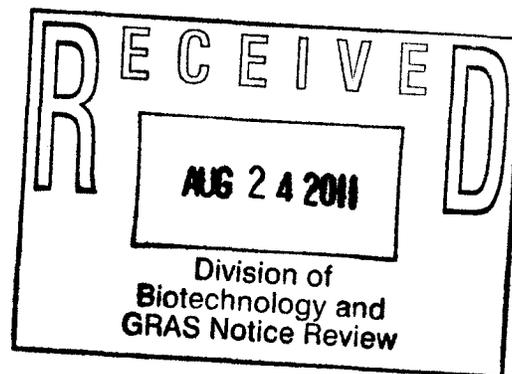


ORIGINAL SUBMISSION

August 18, 2011

Dr. Susan Carlson
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835



Re: GRAS exemption claim for D-psicose as an ingredient in foods

Dear Dr. Carlson,

This is to notify you that CJ Cheiljedang (based in S. Korea) claims that the use of the substance described below (D-psicose) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because CJ America has determined such use to be Generally Recognized As Safe (GRAS).

On behalf of CJ Cheiljedang, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended uses of D-psicose. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. Joanne Slavin (The University of Minnesota, St Paul, MN), and Dr. George Fahey (The University of Illinois, Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of D-psicose (that is manufactured by CJ Cheiljedang, S. Korea) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel also concluded in its August 2011 statement that the intended use of CJ Cheiljedang's D-psicose (to be used as an ingredient in foods ready-to eat breakfast cereals, diet soft drinks, non-diet soft drinks, confectionery, formula diets for meal replacement, meal replacement drink mix (powder), cake, pie, cake mix powder, frostings, ice cream and frozen yogurt, yogurt, frostings, sugar free chewing gum, jelly and pudding, coffee mix powder, biscuits, cookies, and cereal bars) is safe and GRAS for the general population. This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section 170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

Notifier's name and Address: CJ Cheiljedang, Inc.
Attention: Daniel Oh (E mail address: gethero@cj.net)
Address: Namdaemunro 5-ga, Jung-gu, Seoul, Korea
Phone number: +82-2-726-8317; Fax number: +82-2-726-8319

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Address: Namdaemunro 5-ga, Jung-gu, Seoul, Korea
Phone number: +82-2-726-8317; Fax number: +82-2-726-8319

Name of GRAS substance: D-Psicose (Common or trade name: Psicose or pseudo-fructose).

Product description: D-Psicose is a ketohexose, an epimer of D-fructose isomerized at C-3. D-Psicose differs from fructose only in the positioning of the hydroxyl group on the third carbon. D-Psicose is 70% as sweet as sucrose. D-psicose can be used as a sugar substitute or a food formulation aid. D-Psicose provides several health benefits to consumers: 1) it provides approximately 0.2 kcal/g to the diet, and 2) it attenuates a glycemic response. D-Psicose has a history of use in foods with no reported adverse effects.

The LD₅₀ value of D-psicose, 15.9-16.3 g/kg, is comparable to those of fructose (14.7 g/kg) and erythritol (15.3 g/kg) and is much higher than that of table salt (3.0 g/kg). These high LD₅₀ values (over 15 g/kg BW) belong to the “relatively harmless” category (the lowest toxicity rating), according to a toxicity rating chart. Thus, D-psicose is classified as an ordinary carbohydrate substance and the use of psicose in foods and beverages is not expected to pose a safety concern.

Specifications:

Table 1. Specifications of D-psicose

Composition	Specification
D-Psicose	>98.5% (wt/wt)
D-Fructose and other sugars	<1% (wt/wt)
Moisture	<1% (wt/wt)
Ash	<0.1% (wt/wt)
Total plate count	<10,000 CFU/g
Coliforms	negative
<i>Staphylococcus aureus</i>	negative
<i>Salmonella</i>	negative
Heavy metals	<1.0 ppm
Lead	<0.5 ppm
As	<1.0 ppm
Physical appearance	White crystal

Applicable conditions of use of the notified substance

Intended food applications include sugar substitutes (carrier), coffee mix, medical foods, and various low-calorie or dietetic foods including low-calorie rolls, cake, pie, pastries, and cookies, fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, soft candies, frozen dairy desserts (ice cream, soft serve, sorbet), carbonated beverages, non-carbonated beverages, reduced- and low-calorie, soft candies (non-chocolate, plain chocolate, chocolate coated), yogurt (regular and frozen), ready-to-eat cereals (<5% sugar) and chewing gum. The proposed use levels of D-psicose are presented in Table 2.

Table 2. Proposed food application of D-psicose and maximum levels of use

Food category	Maximum level, %
Rolls, cake, pie, pastries, and cookies, dietetic or low calorie	10
Chewing gum	50
Fat-based cream used in modified fat/calorie cookies, cakes, and pastries, low calorie	10
Hard candies, low calorie (including pressed candy, mints)	70
Frozen dairy desserts (regular ice cream, soft serve, sorbet), low-calorie	5
Carbonated beverages, low-calorie	2.1
Non-carbonated beverages, reduced- and low-calorie	2.1
Soft candies, low-calorie (non-chocolate, plain chocolate, chocolate coated)	25
Sugar substitutes (carrier)	100
Yogurt (regular and frozen), low-calorie	5
Medical foods	15
Ready-to-eat cereals (<5% sugar)	10
Coffee mix	30

Exposure estimates

Assuming that 10% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use of D-psicose are estimated to be 1.1 g/d (or 15.4 mg/kg BW/d) for all persons and 2.8 g/d (or 35.8 mg/kg BW/d) for all users of one or more foods. Even if 100% of the foods will be used at the maximum levels under the intended use, although it is far from a realistic situation, the 90th percentile intakes are 11.2 g/d (or 154 mg/kg BW/d) for all persons and 28.5 g/d (or 358 mg/kg BW/d) by all users of one or more foods.

These levels are much lower than the no-observed-adverse-effect level (NOAEL) value (8,530 mg/kg BW/d) that has been found from animal toxicity studies. Also, these estimated daily exposure levels are far below the maximum tolerable value of 500-600 mg/kg BW/d that has been found from human clinical studies.

Basis of GRAS determination

Through scientific procedures.

Review and copying statement

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of CJ Cheiljedang, Inc. or Nutrasource, Inc.

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me.

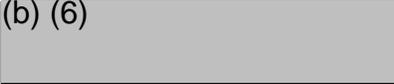
Sincerely,

Susan Cho, Ph.D.
Chief Science Officer
NutraSource, Inc.
6309 Morning Dew Ct.
Clarksville, MD 21029
Phone: 301-875-6454
E mail: susanscho1@yahoo.com

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(b) (6)



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Clarksville, MD 21029
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E mail: susanscho1@yahoo.com

Conclusion of the expert panel:**Generally recognized as safe (GRAS) determination for the addition of D-psicose to foods**

August, 2011

CONCLUSION

We, the undersigned expert panel members, Susan Cho, Ph.D., George Fahey, Ph.D., and Joanne Slavin, Ph.D, have individually and collectively critically evaluated the materials summarized in the D-psicose GRAS report and conclude that D-psicose, a monosaccharide, is safe and GRAS for its intended use in foods and beverages.

There is broad-based and widely disseminated knowledge concerning the chemistry of D-psicose, a monosaccharide. D-Psicose is well characterized and free of chemical and microbial contamination. D-Psicose will be used as a food ingredient. Intended food applications include sugar substitutes (carrier), coffee mix, medical foods, and various low- calorie foods including low-calorie rolls, cake, pie, pastries, and cookies, low calorie fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, soft candies, frozen dairy desserts (ice cream, soft serve, sorbet; low calorie), carbonated and non-carbonated beverages (reduced- and low-calorie), soft candies (non-chocolate, plain chocolate, chocolate coated; low calorie), yogurt (regular and frozen; low calorie), ready-to-eat cereals (<5% sugar) and chewing gum.

Assuming that 10% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use of D-psicose are 1.1 g/d (or 15.4 mg/kg BW/d) for all persons and 2.8 g/d (or 35.8 mg/kg BW/d) for all users of one or more foods. Even if all the foods will be under the intended use, although it is far from a realistic situation, the 90th percentile intakes are 11.2 g/d (or 154 mg/kg BW/d) for all persons and 28.5 g/d (or 358 mg/kg BW/d) by all users of one or more foods. These levels are much lower than the no-observed-adverse-effect level (NOAEL) value (8,530 mg/kg BW/d) that has been found from animal toxicity studies. Also, these estimated daily exposure levels are far below the maximum tolerable value of 500-600 mg/kg BW/d that has been found from human clinical studies. The LD₅₀ value of D-psicose, 15.9-16.3 g/kg, is comparable to those of fructose (14.7 g/kg) and erythritol (15.3 g/kg) and is much higher than that of table salt (3.0 g/kg). These high LD₅₀ values (over 15 g/kg BW) belong to the “relatively harmless” category (the lowest toxicity rating), according to a toxicity rating chart. Thus, D-psicose is classified as an ordinary carbohydrate substance and the use of psicose in foods and beverages is not expected to pose a safety concern.

There are no indications of significant adverse effects related to D-psicose in the publicly available literature. The proposed food use results in exposure at levels significantly below those associated with any adverse effects. Therefore, not only is the proposed use of D-psicose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR).

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

Joanne Slavin, Ph.D.
Professor, University of Minnesota, St Paul, MN

Signature: _____ Date: _____

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: (b) (6) Date: 8/17/2011

George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana, IL 61801

Signature: (b) (6) Date: 8/16/11

Joanne Slavin, Ph.D.
Professor, University of Minnesota, St. Paul, MN 55108

Signature: (b) (6) Date: 8-11-11

Identity of substance

A. Common or trade name: D-psicose, D-allulose, or pseudo-fructose

B. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

C. Background:

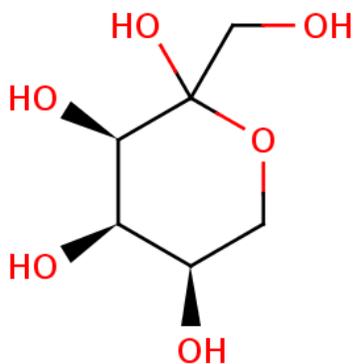
D-Psicose is a monosaccharide, an epimer of D-fructose isomerized at C-3 (Karabinos, 1952).

Chemical name is D-ribo-2-ketohexose

MW=180.16

Molecular formula: C₆H₁₂O₆

CAS Registry ID; 551-68-8



D-Psicose is 70% as sweet as sucrose, but it has just 0.2 kcal/g. Thus, it belongs to the non-digestible carbohydrate category. It is odorless, white or almost white, and non-hygroscopic. D-Psicose is a naturally occurring monosaccharide present in small quantities in food products.

D. Manufacturing Process

- 1) The powder form of fructose is dissolved in clean water (>40% solids concentration) in a reception tank.
- 2) The neutralized syrup is mixed with manganese chloride (1 mM; 50°C) and then subjected to an immobilized cell system (calcium alginate gel bead with *Corynebacterium glutamicum* [non-viable cell] harboring D-psicose 3-epimerase [DPE] originated from *Agrobacterium tumefaciens*). It takes 4-8 h at 50°C to convert D-fructose to D-psicose.
- 3) For decolorization, the D-psicose solution is mixed with 1% active carbon for 30 min in a stirred tank. The liquid undergoes pressure filtration (55-60°C, pH > 4.5) to clarify it.

- 4) The decolorized syrup is cooled to $\leq 40^{\circ}\text{C}$, then treated through an ion exchange process (i.e., cation column with strongly acidic cationic exchange resin; anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly-based acid and strongly basic resins) to remove any impurities (e.g., calcium, manganese, chloride, and other ionic components, including amino acids, peptides, and proteins). The exchange beds are monitored for pH and color every 8 h; real-time conductivity is monitored automatically.
- 5) Following ion exchange purification, the D-psicose solution is concentrated with an evaporator to produce syrup (syrup density of 60° Brix [Bx]).
- 6) This concentrated syrup is pumped into a separation chromatography system to separate D-psicose from other sugars (fructose). This process dilutes the D-psicose solution to a density of $8\text{-}15^{\circ}$ Bx.
- 7) Using an evaporator, the solution is concentrated to the final density of $80\text{-}85^{\circ}$ Bx.
- 8) The final concentrated product is pumped into a batch continuous crystallizer (90 h of retention time).
- 9) The crystalline D-psicose is separated by basket centrifugation for 45 min, washed by spraying distilled water, and finally dried in a rotary dryer.

Quality assurance procedure: Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry. All processing aids used in the manufacturing process are food grades.

Safety of enzymes: The enzyme is non-toxicological and non-pathogenic. Acute toxicity studies showed that NOAEL of the enzyme was 2,000 mg/kg/d, the maximum level tested. No abnormalities were observed from an *in vitro* chromosome aberration test or bacterial mutation tests with *S. typhimium* (TA 98, 100, 1535, and 1357; up to 5,000 ug/plate) and *E. coli* WP2uvrA, with and without S-9 mix activation.

E. Specifications

Table 1. Specifications of D-psicose

Composition	Specification
D-Psicose	>98.5% (wt/wt)
D-Fructose and other sugars	<1% (wt/wt)
Moisture	<1% (wt/wt)
Ash	<0.1% (wt/wt)
Total plate count	<10,000 CFU/g
Coliforms	negative
<i>Staphylococcus aureus</i>	negative
<i>Salmonella</i>	negative
Heavy metals	<1.0 ppm
Lead	<0.5 ppm
As	<1.0 ppm
Physical appearance	White crystal

F. Analytical method for psicose

Psicose is analyzed by HPLC with a refractive index detector.

The analytical conditions are as follows:

- (1) Column : Bio-Rad Carbohydrate Amine®HPX-87C, 300 mm×7.8 mm (Catalog #125-0095) or equivalent
- (2) Detector : Refractive index, RI detector
- (3) Mobile phase : Deionized water (100%)
- (4) Flow rate: 0.6 ml/min
- (5) Column pressure/temperature : 364 psi (26 kg/cm²) / 85°C

II. Natural occurrence and exposure to D-psicose

A. Food sources of D-psicose

D-Psicose is a naturally occurring monosaccharide present in small quantities in natural products, particularly in sweets such as caramel sauce, maple syrup, brown sugar, processed cane and beet molasses, and wheat (Table 2; Matsuo et al., 2001b; Oshima et al., 2006).

Table 2. D-psicose content in foods (adopted from Oshima et al., 2006)

Item	mg/100 g food
Confectionary products	
sponge cake	11.0
Corn-snack	47.0
rice cracker	27.3
cookie	26.7
Brown sugar drop	76.5
fried dough cake	95.6
Chocolate-chip cookie	6.4
Cereal	2.2
Dishes	
Fish broiled with soy	39.1
Simmered dishes of dried radish strips	8.1
Fermented soybeans	7.8
Seasonings and beverages	
Caramel sauce	83.0
Brown sugar	71.1
Meat sauce	15.8
Demiglaze	16.3
Maple syrup	57.9
Ketchup	39.8

Worcester sauce	130.6
Coke	38.3
Coffee	0.5
Fruit juice	21.5
Tomato juice	2.4
Fruits	
Dried fig	29.6
Dried kiwi fruit	9.4
Raisin	38.7
Canned peaches	1.5
Can of mandarin oranges	8.4
Canned cherries	2.0

B. Intended use

D-Psicose is intended to be used as a food ingredient. Intended food uses and use levels are summarized in Table 3. Considering its technological properties (e.g., functions as a sweetener, humectant, flavor enhancer) and nutritional benefits (such as low calorie and glycemic control), D-Psicose is expected to be used as a sugar substitute (carrier). Intended applications include sugar substitutes, coffee mix, medical foods, and various low-calorie foods including low-calorie rolls, cake, pie, pastries, and cookies, fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, soft candies, frozen dairy desserts (ice cream, soft serve, sorbet), carbonated beverages, non-carbonated beverages, soft candies (non-chocolate, plain chocolate, chocolate coated), yogurt (regular and frozen), ready-to-eat cereals (<5% sugar) and chewing gums. Please note: The intended use levels for psicose are much lower than those for erythritol (outlined in the GRN 208).

Table 3. Proposed food applications of D-psicose and maximum levels of use

Food category	Maximum level, %
Rolls, cake, pie, pastries, and cookies, dietetic or low calorie	10
Chewing gum	50
Fat-based cream used in modified fat/calorie cookies, cakes, and pastries	10
Hard candies, low calorie (including pressed candy, mints)	70
Frozen dairy desserts (regular ice cream, soft serve, sorbet), low-calorie	5
Carbonated beverages, low-calorie	2.1
Non-carbonated beverages, reduced- and low-calorie	2.1
Soft candies, low-calorie (non-chocolate, plain chocolate, chocolate coated)	25
Sugar substitutes (carrier)	100
Yogurt (regular and frozen), low-calorie	5
Medical foods	15
Ready-to-eat cereals (<5% sugar)	10
Coffee mix	30

C. Current consumer intake levels

Since the D-psicose level in each food is not listed in the USDA food composition tables and the National Health and Nutrition Examination Survey (NHANES) databases, the current exposure levels from food sources were not estimated.

D. Exposure estimates under the intended use

Using food intake data reported in the 2005-2008 NHANES, exposure levels to D-psicose that will result from the intended uses were estimated (Table 4). The most recent NHANES (2005-2008) compiled by the National Center for Health Statistics and the Nutrition Coordinating Center was used to calculate exposure estimates. The NHANES was conducted between 2005-2008 with non-institutionalized individuals in the U.S. The NHANES provides the most current food consumption data available for the American population. The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations. For this study 1 g is considered equivalent to 1 ml for soft drinks and formula diets for meal replacement. The NHANES does not include consumption levels of chewing gum. Thus, marketing survey data were used in exposure estimates: Average Americans eat 0.815 kg of gum/y (or average daily consumption of 2.29 g/person) and approximately 40% of chewing gums are sugar-free. SUDAAN v10.0 with day 1 dietary weights were used to calculate mean, 90th percentile, and standard errors (SE) for D-psicose exposure.

Even if all the foods will be under the intended use, although it is far from a real world situation, the 90th percentile intakes including D-psicose from the intended use by the population and by users of one or more foods are 11.2 and 28.5 g/d, respectively. These levels correspond to 154 and 358 mg/kg BW/d for the all population, and all users (Tables 4-1 and 4-3).

From a marketing perspective, an assumption that 10% of the product will be used at the maximum levels for each food category is a highly optimistic projection. It is due to the fact that the functional foods (claiming any health benefits of foods) market size is estimated to comprise approximately 5% of total food expenditures in the U.S. (Price Waterhouse Coopers, 2009). Assuming that 10% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes including D-psicose from the intended by all persons and by users of one or more foods are 1.1 and 2.8 g/d, respectively. These levels correspond to 15.4 mg/kg BW/d for the all persons and 35.8 mg/kg BW/d for all users (Tables 4-2 and 4-4). These levels are much lower than the NOAEL values of 10,000 mg/kg BW/d which was found in animal toxicity studies. Also, these estimated daily exposure levels are far below the maximum tolerable value of 500 mg/kg BW/d that has been found from human clinical studies.

Both D-Psicose and erythritol can be used as replacements for sugars. Due to similarity in their attributes (such as a low calorie sweetener), either D-psicose or erythritol may be used in food formulations. The US consumption of all types of sugar alcohols (sorbitol, erythritol, maltitol and xylitol) was estimated at 376,640 tons (or 3.43 g/person/d; Food Navigato5, 2005), of which sorbitol made up the largest percentage, with more than 54% (corresponding to 1.85 g/person/d) of the total sugar alcohol production. Actual US consumption of D-psicose was

estimated to be much lower than the figure for sorbitol. Thus, our exposure estimates (1.1 g/d for all persons and 2.8 g/d for all users) based on the 10% market share assumption might be closer to a real world situation.

Table 4-1. Daily exposure estimates of D-psicose for the all persons: Assuming all the foods will be used at the maximum levels

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	3.23	0.12	11.18	0.56	43.2	1.5	153.8	6.2
1-3 Y	0.54	0.05	1.56	0.19	39.7	3.5	122.4	12.0
0-12 Y	0.74	0.07	1.99	0.19	28.5	2.1	88.0	9.2
13-18 Y	1.18	0.11	3.92	0.93	18.4	1.7	60.0	14.7
19+ Y	4.07	0.15	14.90	0.40	49.7	1.8	180.4	7.1
Males								
0+ Y	3.07	0.14	10.08	0.83	37.4	1.5	135.6	7.0
1-3 Y	0.47	0.06	1.56	0.24	34.6	4.2	121.0	15.9
0-12 Y	0.74	0.10	1.69	0.33	27.6	3.1	80.7	15.8
13-18 Y	1.12	0.14	1.78		16.5	2.2	27.5	
19+ Y	3.90	0.18	14.46	0.61	42.5	2.0	153.4	8.3
Females								
0+ Y	3.39	0.16	12.07	0.67	48.7	2.3	173.9	9.9
1-3 Y	0.62	0.09	1.58	0.31	44.9	6.5	123.6	24.0
0-12 Y	0.75	0.09	2.20	0.25	29.4	2.7	97.1	11.7
13-18 Y	1.24	0.16	4.85	1.17	20.3	2.7	83.7	16.0
19+ Y	4.23	0.20	14.91	0.59	56.3	2.7	199.9	8.8

SE=standard error; Med.=Median; P90=90th percentile

Table 4-2. Daily exposure estimates for all persons; after market share adjustment (assuming 10% of the foods will be used at the maximum levels, i.e., 10% of the market share at the maximum levels)

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	0.32	0.01	1.12	0.06	4.3	0.2	15.4	0.6
1-3 Y	0.05	0.00	0.16	0.02	4.0	0.4	12.2	1.2
0-12 Y	0.07	0.01	0.20	0.02	2.9	0.2	8.8	0.9
13-18 Y	0.12	0.01	0.39	0.09	1.8	0.2	6.0	1.5
19+ Y	0.41	0.01	1.49	0.04	5.0	0.2	18.0	0.7
Males								
0+ Y	0.31	0.01	1.01	0.08	3.7	0.2	13.6	0.7
1-3 Y	0.05	0.01	0.16	0.02	3.5	0.4	12.1	1.6
0-12 Y	0.07	0.01	0.17	0.03	2.8	0.3	8.1	1.6
13-18 Y	0.11	0.01	0.18		1.7	0.2	2.7	
19+ Y	0.39	0.02	1.45	0.06	4.3	0.2	15.3	0.8
Females								
0+ Y	0.34	0.02	1.21	0.07	4.9	0.2	17.4	1.0
1-3 Y	0.06	0.01	0.16	0.03	4.5	0.6	12.4	2.4
0-12 Y	0.07	0.01	0.22	0.02	2.9	0.3	9.7	1.2
13-18 Y	0.12	0.02	0.48	0.12	2.0	0.3	8.4	1.6
19+ Y	0.42	0.02	1.49	0.06	5.6	0.3	20.0	0.9

SE=standard error; P90=90th percentile

Table 4-3. Daily exposure estimate of D-psicose for all users (assuming all the foods will be used at the maximum levels)

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	12.55	0.24	28.48	0.70	167.7	3.2	358.4	9.3
1-3 Y	3.20	0.26	6.58	0.66	237.2	19.5	483.4	91.4
0-12 Y	5.38	0.39	11.92	1.31	208.8	11.6	436.0	36.7
13-18 Y	9.48	0.50	19.21	1.79	147.1	9.0	312.1	21.4
19+ Y	13.49	0.29	29.81	0.57	164.3	3.5	355.5	9.5
Males								
0+ Y	13.59	0.40	30.00	1.10	166.1	4.4	359.9	11.3
1-3 Y	2.84	0.23	6.53	0.69	208.1	16.9	466.9	70.2
0-12 Y	5.98	0.62	12.60	1.40	225.7	16.8	467.7	50.1
13-18 Y	10.05	0.82	20.98	3.42	148.6	13.7	326.8	40.1
19+ Y	14.68	0.49	31.80	1.42	160.1	5.2	348.9	12.6
Females								
0+ Y	11.78	0.28	26.08	0.94	168.8	4.1	356.5	12.1
1-3 Y	3.54	0.46	6.80	2.63	266.4	34.3	558.1	213.9
0-12 Y	4.88	0.41	11.13	1.69	194.8	11.8	390.2	32.9

13-18 Y	9.01	0.68	18.34	1.78	145.8	11.5	308.3	31.4
19+ Y	12.62	0.33	27.36	1.04	167.3	4.4	358.4	14.1

Table 4-4. Daily exposure estimates for all users after market share adjustment (assuming 10% of the foods will be used at the maximum levels, i.e., 10% of the market share at the maximum levels)

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	1.26	0.02	2.85	0.07	16.8	0.3	35.8	0.9
1-3 Y	0.32	0.03	0.66	0.07	23.7	2.0	48.3	9.1
0-12 Y	0.54	0.04	1.19	0.13	20.9	1.2	43.6	3.7
13-18 Y	0.95	0.05	1.92	0.18	14.7	0.9	31.2	2.1
19+ Y	1.35	0.03	2.98	0.06	16.4	0.3	35.5	0.9
Males								
0+ Y	1.36	0.04	3.00	0.11	16.6	0.4	36.0	1.1
1-3 Y	0.28	0.02	0.65	0.07	20.8	1.7	46.7	7.0
0-12 Y	0.60	0.06	1.26	0.14	22.6	1.7	46.8	5.0
13-18 Y	1.00	0.08	2.10	0.34	14.9	1.4	32.7	4.0
19+ Y	1.47	0.05	3.18	0.14	16.0	0.5	34.9	1.3
Females								
0+ Y	1.18	0.03	2.61	0.09	16.9	0.4	35.6	1.2
1-3 Y	0.35	0.05	0.68	0.26	26.6	3.4	55.8	21.4
0-12 Y	0.49	0.04	1.11	0.17	19.5	1.2	39.0	3.3
13-18 Y	0.90	0.07	1.83	0.18	14.6	1.2	30.8	3.1
19+ Y	1.26	0.03	2.74	0.10	16.7	0.4	35.8	1.4

SE=standard error; P90=90th percentile

III. Basis for GRAS determination

A. Current regulatory status

Currently, D-psicose does not have a GRAS (generally recognized as safe) status by the United States Food and Drug Administration. However, other monosaccharides, such as fructose, glucose, galactose, and tagatose, are considered as GRAS substances. Also, erythritol, which has similar metabolism, LD₅₀, NOAEL, and energy values, has been recognized as a GRAS substance by FDA (GRNs 76 and 208). Other low-calorie sweeteners, such as isomaltulose (GRN 184), isomalto-oligosaccharides (GRN 246), mannitol (Food additive permitted on an interim basis pending additional study, 21 CFR 180.25), sorbitol, and xylitol, also are recognized as GRAS.

B. Intended technical effects

D-psicose will be used as a food ingredient for low calorie and/or dietetic foods.

C. Review of safety data

The metabolism, energy value, and toxicity study results for D-psicose are similar to those of erythritol, a GRAS ingredient (GRNs 76 and 208). Both D-psicose and erythritol have an energy

value of approximately 0.2 kcal/g. The LD₅₀ values of the two compounds are comparable; 16.3 g/kg for D-psicose and 15.3 g/kg for erythritol (Matsuo et al., 2002; Yamamoto et al., 1987).

1. Metabolism

Several experiments on the absorption, distribution, metabolism, and excretion of D-psicose in rats have been reported. About 98% of intravenously administered D-psicose is excreted in the urine within 6 h (Whistler et al., 1974). When orally ingested, urinary excretion of unchanged psicose ranged from 11 to 25% (Matsuo et al., 2003). This indicates that D-psicose absorbed in the small intestine may pass into the bloodstream and be excreted in the urine without being significantly metabolized.

Matsuo et al. (2003) investigated the absorption and excretion of D-psicose. The fermentation of D-psicose was measured as cecal short-chain fatty acids (SCFAs) when fed to rats in controlled diets (0, 10, 20, and 30%). Urinary and fecal excretions of D-psicose over 24 h, following a single oral administration, were 11-15% of dosage for the former and 8-13% of dosage for the latter. D-psicose was not detected in urine and feces collected 24-48 h and 48-72 h after administration. Serum D-psicose concentration and D-psicose in the contents of stomach and small intestines decreased progressively after administration. D-Psicose in stomach was 26-37% and 0.4-0.6% of dosage after 1 and 3 h, respectively. D-Psicose in the small intestine was 6-10%, 2-3%, and 1-3% of the dosage after 1, 3, and 7 h, respectively. D-Psicose in the cecum was detected after 3 and 7 h. It was 11-18% and 10-19% of the dosage after 3 and 7 h, respectively. (Matsuo et al., 2003). Continuous administration of D-psicose increased cecal SCFA, as D-psicose is fermented in the cecum by intestinal microflora.

Metabolism of psicose is similar to that of erythritol: A significant portion of erythritol is excreted in urine unmetabolized. In animals and humans, depending on dose, 60-90% of ingested erythritol is rapidly absorbed from the small intestine and excreted unchanged in the urine (from GRN 208; Noda and Oh, 1990,1992; Noda et al., 1996; Oh and Noda, 1992b). No metabolite of erythritol has been found in rats (Noda and Oh, 1992; Noda et al., 1996) or in humans (Noda et al., 1994), indicating that erythritol is not metabolized to a significant extent in the body. Unabsorbed erythritol is fermented to SCFA in the colon (Noda and Oh, 1990, 1992) or is excreted in the feces.

2. Energy values

Based on the results of the plot of breath hydrogen concentration vs. calories ingested, the energy value of d-psicose was predicted to be less than 0.2 kcal/g (Iida et al., 2010). The energy value of erythritol is 0.2 kcal/g (Matsuo et al., 2002b).

3. Animal studies

The LD₅₀ value of D-psicose, 15.8-16.3 g/kg, is comparable to that of other monosaccharides such as fructose (14.7 g/kg) and erythritol (15.3 g/kg), and is much higher than that of table salt (3.0 g/kg). A compound which has a LD₅₀ value of >5 g/kg BW in rats is classified as 'practically non-toxic' and the compound with a LD₅₀ value of >15 g/kg BW as 'relatively harmless' (Altug, 2003). Psicose, like other monosaccharides, belongs to the group which has the lowest toxicity rating.

Subacute and subchronic toxicity studies in rats show that psicose concentration of up to 20% of the diet did not show adverse effects (Table 5). This dietary concentration corresponds to 8,530 mg/kg BW/d (the dose was calculated from the 2009 study of Yagi and Matsuo presenting both % in the diet and corresponding mg/kg BW/d) or 10,000 mg/kg BW/d (the dose was calculated using conversion data from FDA, 1993). Chronic toxicity studies showed that psicose at the dose of 1,280 mg/kg BW/d, the maximum level tested, did not show adverse effects (Yagi and Matsuo, 2009).

The NOAEL values of 8,530 and 1,280 mg/kg BW/d in rats may correspond to 1,376 and 206 mg/kg BW/d, or 82.5 and 12.4 g/d in humans when these animal doses were converted into human equivalent doses as shown in the 2005 FDA's 'Guidance for industry: Estimating for the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers' (FDA, 2005). The data from animal toxicity studies indicate that human daily consumption of psicose up to 1,376 mg/kg BW or 82.5 g would not pose a major safety concern. All the toxicity data indicate that D-psicose is an ordinary monosaccharide and an ordinary carbohydrate.

Table 5. Summary of toxicity studies

Species	Dosage	Length	Primary endpoints and NOAEL	Reference
Male rat	8, 11, 14, 17, and 20 g/kg	Single dose	Acute toxicity-LD ₅₀ , 16.3 g/kg BW	Matsuo et al., 2002
Young rat	10, 20, 30, and 40% in the diet	34 d	Feed intake, wt gain, organ wt; up to 20% in the diet (corresponding to 10,000 mg/kg BW/d)	Matsuo et al., 2002
Male rat	1,280 mg/kg BW/d	12-18 mo	Feed intake, wt gain, organ wt, serum biochemistry, hematology, histology, 1,280 mg/kg BW/d	Yagi and Matsuo 2009

3.1. Acute toxicity

In the acute administration test (Matsuo et al., 2002), five groups of 8 male Wistar rats (3 wk old) were orally given D-psicose in doses of 8, 11, 14, 17, and 20 g/kg BW. Three rats receiving 14 g/kg, three rats receiving 17 g/kg and eight rats receiving 20 g/kg of D-psicose died within 2 d after administration. The calculated LD₅₀ values were 16.3 g/kg by the Behrens-Karber method and 15.8 g/kg by the Litchfield-Wilcoxon method. As shown in Table 6, the LD₅₀ value of psicose is comparable to those of fructose (14.7 g/kg) and erythritol (15.3 g/kg), and much higher than that of table salt (3 g/kg; Sax 1984). A compound that has a LD₅₀ value of 5 g/kg BW or higher in rats is classified as 'practically non-toxic' and the compound with a LD₅₀ value of 15 g/kg BW or higher as 'relatively harmless' (Altug, 2003). Psicose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is classified as an ordinary carbohydrate substance. Thus, the use of psicose in foods and beverages is not expected to pose a safety concern.

Table 6. Comparison of LD₅₀ values in rats

	LD ₅₀ , g/kg BW	Reference
Psicose	16.3	Matsuo et al., 2002
Erythritol (sugar alcohol)	15.3	Yamamoto et al., 1987
Beta-D-fructose	14.7	Sax, 1984
Alpha-D-glucose	25.8	Sax, 1984
D-galactose	Not available	
Sucrose	29.7	Sax, 1984
Maltose	34.8	Sax, 1984
Table salt	3.0	Sax, 1984
Alcohol	7.1	Sax, 1984

3.2. Subacute toxicity in rats

Subacute (34 d) feeding of several concentrations of D-psicose were studied in 4 wk-old Wistar rats. In the subchronic feeding test, eight groups of seven male Wistar rats (3 wk old) were fed diets containing 0 (control), 10, 20, 30, and 40% for 34 d (Matsuo et al., 2002). One rat fed the 30% D-psicose diet and five rats fed the 40% D-psicose diet died during the experimental period. Body weight gain and food efficiency were suppressed by the higher D-psicose concentration. It was concluded that the decreased body weight gain in the 10 and 20% group was attributable to a decrease in food intake, and this was not considered to be of toxicological significance. Surviving rats seemed to be able to adapt, to some extent, to D-psicose feeding, since rats fed the 30 and 40% diet were able to show a recovery in body weight, food intake, and laxation during the first 7 d feeding period. The laxative effect was transient and was not observed after 4 days. Reduced weight gain associated with psicose intakes is not a toxicological concern.

It is well known that nondigestible carbohydrate intakes are associated with body weight reduction or reduced weight gain. The Institute of Medicine report on carbohydrates (IOM, 2002), American Dietetic Association's position paper (ADA, 2002), and USDA Dietary Guideline Committee report (USDA, 2010) acknowledge the efficacy of non-digestible carbohydrates in body weight reduction as a positive attribute that can significantly improve public health in the U.S. Thus, a non-digestible carbohydrate such as psicose also can contribute to improving public health without any adverse effects.

The relative weights of heart, spleen and abdominal adipose tissue were lower as the dietary D-psicose concentration increased. It is due to weight gain reduction associated with D-psicose intakes and is not a toxicological concern. Cecal weight increased with increasing D-psicose concentration in the diets. Cecal hypertrophy was observed in rats fed 10-40% D-psicose diets. However, it should be noted that it is not a toxicological concern. Intake of any dietary fiber in large quantities also results in cecal hypertrophy. Many of the effects were assumed to be secondary to a decrease in food consumption or the consumption of large amounts of a non-nutritive, poorly absorbed, osmotically active substance. The WHO has looked at the relationship

between the consumption of non-nutritive substances in the diet as a cause of decreased weight gain and also reported an association with cecal enlargement (Lu and Sleken, 1991). This was considered to have a causal relationship associated with the physiological response of cecum enlargement induced by diet containing high concentrations of poorly absorbable substances (Cassidy et al., 1981). Enlargement of the cecum is reported to occur frequently in response to feeding poorly-absorbable, osmotically-active substances, such as xylitol and sorbitol to rats (Leegwater et al., 1974), and it is considered that the toxicological significance of this might be minimal. Overall, the NOAEL was found to be 20% in the diet (corresponding to 8,530-10,000 mg/kg BW/d) in rats.

3.3. Chronic toxicity study in rats

Yagi and Matsuo (2009) studied long-term toxicity of D-psicose in male Wistar rats (3 weeks old) fed diets containing 3% D-psicose (or 1,280 mg/kg BW/ d) or 3% sucrose (1,220 mg/kg BW/d) for 12-18 mo. Body weight gain and intra-abdominal adipose tissue weight of rats fed the D-psicose diet for 18 mo were significantly lower than those in rats fed the sucrose diet. Relative weights of liver and kidney were significantly higher in the D-psicose group than in the sucrose group, but it was not considered to be of toxicological significance. It is due to the fact that dietary D-psicose decrease body fat accumulation and increases liver glycogen as a consequence of serum glucose decline and serum insulin elevation. Increased relative weight of liver has been observed in animals fed other type of sugars such as fructose and sucralose.

General hematology or serum chemistry tests were in the normal ranges. All values related to serum chemistry did not differ between the sucrose and D-psicose groups. Mean corpuscular hemoglobin (MCH) at 12 mo was significantly lower in the D-psicose group than in the sucrose group, but no differences were observed in any of the related hematology values. Hemoglobin (Hb) and mean corpuscular volume (MCV) at 18 mo were significantly greater in the D-psicose group than in the sucrose group, but no differences were observed in any of the related hematology values.

The histopathological data demonstrated that there were no toxicologically significant findings in rats given D-tagatose at levels of 3% in the diet for 12-18 mo. No gross pathological findings were evident at dietary doses of 3% D-psicose. The authors concluded that administration of D-psicose at 3% in the diet (or 1,280 mg/kg BW/d) did not result in any adverse effects in rats.

3.4. Animal efficacy studies showing no adverse effects of D-psicose

As shown in Table 7, several animal studies reported no adverse effects of psicose. These animal studies showed that psicose at the level of 5% in the diet (corresponding to up to 2,500 mg/kg BW/d) did not cause any adverse effects.

Table 7. Animal efficacy studies showing no adverse effects of D-psicose

Species	Dosage	Length	Primary endpoints and NOAEL	Reference
Male mice	0.2 g/kg BW/d	4 wk	Glycemic responses, insulin release, and blood lipid profiles, 0.2 g/kg BW/d	Baek et al., 2010
Male rat	5% in the diet	3 wk	Body fat and lipid metabolism, 5% in the diet	Matsuo et al., 2001a
Male rat	5% in the diet	4 wk	Body fat and lipid metabolism, 5% in the diet	Matsuo et al., 2001b
Male rat	5% in the diet	8 wk	Body fat and glycemic responses, 5% in the diet	Matsuo and Izumori, 2006
Male rat	2,000 mg/kg	Single dose	Body fat and glycemic responses, 2 g/kg	Matsuo and Izumori, 2009

3.4.1. A study of Baek et al. (2010)

In the study of Baek et al. (2010), the effects of D-psicose on glycemic responses, insulin release, and lipid profiles were compared with those of D-glucose and D-fructose in a genetic diabetes model. C57BL/6J db/db mice were orally supplemented with 200 mg/kg BW of D-psicose, D-glucose, D-fructose, or water (control), respectively, for 28 d. D-psicose sustained weight gain by about 10% compared to other groups. The initial blood glucose level was maintained at 276 to 305 mg/dL during the 28 d for the D-psicose group, whereas a 2-fold increase was found in the other groups ($P < 0.05$) among diabetic mice. D-psicose significantly improved glucose tolerance and the areas under the curve (AUC) for glucose ($P < 0.05$), but had no effect on serum insulin concentration. The plasma lipid profile was not changed by supplemental monosaccharides. The administration of D-psicose reversed hepatic concentrations of triglyceride (TG) and total cholesterol (TC) by 37.9% and 62.9%, respectively, compared to the diabetic control ($P < 0.05$). No adverse effects were noted.

3.4.2. A study of Matsuo et al. (2001a)

Matsuo et al. (2001a) studied the effects on body fat accumulation of D-psicose compared with cellulose or D-fructose in rats. Wistar male rats were fed experimental diets including 5% D-psicose, cellulose or D-fructose for 21 d. Abdominal adipose tissue weight was lower ($P < 0.05$) in rats fed D-psicose than in those fed D-fructose. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were lower ($P < 0.05$) in rats fed D-psicose, whereas lipoprotein lipase activities in the heart, soleus muscle, perirenal adipose tissue, and subcutaneous adipose tissue did not differ. These results suggest that supplementation of D-psicose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed D-psicose might have resulted from lower lipogenesis in the liver. No adverse effects were reported. The authors concluded that D-psicose could prove to be a good sugar substitute.

3.4.3. A study of Matsuo et al. (2001b).

Wistar male rats were fed experimental diets that consisted of 5% D-psicose, cellulose, D-fructose, or D-glucose for 28 d (Matsuo et al., 2001b). Abdominal adipose tissue weight was lower ($P < 0.05$) in rats fed the D-psicose diet than in rats fed D-fructose and D-glucose diets, even though the four dietary groups were offered the same amount throughout the experimental period. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were lower ($P < 0.05$) in rats fed the D-psicose diet than in rats fed the D-fructose and D-glucose diets. However, lipoprotein lipase activities in the heart, soleus muscle, and perirenal adipose tissue were the same. These results suggest that a supplement of D-psicose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed the D-psicose diet might result from lower lipogenesis in the liver. No adverse effects were reported.

3.4.4. A study of Matsuo and Izumori (2006)

Matsuo and Izumori (2006) studied the effects of supplemental D-psicose in the diet on diurnal variation in plasma glucose and insulin concentrations in rats. Forty-eight male Wistar rats were divided into four groups. Each group except for the control group was fed a diet of 5% D-fructose, D-psicose, or psico-rare sugar (3:1 mixture of D-fructose and D-psicose) for 8 wk. Plasma glucose concentrations were lower and plasma insulin concentrations were higher at all times of the day in the psicose and psico-rare sugar groups than in the control and fructose groups. Weight gain was lower ($P < 0.05$) in the psicose group than in the control and fructose groups. Liver glycogen content, both before and after meals was higher in the psicose group than in the control and fructose groups. These results suggest that supplemental D-psicose can lower plasma glucose concentrations and reduce body fat accumulation. Hence, the authors concluded that D-psicose might be useful in preventing postprandial hyperglycemia in diabetic patients.

3.4.5. A study of Matsuo and Izumori (2009)

Matsuo and Izumori (2009) investigated the effects of D-psicose on the activities of alpha-amylases and alpha-glucosidases *in vitro*, and evaluated the effects of D-psicose on the *in vivo* postprandial glycemic response of rats. Male Wistar rats (6 mo old) were administered 2 g/kg of sucrose, maltose, or soluble starch together with 0.2 g/kg of D-psicose or D-fructose. The D-psicose significantly inhibited the increment of plasma glucose concentration induced by sucrose or maltose. The starch-induced glycemic response tended to be suppressed by D-psicose; however, the suppression was not significant. These results suggest that d-psicose inhibited intestinal sucrase and maltase activities and suppressed the plasma glucose increase that normally occurs after sucrose and maltose ingestion. Thus, D-psicose may be useful in glycemic control. No adverse effects were reported.

3.5. *In vitro* mutagenicity/genotoxicity studies

Results from Ames tests, micronucleus test, and chromosomal aberration test indicate that was not mutagenic or genotoxic (Table 8). D-Psicose also showed neuroprotective effects in hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells, the *in vitro* model of Parkinson's disease (Huntington lab, 2011; Tanaka et al., 2005).

Table 8. Summary of *in vitro* Mutagenicity/Genotoxicity studies

Test	Concentration	Reference
Conventional mutagenicity/genotoxicity studies		
Four histidine-dependent strains of <i>Salmonella typhimurium</i> (TA98, TA100, TA1535, and TA1537) and a tryptophan-dependent strain of <i>Escherichia coli</i> (WP2 urvA(pKM101))	5,000 ug/ml	Huntington lab, 2011
Micronucleus test using CD1 mice	2,000 mg/kg/d	Huntington lab, 2011
Chromosomal aberration test	1,800 ug/ml	Huntington lab, 2011
Apoptosis related genotoxicity effects		
Hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells	50 mM	Takata et al., 2005

3.5.1. Ames test: Four histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and a tryptophan-dependent strain of *Escherichia coli* (WP2 urvA(pKM101)) were used to evaluate the mutagenic potential of D-psicose (up to 5,000 ug/plate). No mutagenic potential of D-psicose was observed.

3.5.2. Micronucleus test: In the micronucleus test using CD1 mice, no significant increase was observed in micronucleated polychromatic erythrocytes (MPCs) at any concentration up to 2,000 mg/kg/d) of D-psicose compared with vehicle control.

3.5.3. Chromosomal aberration test: D-psicose at a dosage of 1,800 ug/mL did not induce an increase in the number of chromosomal aberrations.

3.5.4. Neuroprotective effect of D-psicose on 6-hydroxydopamine-induced apoptosis in rat pheochromocytoma (PC12) cells.

Takata et al. (2005) evaluated the neuroprotective effects of D-psicose on 6-hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells, the *in vitro* model of Parkinson's disease (PD). Apoptotic characteristics of PC12 cells were assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL) assay. The results showed that D-psicose at a concentration of 50 mM exerted significant protective effects against 6-OHDA (200 µM)-induced PC12 cell apoptosis, while other sugars had little or no protective effects. A significant increase was observed in the level of intracellular glutathione after 24 h in 6-OHDA (200 µM) treated cells, while a decrease in the level was observed at 3 h and 6 h. Also, a synergistic exposure to D-psicose and 6-OHDA for 24 h showed a significant increase in intracellular glutathione level. Therefore, these results suggest that D-psicose may play a potential role as a neuroprotective agent by inducing an up-regulation of intracellular glutathione. Antioxidant properties of psicose have been demonstrated in several food systems (Sun 2004, 2007).

3.6. In vivo carcinogenicity and genotoxicity studies

In vivo carcinogenicity and genotoxicity studies indicate that psicose is not genotoxic or carcinogenic (Table 9). In particular, psicose did not negatively alter hepatic gene expression (Shirai et al., 2007) and was protective against diethylnitrosamine (DEN)-induced hepatocarcinogenesis (Zeng et al., 2005).

Table 9. *In vivo* genotoxicity and carcinogenicity studies showing no adverse effects of D-psicose

Species	Dosage	Length	Primary endpoints	Reference
Genotoxicity and carcinogenicity				
Male rat	5% in the diet	3-8 wk	Gene expressions of rat liver and skeletal muscle	Shirai et al., 2007
Male rat, 4-wk old, F344	Up to 1% in diet	a rat medium-term bioassay	Diethylnitrosamine (DEN)-induced hepatocarcinogenesis	Zeng et al., 2005
Male rat, S-D	2% in the diet	2 wk	di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury	Suna et al., 2007

3.6.1. Hepatocarcinogenicity bioassay

The effects of D-psicose on diethylnitrosamine (DEN)-induced hepatocarcinogenesis were examined in male F344 rats using a rat medium-term bioassay based on the two-step model of hepatocarcinogenesis (Zeng et al., 2005). The modifying potential was determined by comparing the numbers and areas/cm² of induced glutathione *S*-transferase placental form (GST-P) positive foci in the liver with those of a corresponding group (control) of rats given DEN alone. Increased relative liver weights were found in the 1% D-psicose treatment group as compared with the basal diet group, while no significant change occurred in the 0.1% D-psicose, 0.01% D-psicose, and 1% D-fructose groups. D-psicose did not significantly alter the numbers and area/cm² of GST-P positive liver cell foci observed after DEN initiation. The results demonstrate that D-psicose shows neither promoting nor preventive potential for liver carcinogenesis in a medium-term bioassay, which has been correlated well with those from long-term tests in rats (Ogiso, 1990). The results also indicate that increased relative liver weights are not associated with liver carcinogenicity.

3.6.2. Effects of D-psicose on gene expressions of rat liver and skeletal muscle

Shirai et al. (2007) evaluated gene expression of liver and skeletal muscle in rats after long-term feeding of D-psicose. Thirty-six male Wistar rats were divided into four groups. Each group except for the control group was fed a diet of 5% D-fructose, D-psicose, or a 1:1 mixture of D-fructose and D-psicose for 3-8 wk. Within 7wk of D-psicose intake no significant change in diurnal variation in plasma glucose and insulin concentrations were observed. Lower plasma insulin concentrations and higher liver glycogen contents were observed in the psicose group. In the liver, glucose transporter protein (GLUT) 2 and glucokinase mRNA expression markedly increased in the psicose group compared with the other groups. No significant changes in

GLUT4 and LKB1 expression of gastrocnemius muscle were noted. These results suggest that improvements in serum and liver components by dietary D-psicose were partly influenced by alteration in hepatic gene expression.

3.6.3. Preventive effects of d-psicose on di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury in rats

Suna et al. (2007) investigated the preventive effects of D-psicose on di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury in prepubertal male Sprague-Dawley rats. The rats given a diet-containing 1% DEHP alone for 7-14 d showed severe testicular atrophy accompanied by aspermatogenesis. Pre-treatment with D-psicose at concentrations of 2 and 4% resulted in an almost complete but not absolute suppression of testicular malondialdehyde production for rats administered 2 g/kg of DEHP. The microarray analysis showed the induction of oxidative stress-related genes including the thioredoxin, glutathione peroxidase 1 and 2, and glutaredoixn 1 after 24 h of the DEHP treatment in the testis. These results show that D-psicose prevents DEHP-induced testicular injury by suppressing the generation of reactive oxygen species in the rat testis.

4. Human clinical studies

As shown in Table 10, several human clinical studies reported no adverse effects of D-psicose. Like non-digestible oligosaccharide and fiber ingredients, the only side effect of D-psicose is gastrointestinal discomfort when ingested in large quantities. It is well-known that this type of side effect is transient. Studies done in the early 1900s showed that inulin (a nondigestible carbohydrate) intakes of up to 120-160 g/d were well tolerated (Carpenter and Root, 1928; Leach and Sobolik, 2010; Lewis, 1912; Root and Baker, 1925; Shoemaker, 1927), although recent reports show daily maximum tolerance limits of the same compound have been reduced to 20-40 g (Garleb et al., 1996; Kleesen et al. 1997; Roberfroid and Slavin, 2000). People in the early 20th century consumed large quantities of nondigestible carbohydrates; thus, their gastrointestinal systems were adapted to handle high loads with no major side effects. As consumption levels of nondigestible carbohydrates decreased, human tolerance levels also decreased. Thus, the gastrointestinal symptoms associated with high intakes of non-digestible carbohydrates are considered as a transient symptom which can be improved over time. Recent clinical studies showed that daily D-psicose intakes of up to 31-33 g were well tolerated (Matsuo et al., 2002). The history of non-digestible carbohydrate intakes suggests that humans may be able to adapt to much higher levels of D-psicose without major gastrointestinal symptoms.

Despite potential gastrointestinal discomfort associated with high fiber intakes, the U.S. Institute of Medicine has established Adequate Intake (AI) of total fiber to 14 g/1,000 kcal (or 38 g/d for adult men) to help reduce the risk of chronic diseases of the U.S. population (IOM, 2002). This type of symptom is usually transient and is not considered to be of toxicological significance.

Table 10. Human clinical trials with D-psicose

Dosage	Length	Results	Reference
Up to 0.9 g/kg BW/d	6 d	No gastrointestinal symptoms up to 0.5 g/kg BW/d	Iida et al., 2007
15 g/d (5g, three times a day)	12 wk	Positive impact on glycemic responses; no adverse effects were noted.	Hayashi et al., 2010
7.5 g	Single dose	Positive impact on glycemic and insulinemic responses; No adverse effects were noted.	Iida et al., 2008
Up to 340 mg/kg BW	Single dose	Metabolism study; no adverse effects were noted.	Iida et al., 2010

4.1. A study Hayashi et al. (2010)

Hayashi et al. (2010) conducted a clinical study to investigate the safety and effect of D-psicose on postprandial blood glucose concentrations in adult men and women. A randomized double-blind placebo-controlled crossover experiment was conducted on 17 subjects who consumed 5 g of D-psicose or D-glucose with meals three times a day (or 15 g/d) for 12 weeks. No abnormal effects or clinical problems caused by the continuous ingestion of D-psicose were observed.

4.2. Acute tolerance test in normal adults

Iida et al. (2008) studied the effects of D-psicose on glycemic and insulinemic responses in an oral maltodextrin tolerance test with healthy adults in a crossover study. Twenty subjects aged 20-39 y, 11 males and 9 females, were recruited. A load test of oral maltodextrin was conducted as a randomized single blind study. The subjects took one of five test beverages (7.5 g D-psicose alone, 75 g maltodextrin alone, 75 g maltodextrin +2.5, 5, or 7.5 g D-psicose). Independent administration of 7.5 g D-psicose had no influence on blood glucose or insulin concentrations. No adverse effects of D-psicose were reported.

4.3. Study of Iida et al. 2007

Iida et al. (2007) investigated the effects of D-psicose on gastrointestinal symptoms in healthy volunteers (5 males and 5 females) aged 20-30 y. All subjects ingested 0.4 g/kg BW/d of D-psicose for the first dose. The dosage was increased from by 0.1 g/kg BW/d to 0.9 g/kg BW/d, the maximum dose level. Diarrhea occurred in one male at the dosage of 0.6 g/kg BW/d, 2 females at the dosage of 0.7 g/kg BW/d, and 2 males and 3 females at the dosage of 0.8 g/kg BW/d. Two males did not suffer from diarrhea even at 0.9 g/kg BW/d. Authors concluded that the maximum tolerable levels in humans were 0.5 g/kg BW/d for males and 0.6 g/kg BW/d for females, with the mean value of 0.55 g/ g/kg BW/d. These dosages correspond to 33.3 g/d for males and 31.0 g/d for females. These maximum tolerable levels of D-psicose are similar to that of erythritol (0.66 g/kg BW/d).

IV. Conclusions

The information/data provided by CJ CheilJedang (specifications, manufacturing process, and intended use) in this report and supplemented by the publicly available literature/toxicity data on D-psicose provide a sufficient basis for an assessment of the safety of D-psicose for the proposed use as an ingredient in foods and beverages prepared according to appropriate specifications and used according to GMP.

Key findings are summarized here:

1. D-psicose is well characterized and free from chemical and microbial contamination.
2. The safety and nutritional benefits of D-psicose are well established by human clinical trials and animal studies of D-psicose.
3. Intended use of D-psicose as part of the proposed food use, even at the 90th percentile, results in levels of exposure significantly below those associated with any adverse effects and provides a reasonable certainty of safety.

Therefore, not only is the proposed use of D-psicose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS).

V. Discussion of information inconsistent with GRAS determination

We are not aware of information that would be inconsistent with a finding that the proposed use of D-psicose preparations in foods and beverages, meeting appropriate specifications and used according to GMP, is GRAS.

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Appendix I Continued

Table 14

Conversion Table^a for Test Chemical Treatment Doses Used in PAFA

Animal	BW ^b (Avg.) (kg)	Conc. in Food		Conc. in Water	
		1 ppm	1%	1 ppm	1%
		(mg/kg-bw/day)		(mg/kg-bw/day)	
Mouse	.0200	.150	1500	.250	2500
Rat (young)	.100	.100	1000	.100	1000
Rat (old ^c)	.400	.050	500.	.100	1000
Hamster	.125	.120	1200	.080.	800.
Guinea Pig	.750	.040	400.	.113	1130
Rabbit	2.00	.030	300.	.175	1750
Dog	10.0	.025	250.	.050	500.
Dog ^d	10.0	.075	750.		
Human ^d	60.0	.025	250.		

^a REF: Appraisal of the safety of chemicals in foods, drugs, and cosmetics (1975). Publ. Assoc. Food & Drug Officials of the U.S.

^b BW: Abbreviation for body weight

^c Old: Use if rats are greater than 90 days of age

^d Dog & Human: Moist diet (all others dry weight).

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Guidance for Industry

Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

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TABLE OF CONTENTS

I.	INTRODUCTION.....	1
II.	BACKGROUND	2
III.	OVERVIEW OF THE ALGORITHM	3
IV.	STEP 1: NO OBSERVED ADVERSE EFFECT LEVEL DETERMINATION.....	5
V.	STEP 2: HUMAN EQUIVALENT DOSE CALCULATION.....	6
	A. Conversion Based on Body Surface Area	6
	B. Basis for Using mg/kg Conversions	7
	C. Other Exceptions to mg/m² Scaling Between Species	8
VI.	STEP 3: MOST APPROPRIATE SPECIES SELECTION.....	9
VII.	STEP 4: APPLICATION OF SAFETY FACTOR.....	9
	A. Increasing the Safety Factor	10
	B. Decreasing the Safety Factor	11
VIII.	STEP 5: CONSIDERATION OF THE PHARMACOLOGICALLY ACTIVE DOSE.....	12
IX.	SUMMARY	12
	REFERENCES.....	13
	GLOSSARY.....	15
	APPENDIX A:.....	16
	Analysis of Allometric Exponent on HED Calculations.....	16
	APPENDIX B:.....	18
	Analysis of Body Weight Effects on HED Calculations	18
	APPENDIX C:.....	24
	Derivation of the Interspecies Scaling Factor (W_a/W_h)^(1-b).....	24
	APPENDIX D:.....	25
	Examples of Calculations for Converting Animal Doses to Human Equivalent Doses.....	25
	APPENDIX E:.....	27
	Selection of Maximum Recommended Starting Dose for Drugs Administered Systemically to Normal Volunteers	27

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Guidance for Industry¹ Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance outlines a process (algorithm) and vocabulary for deriving the maximum recommended starting dose (MRSD) for *first-in-human* clinical trials of new molecular entities in adult healthy volunteers, and recommends a standardized process by which the MRSD can be selected. The purpose of this process is to ensure the safety of the human volunteers.

The goals of this guidance are to: (1) establish a consistent terminology for discussing the starting dose; (2) provide common conversion factors for deriving a human equivalent dose (HED); and (3) delineate a strategy for selecting the MRSD for adult healthy volunteers, regardless of the projected clinical use. This process is depicted in a flow chart that presents the decisions and calculations used to generate the MRSD from animal data (see Appendix E).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

¹ This guidance has been prepared by the Office of New Drugs in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

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II. BACKGROUND

The process identified in this guidance pertains to determining the MRSD for adult healthy subjects when beginning a clinical investigation of any new drug or biological therapeutic that has been studied in animals. This guidance is not pertinent to endogenous hormones and proteins (e.g., recombinant clotting factors) used at physiologic concentrations or prophylactic vaccines. The process outlined in this guidance pertains primarily to drug products for which systemic exposure is intended; it does not address dose escalation or maximum allowable doses in clinical trials.

Although the process outlined in this guidance uses administered doses, observed toxicities, and an algorithmic approach to calculate the MRSD, an alternative approach could be proposed that places primary emphasis on animal pharmacokinetics and modeling rather than dose (Mahmood et al. 2003; Reigner and Blesch 2002). In a limited number of cases, animal pharmacokinetic data can be useful in determining initial clinical doses.² However, in the majority of investigational new drug applications (INDs), animal data are not available in sufficient detail to construct a scientifically valid, pharmacokinetic model whose aim is to accurately project an MRSD.

Toxicity should be avoided at the initial clinical dose. However, doses should be chosen that allow reasonably rapid attainment of the phase 1 trial objectives (e.g., assessment of the therapeutic's tolerability, pharmacodynamic or pharmacokinetic profile). All of the relevant preclinical data, including information on the pharmacologically active dose, the full toxicologic profile of the compound, and the pharmacokinetics (absorption, distribution, metabolism, and excretion) of the therapeutic, should be considered when determining the MRSD. Starting with doses lower than the MRSD is always an option and can be particularly appropriate to meet some clinical trial objectives.

² If the parent drug is measured in the plasma at multiple times and is within the range of toxic exposures for two or more animal species, it may be possible to develop a pharmacokinetic model predicting human doses and concentrations and to draw inferences about safe human plasma levels in the absence of prior human data. Although quantitative modeling for this purpose may be straightforward, the following points suggest this approach can present a number of difficulties when estimating a safe starting dose. Generally, at the time of IND initiation, there are a number of unknowns regarding animal toxicity and comparability of human and animal pharmacokinetics and metabolism: (1) human bioavailability and metabolism may differ significantly from that of animals; (2) mechanisms of toxicity may not be known (e.g., toxic accumulation in a peripheral compartment); and/or (3) toxicity may be due to an unidentified metabolite, not the parent drug. Therefore, relying on pharmacokinetic models (based on the parent drug in plasma) to gauge starting doses would require multiple untested assumptions. Modeling can be used with greatest validity to estimate human starting doses in special cases where few underlying assumptions would be necessary. Such cases are exemplified by large molecular weight proteins (e.g., humanized monoclonal antibodies) that are intravenously administered, are removed from circulation by endocytosis rather than metabolism, have immediate and detectable effects on blood cells, and have a volume of distribution limited to the plasma volume. In these cases, allometric, pharmacokinetic, and pharmacodynamic models have been useful in identifying the human mg/kg dose that would be predicted to correlate with safe drug plasma levels in nonhuman primates. Even in these cases, uncertainties (such as differences between human and animal receptor sensitivity or density) have been shown to affect human pharmacologic or toxicologic outcomes, and the use of safety factors as described in this guidance is still warranted.

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The remainder of this guidance focuses on the recommended algorithmic process for starting dose extrapolation from animals to humans based on administered doses, since this method will likely be useful for the majority of INDs seeking to investigate new drugs in healthy volunteers. Some classes of drugs (e.g., many cytotoxic or biological agents) are commonly introduced into initial clinical trials in patient volunteers rather than healthy volunteers. Typically, patients are used instead of healthy volunteers when a drug is suspected or known to be unavoidably toxic. This guidance does not address starting doses in patients. However, many principles and some approaches recommended here may be applicable to designing such trials.

III. OVERVIEW OF THE ALGORITHM

The recommended process for selecting the MRSD is presented in Appendix E and described in this section. The major elements (i.e., the determination of the no observed adverse effect levels (NOAELs) in the tested animal species, conversion of NOAELs to HED, selection of the most appropriate animal species, and application of a safety factor) are all discussed in greater detail in subsequent sections. Situations are also discussed in which the algorithm should be modified. The algorithm is intended to be used for systemically administered therapeutics. Topical, intranasal, intratissue, and compartmental administration routes and depot formulations can have additional considerations, but similar principles should apply.

The process of calculating the MRSD should begin after the toxicity data have been analyzed. Although only the NOAEL should be used directly in the algorithm for calculating an MRSD, other data (exposure/toxicity relationships, pharmacologic data, or prior clinical experience with related drugs) can affect the choice of most appropriate species, scaling, and safety factors.

The NOAEL for each species tested should be identified, and then converted to the HED using appropriate scaling factors. For most systemically administered therapeutics, this conversion should be based on the normalization of doses to body surface area. Although body surface area conversion is the standard way to approximate equivalent exposure if no further information is available, in some cases extrapolating doses based on other parameters may be more appropriate. This decision should be based on the data available for the individual case. The body surface area normalization and the extrapolation of the animal dose to human dose should be done in one step by dividing the NOAEL in each of the animal species studied by the appropriate body surface area conversion factor (BSA-CF). This conversion factor is a unitless number that converts mg/kg dose for each animal species to the mg/kg dose in humans, which is equivalent to the animal's NOAEL on a mg/m² basis. The resulting figure is called a human equivalent dose (HED). The species that generates the lowest HED is called the most sensitive species.

When information indicates that a particular species is more relevant for assessing human risk (and deemed the *most appropriate species*), the HED for that species may be used in subsequent calculations, regardless of whether this species is the most sensitive. This situation is more applicable to biologic therapies, many of which have high selectivity for binding to human target

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proteins and limited reactivity in species commonly used for toxicity testing. In such cases, in vitro binding and functional studies should be conducted to select an appropriate, relevant species before toxicity studies are designed (refer to ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* for more details³). (However, if serious toxicities are observed in an animal species considered less relevant, those toxicities should be taken into consideration in determining the species to be used to calculate an HED. For example, in one particular case, dog was selected as the animal species used for calculation of an HED because of unmonitorable cardiac lesions, even though the rat was considered the most relevant species based on pharmacological activity data.) Additionally, a species might be considered an inappropriate toxicity model for a given drug if the dose-limiting toxicity in that species was concluded to be of limited value for human risk assessment, based on historical comparisons of toxicities in the animal species to those in humans across a therapeutic class (i.e., the dose-limiting toxicity is species-specific). In this case, data from that species should not be used to derive the HED. Without any additional information to guide the choice of the most appropriate species for assessing human risk, the most sensitive species is designated the *most appropriate*, because using the lowest HED would generate the most conservative starting dose.

A safety factor should then be applied to the HED to increase assurance that the first dose in humans will not cause adverse effects. The use of the safety factor should be based on the possibility that humans may be more sensitive to the toxic effects of a therapeutic agent than predicted by the animal models, that bioavailability may vary across species, and that the models tested do not evaluate all possible human toxicities. For example, ocular disturbances or pain (e.g., severe headaches) in humans can be significant dose-limiting toxicities that may go undetected in animal studies.

In general, one should consider using a safety factor of at least 10. The MRSD should be obtained by dividing the HED by the safety factor. Safety concerns or design shortcomings noted in animal studies may increase the safety factor, and thus reduce the MRSD further. Alternatively, information about the pharmacologic class (well-characterized classes of therapeutics with extensive human clinical and preclinical experience) may allay concerns and form the basis for reducing the magnitude of the default safety factor and increasing the MRSD. Although a dose lower than the MRSD can be used as the actual starting dose, the process described in this guidance will derive the maximum recommended starting dose. This algorithm generates an MRSD in units of mg/kg, a common method of dosing used in phase 1 trials, but the equations and conversion factors provided in this guidance (Table 1, second column) can be used to generate final dosing units in the mg/m² form if desired.

As previously stated, for purposes of initial clinical trials in adult healthy volunteers, the HED should ordinarily be calculated from the animal NOAEL. If the HED is based on an alternative index of effect, such as the pharmacologically active dose (PAD), this exception should be prominently stipulated in descriptions of starting dose calculations.

³ We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at <http://www.fda.gov/cder/guidance/index.htm>.

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The remainder of this guidance provides a description of the individual steps in the recommended process and the reasoning behind each step.

IV. STEP 1: NO OBSERVED ADVERSE EFFECT LEVEL DETERMINATION

The first step in determining the MRSD is to review and evaluate the available animal data so that a NOAEL can be determined for each study. Several definitions of NOAEL exist, but for selecting a starting dose, the following is used: the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group. In this context, adverse effects that are biologically significant (even if they are not statistically significant) should be considered in the determination of the NOAEL. The NOAEL is a generally accepted benchmark for safety when derived from appropriate animal studies and can serve as the starting point for determining a reasonably safe starting dose of a new therapeutic in healthy (or asymptomatic) human volunteers.

The NOAEL is not the same as the *no observed effect level (NOEL)*, which refers to any effect, not just an adverse one, although in some cases the two might be identical. The definition of the NOAEL, in contrast to that of the NOEL, reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic and may not raise a safety concern. The NOAEL should also not be confused with *lowest observed adverse effect level (LOAEL)* or *maximum tolerated dose (MTD)*. Both of the latter concepts are based on findings of adverse effects and are not generally used as benchmarks for establishing safe starting doses in adult healthy volunteers. (The term *level* refers to dose or dosage, generally expressed as mg/kg or mg/kg/day.)

Initial IND submissions for first-in-human studies by definition lack in vivo human data or formal allometric comparison of pharmacokinetics. Measurements of systemic levels or exposure (i.e., AUC or C_{max}) cannot be employed for setting a safe starting dose in humans, and it is critical to rely on dose and observed toxic response data from adequate and well-conducted toxicology studies. However, there are cases where nonclinical data on bioavailability, metabolite profile, and plasma drug levels associated with toxicity may influence the choice of the NOAEL. One such case is when saturation of drug absorption occurs at a dose that produces no toxicity. In this instance, the lowest saturating dose, not the highest (nontoxic) dose, should be used for calculating the HED.

There are essentially three types of findings in nonclinical toxicology studies that can be used to determine the NOAEL: (1) overt toxicity (e.g., clinical signs, macro- and microscopic lesions); (2) surrogate markers of toxicity (e.g., serum liver enzyme levels); and (3) exaggerated pharmacodynamic effects. Although the nature and extent of adverse effects can vary greatly with different types of therapeutics, and it is anticipated that in many instances, experts will disagree on the characterization of effects as being adverse or not, the use of NOAEL as a benchmark for dose-setting in healthy volunteers should be acceptable to all responsible investigators. As a general rule, an adverse effect observed in nonclinical toxicology studies

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used to define a NOAEL for the purpose of dose-setting should be based on an effect that would be unacceptable if produced by the initial dose of a therapeutic in a phase 1 clinical trial conducted in adult healthy volunteers.

V. STEP 2: HUMAN EQUIVALENT DOSE CALCULATION

A. Conversion Based on Body Surface Area

After the NOAELs in the relevant animal studies have been determined, they are converted to HEDs. A decision should be made regarding the most appropriate method for extrapolating the animal dose to the equivalent human dose. Toxic endpoints for therapeutics administered systemically to animals, such as the MTD, are usually assumed to scale well between species when doses are normalized to body surface area (i.e., mg/m²) (EPA 1992; Lowe and Davis 1998). The basis for this assumption lies primarily with the work of Freireich et al. (1966) and Schein et al. (1970). These investigators reported that, for antineoplastic drugs, doses lethal to 10 percent of rodents (LD₁₀s) and MTDs in nonrodents both correlated with the human MTD when the doses were normalized to the same administration schedule and expressed as mg/m². Despite the subsequent analyses showing that the MTDs for this set of drugs scale best between species when doses are normalized to W^{0.75} rather than W^{0.67} (inherent in body surface area normalization) (Travis and White 1988; Watanabe et al. 1992), normalization to body surface area has remained a widespread practice for estimating an HED based on an animal dose.

An analysis of the affect of the allometric exponent on the conversion of an animal dose to the HED was conducted (see Appendix A). Based on this analysis and on the fact that correcting for body surface area increases clinical trial safety by resulting in a more conservative starting dose estimate, it was concluded that the approach of converting NOAEL doses to an HED based on body surface area correction factors (i.e., W^{0.67}) should be maintained for selecting starting doses for initial studies in adult healthy volunteers. Nonetheless, use of a different dose normalization approach, such as directly equating the human dose to the NOAEL in mg/kg, may be appropriate in some circumstances. Deviations from the body surface area approach, when describing the conversion of animal dose to HED, should be justified. The basis for justifying direct mg/kg conversion and examples in which other normalization methods are appropriate are described in the following subsection.

Although normalization to body surface area is an appropriate method for extrapolating doses between species, consistent factors for converting doses from mg/kg to mg/m² have not always been used. Given that body surface area normalization provides a reasonable approach for estimating an HED, the factors used for converting doses for each species should be standardized. Since body surface area varies with W^{0.67}, the conversion factors are dependent on the weight of the animals in the studies. However, analyses conducted to address the effect of body weight on the actual BSA-CF demonstrated that a standard factor provides a reasonable estimate of the HED over a broad range of human and animal weights (see Appendix B). The conversion factors and divisors shown in Table 1 are therefore recommended as the standard

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values to be used for interspecies dose conversions for NOAELs. (These factors may also be applied when comparing safety margins for other toxicity endpoints (e.g., reproductive toxicity and carcinogenicity) when other data for comparison (i.e., AUCs) are unavailable or are otherwise inappropriate for comparison.)

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area			
Species	To Convert Animal Dose in mg/kg to Dose in mg/m ² , Multiply by k _m	To Convert Animal Dose in mg/kg to HED ^a in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg) ^b	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys ^c	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

^a Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

$$\text{HED} = \text{animal dose in mg/kg} \times (\text{animal weight in kg/human weight in kg})^{0.33}$$

^b This k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

^c For example, cynomolgus, rhesus, and stump-tail.

B. Basis for Using mg/kg Conversions

The factors in Table 1 for scaling animal NOAEL to HEDs are based on the assumption that doses scale 1:1 between species when normalized to body surface area. However, there are occasions for which scaling based on body weight (i.e., setting the HED (mg/kg) = NOAEL (mg/kg)) may be more appropriate. To consider mg/kg scaling for a therapeutic, the available data should show that the NOAEL occurs at a similar mg/kg dose across species. The following circumstances should exist before extrapolating to the HED on a mg/kg basis rather than using

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the mg/m² approach. Note that mg/kg scaling will give a twelve-, six-, and twofold higher HED than the default mg/m² approach for mice, rats, and dogs, respectively. If these circumstances do not exist, the mg/m² scaling approach for determining the HED should be followed as it will lead to a safer MRSD.

1. NOAELs occur at a similar mg/kg dose across test species (for the studies with a given dosing regimen relevant to the proposed initial clinical trial). (However, it should be noted that similar NOAELs on a mg/kg basis can be obtained across species because of differences in bioavailability alone.)
2. If only two NOAELs from toxicology studies in separate species are available, one of the following should also be true:
 - The therapeutic is administered orally and the dose is limited by local toxicities. Gastrointestinal (GI) compartment weight scales by $W^{0.94}$ (Mordenti 1986). GI volume determines the concentration of the therapeutic in the GI tract. It is then reasonable that the toxicity of the therapeutic would scale by mg/kg ($W^{1.0}$).
 - The toxicity in humans (for a particular class) is dependent on an exposure parameter that is highly correlated across species with dose on a mg/kg basis. For example, complement activation by systemically administered antisense oligonucleotides in humans is believed to be dependent upon C_{max} (Geary et al. 1997). For some antisense drugs, the C_{max} correlates across nonclinical species with mg/kg dose and in such instances mg/kg scaling would be justified.
 - Other pharmacologic and toxicologic endpoints also scale between species by mg/kg for the therapeutic. Examples of such endpoints include the MTD, lowest lethal dose, and the pharmacologically active dose.
 - There is a robust correlation between plasma drug levels (C_{max} and AUC) and dose in mg/kg.

C. Other Exceptions to mg/m² Scaling Between Species

Scaling between species based on mg/m² is not recommended for the following categories of therapeutics:

1. Therapeutics administered by alternative routes (e.g., topical, intranasal, subcutaneous, intramuscular) for which the dose is limited by local toxicities. Such therapeutics should be normalized to concentration (e.g., mg/area of application) or amount of drug (mg) at the application site.
2. Therapeutics administered into anatomical compartments that have little subsequent distribution outside of the compartment. Examples are intrathecal, intravesical,

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intraocular, or intrapleural administration. Such therapeutics should be normalized between species according to the compartmental volumes and concentrations of the therapeutic.

3. Proteins administered intravascularly with $M_r > 100,000$ daltons. Such therapeutics should be normalized to mg/kg.

VI. STEP 3: MOST APPROPRIATE SPECIES SELECTION

After the HEDs have been determined from the NOAELs from all toxicology studies relevant to the proposed human trial, the next step is to pick one HED for subsequent derivation of the MRSD. This HED should be chosen from the most appropriate species. In the absence of data on species relevance, a default position is that the most appropriate species for deriving the MRSD for a trial in adult healthy volunteers is the most sensitive species (i.e., the species in which the lowest HED can be identified).

Factors that could influence the choice of the most appropriate species rather than the default to the most sensitive species include: (1) differences in the absorption, distribution, metabolism, and excretion (ADME) of the therapeutic between the species, and (2) class experience that may indicate a particular animal model is more predictive of human toxicity. Selection of the most appropriate species for certain biological products (e.g., human proteins) involves consideration of various factors unique to these products. Factors such as whether an animal species expresses relevant receptors or epitopes may affect species selection (refer to ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* for more details).

When determining the MRSD for the first dose of a new therapeutic in humans, absorption, distribution, and elimination parameters will not be known for humans. Comparative metabolism data, however, might be available based on in vitro studies. These data are particularly relevant when there are marked differences in both the in vivo metabolite profiles and HEDs in animals. Class experience implies that previous studies have demonstrated that a particular animal model is more appropriate for the assessment of safety for a particular class of therapeutics. For example, in the nonclinical safety assessment of the phosphorothioate antisense drugs, the monkey is considered the most appropriate species because monkeys experience the same dose limiting toxicity as humans (e.g., complement activation) whereas rodents do not. For this class of therapeutics, the MRSD would usually be based on the HED for the NOAEL in monkeys regardless of whether it was lower than that in rodents, unless unique dose limiting toxicities were observed with the new antisense compound in the rodent species.

VII. STEP 4: APPLICATION OF SAFETY FACTOR

Once the HED of the NOAEL in the most appropriate species has been determined, a safety factor should then be applied to provide a margin of safety for protection of human subjects receiving the initial clinical dose. This safety factor allows for variability in extrapolating from

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animal toxicity studies to studies in humans resulting from: (1) uncertainties due to enhanced sensitivity to pharmacologic activity in humans versus animals; (2) difficulties in detecting certain toxicities in animals (e.g., headache, myalgias, mental disturbances); (3) differences in receptor densities or affinities; (4) unexpected toxicities; and (5) interspecies differences in ADME of the therapeutic. These differences can be accommodated by lowering the human starting dose from the HED of the selected species NOAEL.

In practice, the MRSD for the clinical trial should be determined by dividing the HED derived from the animal NOAEL by the safety factor. The default safety factor that should normally be used is 10. This is a historically accepted value, but, as described below, should be evaluated based on available information.

A safety factor of 10 may not be appropriate for all cases. The safety factor should be raised when there is reason for increased concern, and lowered when concern is reduced because of available data that provide added assurance of safety. This can be visualized as a sliding scale, balancing findings that mitigate the concern for harm to healthy volunteers with those that suggest greater concern is warranted. The extent of the increase or decrease is largely a matter of judgment, using the available information. It is incumbent on the evaluator to clearly explain the reasoning behind the applied safety factor when it differs from the default value of 10, particularly if it is less than 10.

A. Increasing the Safety Factor

The following considerations indicate a safety concern that might warrant increasing the safety factor. In these circumstances, the MRSD would be calculated by dividing the HED by a safety factor that is greater than 10. If any of the following concerns are defined in review of the nonclinical safety database, an increase in the safety factor may be called for. If multiple concerns are identified, the safety factor should be increased accordingly.

- **Steep dose response curve.** A steep dose response curve for significant toxicities in the most appropriate species or in multiple species may indicate a greater risk to humans.
- **Severe toxicities.** Qualitatively severe toxicities or damage to an organ system (e.g., central nervous system (CNS)) indicate increased risk to humans.
- **Nonmonitorable toxicity.** Nonmonitorable toxicities may include histopathologic changes in animals that are not readily monitored by clinical pathology markers.
- **Toxicities without premonitory signs.** If the onset of significant toxicities is not reliably associated with premonitory signs in animals, it may be difficult to know when toxic doses are approached in human trials.

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- **Variable bioavailability.** Widely divergent or poor bioavailability in the several animal species, or poor bioavailability in the test species used to derive the HED, suggest a greater possibility for underestimating the toxicity in humans.
- **Irreversible toxicity.** Irreversible toxicities in animals suggest the possibility of permanent injury in human trial participants.
- **Unexplained mortality.** Mortality that is not predicted by other parameters raises the level of concern.
- **Large variability in doses or plasma drug levels eliciting effect.** When doses or exposure levels that produce a toxic effect differ greatly across species or among individual animals of a species, the ability to predict a toxic dose in humans is reduced and a greater safety factor may be needed.
- **Nonlinear pharmacokinetics.** When plasma drug levels do not increase in a dose-related manner, the ability to predict toxicity in humans in relation to dose is reduced and a greater safety factor may be needed.
- **Inadequate dose-response data.** Poor study design (e.g., few dose levels, wide dosing intervals) or large differences in responses among animals within dosing groups may make it difficult to characterize the dose-response curve.
- **Novel therapeutic targets.** Therapeutic targets that have not been previously clinically evaluated may increase the uncertainty of relying on the nonclinical data to support a safe starting dose in humans.
- **Animal models with limited utility.** Some classes of therapeutic biologics may have very limited interspecies cross-reactivity or pronounced immunogenicity, or may work by mechanisms that are not known to be conserved between (nonhuman) animals and humans; in these cases, safety data from any animal studies may be very limited in scope and interpretability.

B. Decreasing the Safety Factor

Safety factors of less than 10 may be appropriate under some conditions. The toxicologic testing in these cases should be of the highest caliber in both conduct and design. Most of the time, candidate therapeutics for this approach would be members of a well-characterized class. Within the class, the therapeutics should be administered by the same route, schedule, and duration of administration; should have a similar metabolic profile and bioavailability; and should have similar toxicity profiles across all the species tested including humans. A smaller safety factor might also be used when toxicities produced by the therapeutic are easily monitored, reversible, predictable, and exhibit a moderate-to-shallow dose-response relationship with toxicities that are

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consistent across the tested species (both qualitatively and with respect to appropriately scaled dose and exposure).

A safety factor smaller than 10 could be justified when the NOAEL was determined based on toxicity studies of longer duration compared to the proposed clinical schedule in healthy volunteers. In this case, a greater margin of safety should be built into the NOAEL, as it was associated with a longer duration of exposure than that proposed in the clinical setting. This assumes that toxicities are cumulative, are not associated with acute peaks in therapeutic concentration (e.g., hypotension), and did not occur early in the repeat dose study.

VIII. STEP 5: CONSIDERATION OF THE PHARMACOLOGICALLY ACTIVE DOSE

Selection of a PAD depends upon many factors and differs markedly among pharmacological drug classes and clinical indications; therefore, selection of a PAD is beyond the scope of this guidance. However, once the MRSD has been determined, it may be of value to compare it to the PAD derived from appropriate pharmacodynamic models. If the PAD is from an in vivo study, an HED can be derived from a PAD estimate by using a BSA-CF. This HED value should be compared directly to the MRSD. If this *pharmacologic* HED is lower than the MRSD, it may be appropriate to decrease the clinical starting dose for pragmatic or scientific reasons. Additionally, for certain classes of drugs or biologics (e.g., vasodilators, anticoagulants, monoclonal antibodies, or growth factors), toxicity may arise from *exaggerated pharmacologic* effects. The PAD in these cases may be a more sensitive indicator of potential toxicity than the NOAEL and might therefore warrant lowering the MRSD.

IX. SUMMARY

A strategy has been proposed to determine the maximum recommended starting dose for clinical trials of new therapeutics in adult healthy volunteers. In summary, usually NOAELs from the relevant animal studies should be converted to the HEDs using the standard factors presented in Table 1. Using sound scientific judgment, a safety factor should be applied to the HED from the most appropriate species to arrive at the MRSD. This process is meant to define the upper limit of recommended starting doses and, in general, lower starting doses can be appropriate. The process described in this guidance should foster consistency among sponsors and Agency reviewers.

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ICH guidance for industry *S3A Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies*

ICH guidance for industry *M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals*

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GLOSSARY

b: Allometric exponent

Body surface area conversion factor (BSA-CF): A factor that converts a dose (mg/kg) in an animal species to the equivalent dose in humans (also known as the *human equivalent dose*), based on differences in body surface area. A BSA-CF is the ratio of the body surface areas in the tested species to that of an average human.

Human equivalent dose (HED): A dose in humans anticipated to provide the same degree of effect as that observed in animals at a given dose. In this guidance, as in many communications from sponsors, the term HED is usually used to refer to the human equivalent dose of the NOAEL. When reference is made to the human equivalent of a dose other than the NOAEL (e.g., the PAD), sponsors should explicitly and prominently note this usage.

K: A dimensionless factor that adjusts for differences in the surface area to weight ratio of species because of their different body shapes.

k_m: Factor for converting mg/kg dose to mg/m² dose

Lowest observed adverse effect level (LOAEL): The lowest dose tested in an animal species with adverse effects.

Maximum recommended starting dose (MRSD): The highest dose recommended as the initial dose in a clinical trial. In clinical trials of adult healthy volunteers, the MRSD is predicted to cause no adverse reactions. The units of the dose (e.g., mg/kg or mg/m²) may vary depending on practices employed in the area being investigated.

Maximum tolerated dose (MTD): In a toxicity study, the highest dose that does not produce unacceptable toxicity.

No observed adverse effect level (NOAEL): The highest dose tested in an animal species that does not produce a significant increase in adverse effects in comparison to the control group. Adverse effects that are biologically significant, even if not statistically significant, should be considered in determining an NOAEL.

No observed effect level (NOEL): The highest dose tested in an animal species with no detected effects.

Pharmacologically active dose (PAD): The lowest dose tested in an animal species with the intended pharmacologic activity.

Safety factor (SF): A number by which the HED is divided to introduce a margin of safety between the HED and the *maximum recommended starting dose*.

W: Body weight in kg

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**APPENDIX A:
Analysis of Allometric Exponent on HED Calculations**

An analysis was conducted to determine the effect of the allometric exponent on the conversion of an animal dose to the HED. One can derive the following equation (see Appendix C) for converting animal doses to the HED based on body weights and the allometric exponent (b):

$$\text{HED} = \text{animal NOAEL} \times (\text{W}_{\text{animal}}/\text{W}_{\text{human}})^{(1-b)}$$

Conventionally, for a mg/m² normalization *b* would be 0.67, but a number of studies (including the original Freireich data) have shown that MTDs scale best across species when *b* = 0.75. The Interagency Pharmacokinetics Group has recommended that W^{0.75} be used for interspecies extrapolation of doses in carcinogenicity studies (EPA 1992). There are no data, however, to indicate the optimal method for converting NOAELs to HEDs. Conversion factors were calculated over a range of animal and human weights using (W_{animal}/W_{human})^{0.33} or (W_{animal}/W_{human})^{0.25} to assess the effect on starting dose selection of using *b* = 0.75 instead of *b* = 0.67. The results are shown in Table 2. Using an allometric exponent of 0.75 had a big effect on the conversion factor for the smaller species mice and rats. Nonetheless, mice are not commonly used for toxicology studies to support the first-in-human clinical trials. In addition, there is evidence that the area under the plasma concentration versus time curves in rats and humans correlates reasonably well when doses are normalized to mg/m² (Contrera et al. 1995). We conclude that the approach of converting NOAEL doses to an HED based on body surface area correction factors (i.e., *b* = 0.67) should be maintained for selecting starting doses for initial studies in healthy volunteers since: (1) mg/m² normalization is widely used throughout the toxicology and pharmacokinetic research communities; (2) mg/m² normalization provides a more conservative conversion; (3) there are no data to suggest a superior method for converting NOAELs; and (4) CDER has significant experience in establishing safe starting doses based on mg/m², and it is readily calculated.

Species	Weight Range ^b (kg)	Conversion Factors ^c			Ratio of 0.75 to 0.67
		Standard	<i>b</i> = 0.67	<i>b</i> = 0.75	
Mouse	0.018-0.033	0.081	0.075	0.141	1.88
Rat	0.09-0.40	0.162	0.156	0.245	1.57
Rabbit	1.5-3	0.324	0.33	0.43	1.30
Monkey	1.5-4	0.324	0.37	0.47	1.27
Dog	6.5-13.0	0.541	0.53	0.62	1.17

^a conversion factor = (W_{animal}/W_{human})^(1-b)

^b human weight range used was 50-80 kg (110-176 lb)

^c mean conversion factor calculated across entire animal weight range and human weight range

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The following summarizes the analysis of the effects of the allometric exponent on HED calculations:

- Changing the allometric exponent from 0.67 to 0.75 had a big effect on the conversion factor for the smaller rodent species; for mice the conversion factors differed by a factor of almost 2.
- Converting doses based on an exponent of 0.75 would lead to higher, more aggressive and potentially more toxic starting doses.
- The limited data available suggest that the most accurate allometric exponent for normalizing MTDs of antineoplastic agents for interspecies extrapolation is $b = 0.75$, but there are no data to indicate the optimal normalization method for interspecies extrapolation of NOAELs in a broad range of therapeutic classes. Using mg/m^2 is widely adopted throughout the drug development community.
- Unless evidence is provided to the contrary, HED calculations should be based on $b = 0.67$ (i.e., the standard conversions based on mg/m^2 relationships).
- There was no notable effect of body weight on calculation of the HED within the weight ranges examined.

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APPENDIX B: Analysis of Body Weight Effects on HED Calculations

Accurate conversion of a mg/kg dose to a mg/m² dose depends on the actual weight (and surface area) of the test species. A popular formula for converting doses is:

- (i) $\text{mg/m}^2 = k_m \times \text{mg/kg}$
where $k_m = 100/K \times W^{0.33}$ where K is a value unique to each species (Freireich et al. 1966)
or $k_m = 9.09 \times W^{0.35}$ where a K value unique to each species is not needed (Boxenbaum and DiLea 1995; Burtles et al. 1995; Stahl 1956).

The k_m value is not truly constant for any species, but increases within a species as body weight increases. The increase is not linear, but increases approximately proportional to $W^{2/3}$. For example, the k_m value in rats varies from 5.2 for a 100 g rat to 7.0 for a 250 g rat. Strictly speaking, the k_m value of 6 applies only to rats at the *reference weight* of 150 g. For standardization and practical purposes, a fixed k_m factor for each species is preferred. An analysis was undertaken to determine the effect of different body weights within a species on the conversion of an animal dose to the HED using k_m factors. The k_m factor was calculated for a range of body weights using $k_m = 100/K \times W^{0.33}$. In Table 3, a working weight range is shown next to the reference body weight. This is the range within which the HED calculated by using the standard k_m value will not vary more than ± 20 percent from that which would be calculated using a k_m value based on exact animal weight. This is a relatively small variance considering dose separation generally used in deriving the NOAEL, in toxicology studies, which are often twofold separations. For example, suppose a NOAEL in rats is 75 mg/kg and the average rat weight is 250 g. The k_m value for a 250 g rat is 7.0.

$$\begin{aligned} \text{HED} &= 75 \times (7/37) = 14 \text{ mg/kg in humans.} \\ \text{Using the standard } k_m \text{ value of 6 for rats,} \\ \text{HED} &= 75 \times (6/37) = 12 \text{ mg/kg in humans.} \end{aligned}$$

The HED calculated with the standard k_m value of 6 is within 15 percent of the value calculated using the actual k_m value of 7. As shown in Table 3, the body weights producing k_m factors for which the nominal, integer conversion factor was within 20 percent of the calculated factor covered a broad range. This working weight range encompassed the animal weights expected for the majority of studies used to support starting doses in humans.

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Table 3: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area						
Species	Reference Body Weight (kg)	Working Weight Range ^a (kg)	Body Surface Area (m ²)	To Convert Dose in mg/kg to Dose in mg/m ² Multiply by k _m	To Convert Animal Dose in mg/kg to HED ^b in mg/kg, Either	
					Divide Animal Dose By	Multiply Animal Dose By
Human	60	---	1.62	37	---	---
Child ^c	20	---	0.80	25	---	---
Mouse	0.020	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.080	0.047-0.157	0.016	5	7.4	0.135
Rat	0.150	0.080-0.270	0.025	6	6.2	0.162
Ferret	0.300	0.160-0.540	0.043	7	5.3	0.189
Guinea pig	0.400	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.9-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Primates:						
Monkeys ^d	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.350	0.140-0.720	0.06	6	6.2	0.162
Squirrel monkey	0.600	0.290-0.970	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro-pig	20	10-33	0.74	27	1.4	0.730
Mini-pig	40	25-64	1.14	35	1.1	0.946

^a For animal weights within the specified ranges, the HED for a 60 kg human calculated using the standard k_m value will not vary more than ±20 percent from the HED calculated using a k_m value based on the exact animal weight.

^b Assumes 60 kg human. For species not listed or for weights outside the standard ranges, human equivalent dose can be calculated from the formula: HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)^{0.33}.

^c The k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

^d For example, cynomolgus, rhesus, and stump-tail.

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For the typical species used in nonclinical safety studies, Table 3 also shows the body surface area in m^2 for an animal at a particular *reference* weight. For example, a 400 g guinea pig has a body surface area of approximately $0.05 m^2$. These values come from published sources with surface area determined experimentally by various methods. Compilations of this type of data can be found in published references (Spector 1956).

For animal weights outside the working weight range in Table 3, or for species not included in the table, an alternative method is available for calculating the HED. In these cases the following formula can be used:

$$\text{HED} = \text{Animal dose (mg/kg)} \times [\text{animal weight (kg)} \div \text{human weight (kg)}]^{0.33}$$

For example, assume that a NOAEL of 25 mg/kg was determined in a study using rabbits weighing 4.0 kg. The 4.0 kg animals are outside the working range for rabbits of 0.9 to 3.0 kg indicated in Table 3.

$$\text{HED} = 25 \text{ mg/kg} \times (4.0 \div 60)^{0.33} = 25 \times (0.41) = 10 \text{ mg/kg}$$

Alternatively, if the standard conversion factor was used to calculate the HED

$$\text{HED} = 25 \text{ mg/kg} \div 3.1 = 8.1 \text{ mg/kg}$$

The value of 10 mg/kg for the HED is 25 percent greater than the value of 8.1 mg/kg that would be calculated using the standard conversion factor. For example, assume that a NOAEL of 25 mg/kg was determined in a study using rabbits weighing 4.0 kg. The 4.0 kg animals are outside the working range for rabbits of 0.9 to 3.0 kg indicated in Table 3.

$$\text{HED} = 25 \text{ mg/kg} \times (4.0 \div 60)^{0.33} = 25 \times (0.41) = 10 \text{ mg/kg}$$

Alternatively, if the standard conversion factor was used to calculate the HED

$$\text{HED} = 25 \text{ mg/kg} \div 3.1 = 8.1 \text{ mg/kg}$$

The value of 10 mg/kg for the HED is 25 percent greater than the value of 8.1 mg/kg that would be calculated using the standard conversion factor.

The k_m analysis addresses only half of the HED conversion process. The range of human sizes should also be considered to convert the mg/m^2 dose back to an HED dose in mg/kg. To examine the effect of both animal and human weights on the conversion factor, the principle of allometry was used. Interspecies biologic parameters are often related by the power function $Y = aW^b$ where W is body weight and b (allometric exponent) is the slope of the log-log plot, $\log y = b \times \log W + C$. Using algebraic manipulation (see Appendix C), one can derive an equation for converting an animal dose to the HED based on the body weights of the human and the animals for a given allometric exponent. For converting an animal NOAEL in mg/kg to the HED in mg/kg, the equation is:

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(ii) $HED = \text{animal NOAEL} \times (W_{\text{animal}}/W_{\text{human}})^{(1-b)}$

Since body surface area is believed to scale with an allometric exponent (b) of 0.67, one can explore how the animal and human body weights affect the conversion factor $(W_{\text{animal}}/W_{\text{human}})^{0.33}$.

The conversion factor was calculated over a range of animal weights and a range of human weights from 50-80 kg. The results are summarized in Table 4. Column B is the weight range of the animals used to calculate, in conjunction with the 50-80 kg range in humans, the conversion factor. The extremes of the conversion factors for the permutations chosen are shown in columns C and D. The proposed standard conversion factors are shown in column E. The percentage difference of these extremes from the standard is shown in column F. Finally, the range of animal weights that produced a conversion factor for a 60 kg human within 20 percent of the standard factor is shown in column G. The ± 10 percent and ± 20 percent intervals across the entire range of weights are graphically illustrated for rats in Table 5.

Table 4: Effect of Body Weight on Human Equivalent Dose Conversions^a						
A	B	C	D	E	F	G
Species	Animal Weight Range ^b (kg)	Conversion Factor ^c			% Difference of Extreme ^e from Standard	$\pm 20\%$ Range ^f for 60 kg Human (kg)
		sm animal lg human	lg animal sm human	Standard ^d		
Mouse	0.018-0.033	0.060	0.089	0.081	-22%	0.015-0.051
Rat	0.090-0.400	0.106	0.213	0.162	-35%	0.123-0.420
Rabbit	1.5-3.0	0.269	0.395	0.324	+22%	1.0-3.4
Monkey	1.5-4.0	0.319	0.435	0.324	+34%	1.0-3.4
Dog	6.5-13.0	0.437	0.641	0.541	-19%	4.7-16.2

^a conversion factor = $(W_{\text{animal}}/W_{\text{human}})^{0.33}$

^b human weight range used was 50-80 kg (110-176 lb)

^c HED in mg/kg equals animal dose in mg/kg multiplied by this value

^d See Table 1

^e extreme from column C or D

^f range of animal weights that produced a calculated conversion factor within 20 percent of the standard factor (column E) when human weight was set at 60 kg

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Table 5: Human and Rat Body Weights Producing Body Surface Area Dose Conversion Factors Within 10 Percent and 20 Percent of the Standard Factor (0.162)

EFFECT OF BODY WEIGHT ON BSA-CF							
HED = animal NOAEL · (W_{animal}/W_{human})^bexp(1-b), b = 0.67 for mg/m² conversion							
Standard conversion to mg/kg = 0.162				± 10%	0.146-0.178		
				± 20%	0.130-0.194		
Rat Body Weight (kg)	Human Body Weight (kg)						
	50	55	60	65	70	75	80
0.090	0.124	0.120	0.117	0.114	0.111	0.109	0.106
0.100	0.129	0.125	0.121	0.118	0.115	0.113	0.110
0.110	0.133	0.129	0.125	0.122	0.119	0.116	0.114
0.120	0.137	0.132	0.129	0.125	0.122	0.119	0.117
0.130	0.140	0.136	0.132	0.129	0.126	0.123	0.120
0.140	0.144	0.139	0.135	0.132	0.129	0.126	0.123
0.150	0.147	0.142	0.138	0.135	0.132	0.129	0.126
0.160	0.150	0.146	0.141	0.138	0.134	0.131	0.129
0.170	0.153	0.149	0.144	0.141	0.137	0.134	0.131
0.180	0.156	0.151	0.147	0.143	0.140	0.137	0.134
0.190	0.159	0.154	0.150	0.146	0.142	0.139	0.136
0.200	0.162	0.157	0.152	0.148	0.145	0.141	0.138
0.210	0.164	0.159	0.155	0.151	0.147	0.144	0.141
0.220	0.167	0.162	0.157	0.153	0.149	0.146	0.143
0.230	0.169	0.164	0.159	0.155	0.152	0.148	0.145
0.240	0.172	0.166	0.162	0.157	0.154	0.150	0.147
0.250	0.174	0.169	0.164	0.160	0.156	0.152	0.149
0.260	0.176	0.171	0.166	0.162	0.158	0.154	0.151
0.270	0.179	0.173	0.168	0.164	0.160	0.156	0.153
0.280	0.181	0.175	0.170	0.166	0.162	0.158	0.155
0.290	0.183	0.177	0.172	0.168	0.164	0.160	0.157
0.300	0.185	0.179	0.174	0.179	0.165	0.162	0.158
0.310	0.187	0.181	0.176	0.171	0.167	0.163	0.160
0.320	0.189	0.183	0.178	0.173	0.169	0.165	0.162
0.330	0.191	0.185	0.180	0.175	0.171	0.167	0.163
0.340	0.193	0.187	0.181	0.177	0.172	0.169	0.165
0.350	0.194	0.188	0.183	0.178	0.174	0.170	0.167
0.360	0.196	0.190	0.185	0.180	0.176	0.172	0.168
0.370	0.198	0.192	0.187	0.182	0.177	0.173	0.170
0.380	0.200	0.194	0.188	0.183	0.179	0.175	0.171
0.390	0.202	0.195	0.190	0.185	0.180	0.176	0.173
0.400	0.203	0.197	0.191	0.186	0.182	0.178	0.174
0.410	0.205	0.199	0.193	0.188	0.183	0.179	0.175
0.420	0.207	0.200	0.194	0.189	0.185	0.181	0.177
0.430	0.208	0.202	0.196	0.191	0.186	0.182	0.178
0.440	0.210	0.203	0.197	0.192	0.188	0.183	0.180
0.450	0.211	0.205	0.199	0.194	0.189	0.185	0.181
0.460	0.213	0.206	0.200	0.195	0.190	0.186	0.182

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The following are conclusions from these analyses:

- The ± 20 percent interval around the standard conversion factor includes a broad range of animal and human weights.
- Given that the human weights will vary broadly, it is not usually necessary to be concerned about the affect of the variation of animal weights within a species on the HED calculation.
- If an extreme animal weight is encountered in a toxicology study, one can calculate an accurate conversion factor using $(W_{\text{animal}}/W_{\text{human}})^{0.33}$.

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**APPENDIX C:
Derivation of the Interspecies Scaling Factor $(W_a/W_h)^{(1-b)}$**

Power equation $(mg) = aW^b$
 $\log(mg) = \log(a) + b \cdot \log(W) = b \cdot \log(W) + c$

Given the weights of animal and human, and animal dose in mg/kg, solve for HED in mg/kg:

Let H = mg/kg dose in humans
 A = mg/kg dose in animals
 W_h = weight of human
 W_a = weight of animal

for animal $\log(mg) = \log(a) + b \cdot \log(W_a) = b \cdot \log(W_a) + c$
replace mg $\log(A \cdot W_a) = b \cdot \log(W_a) + c$
solve for c $c = \log(A \cdot W_a) - b \cdot \log(W_a)$
 $= \log(A) + \log(W_a) - b \cdot \log(W_a)$
 $= \log(A) + (1-b)\log(W_a)$

likewise for human $c = \log(H) + (1-b)\log(W_h)$

equate two equations $\log(A) + (1-b)\log(W_a) = \log(H) + (1-b)\log(W_h)$
solve for $\log(H)$ $\log(H) = \log(A) + (1-b)\log(W_a) - (1-b)\log(W_h)$
 $= \log(A) + (1-b)[\log(W_a) - \log(W_h)]$
 $= \log(A) + \log[(W_a/W_h)^{(1-b)}]$
 $\log(H) = \log[A \cdot (W_a/W_h)^{(1-b)}]$

solve for H $H = A \cdot (W_a/W_h)^{(1-b)}$

For example, using mg/m^2 normalization ($b = 0.67$) the predicted human MTD in mg/kg based on a rat LD_{10} in mg/kg is $MTD = LD_{10} \cdot (W_a/W_h)^{0.33}$.

Likewise the HED in mg/kg based on a surface area conversion given an animal NOAEL is $HED = NOAEL \cdot (W_a/W_h)^{0.33}$.

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**APPENDIX D:
Examples of Calculations for Converting Animal Doses
to Human Equivalent Doses**

This appendix provides examples of specific calculations to be taken in deriving an HED based on standardized factors.

Tables 1 and 3 provide standardized conversion factors for changing animal or human doses expressed as mg/kg to doses expressed as mg/m². Tables 1 and 3 also have factors (and divisors) for converting animal doses in mg/kg to the human dose in mg/kg that is equivalent to the animal dose if both were expressed on a mg/m² basis. This human dose in mg/kg is referred to as the HED.

Example 1: Converting to mg/m² HED

To convert an animal or human dose from mg/kg to mg/m², the dose in mg/kg is multiplied by the conversion factor indicated as k_m (for mass constant). The k_m factor has units of kg/m²; it is equal to the body weight in kg divided by the surface area in m².

formula:	$\text{mg/kg} \times k_m = \text{mg/m}^2$
to convert a dose of 30 mg/kg in a dog:	$30 \times 20 = 600 \text{ mg/m}^2$
to convert a dose of 2.5 mg/kg in a human:	$2.5 \times 37 = 92.5 \text{ mg/m}^2$

Example 2: Converting to mg/kg HED in two steps

To calculate the HED for a particular dose in animals, one can calculate the animal dose in mg/m² by **multiplying** the dose in mg/kg by the k_m factor for that species as described in Example 1. The dose can then be converted back to mg/kg in humans by **dividing** the dose in mg/m² by the k_m factor for humans.

formula:	$(\text{animal mg/kg dose} \times \text{animal } k_m) \div \text{human } k_m = \text{human mg/kg dose}$
to calculate the HED for a 15 mg/kg dose in dogs:	$(15 \times 20) \div 37 = 300 \text{ mg/m}^2 \div 37 = 8 \text{ mg/kg}$

Example 3: Converting to mg/kg HED in one step

The calculation in Example 2 can be simplified by combining the two steps. The HED can be calculated directly from the animal dose by **dividing** the animal dose by the ratio of the human/animal k_m factor (third column in Table 1) or by **multiplying** by the ratio of the animal/human k_m factor (fourth column in Table 1).

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Division method

NOAEL	calculation	HED
15 mg/kg in dogs	$\text{mg/kg} \div [k_{\text{mhuman}}/k_{\text{manimal}}]$ $15 \text{ mg/kg} \div 1.8 =$	8 mg/kg
50 mg/kg in rats	$50 \text{ mg/kg} \div 6.2 =$	8 mg/kg
50 mg/kg in monkeys	$50 \text{ mg/kg} \div 3.1 =$	16 mg/kg

Multiplication method

NOAEL	calculation	HED
15 mg/kg in dogs	$\text{mg/kg} \times [k_{\text{manimal}}/k_{\text{mhuman}}]$ $15 \text{ mg/kg} \times 0.541 =$	8 mg/kg
50 mg/kg in rats	$50 \text{ mg/kg} \times 0.162 =$	8 mg/kg
50 mg/kg in monkeys	$50 \text{ mg/kg} \times 0.324 =$	16 mg/kg

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**APPENDIX E:
Selection of Maximum Recommended Starting Dose
for Drugs Administered Systemically to Normal Volunteers**

Step 1

Determine NOAELs
(mg/kg) in toxicity
studies

Is there justification for extrapolating
animal NOAELs to human equivalent dose
(HED) based on mg/kg (or other
appropriate normalization)?

Yes

HED (mg/kg) = NOAEL (mg/kg)
(or other appropriate
normalization)

No

Convert each animal NOAEL
to HED (based on body
surface area; see Table 1)

Step 2

Step 3

Select HED from most
appropriate species

Step 4

Choose safety factor and
divide HED by that factor

**Maximum Recommended
Starting Dose (MRSD)**

Step 5

Consider lowering dose based on a
variety of factors, e.g., PAD



Home > Food > Food Ingredients & Packaging > Generally Recognized as Safe (GRAS)

Food

Agency Response Letter GRAS Notice No. GRN 000076

CFSAN/Office of Food Additive Safety

September 11, 2001

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

Re: GRAS Notice No. GRN 000076

Dear Ms. McColi:

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

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 effect 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/d (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.

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.

000067

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

⁽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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[Home](#) > [Food](#) > [Food Ingredients & Packaging](#) > [Generally Recognized as Safe \(GRAS\)](#)

Food

Agency Response Letter GRAS Notice No. GRN 000208

CFSAN/Office of Food Additive Safety
November 20, 2007

David R. Joy
Keller and Heckman LLP
1001 G Street, N.W.
Suite 500 West
Washington, DC 20001

Re: GRAS Notice No. GRN 000208

Dear Mr. Joy:

This letter corrects a propagated error in the letter issued to you on January 25, 2007, in response to GRAS Notice No. 000208. In that letter, in the middle of the sixth paragraph (now the seventh paragraph in this letter), an unpublished acute study of the fermentation broth is cited. The broth is erroneously described as containing the organism. This letter repeats the text of our letter dated January 25, 2007, with the exception of the description of the fermentation broth, which is corrected.

The Food and Drug Administration (FDA) is responding to the notice, dated July 27, 2006, that you submitted on behalf of Mitsubishi-Kagaku Foods Corporation (Mitsubishi-Kagaku) 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); the GRAS proposal). FDA received the notice on July 31, 2006, filed it on August 3, 2006, and designated it as GRAS Notice No. GRN 000208.

The subject of the notice is erythritol produced through fermentation of glucose by a microorganism known as *Trichosporonoides megachiliensis*. The notice informs FDA of the view of Mitsubishi-Kagaku 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).

Table 1
Mitsubishi-Kagaku's intended conditions of use

Food	Level of Use
Reduced and low-calorie 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

Mitsubishi-Kagaku's erythritol is manufactured by pure culture fermentation of glucose using the non-toxic and non-pathogenic microorganism *T. megachiliensis*. The fermentation broth is heated to kill the culture organisms, and dead cells are separated from the broth by filtration. The supernatant is passed first through ion-exchange resins to remove salts, impurities, and colorants, and then through activated charcoal. The resulting solution is further purified through ultrafiltration, concentrated, crystallized, centrifuged, washed, and air-dried. The resulting erythritol is at least 99.5% pure by high-performance liquid chromatography analysis, and complies with the specifications for erythritol set forth in the Food Chemicals Codex (FCC), 5th Edition⁽¹⁾ (2003). Mitsubishi-Kagaku includes a summary analysis of five batches of its product that are in compliance with the FCC specifications.

Mitsubishi-Kagaku's notification incorporates by reference GRAS Affirmation Petition GRP No. 7G0422 and also relies on data and information previously submitted to FDA to support its evaluation of GRN 000076. Mitsubishi-Kagaku and the notifier for GRN 000076 (Cerestar Holding B.V.; Cerestar) were joint petitioners for GRP 7G0422, which requested that FDA affirm as GRAS certain food uses of erythritol produced using either of the microorganisms *T. megachiliensis* or *Moniliella pollinis*. Cerestar subsequently submitted GRN 000076 to the agency and included expanded uses of erythritol produced using only the microorganism *M. pollinis*.² FDA had no questions about Cerestar's determination that the intended uses of erythritol (produced using *M. pollinis*) are GRAS.

Mitsubishi-Kagaku's current notification, GRN 000208, states that erythritol produced using *T. megachiliensis* is GRAS under the same conditions of use (Table 1) as described in GRN 000076. Mitsubishi-Kagaku considers that the estimated exposure of erythritol from the intended uses in GRN 000208 is therefore identical to the exposure calculated in GRN 000076. Mitsubishi-Kagaku notes that, in FDA's response to GRN 000076, the agency indicated that its own calculations of the estimated daily intake for erythritol under the conditions of use in GRN 000076 are 13 grams per person per day (g/p/d) at the mean and 30 g/p/d at the 90th percentile.

Mitsubishi-Kagaku states that a panel of individuals evaluated the data and information that were the basis for Cerestar's GRAS determination (Cerestar's GRAS panel). Cerestar's GRAS panel had previously published a review accompanying an entire issue of the journal *Regulatory Toxicology and Pharmacology* in 1996 on the safety of erythritol. The published studies in this issue included acute, subchronic, and chronic oral toxicity studies in rats, mice, and dogs, teratogenicity, reproductive, genotoxicity, and metabolic studies, as well as human tolerance studies. Members of Cerestar's GRAS panel were also among the authors of a published 1998 review article on the safety of erythritol. Mitsubishi-Kagaku notes that Cerestar's GRAS panel subsequently reviewed information on expanded levels of use of the ingredient as described in GRN 000076. In addition to the evidence described above, Mitsubishi-Kagaku provides (by reference to GRP 7G0422) an unpublished acute toxicity study in which erythritol fermentation broth (filtered to remove *T. megachiliensis*) was fed to rats. Finally, Mitsubishi-Kagaku notes that erythritol (including that produced by *T. megachiliensis*) has been evaluated as a food ingredient by both the Joint FAO/WHO Expert Committee on Food Additives and the Scientific Committee on Food of the European Union and that neither organization considered it necessary to establish an acceptable daily intake limiting erythritol consumption.

Standards of Identity

In the notice, Mitsubishi-Kagaku states its intention to use erythritol in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Conclusion

Based on the information provided by Mitsubishi-Kagaku, as well as other information available to FDA, the agency has no questions at this time regarding Mitsubishi-Kagaku's conclusion that erythritol (produced by *T. megachiliensis*) 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 Mitsubishi-Kagaku 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 responding to GRN 000208, as well as a copy of the information in this notice that conforms to the information in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying on the homepage of OFAS (on the Internet at <http://www.cfsan.fda.gov/~ird/foodadd.html>).

000069

Sincerely,
Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

⁽¹⁾The FCC monograph for erythritol mentions both *T. megachiliensis* and *Moniliella pollinis*

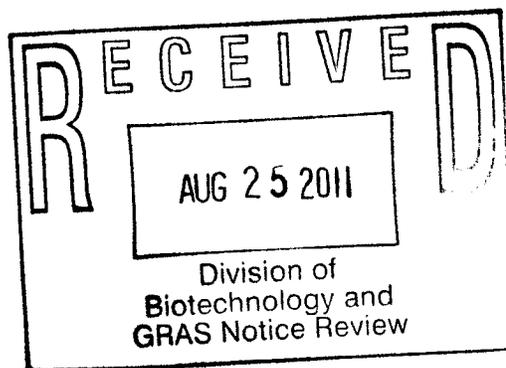
⁽²⁾Cerestar cites the erythritol monograph published in the second supplement to the fourth edition of the FCC. Mitsubishi-Kagaku relies on the monograph published in the fifth edition, which is essentially identical but contains some minor changes in the descriptions of the tests associated with the specification.

Links on this page:

Pages 000071-000294 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

August 24, 2011

Ms. Moraima J. Ramos Valle
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835



Re: GRAS notice for D-psicose as an ingredient in foods

Dear Ms. Ramos Valle,

Thank you very much for your kind phone call of today.

We have modified the document for clarifications. Please replace the first 10 pages with the enclosed printouts to file the GRAS notice. I am also sending you 3 CD Roms which contain modified documents and references. I apologize for the inconvenience.

If you have any questions or concerns, please contact me.

Sincerely,

(b) (6)

Susan Cho, Ph.D.
Chief Science Officer
NutraSource, Inc.
6309 Morning Dew Ct.
Clarksville, MD 21029
Phone: 301-875-6454
E mail: susanscho1@yahoo.com

August 25, 2011

Dr. Susan Carlson
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835

Re: GRAS exemption claim for D-psicose as an ingredient in foods

Dear Dr. Carlson,

This is to notify you that CJ Cheiljedang (based in S. Korea) claims that the use of the substance described below (D-psicose) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because CJ Cheiljedang has determined such use to be Generally Recognized As Safe (GRAS).

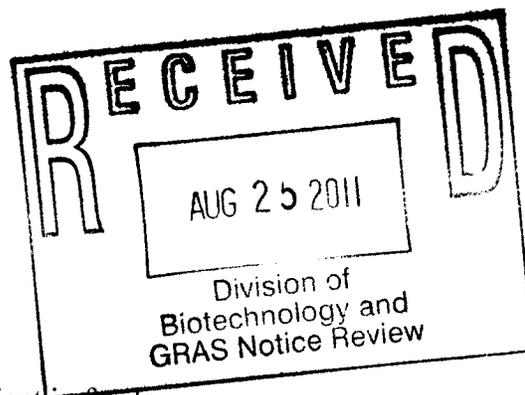
On behalf of CJ Cheiljedang, NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended uses of D-psicose. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. Joanne Slavin (The University of Minnesota, St Paul, MN), and Dr. George Fahey (The University of Illinois, Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of D-psicose (that is manufactured by CJ Cheiljedang, S. Korea) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel also concluded in its August 2011 statement that the intended use of CJ Cheiljedang's D-psicose (to be used as an ingredient in foods such as sugar substitutes [carrier], coffee mix, medical foods, and various low-calorie foods including low-calorie rolls, cake, pie, pastries, and cookies, low-calorie fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, frozen dairy desserts [ice cream, soft serve, sorbet; low-calorie], carbonated and non-carbonated beverages [reduced- and low-calorie], soft candies [non-chocolate, plain chocolate, chocolate coated; low-calorie], yogurt [regular and frozen; low-calorie], ready-to-eat cereals [<5% sugar], and chewing gum) is safe and GRAS for the general population. This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section 170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

Notifier's name and Address: CJ Cheiljedang, Inc.
Attention: Daniel Oh (E mail address: gethero@cj.net)
Address: Namdaemunro 5-ga, Jung-gu, Seoul, Korea
Phone number: +82-2-726-8317; Fax number: +82-2-726-8319

August 25, 2011

Dr. Susan Carlson
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835



Re: GRAS exemption claim for D-psicose as an ingredient in foods

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Notifier's name and Address: CJ Cheiljedang, Inc.
Attention: Daniel Oh (E mail address: gethero@cj.net)
Address: Namdaemunro 5-ga, Jung-gu, Seoul, Korea
Phone number: +82-2-726-8317; Fax number: +82-2-726-8319

Name of GRAS substance: D-Psicose (Common or trade name: Psicose or pseudo-fructose).

Product description: D-Psicose is a ketohexose, an epimer of D-fructose isomerized at C-3. D-Psicose differs from fructose only in the positioning of the hydroxyl group on the third carbon. D-Psicose is 70% as sweet as sucrose. D-psicose can be used as a sugar substitute or a food formulation aid. D-Psicose provides several health benefits to consumers: 1) it provides approximately 0.2 kcal/g to the diet, and 2) it attenuates a glycemic response. D-Psicose has a history of use in foods with no reported adverse effects.

The LD₅₀ value of D-psicose, 15.9-16.3 g/kg, is comparable to those of fructose (14.7 g/kg) and erythritol (15.3 g/kg) and is much higher than that of table salt (3.0 g/kg). These high LD₅₀ values (over 15 g/kg BW) belong to the “relatively harmless” category (the lowest toxicity rating), according to a toxicity rating chart. Thus, D-psicose is classified as an ordinary carbohydrate substance and the use of psicose in foods and beverages is not expected to pose a safety concern.

Specifications:

Table 1. Specifications of D-psicose

Composition	Specification
D-Psicose	>98.5% (wt/wt)
D-Fructose and other sugars	<1% (wt/wt)
Moisture	<1% (wt/wt)
Ash	<0.1% (wt/wt)
Total plate count	<10,000 CFU/g
Coliforms	negative
<i>Salmonella</i>	negative
Lead	<1.0 ppm
As	<1.0 ppm
Physical appearance	White crystal

Applicable conditions of use of the notified substance

Intended food applications include sugar substitutes (carrier), coffee mix, medical foods, and various low- calorie or dietetic foods including low-calorie rolls, cake, pie, pastries, and cookies, fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, frozen dairy desserts (ice cream, soft serve, sorbet), carbonated beverages, non-carbonated beverages, reduced- and low-calorie, soft candies (non-chocolate, plain chocolate, chocolate coated), yogurt (regular and frozen), ready-to-eat cereals (<5% sugar), and chewing gum. The proposed use levels of D-psicose are presented in Table 2.

Table 2. Proposed food application of D-psicose and maximum levels of use

Food category	Maximum level, %
Rolls, cake, pie, pastries, and cookies, dietetic or low calorie	10
Chewing gum	50
Fat-based cream used in modified fat/calorie cookies, cakes, and pastries, low calorie	10
Hard candies, low calorie (including pressed candy, mints)	70
Frozen dairy desserts (regular ice cream, soft serve, sorbet), low-calorie	5
Carbonated beverages, low-calorie	2.1
Non-carbonated beverages, reduced- and low-calorie	2.1
Soft candies, low-calorie (non-chocolate, plain chocolate, chocolate coated)	25
Sugar substitutes (carrier)	100
Yogurt (regular and frozen), low-calorie	5
Medical foods	15
Ready-to-eat cereals (<5% sugar)	10
Coffee mix	30

Exposure estimates

Assuming that 10% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use of D-psicose are estimated to be 1.1 g/d (or 15.4 mg/kg BW/d) for all persons and 2.8 g/d (or 35.8 mg/kg BW/d) for all users of one or more foods. Even if 100% of the foods will be used at the maximum levels under the intended use, although it is far from a realistic situation, the 90th percentile intakes are 11.2 g/d (or 154 mg/kg BW/d) for all persons and 28.5 g/d (or 358 mg/kg BW/d) by all users of one or more foods.

These levels are much lower than the no-observed-adverse-effect level (NOAEL) value (8,530 mg/kg BW/d) that has been found from animal toxicity studies. Also, these estimated daily exposure levels are far below the maximum tolerable value of 500-600 mg/kg BW/d that has been found from human clinical studies.

Basis of GRAS determination

Through scientific procedures.

Review and copying statement

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of CJ Cheiljedang, Inc. or Nutrasource, Inc.

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me.

Sincerely,

Susan Cho, Ph.D.
Chief Science Officer
NutraSource, Inc.
6309 Morning Dew Ct.
Clarksville, MD 21029
Phone: 301-875-6454
E mail: susanscho1@yahoo.com

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(b) (6)

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E mail: susanscho1@yahoo.com

Conclusion of the expert panel:**Generally recognized as safe (GRAS) determination for the addition of D-psicose to foods**

August, 2011

CONCLUSION

We, the undersigned expert panel members, Susan Cho, Ph.D., George Fahey, Ph.D., and Joanne Slavin, Ph.D, have individually and collectively critically evaluated the materials summarized in the D-psicose GRAS report and conclude that D-psicose, a monosaccharide, is safe and GRAS for its intended use in foods and beverages.

There is broad-based and widely disseminated knowledge concerning the chemistry of D-psicose, a monosaccharide. D-Psicose is well characterized and free of chemical and microbial contamination. D-Psicose will be used as a food ingredient. Intended food applications include sugar substitutes (carrier), coffee mix, medical foods, and various low- calorie foods including low-calorie rolls, cake, pie, pastries, and cookies, low calorie fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, frozen dairy desserts (ice cream, soft serve, sorbet; low calorie), carbonated and non-carbonated beverages (reduced- and low-calorie), soft candies (non-chocolate, plain chocolate, chocolate coated; low calorie), yogurt (regular and frozen; low calorie), ready-to-eat cereals (<5% sugar), and chewing gum.

Assuming that 10% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes from the intended use of D-psicose are 1.1 g/d (or 15.4 mg/kg BW/d) for all persons and 2.8 g/d (or 35.8 mg/kg BW/d) for all users of one or more foods. Even if all the foods will be under the intended use, although it is far from a realistic situation, the 90th percentile intakes are 11.2 g/d (or 154 mg/kg BW/d) for all persons and 28.5 g/d (or 358 mg/kg BW/d) by all users of one or more foods. These levels are much lower than the no-observed-adverse-effect level (NOAEL) value (8,530 mg/kg BW/d) that has been found from animal toxicity studies. Also, these estimated daily exposure levels are far below the maximum tolerable value of 500-600 mg/kg BW/d that has been found from human clinical studies. The LD₅₀ value of D-psicose, 15.9-16.3 g/kg, is comparable to those of fructose (14.7 g/kg) and erythritol (15.3 g/kg) and is much higher than that of table salt (3.0 g/kg). These high LD₅₀ values (over 15 g/kg BW) belong to the “relatively harmless” category (the lowest toxicity rating), according to a toxicity rating chart. Thus, D-psicose is classified as an ordinary carbohydrate substance and the use of psicose in foods and beverages is not expected to pose a safety concern.

There are no indications of significant adverse effects related to D-psicose in the publicly available literature. The proposed food use results in exposure at levels significantly below those associated with any adverse effects. Therefore, not only is the proposed use of D-psicose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR).

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

Signature: _____ Date: _____

George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana, IL 61801

Signature: _____ Date: _____

Joanne Slavin, Ph.D.
Professor, University of Minnesota, St Paul, MN

Signature: _____ Date: _____

Susan Cho, Ph.D.
President, NutraSource, Inc., Clarksville, MD 21029

(b) (6)
Signature: _____ Date: 8/17/2011

George C. Fahey, Jr., Ph.D.
Professor, University of Illinois, Urbana, IL 61801

(b) (6)
Signature _____ Date: 8/10/11

Joanne Slavin, Ph.D.
Professor, University of Minnesota, St. Paul, MN 55108

(b) (6)
Signature _____ Date: 8-11-11

Identity of substance

A. Common or trade name: D-psicose, D-allulose, or pseudo-fructose

B. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

C. Background:

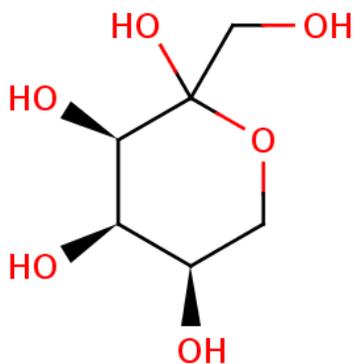
D-Psicose is a monosaccharide, an epimer of D-fructose isomerized at C-3 (Karabinos, 1952).

Chemical name is D-ribo-2-ketohexose

MW=180.16

Molecular formula: C₆H₁₂O₆

CAS Registry ID; 551-68-8



D-Psicose is 70% as sweet as sucrose, but it has just 0.2 kcal/g. Thus, it belongs to the non-digestible carbohydrate category. It is odorless, white or almost white, and non-hygroscopic. D-Psicose is a naturally occurring monosaccharide present in small quantities in food products.

D. Manufacturing Process

- 1) The powder or syrup form of fructose is dissolved in clean water (>40% solids concentration) in a reception tank.
- 2) The neutralized syrup is mixed with manganese chloride (1 mM; 50°C) and then subjected to an immobilized cell system (calcium alginate gel bead with *Corynebacterium glutamicum* [non-viable cell] harboring D-psicose 3-epimerase [DPE] originated from *Agrobacterium tumefaciens*). It takes 4-8 h at 50°C to convert D-fructose to D-psicose.
- 3) For decolorization, the D-psicose solution is mixed with 1% active carbon for 30 min in a stirred tank. The liquid undergoes pressure filtration (55-60°C, pH > 4.5) to clarify it.

- 4) The decolorized syrup is cooled to $\leq 40^{\circ}\text{C}$, then treated through an ion exchange process (i.e., cation column with strongly acidic cationic exchange resin; anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly-based acid and strongly basic resins) to remove any impurities (e.g., calcium, manganese, chloride, and other ionic components, including amino acids, peptides, and proteins). The exchange beds are monitored for pH and color every 8 h; real-time conductivity is monitored automatically.
- 5) Following ion exchange purification, the D-psicose solution is concentrated with an evaporator to produce syrup (syrup density of 60° Brix [Bx]).
- 6) This concentrated syrup is pumped into a separation chromatography system to separate D-psicose from other sugars (fructose). This process dilutes the D-psicose solution to a density of $8\text{-}15^{\circ}$ Bx.
- 7) Using an evaporator, the solution is concentrated to the final density of $80\text{-}85^{\circ}$ Bx.
- 8) The final concentrated product is pumped into a batch continuous crystallizer (90 h of retention time).
- 9) The crystalline D-psicose is separated by basket centrifugation for 45 min, washed by spraying distilled water, and finally dried in a rotary dryer.

Quality assurance procedure: Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry. All processing aids used in the manufacturing process are food grades.

Safety of enzymes: The enzyme is non-toxicological and non-pathogenic. Acute toxicity studies showed that NOAEL of the enzyme was 2,000 mg/kg/d, the maximum level tested. No abnormalities were observed from an *in vitro* chromosome aberration test or bacterial mutation tests with *S. typhimium* (TA 98, 100, 1535, and 1357; up to 5,000 ug/plate) and *E. coli* WP2uvrA, with and without S-9 mix activation.

E. Specifications

Table 1. Specifications of D-psicose

Composition	Specification
D-Psicose	>98.5% (wt/wt)
D-Fructose and other sugars	<1% (wt/wt)
Moisture	<1% (wt/wt)
Ash	<0.1% (wt/wt)
Total plate count	<10,000 CFU/g
Coliforms	negative
<i>Salmonella</i>	negative
Lead	<1.0 ppm
As	<1.0 ppm
Physical appearance	White crystal

F. Analytical method for psicose

Psicose is analyzed by HPLC with a refractive index detector.

The analytical conditions are as follows:

- (1) Column : Bio-Rad Carbohydrate Amine®HPX-87C, 300 mm×7.8 mm (Catalog #125-0095) or equivalent
- (2) Detector : Refractive index, RI detector
- (3) Mobile phase : Deionized water (100%)
- (4) Flow rate: 0.6 ml/min
- (5) Column pressure/temperature : 364 psi (26 kg/cm²) / 85°C

II. Natural occurrence and exposure to D-psicose

A. Food sources of D-psicose

D-Psicose is a naturally occurring monosaccharide present in small quantities in natural products, particularly in sweets such as caramel sauce, maple syrup, brown sugar, processed cane and beet molasses, and wheat (Table 2; Matsuo et al., 2001b; Oshima et al., 2006).

Table 2. D-psicose content in foods (adopted from Oshima et al., 2006)

Item	mg/100 g food
Confectionary products	
sponge cake	11.0
Corn-snack	47.0
rice cracker	27.3
cookie	26.7
Brown sugar drop	76.5
fried dough cake	95.6
Chocolate-chip cookie	6.4
Cereal	2.2
Dishes	
Fish broiled with soy	39.1
Simmered dishes of dried radish strips	8.1
Fermented soybeans	7.8
Seasonings and beverages	
Caramel sauce	83.0
Brown sugar	71.1
Meat sauce	15.8
Demiglace	16.3
Maple syrup	57.9
Ketchup	39.8
Worcester sauce	130.6
Coke	38.3
Coffee	0.5

Fruit juice	21.5
Tomato juice	2.4
Fruits	
Dried fig	29.6
Dried kiwi fruit	9.4
Raisin	38.7
Canned peaches	1.5
Can of mandarin oranges	8.4
Canned cherries	2.0

B. Intended use

D-Psicose is intended to be used as a food ingredient. Intended food uses and use levels are summarized in Table 3. Considering its technological properties (e.g., functions as a sweetener, humectant, flavor enhancer) and nutritional benefits (such as low calorie and glycemic control), D-Psicose is expected to be used as a sugar substitute (carrier). Intended applications include sugar substitutes, coffee mix, medical foods, and various low-calorie foods including low-calorie rolls, cake, pie, pastries, and cookies, fat-based cream used in modified fat/calorie cookies, cakes and pastries, hard candies including pressed candy, mints, frozen dairy desserts (ice cream, soft serve, sorbet), carbonated beverages, non-carbonated beverages, soft candies (non-chocolate, plain chocolate, chocolate coated), yogurt (regular and frozen), ready-to-eat cereals (<5% sugar) and chewing gums. Please note: The intended use levels for psicose are much lower than those for erythritol (outlined in the GRN 208).

Table 3. Proposed food applications of D-psicose and maximum levels of use

Food category	Maximum level, %
Rolls, cake, pie, pastries, and cookies, dietetic or low calorie	10
Chewing gum	50
Fat-based cream used in modified fat/calorie cookies, cakes, and pastries	10
Hard candies, low calorie (including pressed candy, mints)	70
Frozen dairy desserts (regular ice cream, soft serve, sorbet), low-calorie	5
Carbonated beverages, low-calorie	2.1
Non-carbonated beverages, reduced- and low-calorie	2.1
Soft candies, low-calorie (non-chocolate, plain chocolate, chocolate coated)	25
Sugar substitutes (carrier)	100
Yogurt (regular and frozen), low-calorie	5
Medical foods	15
Ready-to-eat cereals (<5% sugar)	10
Coffee mix	30

C. Current consumer intake levels

Since the D-psicose level in each food is not listed in the USDA food composition tables and the National Health and Nutrition Examination Survey (NHANES) databases, the current exposure levels from food sources were not estimated.

D. Exposure estimates under the intended use

Using food intake data reported in the 2005-2008 NHANES, exposure levels to D-psicose that will result from the intended uses were estimated (Table 4). The most recent NHANES (2005-2008) compiled by the National Center for Health Statistics and the Nutrition Coordinating Center was used to calculate exposure estimates. The NHANES was conducted between 2005-2008 with non-institutionalized individuals in the U.S. The NHANES provides the most current food consumption data available for the American population. The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations. For this study 1 g is considered equivalent to 1 ml for soft drinks and formula diets for meal replacement. The NHANES does not include consumption levels of chewing gum. Thus, marketing survey data were used in exposure estimates: Average Americans eat 0.815 kg of gum/y (or average daily consumption of 2.29 g/person) and approximately 40% of chewing gums are sugar-free. SUDAAN v10.0 with day 1 dietary weights were used to calculate mean, 90th percentile, and standard errors (SE) for D-psicose exposure.

Even if all the foods will be under the intended use, although it is far from a real world situation, the 90th percentile intakes including D-psicose from the intended use by the population and by users of one or more foods are 11.2 and 28.5 g/d, respectively. These levels correspond to 154 and 358 mg/kg BW/d for the all population, and all users (Tables 4-1 and 4-3).

From a marketing perspective, an assumption that 10% of the product will be used at the maximum levels for each food category is a highly optimistic projection. It is due to the fact that the functional foods (claiming any health benefits of foods) market size is estimated to comprise approximately 5% of total food expenditures in the U.S. (Price Waterhouse Coopers, 2009). Assuming that 10% of the product will be used at the maximum levels under the intended use, the 90th percentile intakes including D-psicose from the intended by all persons and by users of one or more foods are 1.1 and 2.8 g/d, respectively. These levels correspond to 15.4 mg/kg BW/d for the all persons and 35.8 mg/kg BW/d for all users (Tables 4-2 and 4-4). These levels are much lower than the NOAEL values of 10,000 mg/kg BW/d which was found in animal toxicity studies. Also, these estimated daily exposure levels are far below the maximum tolerable value of 500 mg/kg BW/d that has been found from human clinical studies.

Both D-Psicose and erythritol can be used as replacements for sugars. Due to similarity in their attributes (such as a low calorie sweetener), either D-psicose or erythritol may be used in food formulations. The US consumption of all types of sugar alcohols (sorbitol, erythritol, maltitol and xylitol) was estimated at 376,640 tons (or 3.43 g/person/d; Food Navigato5, 2005), of which sorbitol made up the largest percentage, with more than 54% (corresponding to 1.85 g/person/d) of the total sugar alcohol production. Actual US consumption of D-psicose was estimated to be much lower than the figure for sorbitol. Thus, our exposure estimates (1.1 g/d for all persons and 2.8 g/d for all users) based on the 10% market share assumption might be closer to a real world situation.

Table 4-1. Daily exposure estimates of D-psicose for the all persons: Assuming all the foods will be used at the maximum levels

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	3.23	0.12	11.18	0.56	43.2	1.5	153.8	6.2
1-3 Y	0.54	0.05	1.56	0.19	39.7	3.5	122.4	12.0
0-12 Y	0.74	0.07	1.99	0.19	28.5	2.1	88.0	9.2
13-18 Y	1.18	0.11	3.92	0.93	18.4	1.7	60.0	14.7
19+ Y	4.07	0.15	14.90	0.40	49.7	1.8	180.4	7.1
Males								
0+ Y	3.07	0.14	10.08	0.83	37.4	1.5	135.6	7.0
1-3 Y	0.47	0.06	1.56	0.24	34.6	4.2	121.0	15.9
0-12 Y	0.74	0.10	1.69	0.33	27.6	3.1	80.7	15.8
13-18 Y	1.12	0.14	1.78		16.5	2.2	27.5	
19+ Y	3.90	0.18	14.46	0.61	42.5	2.0	153.4	8.3
Females								
0+ Y	3.39	0.16	12.07	0.67	48.7	2.3	173.9	9.9
1-3 Y	0.62	0.09	1.58	0.31	44.9	6.5	123.6	24.0
0-12 Y	0.75	0.09	2.20	0.25	29.4	2.7	97.1	11.7
13-18 Y	1.24	0.16	4.85	1.17	20.3	2.7	83.7	16.0
19+ Y	4.23	0.20	14.91	0.59	56.3	2.7	199.9	8.8

SE=standard error; Med.=Median; P90=90th percentile

Table 4-2. Daily exposure estimates for all persons; after market share adjustment (assuming 10% of the foods will be used at the maximum levels, i.e., 10% of the market share at the maximum levels)

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	0.32	0.01	1.12	0.06	4.3	0.2	15.4	0.6
1-3 Y	0.05	0.00	0.16	0.02	4.0	0.4	12.2	1.2
0-12 Y	0.07	0.01	0.20	0.02	2.9	0.2	8.8	0.9
13-18 Y	0.12	0.01	0.39	0.09	1.8	0.2	6.0	1.5
19+ Y	0.41	0.01	1.49	0.04	5.0	0.2	18.0	0.7
Males								
0+ Y	0.31	0.01	1.01	0.08	3.7	0.2	13.6	0.7
1-3 Y	0.05	0.01	0.16	0.02	3.5	0.4	12.1	1.6
0-12 Y	0.07	0.01	0.17	0.03	2.8	0.3	8.1	1.6
13-18 Y	0.11	0.01	0.18		1.7	0.2	2.7	
19+ Y	0.39	0.02	1.45	0.06	4.3	0.2	15.3	0.8
Females								
0+ Y	0.34	0.02	1.21	0.07	4.9	0.2	17.4	1.0
1-3 Y	0.06	0.01	0.16	0.03	4.5	0.6	12.4	2.4
0-12 Y	0.07	0.01	0.22	0.02	2.9	0.3	9.7	1.2
13-18 Y	0.12	0.02	0.48	0.12	2.0	0.3	8.4	1.6
19+ Y	0.42	0.02	1.49	0.06	5.6	0.3	20.0	0.9

SE=standard error; P90=90th percentile

Table 4-3. Daily exposure estimate of D-psicose for all users (assuming all the foods will be used at the maximum levels)

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	12.55	0.24	28.48	0.70	167.7	3.2	358.4	9.3
1-3 Y	3.20	0.26	6.58	0.66	237.2	19.5	483.4	91.4
0-12 Y	5.38	0.39	11.92	1.31	208.8	11.6	436.0	36.7
13-18 Y	9.48	0.50	19.21	1.79	147.1	9.0	312.1	21.4
19+ Y	13.49	0.29	29.81	0.57	164.3	3.5	355.5	9.5
Males								
0+ Y	13.59	0.40	30.00	1.10	166.1	4.4	359.9	11.3
1-3 Y	2.84	0.23	6.53	0.69	208.1	16.9	466.9	70.2
0-12 Y	5.98	0.62	12.60	1.40	225.7	16.8	467.7	50.1
13-18 Y	10.05	0.82	20.98	3.42	148.6	13.7	326.8	40.1
19+ Y	14.68	0.49	31.80	1.42	160.1	5.2	348.9	12.6
Females								
0+ Y	11.78	0.28	26.08	0.94	168.8	4.1	356.5	12.1
1-3 Y	3.54	0.46	6.80	2.63	266.4	34.3	558.1	213.9
0-12 Y	4.88	0.41	11.13	1.69	194.8	11.8	390.2	32.9

13-18 Y	9.01	0.68	18.34	1.78	145.8	11.5	308.3	31.4
19+ Y	12.62	0.33	27.36	1.04	167.3	4.4	358.4	14.1

Table 4-4. Daily exposure estimates for all users after market share adjustment (assuming 10% of the foods will be used at the maximum levels, i.e., 10% of the market share at the maximum levels)

	g/d				mg/kg BW/d			
	Mean	SE	P 90	SE	Mean	SE	P 90	SE
All gender								
0+ Y	1.26	0.02	2.85	0.07	16.8	0.3	35.8	0.9
1-3 Y	0.32	0.03	0.66	0.07	23.7	2.0	48.3	9.1
0-12 Y	0.54	0.04	1.19	0.13	20.9	1.2	43.6	3.7
13-18 Y	0.95	0.05	1.92	0.18	14.7	0.9	31.2	2.1
19+ Y	1.35	0.03	2.98	0.06	16.4	0.3	35.5	0.9
Males								
0+ Y	1.36	0.04	3.00	0.11	16.6	0.4	36.0	1.1
1-3 Y	0.28	0.02	0.65	0.07	20.8	1.7	46.7	7.0
0-12 Y	0.60	0.06	1.26	0.14	22.6	1.7	46.8	5.0
13-18 Y	1.00	0.08	2.10	0.34	14.9	1.4	32.7	4.0
19+ Y	1.47	0.05	3.18	0.14	16.0	0.5	34.9	1.3
Females								
0+ Y	1.18	0.03	2.61	0.09	16.9	0.4	35.6	1.2
1-3 Y	0.35	0.05	0.68	0.26	26.6	3.4	55.8	21.4
0-12 Y	0.49	0.04	1.11	0.17	19.5	1.2	39.0	3.3
13-18 Y	0.90	0.07	1.83	0.18	14.6	1.2	30.8	3.1
19+ Y	1.26	0.03	2.74	0.10	16.7	0.4	35.8	1.4

SE=standard error; P90=90th percentile

III. Basis for GRAS determination

A. Current regulatory status

Currently, D-psicose does not have a GRAS (generally recognized as safe) status by the United States Food and Drug Administration. However, other monosaccharides, such as fructose, glucose, galactose, and tagatose, are considered as GRAS substances. Also, erythritol, which has similar metabolism, LD₅₀, NOAEL, and energy values, has been recognized as a GRAS substance by FDA (GRNs 76 and 208). Other low-calorie sweeteners, such as isomaltulose (GRN 184), isomalto-oligosaccharides (GRN 246), mannitol (Food additive permitted on an interim basis pending additional study, 21 CFR 180.25), sorbitol, and xylitol, also are recognized as GRAS.

B. Intended technical effects

D-psicose will be used as a food ingredient for low calorie and/or dietetic foods.

C. Review of safety data

The metabolism, energy value, and toxicity study results for D-psicose are similar to those of erythritol, a GRAS ingredient (GRNs 76 and 208). Both D-psicose and erythritol have an energy

value of approximately 0.2 kcal/g. The LD₅₀ values of the two compounds are comparable; 16.3 g/kg for D-psicose and 15.3 g/kg for erythritol (Matsuo et al., 2002; Yamamoto et al., 1987).

1. Metabolism

Several experiments on the absorption, distribution, metabolism, and excretion of D-psicose in rats have been reported. About 98% of intravenously administered D-psicose is excreted in the urine within 6 h (Whistler et al., 1974). When orally ingested, urinary excretion of unchanged psicose ranged from 11 to 25% (Matsuo et al., 2003). This indicates that D-psicose absorbed in the small intestine may pass into the bloodstream and be excreted in the urine without being significantly metabolized.

Matsuo et al. (2003) investigated the absorption and excretion of D-psicose. The fermentation of D-psicose was measured as cecal short-chain fatty acids (SCFAs) when fed to rats in controlled diets (0, 10, 20, and 30%). Urinary and fecal excretions of D-psicose over 24 h, following a single oral administration, were 11-15% of dosage for the former and 8-13% of dosage for the latter. D-psicose was not detected in urine and feces collected 24-48 h and 48-72 h after administration. Serum D-psicose concentration and D-psicose in the contents of stomach and small intestines decreased progressively after administration. D-Psicose in stomach was 26-37% and 0.4-0.6% of dosage after 1 and 3 h, respectively. D-Psicose in the small intestine was 6-10%, 2-3%, and 1-3% of the dosage after 1, 3, and 7 h, respectively. D-Psicose in the cecum was detected after 3 and 7 h. It was 11-18% and 10-19% of the dosage after 3 and 7 h, respectively. (Matsuo et al., 2003). Continuous administration of D-psicose increased cecal SCFA, as D-psicose is fermented in the cecum by intestinal microflora.

Metabolism of psicose is similar to that of erythritol: A significant portion of erythritol is excreted in urine unmetabolized. In animals and humans, depending on dose, 60-90% of ingested erythritol is rapidly absorbed from the small intestine and excreted unchanged in the urine (from GRN 208; Noda and Oh, 1990,1992; Noda et al., 1996; Oh and Noda, 1992b). No metabolite of erythritol has been found in rats (Noda and Oh, 1992; Noda et al., 1996) or in humans (Noda et al., 1994), indicating that erythritol is not metabolized to a significant extent in the body. Unabsorbed erythritol is fermented to SCFA in the colon (Noda and Oh, 1990, 1992) or is excreted in the feces.

2. Energy values

Based on the results of the plot of breath hydrogen concentration vs. calories ingested, the energy value of d-psicose was predicted to be less than 0.2 kcal/g (Iida et al., 2010). The energy value of erythritol is 0.2 kcal/g (Matsuo et al., 2002b).

3. Animal studies

The LD₅₀ value of D-psicose, 15.8-16.3 g/kg, is comparable to that of other monosaccharides such as fructose (14.7 g/kg) and erythritol (15.3 g/kg), and is much higher than that of table salt (3.0 g/kg). A compound which has a LD₅₀ value of >5 g/kg BW in rats is classified as 'practically non-toxic' and the compound with a LD₅₀ value of >15 g/kg BW as 'relatively harmless' (Altug, 2003). Psicose, like other monosaccharides, belongs to the group which has the lowest toxicity rating.

Subacute and subchronic toxicity studies in rats show that psicose concentration of up to 20% of the diet did not show adverse effects (Table 5). This dietary concentration corresponds to 8,530 mg/kg BW/d (the dose was calculated from the 2009 study of Yagi and Matsuo presenting both % in the diet and corresponding mg/kg BW/d) or 10,000 mg/kg BW/d (the dose was calculated using conversion data from FDA, 1993). Chronic toxicity studies showed that psicose at the dose of 1,280 mg/kg BW/d, the maximum level tested, did not show adverse effects (Yagi and Matsuo, 2009).

The NOAEL values of 8,530 and 1,280 mg/kg BW/d in rats may correspond to 1,376 and 206 mg/kg BW/d, or 82.5 and 12.4 g/d in humans when these animal doses were converted into human equivalent doses as shown in the 2005 FDA's 'Guidance for industry: Estimating for the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers' (FDA, 2005). The data from animal toxicity studies indicate that human daily consumption of psicose up to 1,376 mg/kg BW or 82.5 g would not pose a major safety concern. All the toxicity data indicate that D-psicose is an ordinary monosaccharide and an ordinary carbohydrate.

Table 5. Summary of toxicity studies

Species	Dosage	Length	Primary endpoints and NOAEL	Reference
Male rat	8, 11, 14, 17, and 20 g/kg	Single dose	Acute toxicity-LD ₅₀ , 16.3 g/kg BW	Matsuo et al., 2002
Young rat	10, 20, 30, and 40% in the diet	34 d	Feed intake, wt gain, organ wt; up to 20% in the diet (corresponding to 10,000 mg/kg BW/d)	Matsuo et al., 2002
Male rat	1,280 mg/kg BW/d	12-18 mo	Feed intake, wt gain, organ wt, serum biochemistry, hematology, histology, 1,280 mg/kg BW/d	Yagi and Matsuo 2009

3.1. Acute toxicity

In the acute administration test (Matsuo et al., 2002), five groups of 8 male Wistar rats (3 wk old) were orally given D-psicose in doses of 8, 11, 14, 17, and 20 g/kg BW. Three rats receiving 14 g/kg, three rats receiving 17 g/kg and eight rats receiving 20 g/kg of D-psicose died within 2 d after administration. The calculated LD₅₀ values were 16.3 g/kg by the Behrens-Karber method and 15.8 g/kg by the Litchfield-Wilcoxon method. As shown in Table 6, the LD₅₀ value of psicose is comparable to those of fructose (14.7 g/kg) and erythritol (15.3 g/kg), and much higher than that of table salt (3 g/kg; Sax 1984). A compound that has a LD₅₀ value of 5 g/kg BW or higher in rats is classified as 'practically non-toxic' and the compound with a LD₅₀ value of 15 g/kg BW or higher as 'relatively harmless' (Altug, 2003). Psicose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is classified as an ordinary carbohydrate substance. Thus, the use of psicose in foods and beverages is not expected to pose a safety concern.

Table 6. Comparison of LD₅₀ values in rats

	LD ₅₀ , g/kg BW	Reference
Psicose	16.3	Matsuo et al., 2002
Erythritol (sugar alcohol)	15.3	Yamamoto et al., 1987
Beta-D-fructose	14.7	Sax, 1984
Alpha-D-glucose	25.8	Sax, 1984
D-galactose	Not available	
Sucrose	29.7	Sax, 1984
Maltose	34.8	Sax, 1984
Table salt	3.0	Sax, 1984
Alcohol	7.1	Sax, 1984

3.2. Subacute toxicity in rats

Subacute (34 d) feeding of several concentrations of D-psicose were studied in 4 wk-old Wistar rats. In the subchronic feeding test, eight groups of seven male Wistar rats (3 wk old) were fed diets containing 0 (control), 10, 20, 30, and 40% for 34 d (Matsuo et al., 2002). One rat fed the 30% D-psicose diet and five rats fed the 40% D-psicose diet died during the experimental period. Body weight gain and food efficiency were suppressed by the higher D-psicose concentration. It was concluded that the decreased body weight gain in the 10 and 20% group was attributable to a decrease in food intake, and this was not considered to be of toxicological significance. Surviving rats seemed to be able to adapt, to some extent, to D-psicose feeding, since rats fed the 30 and 40% diet were able to show a recovery in body weight, food intake, and laxation during the first 7 d feeding period. The laxative effect was transient and was not observed after 4 days. Reduced weight gain associated with psicose intakes is not a toxicological concern.

It is well known that nondigestible carbohydrate intakes are associated with body weight reduction or reduced weight gain. The Institute of Medicine report on carbohydrates (IOM, 2002), American Dietetic Association's position paper (ADA, 2002), and USDA Dietary Guideline Committee report (USDA, 2010) acknowledge the efficacy of non-digestible carbohydrates in body weight reduction as a positive attribute that can significantly improve public health in the U.S. Thus, a non-digestible carbohydrate such as psicose also can contribute to improving public health without any adverse effects.

The relative weights of heart, spleen and abdominal adipose tissue were lower as the dietary D-psicose concentration increased. It is due to weight gain reduction associated with D-psicose intakes and is not a toxicological concern. Cecal weight increased with increasing D-psicose concentration in the diets. Cecal hypertrophy was observed in rats fed 10-40% D-psicose diets. However, it should be noted that it is not a toxicological concern. Intake of any dietary fiber in large quantities also results in cecal hypertrophy. Many of the effects were assumed to be secondary to a decrease in food consumption or the consumption of large amounts of a non-nutritive, poorly absorbed, osmotically active substance. The WHO has looked at the relationship

between the consumption of non-nutritive substances in the diet as a cause of decreased weight gain and also reported an association with cecal enlargement (Lu and Sleken, 1991). This was considered to have a causal relationship associated with the physiological response of cecum enlargement induced by diet containing high concentrations of poorly absorbable substances (Cassidy et al., 1981). Enlargement of the cecum is reported to occur frequently in response to feeding poorly-absorbable, osmotically-active substances, such as xylitol and sorbitol to rats (Leegwater et al., 1974), and it is considered that the toxicological significance of this might be minimal. Overall, the NOAEL was found to be 20% in the diet (corresponding to 8,530-10,000 mg/kg BW/d) in rats.

3.3. Chronic toxicity study in rats

Yagi and Matsuo (2009) studied long-term toxicity of D-psicose in male Wistar rats (3 weeks old) fed diets containing 3% D-psicose (or 1,280 mg/kg BW/ d) or 3% sucrose (1,220 mg/kg BW/d) for 12-18 mo. Body weight gain and intra-abdominal adipose tissue weight of rats fed the D-psicose diet for 18 mo were significantly lower than those in rats fed the sucrose diet. Relative weights of liver and kidney were significantly higher in the D-psicose group than in the sucrose group, but it was not considered to be of toxicological significance. It is due to the fact that dietary D-psicose decrease body fat accumulation and increases liver glycogen as a consequence of serum glucose decline and serum insulin elevation. Increased relative weight of liver has been observed in animals fed other type of sugars such as fructose and sucralose.

General hematology or serum chemistry tests were in the normal ranges. All values related to serum chemistry did not differ between the sucrose and D-psicose groups. Mean corpuscular hemoglobin (MCH) at 12 mo was significantly lower in the D-psicose group than in the sucrose group, but no differences were observed in any of the related hematology values. Hemoglobin (Hb) and mean corpuscular volume (MCV) at 18 mo were significantly greater in the D-psicose group than in the sucrose group, but no differences were observed in any of the related hematology values.

The histopathological data demonstrated that there were no toxicologically significant findings in rats given D-tagatose at levels of 3% in the diet for 12-18 mo. No gross pathological findings were evident at dietary doses of 3% D-psicose. The authors concluded that administration of D-psicose at 3% in the diet (or 1,280 mg/kg BW/d) did not result in any adverse effects in rats.

3.4. Animal efficacy studies showing no adverse effects of D-psicose

As shown in Table 7, several animal studies reported no adverse effects of psicose. These animal studies showed that psicose at the level of 5% in the diet (corresponding to up to 2,500 mg/kg BW/d) did not cause any adverse effects.

Table 7. Animal efficacy studies showing no adverse effects of D-psicose

Species	Dosage	Length	Primary endpoints and NOAEL	Reference
Male mice	0.2 g/kg BW/d	4 wk	Glycemic responses, insulin release, and blood lipid profiles, 0.2 g/kg BW/d	Baek et al., 2010
Male rat	5% in the diet	3 wk	Body fat and lipid metabolism, 5% in the diet	Matsuo et al., 2001a
Male rat	5% in the diet	4 wk	Body fat and lipid metabolism, 5% in the diet	Matsuo et al., 2001b
Male rat	5% in the diet	8 wk	Body fat and glycemic responses, 5% in the diet	Matsuo and Izumori, 2006
Male rat	2,000 mg/kg	Single dose	Body fat and glycemic responses, 2 g/kg	Matsuo and Izumori, 2009

3.4.1. A study of Baek et al. (2010)

In the study of Baek et al. (2010), the effects of D-psicose on glycemic responses, insulin release, and lipid profiles were compared with those of D-glucose and D-fructose in a genetic diabetes model. C57BL/6J db/db mice were orally supplemented with 200 mg/kg BW of D-psicose, D-glucose, D-fructose, or water (control), respectively, for 28 d. D-psicose sustained weight gain by about 10% compared to other groups. The initial blood glucose level was maintained at 276 to 305 mg/dL during the 28 d for the D-psicose group, whereas a 2-fold increase was found in the other groups ($P < 0.05$) among diabetic mice. D-psicose significantly improved glucose tolerance and the areas under the curve (AUC) for glucose ($P < 0.05$), but had no effect on serum insulin concentration. The plasma lipid profile was not changed by supplemental monosaccharides. The administration of D-psicose reversed hepatic concentrations of triglyceride (TG) and total cholesterol (TC) by 37.9% and 62.9%, respectively, compared to the diabetic control ($P < 0.05$). No adverse effects were noted.

3.4.2. A study of Matsuo et al. (2001a)

Matsuo et al. (2001a) studied the effects on body fat accumulation of D-psicose compared with cellulose or D-fructose in rats. Wistar male rats were fed experimental diets including 5% D-psicose, cellulose or D-fructose for 21 d. Abdominal adipose tissue weight was lower ($P < 0.05$) in rats fed D-psicose than in those fed D-fructose. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were lower ($P < 0.05$) in rats fed D-psicose, whereas lipoprotein lipase activities in the heart, soleus muscle, perirenal adipose tissue, and subcutaneous adipose tissue did not differ. These results suggest that supplementation of D-psicose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed D-psicose might have resulted from lower lipogenesis in the liver. No adverse effects were reported. The authors concluded that D-psicose could prove to be a good sugar substitute.

3.4.3. A study of Matsuo et al. (2001b).

Wistar male rats were fed experimental diets that consisted of 5% D-psicose, cellulose, D-fructose, or D-glucose for 28 d (Matsuo et al., 2001b). Abdominal adipose tissue weight was lower ($P < 0.05$) in rats fed the D-psicose diet than in rats fed D-fructose and D-glucose diets, even though the four dietary groups were offered the same amount throughout the experimental period. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were lower ($P < 0.05$) in rats fed the D-psicose diet than in rats fed the D-fructose and D-glucose diets. However, lipoprotein lipase activities in the heart, soleus muscle, and perirenal adipose tissue were the same. These results suggest that a supplement of D-psicose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed the D-psicose diet might result from lower lipogenesis in the liver. No adverse effects were reported.

3.4.4. A study of Matsuo and Izumori (2006)

Matsuo and Izumori (2006) studied the effects of supplemental D-psicose in the diet on diurnal variation in plasma glucose and insulin concentrations in rats. Forty-eight male Wistar rats were divided into four groups. Each group except for the control group was fed a diet of 5% D-fructose, D-psicose, or psico-rare sugar (3:1 mixture of D-fructose and D-psicose) for 8 wk. Plasma glucose concentrations were lower and plasma insulin concentrations were higher at all times of the day in the psicose and psico-rare sugar groups than in the control and fructose groups. Weight gain was lower ($P < 0.05$) in the psicose group than in the control and fructose groups. Liver glycogen content, both before and after meals was higher in the psicose group than in the control and fructose groups. These results suggest that supplemental D-psicose can lower plasma glucose concentrations and reduce body fat accumulation. Hence, the authors concluded that D-psicose might be useful in preventing postprandial hyperglycemia in diabetic patients.

3.4.5. A study of Matsuo and Izumori (2009)

Matsuo and Izumori (2009) investigated the effects of D-psicose on the activities of alpha-amylases and alpha-glucosidases *in vitro*, and evaluated the effects of D-psicose on the *in vivo* postprandial glycemic response of rats. Male Wistar rats (6 mo old) were administered 2 g/kg of sucrose, maltose, or soluble starch together with 0.2 g/kg of D-psicose or D-fructose. The D-psicose significantly inhibited the increment of plasma glucose concentration induced by sucrose or maltose. The starch-induced glycemic response tended to be suppressed by D-psicose; however, the suppression was not significant. These results suggest that d-psicose inhibited intestinal sucrase and maltase activities and suppressed the plasma glucose increase that normally occurs after sucrose and maltose ingestion. Thus, D-psicose may be useful in glycemic control. No adverse effects were reported.

3.5. *In vitro* mutagenicity/genotoxicity studies

Results from Ames tests, micronucleus test, and chromosomal aberration test indicate that was not mutagenic or genotoxic (Table 8). D-Psicose also showed neuroprotective effects in hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells, the *in vitro* model of Parkinson's disease (Huntington lab, 2011; Tanaka et al., 2005).

Table 8. Summary of *in vitro* Mutagenicity/Genotoxicity studies

Test	Concentration	Reference
Conventional mutagenicity/genotoxicity studies		
Four histidine-dependent strains of <i>Salmonella typhimurium</i> (TA98, TA100, TA1535, and TA1537) and a tryptophan-dependent strain of <i>Escherichia coli</i> (WP2 urvA(pKM101))	5,000 ug/ml	Huntington lab, 2011
Micronucleus test using CD1 mice	2,000 mg/kg/d	Huntington lab, 2011
Chromosomal aberration test	1,800 ug/ml	Huntington lab, 2011
Apoptosis related genotoxicity effects		
Hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells	50 mM	Takata et al., 2005

3.5.1. Ames test: Four histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and a tryptophan-dependent strain of *Escherichia coli* (WP2 urvA(pKM101)) were used to evaluate the mutagenic potential of D-psicose (up to 5,000 ug/plate). No mutagenic potential of D-psicose was observed.

3.5.2. Micronucleus test: In the micronucleus test using CD1 mice, no significant increase was observed in micronucleated polychromatic erythrocytes (MPCs) at any concentration up to 2,000 mg/kg/d) of D-psicose compared with vehicle control.

3.5.3. Chromosomal aberration test: D-psicose at a dosage of 1,800 ug/mL did not induce an increase in the number of chromosomal aberrations.

3.5.4. Neuroprotective effect of D-psicose on 6-hydroxydopamine-induced apoptosis in rat pheochromocytoma (PC12) cells.

Takata et al. (2005) evaluated the neuroprotective effects of D-psicose on 6-hydroxydopamine (6-OHDA)-induced apoptosis in catecholaminergic PC12 cells, the *in vitro* model of Parkinson's disease (PD). Apoptotic characteristics of PC12 cells were assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL) assay. The results showed that D-psicose at a concentration of 50 mM exerted significant protective effects against 6-OHDA (200 µM)-induced PC12 cell apoptosis, while other sugars had little or no protective effects. A significant increase was observed in the level of intracellular glutathione after 24 h in 6-OHDA (200 µM) treated cells, while a decrease in the level was observed at 3 h and 6 h. Also, a synergistic exposure to D-psicose and 6-OHDA for 24 h showed a significant increase in intracellular glutathione level. Therefore, these results suggest that D-psicose may play a potential role as a neuroprotective agent by inducing an up-regulation of intracellular glutathione. Antioxidant properties of psicose have been demonstrated in several food systems (Sun 2004, 2007).

3.6. In vivo carcinogenicity and genotoxicity studies

In vivo carcinogenicity and genotoxicity studies indicate that psicose is not genotoxic or carcinogenic (Table 9). In particular, psicose did not negatively alter hepatic gene expression (Shirai et al., 2007) and was protective against diethylnitrosamine (DEN)-induced hepatocarcinogenesis (Zeng et al., 2005).

Table 9. *In vivo* genotoxicity and carcinogenicity studies showing no adverse effects of D-psicose

Species	Dosage	Length	Primary endpoints	Reference
Genotoxicity and carcinogenicity				
Male rat	5% in the diet	3-8 wk	Gene expressions of rat liver and skeletal muscle	Shirai et al., 2007
Male rat, 4-wk old, F344	Up to 1% in diet	a rat medium-term bioassay	Diethylnitrosamine (DEN)-induced hepatocarcinogenesis	Zeng et al., 2005
Male rat, S-D	2% in the diet	2 wk	di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury	Suna et al., 2007

3.6.1. Hepatocarcinogenicity bioassay

The effects of D-psicose on diethylnitrosamine (DEN)-induced hepatocarcinogenesis were examined in male F344 rats using a rat medium-term bioassay based on the two-step model of hepatocarcinogenesis (Zeng et al., 2005). The modifying potential was determined by comparing the numbers and areas/cm² of induced glutathione *S*-transferase placental form (GST-P) positive foci in the liver with those of a corresponding group (control) of rats given DEN alone. Increased relative liver weights were found in the 1% D-psicose treatment group as compared with the basal diet group, while no significant change occurred in the 0.1% D-psicose, 0.01% D-psicose, and 1% D-fructose groups. D-psicose did not significantly alter the numbers and area/cm² of GST-P positive liver cell foci observed after DEN initiation. The results demonstrate that D-psicose shows neither promoting nor preventive potential for liver carcinogenesis in a medium-term bioassay, which has been correlated well with those from long-term tests in rats (Ogiso, 1990). The results also indicate that increased relative liver weights are not associated with liver carcinogenicity.

3.6.2. Effects of D-psicose on gene expressions of rat liver and skeletal muscle

Shirai et al. (2007) evaluated gene expression of liver and skeletal muscle in rats after long-term feeding of D-psicose. Thirty-six male Wistar rats were divided into four groups. Each group except for the control group was fed a diet of 5% D-fructose, D-psicose, or a 1:1 mixture of D-fructose and D-psicose for 3-8 wk. Within 7wk of D-psicose intake no significant change in diurnal variation in plasma glucose and insulin concentrations were observed. Lower plasma insulin concentrations and higher liver glycogen contents were observed in the psicose group. In the liver, glucose transporter protein (GLUT) 2 and glucokinase mRNA expression markedly increased in the psicose group compared with the other groups. No significant changes in

GLUT4 and LKB1 expression of gastrocnemius muscle were noted. These results suggest that improvements in serum and liver components by dietary D-psicose were partly influenced by alteration in hepatic gene expression.

3.6.3. Preventive effects of d-psicose on di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury in rats

Suna et al. (2007) investigated the preventive effects of D-psicose on di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury in prepubertal male Sprague-Dawley rats. The rats given a diet-containing 1% DEHP alone for 7-14 d showed severe testicular atrophy accompanied by aspermatogenesis. Pre-treatment with D-psicose at concentrations of 2 and 4% resulted in an almost complete but not absolute suppression of testicular malondialdehyde production for rats administered 2 g/kg of DEHP. The microarray analysis showed the induction of oxidative stress-related genes including the thioredoxin, glutathione peroxidase 1 and 2, and glutaredoixn 1 after 24 h of the DEHP treatment in the testis. These results show that D-psicose prevents DEHP-induced testicular injury by suppressing the generation of reactive oxygen species in the rat testis.

4. Human clinical studies

As shown in Table 10, several human clinical studies reported no adverse effects of D-psicose. Like non-digestible oligosaccharide and fiber ingredients, the only side effect of D-psicose is gastrointestinal discomfort when ingested in large quantities. It is well-known that this type of side effect is transient. Studies done in the early 1900s showed that inulin (a nondigestible carbohydrate) intakes of up to 120-160 g/d were well tolerated (Carpenter and Root, 1928; Leach and Sobolik, 2010; Lewis, 1912; Root and Baker, 1925; Shoemaker, 1927), although recent reports show daily maximum tolerance limits of the same compound have been reduced to 20-40 g (Garleb et al., 1996; Kleesen et al. 1997; Roberfroid and Slavin, 2000). People in the early 20th century consumed large quantities of nondigestible carbohydrates; thus, their gastrointestinal systems were adapted to handle high loads with no major side effects. As consumption levels of nondigestible carbohydrates decreased, human tolerance levels also decreased. Thus, the gastrointestinal symptoms associated with high intakes of non-digestible carbohydrates are considered as a transient symptom which can be improved over time. Recent clinical studies showed that daily D-psicose intakes of up to 31-33 g were well tolerated (Matsuo et al., 2002). The history of non-digestible carbohydrate intakes suggests that humans may be able to adapt to much higher levels of D-psicose without major gastrointestinal symptoms.

Despite potential gastrointestinal discomfort associated with high fiber intakes, the U.S. Institute of Medicine has established Adequate Intake (AI) of total fiber to 14 g/1,000 kcal (or 38 g/d for adult men) to help reduce the risk of chronic diseases of the U.S. population (IOM, 2002). This type of symptom is usually transient and is not considered to be of toxicological significance.

Table 10. Human clinical trials with D-psicose

Dosage	Length	Results	Reference
Up to 0.9 g/kg BW/d	6 d	No gastrointestinal symptoms up to 0.5 g/kg BW/d	Iida et al., 2007
15 g/d (5g, three times a day)	12 wk	Positive impact on glycemic responses; no adverse effects were noted.	Hayashi et al., 2010
7.5 g	Single dose	Positive impact on glycemic and insulinemic responses; No adverse effects were noted.	Iida et al., 2008
Up to 340 mg/kg BW	Single dose	Metabolism study; no adverse effects were noted.	Iida et al., 2010

4.1. A study Hayashi et al. (2010)

Hayashi et al. (2010) conducted a clinical study to investigate the safety and effect of D-psicose on postprandial blood glucose concentrations in adult men and women. A randomized double-blind placebo-controlled crossover experiment was conducted on 17 subjects who consumed 5 g of D-psicose or D-glucose with meals three times a day (or 15 g/d) for 12 weeks. No abnormal effects or clinical problems caused by the continuous ingestion of D-psicose were observed.

4.2. Acute tolerance test in normal adults

Iida et al. (2008) studied the effects of D-psicose on glycemic and insulinemic responses in an oral maltodextrin tolerance test with healthy adults in a crossover study. Twenty subjects aged 20-39 y, 11 males and 9 females, were recruited. A load test of oral maltodextrin was conducted as a randomized single blind study. The subjects took one of five test beverages (7.5 g D-psicose alone, 75 g maltodextrin alone, 75 g maltodextrin +2.5, 5, or 7.5 g D-psicose). Independent administration of 7.5 g D-psicose had no influence on blood glucose or insulin concentrations. No adverse effects of D-psicose were reported.

4.3. Study of Iida et al. 2007

Iida et al. (2007) investigated the effects of D-psicose on gastrointestinal symptoms in healthy volunteers (5 males and 5 females) aged 20-30 y. All subjects ingested 0.4 g/kg BW/d of D-psicose for the first dose. The dosage was increased from by 0.1 g/kg BW/d to 0.9 g/kg BW/d, the maximum dose level. Diarrhea occurred in one male at the dosage of 0.6 g/kg BW/d, 2 females at the dosage of 0.7 g/kg BW/d, and 2 males and 3 females at the dosage of 0.8 g/kg BW/d. Two males did not suffer from diarrhea even at 0.9 g/kg BW/d. Authors concluded that the maximum tolerable levels in humans were 0.5 g/kg BW/d for males and 0.6 g/kg BW/d for females, with the mean value of 0.55 g/ g/kg BW/d. These dosages correspond to 33.3 g/d for males and 31.0 g/d for females. These maximum tolerable levels of D-psicose are similar to that of erythritol (0.66 g/kg BW/d).

IV. Conclusions

The information/data provided by CJ CheilJedang (specifications, manufacturing process, and intended use) in this report and supplemented by the publicly available literature/toxicity data on D-psicose provide a sufficient basis for an assessment of the safety of D-psicose for the proposed use as an ingredient in foods and beverages prepared according to appropriate specifications and used according to GMP.

Key findings are summarized here:

1. D-psicose is well characterized and free from chemical and microbial contamination.
2. The safety and nutritional benefits of D-psicose are well established by human clinical trials and animal studies of D-psicose.
3. Intended use of D-psicose as part of the proposed food use, even at the 90th percentile, results in levels of exposure significantly below those associated with any adverse effects and provides a reasonable certainty of safety.

Therefore, not only is the proposed use of D-psicose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS).

V. Discussion of information inconsistent with GRAS determination

We are not aware of information that would be inconsistent with a finding that the proposed use of D-psicose preparations in foods and beverages, meeting appropriate specifications and used according to GMP, is GRAS.

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