed at doses up to 8 g/kg bw/day in mice or at doses representing up to 100 g/kg of feed in rats.

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In the May 2000 panel report, Cerestar's GRAS panel discusses dietary exposure to erythritol for uses that were expanded compared to those described in both the 1996 panel report and in a GRAS affirmation petition (GRP 7G0422) that is pending at FDA. Cerestar's GRAS panel also discusses the toxicological significance of some effects that were described in the previous reports and considers the handling of dietary erythritol by the renal system of young children. Cerestar's GRAS panel unanimously concludes that its detailed analysis of the data and information provides no evidence that erythritol would be associated with adverse health effects under the conditions of its intended use.

Based on the information provided by Cerestar, as well as other information available to FDA, the agency has no questions at this time regarding Cerestar's conclusion that erythritol is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of erythritol. As always, it is the continuing responsibility of Cerestar to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36 (f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet (at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,
/s/
Alan M. Rulis, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

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(1) As we discussed with you by telephone on July 24, 2001, FDA does not concur with the methodology used by Cerestar to estimate the dietary intake of erythritol. FDA's own calculations of the EDI for erythritol under the conditions of use proposed by Cerestar are 13 g/p/d at the mean, and 30 g/p/d at the 90th percentile.

(2) In an addendum to the notice, Cerestar informed FDA that the lead specification for erythritol manufactured by Cerestar is 0.1 milligrams per kilogram (mg/kg; equivalent to 0.1 parts per million). This limit is 10-fold lower than that specified in the Food Chemicals Codex (1 part per million).

(3) In 2000, as part of its 53rd meeting, JECFA published a technical report (Series No. 896) and a toxicological monograph (Series No. 44) on erythritol. The monograph discusses the studies reviewed by Cerestar's GRAS panel with comments on the EDI and the toxicological significance of the effects, such as laxation, observed with high intake levels of erythritol. In the monograph, JECFA establishes an Acceptable Daily Intake (ADI) of "not specified." JECFA describes "ADI not specified" as a term applicable to a food component of very low toxicity for which the total dietary intake of the substance does not, in the opinion of the Committee, represent a hazard to health.

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