APPLICATION FOR THE APPROVAL OF ISOMALTULOSE

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

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ADMINISTRATIVE DATA

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GENERAL DESCRIPTION

Isomaltulose, an isomer of sucrose, is a reducing disaccharide, composed of a glucose and fructose molecule, joined by a 1,6-glycosidic bond. It is reported to occur naturally at low levels in honey as well as in sugar cane extract (Siddiqua and Furhala, 1967; Takazoe, 1985). In Japan, isomaltulose has been used in yoghurt, chewing gum, and candy since 1985. Annual sales of isomaltulose approximate 3,000 tons. Moreover, isomaltulose is contained on the list of FOSHU approved foods (*i.e.*, foods for a specific health use, also termed functional foods). Commercially, isomaltulose is prepared by the enzymatic rearrangement of sucrose. The biocatalyst sucrose-glucosylmutase, which is utilized to carry out the conversion, is obtained

from the microorganism *Protaminobacter rubrum* (CBS 574.44). Relative to sucrose, it is characterized by a sweetening potential of 42%.

Cerestar proposes to market isomaltulose, for use as a novel food ingredient in Europe. Approval is sought under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredient*s (hereafter referred to as EC 258/97), and accordingly, this submission has been prepared pursuant to the *Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients* (hereafter referred to as the Commission Recommendation of 1997).

Article 1(2) of EC 258/97 states that the regulation "…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community…" and which fall under one of six categories of novel foods and novel food ingredients. The lack of a significant prior history of human consumption in the European Community dictates that isomaltulose will be considered under category (c), pertaining to "foods and food ingredients with a new or intentionally modified primary molecular structure". Isomaltulose is thus considered a novel food/food ingredient.

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee for Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the six classes identified, isomaltulose would be allocated a Class 1 designation (pure chemicals or simple mixtures from non-GM sources), since it is manufactured by conventional methods as a pure chemical, with no use of genetic modification. Isomaltulose is further classified under sub-class 1.2 (the source of the NF has no history of food use in the Community) of the SCF categorization. However, isomaltulose occurs as an intermediate product in the production of isomalt (E953), an additive permitted for use in food, which is manufactured by both Cerestar and Südzucker and involves use of the same microorganism as that used in the preparation of isomaltulose.

IDENTIFICATION OF ESSENTIAL INFORMATION REQUIREMENTS

The structured schemes outlined for the assessment of a class 1.2 novel food ingredient, such as isomaltulose, are listed below and discussed in detail in subsequent sections (Sections I through XIII).

I. Specification of the novel food

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

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

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

XI. Nutritional information on the novel food

XII. Microbiological information on the novel food

XIII. Toxicological information on the novel food

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.

I. SPECIFICATIONS OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- "Is the information representative of the novel food when produced on a commercial scale?"
- "Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These questions have been addressed collectively in Sections I.1 through I.8.

I.1 Common or Usual Name

Isomaltulose

I.2 Chemical Names

6-*O*-α-D-glucopyranosyl-D-fructofuranose, monohydrate

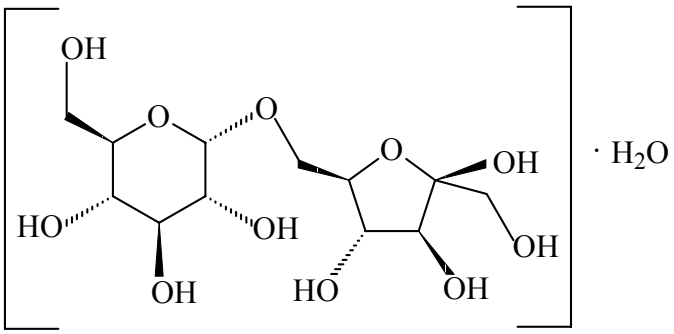
I.3 Trade Names

Palatinose; Lylose

I.4 Chemical Abstract Service (CAS) Number

13718-94-0

I.5 Chemical Structure



I.6 Molecular Formula and Weight

$C_{12}H_{22}O_{11}\cdot H_2O$ 360.3

I.7 Chemical and Physical Properties

Isomaltulose is a white crystalline substance, characterized by a sweetness quality similar to that of sucrose and a melting point of 123 to 124°C (Irwin and Sträter, 1991). Specifically, isomaltulose has about 42% of the sweetness of sucrose, and has been identified to occur naturally at low levels in honey and sugar cane extract (Siddiqua and Furhala, 1967; Takazoe, 1985).

I.8 Product Specifications and Analysis

I.8.1 Product Specifications

The chemical and physical specifications for isomaltulose are presented in Table I.8.1-1. The content of isomaltulose is determined by High Performance Liquid Chromatography (HPLC), loss on drying is determined by the Karl Fischer method, and lead content is determined by Atomic Absorption Spectroscopy.

Table I.8.1-1 Chemical and Physical Specifications for Isomaltulose

Specification Parameter	Specification
Appearance	White crystalline powder
Assay (dry basis)	Not less than 98% isomaltulose
Loss on Drying	Not more than 6.5%
Lead	Not more than 0.1 ppm

I.8.2 Product Analysis

Several lots of the manufactured product were analyzed to verify that the manufacturing process produced a consistent product within the product specifications. A summary of the chemical product analysis for 5 batches of isomaltulose is presented in Table I.8.2-1. See Appendix A for certificates of analysis and analytical methods.

Table I.8.2-1 Chemical and Physical Analyses of Isomaltulose Intended for Use in Food

Analysis	Specifications	Lot Number				
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Appearance	White crystalline powder	Conforms	Conforms	Conforms	Conforms	Conforms
Assay (dry basis)	Not less than 98%	99.06	98.99	98.99	99.11	99.23
Glucose (%)		0.03	0.03	0.03	0.01	0.02
Fructose (%)		0.04	0.04	0.04	0.01	0.03
Isomaltose (%)		0.06	0.06	0.07	0.04	0.07
Trehalose (%)		0.43	0.42	0.39	0.36	0.25
Saccharose (%)		0.01	0.0	0.01	0.0	0.0
Trisaccharides and higher saccharides		0.37	0.46	0.47	0.47	0.40

Table I.8.2-1 Chemical and Physical Analyses of Isomaltulose Intended for Use in Food						
Analysis	**Specifications**	**Lot Number**				
		Batch 1	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**
Loss on Drying	Not more than 6.5%	5.08	5.10	5.10	5.06	5.19
Sulphated Ash		<0.01	<0.01	<0.01	<0.01	<0.01
Lead	Not more than 0.1 ppm	<0.02	<0.02	<0.02	0.021	<0.02

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Does the novel food undergo a production process?"
- "Is there a history of use of the production process for the food?"
- "Does the process result in a significant change in the composition or structure of the NF compared to its traditional counterpart?"

These questions have been addressed collectively in Sections II.1 through II.5.

II.1 Novelty of the Process

Isomaltulose is a reducing disaccharide ($C_{12}H_{22}O_{11}$) produced by an enzymatic conversion of sucrose ($C_{12}H_{22}O_{11}$), whereby the 1,2-glycosidic linkage between glucose and fructose is rearranged to a 1,6-glycosidic linkage. It occurs as an intermediate in the production of isomalt (E953), permitted for use as a nutritive sweetener and marketed under the trade names Palatinit® and C*Isomaltidex® by Südzucker AG (hereafter Südzucker) and Cerestar, respectively. Specifically, the use of isomalt in food was considered acceptable by the Scientific Committee for Food (SCF) in 1984 (SCF, 1984).

The production of isomaltulose by Cerestar is initiated by dissolving food-grade sucrose in water and subsequently treating the resulting solution with a biocatalyst obtained from a non-viable, non-pathogenic strain of *Protaminobacter rubrum* (Porter *et al*., 1991). Prior to the addition of the biocatalyst to the sucrose solution, *P. rubrum* cells are killed by treatment with formaldehyde. Following completion of the conversion of sucrose, residual *P. rubrum* material is removed by filtration. These steps prevent the presence of the production organism in the isomaltulose product. The crude isomaltulose product is then sequentially subjected to several stages of purification, including demineralisation, crystallization, and washing. Drying and cooling of isomaltulose complete the production process, resulting typically in a product of 99% or greater purity.

Isomaltulose was first prepared by Südzucker, as an intermediate compound in the production of isomalt. In particular, the production of isomalt is completed by catalytic hydrogenation following enzymatic rearrangement of the sucrose to isomaltulose. Before progressing to the catalytic hydrogenation, isomaltulose is isolated by concentration of the isomaltulose solution and subsequently purified by crystallization, which in particular removes residual sucrose. On an anhydrous basis this purification method yields isomaltulose, which is typically 98% pure (Irwin and Sträter, 1991; Südzucker, 1996). Südzucker utilizes *P. rubrum* as the source of the biocatalyst required for the rearrangement of sucrose. The microorganism is grown on synthetic media consisting of sucrose, a nitrogen source, and inorganic salts. Subsequently, the cells are isolated, killed by treatment with formaldehyde, and, unlike Cerestar's method, which utilizes filtration to remove any residual cells, immobilized.

More recently, an alternative method for the purification of isomaltulose has been developed by Südzucker, allowing for the marketing of an additional grade of isomalt. Specifically, residual sucrose is removed by enzymatic conversion using *Saccharomyces cerevisiae*. The purity of isomaltulose typically obtained with this method is only approximately 82%. This alternative

method has been submitted for approval in the U.S. (Südzucker, 1996), as well as in Europe, where it was accepted by the SCF (SCF, 1997).

Additionally, isomaltulose is produced and made commercially available by Shin Mitsui Sugar Co. for the Japanese, Korean, and Taiwanese food markets. Furthermore, in Japan, Shin Mitsui Sugar Co. distributes Südzucker's isomalt. The isomaltulose produced by Shin Mitsui Sugar Co. also is prepared from food-grade sucrose *via* enzymatic rearrangement. Similarly to Südzucker, Shin Mitsui Sugar Co. immobilises the biocatalyst (Nakajima, 1984) and there are strong indications that the biocatalyst is obtained from *P. rubrum* (Okuda *et al*., 1986). This is corroborated by Shin Mitsui Sugar Co.'s role as the distributor in Japan of the isomalt produced by Südzucker. The resulting isomaltulose is purified through concentration and crystallization, providing a material of 99% or greater purity on a dry basis (Shin Mitsui Sugar Co., 2003).

A comparison of the chemical characteristics of isomaltulose as produced by Südzucker, Shin Mitsui Sugar Co., and Cerestar is presented below in Table II.1-1.

Table II.1-1 Chemical Comparison of Isomaltulose as Manufactured by Cerestar, Südzucker, and Shin Mitsui Sugar Co.

	Cerestar	**Südzucker (concentration and crystallisation)**	**Südzucker (*Saccharomyces cerevisiae*)**	**Shin Mitsui Sugar Co.**
Isomaltulose (%) (dry basis)	99.0 to 99.5	98.0[1,2]	82.0[2]	≥ 99.15[3]
Trehalose (%)	0.4	1.0[2]	10.0[2]	NS
Isomaltose (%)	0.06			NS
Glucose (%)	0.03	0.5[2]	7.0[2]	NS
Fructose (%)	0.03			NS
Trisaccharides and higher saccharides (%)	0.4	0.5[2]	1.0[2]	NS

NS: Not Specified
[1] Irwin and Sträter, 1991
[2] Südzucker, 1996
[3] Shin Mitsui Sugar Co., 2003; Note: The purity of Palatinose® mentioned in the catalogue of Shin Mitsui Sugar Co. is ≥ 99% (includes crystal water), a purity that is equivalent to 99.15% on an anhydrous basis.

II.2 Sucrose, Biocatalyst Source, Raw Materials, and Chemicals Specifications

II.2.1 Sucrose

Sucrose [>99.7 International Sugar Degrees (°Z)], consistent with Food Chemicals Codex (FCC) food-grade specifications, is utilized as the primary starting material in the manufacturing process of isomaltulose (see Appendix B).

II.2.2 *Protaminobacter Rubrum* CBS 574.77

The non-pathogenic microorganism *P. rubrum* CBS 574.77 (Porter *et al*., 1991) is the source of the biocatalyst responsible for carrying out the reaction in which sucrose is converted to isomaltulose. *P. rubrum* is obtained from the Centralbureau voor Schimmelcultures in Baarn, Netherlands, where it was deposited in relation with the patents of Südzucker on isomalt production (see Appendix B). Processing steps employed to prepare the biocatalyst prior to its use during isomaltulose production effectively kill *P. rubrum* cells. Subsequent purification of the isomaltulose product removes residual *P. rubrum* material. For a summary of the safety evaluation of the microorganism, refer to Section XIII.1.

II.2.3 Substrates for Production of the Biocatalyst

High purity substrates, consistent with FCC food-grade specifications, are used for the growth of *P. rubrum* in the production of the biocatalyst.

II.2.3.1 Demineralised Drinking Water

Demineralised drinking water is considered a human food ingredient and is used as a substrate solution in the production of the biocatalyst.

II.2.4 Formaldehyde, 37% Solution

The 37% solution of formaldehyde is used for sanitation purposes to kill the live organism in the production of isomaltulose. It is also commonly used in the production of beet sugar (see Appendix B).

II.2.5 Methanol

Methanol is not added directly at any stage of the production process; however, residual levels of methanol may be present in the formaldehyde solution.

II.3 Description of the Manufacturing Process

II.3.1 Enzymatic Preparation

The enzymatic conversion of sucrose to isomaltulose is carried out by the biocatalyst sucrose glucosylmutase (EC 5.4.99.10), which is an enzyme contained in non-viable, non-pathogenic *P. rubrum* (Reg. No. CBS 574.77) cells. The microorganism is obtained from the Centralbureau voor Schimmelcultures in Baarn, Netherlands. The purity of the *P. rubrum* strain is routinely verified and samples of the biocatalyst are examined both macro- and microscopically. Absence of contaminating microorganisms (*e.g.,* Listeria, *Escherichia coli*, *Salmonella*) and mycotoxins (*i.e.,* aflatoxins, ochratoxin, zearalenone) has been verified in several samples, and levels of heavy metals, including lead, arsenic, cadmium and mercury, have been determined. Results of several analyses are presented in Appendix A.

II.4 Potential Impurities Resulting from the Production Process

II.4.1 General Considerations

Following completion of the enzymatic rearrangement, the biomass is removed by filtration. Subsequently, several purification steps are employed in order to consistently produce an uncontaminated product of high purity (≥ 98% purity). Substances characterized by anionic or cationic functional groups are removed by passing the crude isomaltulose product through columns of ion exchange resins. Any remaining inorganic impurities are effectively removed by the process of crystallization. Purification is completed by removal of the crystals from the mother liquid by centrifugation and washing with demineralised water.

In light of the use of a microorganism in the manufacture of isomaltulose, which is introduced into the reaction mixture at the initial stages of production, the potential for contamination of the final product with residual protein material was provided special consideration. As described in Section II.4.2, the effectiveness of the purification process was verified by examining samples of wet isomaltulose crystals for nitrogen content, as an indicator of potential biomass residues. Additionally, as formaldehyde is used in the inactivation of the *P. rubrum* cells, prior to their addition to the sucrose solution, the isomaltulose samples also were examined for levels of residual formaldehyde (see Section II.4.3).

II.4.2 Residual Biomass

The inactivated cells containing the biocatalyst are added to sucrose during the production of isomaltulose and subsequently filtered during the purification process. To determine if any significant residual biomass (resulting from potential lysis of the cells) is likely to remain in the

isomaltulose product following filtration, nitrogen levels, as an indicator of protein content, were determined in the starting sucrose material, the isomaltulose syrup, and wet isomaltulose crystals (see Appendix D for sample data).

Protein levels were derived on the basis of the measured nitrogen assuming that the weight of an average amino acid is approximately 6.25 times the weight of the nitrogen it contains. Samples of sucrose, isomaltulose syrup, and wet isomaltulose crystals were analyzed for total and soluble levels of nitrogen. Soluble nitrogen was present in the sucrose at levels ranging from 15.5 to 16.2 ppm, with a mean nitrogen level of 15.7 ppm (equivalent to approximately 98.4 ppm protein). The soluble nitrogen in the sucrose was likely organic nitrogenous remnants from the sugar beet or sugar cane source of the sucrose. Following the addition of the biocatalyst, soluble and insoluble nitrogen were present in the isomaltulose syrup at mean concentrations of 29.1 and 148.5 ppm, respectively. The mean concentration of total nitrogen (soluble plus insoluble) was 177.6 ppm (about 15.9 ppm originating from the sucrose and, at most, 161.7 ppm from the cells). The difference between the level of soluble nitrogen measured in the isomaltulose syrup sample and that present in the sucrose, equivalent to 13.4 ppm (29.1 ppm - 15.7 ppm). This is representative of residual nitrogen levels possibly originating from the lysis of *P. rubrum* cells in combination with soluble nitrogen that may have been present in the water and processing material (*e.g.,* resins) and possibly from the bakers yeast extract used as a growth substrate for the production of the biocatalyst.

Following several purification steps including microfiltration, demineralization, and crystallisation of the product, levels of soluble nitrogen in the wet isomaltulose crystals were substantially reduced to approximately 0.8 ppm (equivalent to approximately 5.2 ppm protein). The considerable reduction in the soluble nitrogen content of the wet isomaltulose crystals compared to the levels originally present in the sucrose indicates that some of the original soluble nitrogen component of the sucrose was removed by filtration along with cellular material. The soluble nitrogen remaining in the wet isomaltulose crystals is likely representative of some of the original soluble nitrogen that was present in the sucrose, some lysed cellular material, and some soluble nitrogen from the water or processing material.

Based on the use of the biocatalyst at levels of 4,500 IU/kg of sucrose, which is equivalent to approximately $4x10^9$ cells added per g of the sucrose starting material, the 0.8 ppm of soluble nitrogen would be equivalent to approximately $2 x10^7$ cells. This assumes that the original number of cells added corresponds to 161.7 ppm total nitrogen as determined from the nitrogen analysis [Therefore: $4x10^9$ cells/161.7 ppm x 0.8 ppm = $2 x10^7$ cells]. Thus, even if it is conservatively assumed that all of the 0.8 ppm soluble nitrogen is residual biomass from the lysed *P. rubrum* cells, the cells were non-viable (*i.e.,* they were killed prior to isomaltulose

production) and should not be of concern given that the intravenous injection of up to $2.23x10^8$ and $2.50x10^9$ viable cells was non-pathogenic in rabbits and mice (see Section XIII.1 for study description).

II.4.3 Residual Formaldehyde

The possibility of formaldehyde remaining in the final isomaltulose product as a consequence of its use in the inactivation of the *P. rubrum* cells also was considered in the evaluation of potential residues. Under the testing conditions allowing for detection of 1 mg formaldehyde/L of watery sample, formaldehyde levels in samples of the mother liquor of crystallised isomaltulose were reported not to exceed the limit of detection. Considering the historical safe use of formaldehyde as a disinfectant in beet sugar production, the significant levels occurring in various food products such as soft drinks (8 ppm), milk and fruit (20 ppm), and vegetable products (2 to 8 ppm), as well as the long-term oral toxicity studies indicating a no-observed-adverse-effect level (NOAEL) of 0.2 mg formaldehyde/kg body weight/day in rodents (IRIS database, U.S. EPA, 1991), the exposure to formaldehyde from isomaltulose consumption would be negligible and of no safety concern. Residual methanol, originating from methanol contained in the formaldehyde solution, if present in the liquid isomaltulose intermediate would be at levels less than those for residual formaldehyde, which as discussed above, are below levels of detection (<1 ppm), and would, therefore, be of no concern. Further details are provided in Appendix D.

II.5 Stability of Isomaltulose

On hydrolysis with acids, isomaltulose was reported to yield 1 mol each of glucose and fructose, and compared to sucrose, was reported to undergo hydrolysis at a reduced rate (see Appendix A).

The stability of isomaltulose was confirmed by retesting sample 16.12.2002 10 months following initial analysis (see Appendix A). A comparison of the results, indicating minimal conversion of the isomaltulose sample over the 10-month period, is presented in Table II.5-1.

Table II.5-1 Stability of Isomaltulose: Comparison of Analysis Results Obtained on December 16, 2002 and October 17, 2003

Description / Testing Date	16.12.02	12.09.03/09.10.2003/13.10.2003[1]
Isomaltulose (%)	99.23	99.29
Dextrose (%)	0.02	0.04
Fructose (%)	0.03	0.03
Isomaltose (%)	0.07	0.10

Table II.5-1 Stability of Isomaltulose: Comparison of Analysis Results Obtained on December 16, 2002 and October 17, 2003

Description / Testing Date	16.12.02	12.09.03/09.10.2003/13.10.2003[1]
Trehalulose (%)	0.25	0.14
Trisaccharides and higher saccharides (%)	0.4	0.42

[1] Retesting of the sample was completed on different days in two laboratories and twice by two different persons.

The acid stability of isomaltulose in cola drinks (110 g isomaltulose/L), adjusted to a pH of 2.3, was evaluated following a 3-month storage period and compared to the stability of sucrose. The results of this study indicate that isomaltulose is not hydrolyzed, whereas 98% of the sucrose was inverted in the same period of time. Therefore, isomaltulose when added to isotonic drinks demonstrates greater stability than sucrose, which will allow maintenance of a lower and more stable osmolality (see Appendix A).

The colour stability of isomaltulose during heating was examined and compared to that of other sugars, including sucrose, dextrose, fructose, and trehalose. Ten-brix sugar solutions, containing 3.3 g of β-alanine, were prepared and adjusted to a final pH of 7.3. In order to assess the effects of the pH, a lower-pH (4.2) isomaltulose solution also was prepared. Solutions were heated at 90°C for 30, 60, and 90 minutes, and following a cooling period were analyzed for measurements of C* value, a quantitative measure of colour. In comparison to the other sugars examined, isomaltulose was determined to be the most heat-sensitive sugar; however, increased heat stability was reported at the more acidic pH (see Appendix A).

Additional trials verifying the stability of isomaltulose in solution of low pH and following heating were conducted in parallel to the determinations of colour-stability. Isomaltulose solutions (pH 3.5) were prepared without β-alanine, heated at 90°C, and allowed to cool. Study results confirmed isomaltulose to be stable when heated for periods of 0, 30, 60, and 90 minutes (see Appendix A).

III HISTORY OF THE ORGANISM USED AS THE SOURCE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- "Is the novel food obtained from a biological source (*i.e*., a plant, animal or microorganism)?"

In the novel production process, as described herein, isomaltulose is produced by enzymatic conversion from food-grade sucrose. The biocatalyst used to convert the sucrose is obtained from is obtained from non-viable, non-pathogenic *P. rubrum* (CBS 574.44) cells. The cells are killed using formaldehyde before they are added to the sucrose. Thus, isomaltulose is not directly obtained from plant, animal, or microorganism. However, as indicated in Section II.1, *P. rubrum* cells are used as the source of the biocatalyst for the production of isomaltulose by both Cerestar and Südzucker AG. In addition, this strain is used as the source of the biocatalyst for the product of isomalt. The use of isomalt in food was considered acceptable by the SCF in 1984 (SCF, 1984).

IX ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Is there information on the anticipated uses of the novel food based on its properties?"
- "Is there information to show anticipated intakes for groups predicted to be at risk?"
- "Will introduction of the novel food be restricted geographically?"
- "Will the novel food replace other foods in the diet?"
- "Are any of the replaced foods significant nutritional sources?"
- "Does the probable level of substitution have a nutritional significance for any population groups?"

These questions have been addressed collectively in Sections IX.1 through IX.3.

IX.1 Intended Uses in Food

Isomaltulose is intended for use in conventional foods as a nutritive sweetener. The individual proposed food-uses and use-levels for isomaltulose in the E.U. are summarized in Table IX.1-1.

Table IX.1-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Isomaltulose in the E.U.

Food Category	Proposed Food-Use	Use-Levels for IM (%)
Beverages	Dilutable Soft Drinks	20.0
	Energy Drinks	5.5
	Energy-Reduced Soft Drinks	7.0
	Regular Soft Drinks	5.5
	Sports and Isotonic Drinks	7.0
Cereals and Cereal Products	Biscuits, Sandwich-Type with Filling	20.0
	Cereal Bars	10.0
	Coated Ready-to-Eat Breakfast Cereals	30.0
	Energy and Diet Meal Bars	15.0
Miscellaneous Beverages	Meal Replacements, Dry Weight	20.0
	Milk Based Meal Replacements, Dry Weight	20.0
Sugar, Preserves, and Confectionery	Candy and Chocolate Bars	25.0
	Energy Tablets	97.0

IX.2 Estimated Consumption of Isomaltulose from Proposed Food Uses in the E.U.

IX.2.1 Estimated Daily Isomaltulose Intake from All Proposed Food-Uses

Estimates for the intake of isomaltulose in the E.U. were based on the proposed use-levels for isomaltulose summarized in Table IX.1-1 and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency's Dietary Survey Programme (DSP). The main component of the DSP is the U.K. National Diet and Nutrition Survey (NDNS) programme commissioned jointly in 1992 by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health, and transferred to the Food Standards Agency on its inception in April 2000. The NDNS programme consists of 4 different surveys for specific age groups, conducted approximately every 3 years in succession. In 2001, an NDNS for adults aged 19 to 64 was completed, but the raw data is not yet available for public use. The National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS, 1992-1993) (UKDA, 1995), the National Diet and Nutrition Survey: Young People Aged 4 to 18 Years (NDNS, 1997) (UKDA, 2001), and the Dietary and Nutritional Survey of British Adults, aged 16 to 64

(DNSBA, 1986-1987) (UKDA, 1991), were used to generate estimates in the current intake analysis. Combined, these surveys provide the most up-to-date data for evaluating food-use, food-consumption patterns, and nutritional status in the U.K., containing 4- or 7-day weighed food records for individuals selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001) or local authority wards (UKDA, 1991) as the primary sampling unit.

NDNS data were collected from individuals and households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighed dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviour due to seasonality. Dietary data were recorded by survey respondents, or in the case of the children's survey, by parents or guardians, for the duration of the survey period. DNSBA 1986-1987 contains 7-day weighed dietary records for more than 2,190 individuals aged 16 to 64, while, NDNS 1992-1993 contributes 4-day data from an additional 1,592 children 1½ to 4½ years of age. NDNS 1997 adds 7-day records for approximately 1,700 youth aged 4 to 18 (UKDA, 1991, 1995, 2001). The overall response rate (assessed as completion of a full dietary record) for individuals selected for participation in the child, youth, and adult surveys, were 81, 64, and 70%, respectively (Gregory *et al*., 1990, 1995; UKDA, 2001).

In addition to collecting information on the types and quantities of foods being consumed, the NDNS programme collects physiological, anthropometric, and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples. Sample weights were developed and incorporated with the youth survey (NDNS, 1997) to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to differential sampling probabilities and differential non-response rates, particularly the lower response obtained from males, aged 15 to 18 years (UKDA, 2001).

To facilitate comparison with the adult and youth 7-day dietary survey data, dietary data from the children's survey (4-day data) was weighted to seven days, based on the assumption that intake patterns on non-recording weekdays were similar to dietary intakes on recorded weekdays; the two weekend days were not reweighed. Accordingly, all food and drink consumed on the two-recorded weekdays were averaged to give a daily intake value, which was multiplied by 5 to approximate intakes for all weekdays. These values were then combined with consumption data from weekend dietary records. Full details of the weighting method applied are provided in

Appendix J of the intake report on the children's diet and nutrition survey (Gregory *et al*., 1995) (for Intake Report see Appendix E).

Consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days, were collated by computer and used to generate estimates for the intake of isomaltulose by the U.K. population. Estimates for the daily intake of isomaltulose represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. All-person intake refers to the estimated intake of isomaltulose averaged over all individuals surveyed regardless of whether they consumed food products in which isomaltulose is currently proposed for use, while all-user intake refers to the estimated intake of isomaltulose by those individuals consuming food products in which the use of isomaltulose is under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which isomaltulose is proposed for use on one of the 7 survey days.

Calculations for the mean and high-level (97.5^{th} percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for isomaltulose. Similar calculations were used to determine the estimated total intake of isomaltulose from all proposed food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

children, ages 1½ to 4½;
young people, ages 4 to 10;
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64;
male adults, ages 16 to 64.

The estimated total consumption of isomaltulose from all proposed food uses is summarized in Tables IX.2.1-1 and IX.2.1-2 on a daily g per person and g per kg body weight basis, respectively. A complete intake report is provided in Appendix E.

As would be expected for a 7-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 85% of the population groups consisted of users of those food products in which isomaltulose is currently proposed for use, though the proportion of users decreased in adults relative to children and young people (Table IX.2.1-1). Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be

discussed in detail. Of the individual population groups, male teenagers were determined to have the greatest mean and 97.5th percentile all-user intakes of isomaltulose on an absolute basis, at 37.8 and 97.8 g/person/day, respectively, while female adults had the lowest intakes of 10.4 and 41.5 g/person/day, respectively (Table IX.2.1-1). When assessed by sex, estimated daily isomaltulose intakes were lower in females relative to males.

Table IX.2.1-1 Summary of the Estimated Daily Intake of Isomaltulose from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (g)	Percentile (g)			Mean (g)	Percentile (g)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.6	1,570	21.8	40.4	50.0	56.8	22.1	40.6	50.1	56.8
Young People	4-10	99.9	836	30.2	50.6	58.5	70.1	30.2	50.6	58.5	70.1
Female Teenagers	11-18	99.6	446	28.0	51.4	60.6	71.5	28.1	51.4	60.9	71.5
Male Teenagers	11-18	99.8	415	37.7	67.2	78.1	97.8	37.8	67.2	78.1	97.8
Female Adults	16-64	88.4	977	9.2	21.8	29.2	38.8	10.4	23.4	29.9	41.5
Male Adults	16-64	85.6	930	10.2	25.4	37.4	46.1	12.0	27.6	39.5	46.9

On a body weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user isomaltulose intakes of 1.6 and 4.0 g/kg body weight/day, respectively, while similar to the case observed for absolute intakes, female adults had the lowest 97.5th percentile intakes on a body weight basis, of 0.6 g/kg body weight/day. Male and female adults had equivalent mean all-user intakes of isomaltulose, 0.2 g/kg body weight/day (Table IX.2.1-2).

Table IX.2.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Isomaltulose from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (g/kg)	Percentile (g/kg)			Mean (g/kg)	Percentile (g/kg)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.6	1,570	1.5	2.8	3.4	4.0	1.6	2.8	3.4	4.0
Young People	4-10	99.9	836	1.2	2.0	2.4	2.7	1.2	2.0	2.4	2.7
Female Teenagers	11-18	99.6	446	0.5	1.0	1.1	1.3	0.5	1.0	1.1	1.3
Male Teenagers	11-18	99.8	415	0.7	1.3	1.6	1.9	0.7	1.3	1.6	1.9
Female Adult	16-64	88.4	977	0.1	0.4	0.5	0.6	0.2	0.4	0.5	0.6
Male Adult	16-64	85.6	930	0.1	0.3	0.5	0.7	0.2	0.4	0.6	0.7

IX.2.2 Estimated Daily Isomaltulose Intake from Individual Proposed Food-Uses in the E.U.

Estimates for the all-user intakes of isomaltulose from each of the individual food-uses demonstrated that (Appendices A and B of the Intake Report; see Appendix E) the highest 97.5th percentile all-user intakes of isomaltulose were identified in female teenagers consuming energy-reduced soft drinks, at 43.7 g/person/day, while the highest mean intakes were observed in male teenagers consuming regular soft drinks, 14.6 g/person/day. On a per kilogram body weight basis, children consuming dilutable soft drinks were identified as having the highest mean and 97.5th percentile all-user intakes of isomaltulose of 0.9 and 2.9 g/kg body weight/day, respectively.

IX.3 Food Product Labelling Information

The designation 'isomaltulose' shall be displayed on the labelling of the product as such or in the list of ingredients of foodstuffs containing it.

In a prominently displayed footnote related to the designation isomaltulose by means of an asterisk (*) the words “Isomaltulose, like sugar, is a source of glucose and fructose which undergoes slower digestion and absorption” shall be displayed.

The words of the footnote shall have a typeface of at least the same size as the list of ingredients itself.

IX.4 Conclusion

Consumption data and information pertaining to the individual proposed food-uses for isomaltulose were used to estimate the all-person and all-user isomaltulose intakes of specific demographic groups in the U.K. population. This type of intake methodology is generally considered to be ‘worst case’ as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the 4-day children’s survey, may overestimate consumption of food products that are consumed relatively infrequently, particularly when weighted to 7 days (Gregory *et al*., 1995).

In summary, on an all-user basis, the highest mean and 97.5th percentile intakes of isomaltulose by the U.K. population from all proposed food-uses in the E.U., as observed in male teenagers, were estimated to be 37.8 g/person/day (0.7 g/kg body weight/day) and 97.8 g/person/day (1.9 g/kg body weight/day), respectively. On a body weight basis, children consumed the greatest amount of isomaltulose, with mean and 97.5th percentile all-user intakes of 1.6 and 4.0 g/kg body weight/day, respectively.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- "Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

This question has been addressed in Sections XI.1.

XI.1 Nutritional Equivalence to Existing Foods

Ingested isomaltulose is metabolised by the isomaltase-sucrase complex in the intestinal mucosa to equal parts glucose and fructose, which are readily absorbed and utilized in carbohydrate metabolic pathways (for further details see Section XIII.2.1). Although the hydrolysis reaction proceeds at a rate one-fifth to one-fourth that of sucrose, the resulting intermediates of isomaltulose metabolism, which upon digestion also produces fructose and glucose, are identical to those of sucrose. Thus, isomaltulose can be considered to be nutritionally equivalent to sucrose as well as providing the same physiological energy value (caloric value) as sucrose (*i.e.*, approximately 4 kcal/g).

In comparison to trials conducted with sucrose, clinical studies with equivalent amounts of isomaltulose (see Section XIII.2.7) consistently demonstrate an attenuated glycaemic and insulin response in both healthy subjects as well as in type II (non-insulin dependent) diabetics (MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989; Liao *et al*., 2001; Achten *et al*., 2003). This difference in response has been attributed to the decreased rate of isomaltulose hydrolysis compared to that of sucrose. Furthermore, in a study evaluating specifically the utilization of isomaltulose during periods of exercise, in which healthy males received an 8.6% isomaltulose solution before and during the trial, Achten *et al*. (2003) noted that in comparison to sucrose, the oxidation rate of isomaltulose was significantly lower (*i.e*., 42%) (see Section XIII.2.7). During the isomaltulose trial only 12% of energy was obtained from isomaltulose oxidation, while during the sucrose trial 24% was derived from exogenous carbohydrate oxidation (*i.e*., sucrose). The remainder of energy was derived from either endogenous carbohydrate supplies or fat. Therefore, during exercise, isomaltulose consumption was associated with a greater utilization of whole body glycogen and fat compared to sucrose.

This apparent difference in the rate of isomaltulose hydrolysis and its reduced utilization immediately after consumption while exercising, is not expected to result in differences in its overall nutritive value compared to that of sucrose, based on results of studies demonstrating complete absorption of the carbohydrate.

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"
- "Is there information to show that the NF is unlikely to contain microorganisms and/or their metabolites of adverse public health significance?"

This question has been addressed collectively below.

The biocatalyst used in the novel production process of isomaltulose is obtained from a non-genetically modified strain of *P. rubrum*. Prior to the addition of the cells to the sucrose solution, the cells are killed with formaldehyde. A dose-effect study has been performed in order to ascertain the level of formaldehyde required to effectively inactivate the *P. rubrum* biomass (see Appendix C). Therefore, no viable microorganisms are present in the reaction mixture. Furthermore, the purity of the stock culture is verified at its preparation, as well as at biocatalyst production, and is examined macro- and microscopically. The absence of contaminating microorganisms also is demonstrated.

Once the enzymatic rearrangement is completed the biomass is separated from the reaction mixture by filtration, followed by several sequential purification steps of the resulting isomaltulose, ensuring a final product, free of residual biomass.

The results of a study by Porter *et al*. (1991) conducted to evaluate the safety of *P. rubrum* indicated that the organism is nonpathogenic and has a low order of toxigenicity. Details pertaining to the safety of the microorganism are presented in Section XIII.1.

XIII TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"
- "Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"
- "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

These questions have been addressed collectively in Sections XIII.1 through XIII.4.

XIII.1 Toxicological Evaluation of *Protaminobacter rubrum*

The safety of the biocatalyst source was addressed by conducting toxicity studies in mice and rabbits in which live *P. rubrum* cells were administered by intravenous injection. The results of these studies indicated that the organism is nonpathogenic and has a low order of toxigenicity (Porter *et al*., 1991).

Specifically, a probe study and a definitive study were conducted in New Zealand white rabbits and Crl:COBS CF1 BR mice to assess the potential pathogenicity and toxigenicity of *P. rubrum*. In the probe study, 24 rabbits (3/sex/group) were injected intravenously with 1 mL of a viable-cell suspension (VCS) containing $2.5x10^{10}$ cells/mL or a cell-free supernatant fluid (CFS) consisting of the medium in which *P. rubrum* had been grown to a concentration of approximately 2.5 to $3.0x10^{10}$ cells/mL. Controls were administered uninoculated culture medium or uninoculated phosphate buffer saline (PBS) solution. Rabbits were monitored for 14 days following treatment for clinical signs of toxicity, and body weights were recorded on days 0, 7, and 14. All surviving rabbits were necropsied at the end of the observation period, and each rabbit was subjected to gross pathological examination. Samples of blood, and liver, spleen and lung sections were obtained from all 6 VCS-treated rabbits and 1 male and 1 female in each of the other 3 groups. Tissues exhibiting lesions, indicative of possible infection were cultured. Clinical signs of toxicity included reduced activity in all VCS- and CFS-treated rabbits, whereas laboured respiration and vocalization were observed only in a single CFS-treated female, which was reported to die at 5 hours post-dosing. The occurrence of dyspnea, diarrhoea, and prostrate posture was limited to rabbits in the group receiving administrations of VCS, and a decrease in body weight gain was reported in 2 of 3 surviving CFS-treated rabbits. In addition to the CFS-treated female, 2 male CFS- and all VCS-treated rabbits died within 40 hours following treatment administration. *P. rubrum* was recovered from all blood, liver, spleen and lung samples. However, as noted by the authors, it is not unexpected that *P. rubrum* cells would be present in the samples following administration of such large concentrations, especially since samples were obtained within the first 24 hours following treatment. At necropsy, post-mortem and agonal changes, including petechial haemorrhages localized in the lungs, were limited to rabbits that died during the study period.

In the definitive study, 9 groups of 3 male and 3 female rabbits received 1 mL intravenous administrations of VCS at concentrations of $2.23x10^2$, 10^4, 10^6, or 10^8 cells/mL, or CFS administered as 1:100, 1:10,000, or 1:1,000,000 dilutions. As described in the probe study, 2 control groups also were provided. With the exception of additionally recording body temperatures before treatment initiation, and at 0.5, 1, 2, and 8 hours thereafter, all other parameters monitored were identical to those detailed in the probe study. Blood and tissue

specimens were obtained from the 6 rabbits in the high-dose VCS-treated group and from 1 male and 1 female rabbit in each of the 8 remaining groups, and all samples taken from the high-dose VCS-treated rabbits were cultured for *P. rubrum*. Additionally, 80 Crl:COBS CF1 BR mice (10/sex/group) were administered intravenously 0.1 mL of VCS containing the microorganism at a concentration of $2.5x10^{10}$ cells/mL, CFS as previously described in the probe study, or uninoculated culture medium or uninoculated PBS. Testing procedures followed those outlined for the probe study, and blood and only liver and spleen samples were taken from 5 male and 5 female VCS-treated mice, as well as from 1 male and 1 female in each of the 3 other groups.

All rabbits were reported to survive the entire duration of the observation period. Rabbits in the 2 highest-dose VCS-treated groups ($2.23x10^6$ and 10^8 cells/mL) and in the 1:100 dilution CFS-treated group demonstrated transient reduction in activity and modest increases in body temperature. Elevated body temperatures were, however, not noted in rabbits receiving VCS at the $2.23x10^6$ cells/mL concentration. No variations in body weight gain were reported, and gross pathological examinations were unremarkable and did not reveal any signs of infection. Furthermore, *P. rubrum* was not identified in the blood and tissue samples. All VCS- and CFS-treated mice were reported to exhibit reduced activity and ptosis, accompanied by a temporary unkempt appearance also observed in all VCS-treated mice but limited only to the males in the CFS-treated group. Decreased body weight gain was observed in VCS-treated mice for the 1st week of the study; however, by day 14, weight gain of treated mice exceeded that of controls, and at days 5 to days 14 all mice were healthy in appearance. Dark discoloration, oedema, and occasional eschar at the site of injection were noted in several VCS-treated mice. Macroscopic evaluations performed at necropsy revealed tail lesions in some VCS-treated mice, enlarged lymph nodes in 2 VCS-treated mice, and a single incidence of a grey-tan focus on the liver in a CFS-treated mouse. None of the blood and tissue samples obtained from the mice tested positive for *P. rubrum.*

The transient signs of infection observed in the mouse study and in rabbits in the 2 highest dose VCS-groups and in the group receiving the highest concentration of CFS are indicative of low order *P. rubrum* toxigenicity possibly induced by the presence of low levels of toxins arising from the lysis of dead cells. Accordingly, it is also suggested by the authors that the deaths reported in the probe study may have occurred as a result of the very high doses of cells and CFS initially administered. However, based on the absence of *P. rubrum* in any of the blood and tissue samples and the survival of all animals in the definitive study, *P. rubrum* is considered non-pathogenic in rabbits and mice receiving intravenous administrations of up to $2.23x10^8$ and $2.50x10^9$ viable cells per animal (approximately $1x10^8$ and $8x10^{10}$ cells/kg body weight respectively), respectively.

XIII.2 Toxicological Evaluation of Isomaltulose

The isomaltulose product manufactured by Cerestar has been evaluated in 4 separate clinical trials (NutriScience, 2002, 2003; Achten *et al.,* 2003; Hespel *et al.,* 2003). Several additional toxicology and clinical trials also have been conducted with isomaltulose produced by Südzucker and Shin Mitsui Sugar Co. (Palatinose®), which, as discussed in Sections II.1 and II.3, is produced by a similar method and is chemically comparable to Cerestar's isomaltulose.

Overall, this toxicological evaluation of the safety of isomaltulose was primarily based on (i) metabolic data in animals (Dahlquist *et al*., 1963; Goda and Hosoya, 1983; MacDonald and Daniel, 1983; Kawai *et al*., 1986; Okuda *et al*., 1986; Tsuji *et al*., 1986; Ziesenitz, 1986; Goda *et al.*, 1991; Würsch, 1991; Hall and Batt, 1996) and humans (Menzies, 1974; MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989; NutriScience, 2003); (ii) clinical data pertaining to the glycaemic response obtained following isomaltulose administration as compared to that obtained with either sucrose or glucose (MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989; Liao *et al*., 2001; NutriScience, 2002); (iii) the results of human studies demonstrating that isomaltulose is well-tolerated (MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989; Spengler and Sommerauer, 1989); (iv) the results of a developmental toxicity study in rats (Lina *et al*., 1997); and (v) supportive animal sub-chronic and chronic toxicity data, including a study involving feeding of isomaltulose to rats at doses of up to 4,500 mg/kg body weight/day for 26 weeks (Yamaguchi *et al*., 1986), a 13-week feeding study in rats in which doses up to 8,100 mg/kg body weight were administered, and two additional oral studies focusing particularly on the effect of isomaltulose on tissue mineral content, also conducted in rats, in which doses up to 15,000 mg/kg body weight/day were administered (Kashimura *et al*., 1990a, 1992; Jonker *et al*., 2002). No treatment-related toxicological relevant effects were observed in any of the animal studies. Results of animal and clinical human trials are summarized in Tables XIII.2.5-1 and XIII.2.7-1, respectively. Additionally, in considering the safety of isomaltulose, clinical study data have been provided for fructose, one of the hydrolysis products of isomaltulose. The other hydrolysis product is glucose, which is the basic metabolic intermediate of carbohydrates.

In addition to the above data, a considerable amount of information has been reported in the scientific literature with respect to the cariogenic potential, or lack thereof, of isomaltulose in comparison to other sugars (Sasaki *et al*., 1985; Takazoe *et al*., 1985; Ooshima *et al*., 1990; Würsch, 1991). Some of these studies also provide reports in support of the fact that isomaltulose is well tolerated in humans without inducing gastrointestinal effects.

XIII.2.1 Absorption, Distribution, Metabolism, and Excretion (ADME)

The enzymatic hydrolysis of isomaltulose to equal parts of glucose and fructose is well documented in published *in vitro* and *in vivo* studies (Cheetham, 1982; Goda and Hosoya, 1983; MacDonald and Daniel, 1983; Ziesenitz, 1986; Goda *et al*., 1991; Würsch, 1991; Günther and Heymann, 1998). Specifically, the hydrolysis is catalyzed by the isomaltase-sucrase complex present in the intestinal mucosa, of which the isomaltase subunit is reported to carry out most of the hydrolysis (Goda and Hosoya, 1983; Goda *et al*., 1991). Results of studies conducted in animals (Dahlquist *et al*., 1963; MacDonald and Daniel, 1983; Kawai *et al*., 1986; Okuda *et al*., 1986; Tsuji *et al*., 1986) as well as in humans (MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989) indicate that while the rate of hydrolysis of isomaltulose is only one-fifth to one-fourth that of sucrose, at dose levels likely to be encountered by humans (*i.e*., in the 1 g/kg body weight/day range), no intact isomaltulose reaches the colon, and thus is not available for fermentation to short-chain fatty acids. An unpublished pig study in which ileal chyme was analyzed following treatment with a diet containing 20% isomaltulose, demonstrated no passage of unhydrolyzed isomaltulose into the large intestine (van Weerden, 1983). In rats only small amounts of radioactivity were detected in the faeces and urine (up to 2.5 and 3.6% in the urine and faeces, respectively) whereas over 50% of the radioactivity was identified in the expired air within the first 72 hours following administration of single oral doses of up to 0.5 g ^{14}C-isomaltulose/kg body weight (MacDonald and Daniel, 1983; Lina *et al*., 2002). The routes of excretion and the amounts of radioactivity recovered following the administration of 0.5 g ^{14}C-sucrose/kg body weight were comparable to those reported for isomaltulose. Therefore, the hydrolysis and absorption of isomaltulose occurs almost completely in the small intestine.

Furthermore, studies performed in which isomaltulose was administered intravenously have demonstrated that any isomaltulose absorbed intact is hydrolyzed in plasma and in peripheral tissues to glucose and fructose, or is alternatively excreted in the urine. In male Wistar rats administered bolus intravenous injections of 0.5 g isomaltulose/kg body weight, elevated blood glucose and insulin levels, indicative of parenteral hydrolysis, were observed (Okuda *et al*., 1986). In a study conducted using 6 one-year-old, clinically healthy beagle dogs, which were administered 2 g isomaltulose (Palatinose®) intravenously; approximately 83% of the dose was excreted in the urine within 24 hours following treatment (Hall and Batt, 1996). In humans, amounts in excess of 88% of a 0.5 g dose of isomaltulose administered intravenously to 5 healthy individuals, were recovered unmetabolized in the urine, with approximately 65% excreted within the first 2.5 hours following infusion. These results are representative of rapid and more complete excretion of intact isomaltulose in humans as compared to that observed in dogs. Collectively these studies indicate that isomaltulose introduced systemically is efficiently eliminated intact in the urine, or alternatively is subject to partial hydrolysis to glucose and

fructose. As a result, exposure to parental isomaltulose is not expected to present a safety concern.

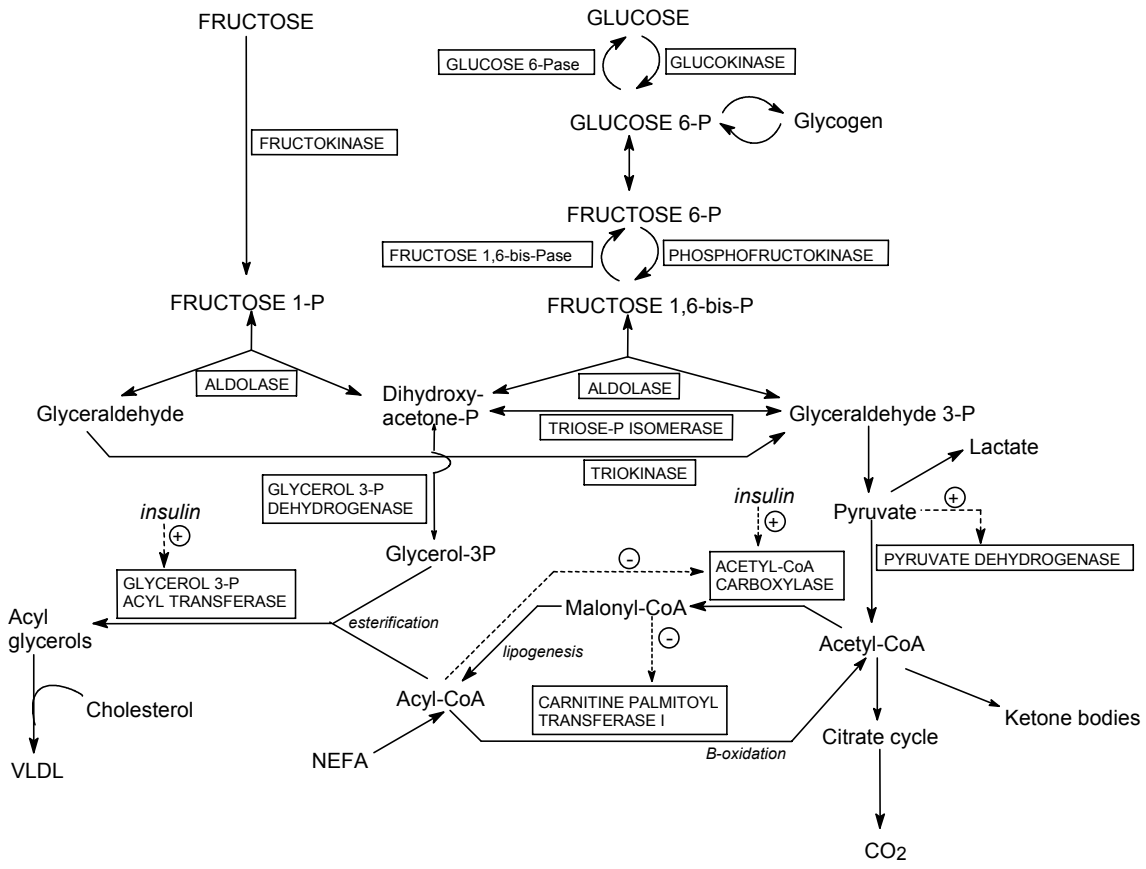
Due to a slower rate of hydrolysis, and hence absorption from the small intestine, a blunted or attenuated increase in serum glucose and insulin levels, in comparison to similar treatment with sucrose, is expected for isomaltulose. This is confirmed by the results of clinical trials (MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989; Liao *et al*., 2001) and several animal studies (MacDonald and Daniel, 1983; Kawai *et al*., 1986). Since isomaltulose is almost completely hydrolyzed in the small intestine, total exposure to glucose is similar to that achieved with sucrose; however, the lower peak glucose concentrations associated with the slower rate of absorption result in a reduction of both peak and total systemic (AUC) insulin levels in comparison to similar treatments with sucrose.

The monosaccharides glucose and fructose are produced by the hydrolysis of isomaltulose in the small intestine. Subsequent to the hydrolysis, both monosaccharides are utilized by well-characterized carbohydrate metabolic pathways. These metabolic processes mirror those of sucrose (Lina *et al*., 2002). In the free monosaccharide form, glucose is rapidly absorbed in the small intestine by an active process, while fructose is absorbed by two separate mechanisms, namely glucose-independent facilitated transport and glucose-dependent fructose co-transport (Rumessen and Gudmand-Høyer, 1986; Smith *et al*., 1995). Of the two mechanisms involved in fructose absorption, the presence of glucose facilitates the transport of fructose across the brush-border membrane of the small intestine in the glucose-dependent fructose co-transport mechanism, thereby increasing the rate at which fructose is absorbed (Rumessen and Gudmand-Høyer, 1986; Smith *et al*., 1995). In addition, it has been hypothesized that monosaccharides resulting from the enzymatic hydrolysis of such disaccharides as isomaltulose and sucrose are absorbed through the brush-border membrane directly without being released into the luminal space by the disaccharidase-related transport system, which further facilitates absorption (Ugolev *et al*., 1986; Fujisawa *et al*., 1991). Upon absorption, glucose and fructose are transported to the liver *via* the portal vein, where they are metabolized and subsequently distributed to all the tissues (Glinsmann *et al*., 1986). In addition to the liver, the small intestine and the kidney contain enzymes necessary for the metabolism of fructose (Van den Berghe, 1986); however, the utilization of fructose in extrahepatic tissues is minimal (Hallfrisch, 1987).

In the liver, fructose is rapidly phosphorylated by adenosine triphosphate (ATP) to form fructose-1-phosphate, a reaction catalyzed by the fructose-specific enzyme, fructokinase (Hers, 1952). The ability of the liver to extract the majority of the fructose that passes through it is primarily due to the high activity of fructokinase (Mayes, 1993). Fructose-1-phosphate is then broken down into glyceraldehyde and dihydroxyacetone phosphate by the liver enzyme aldolase

B (Mayes, 1993; Levi and Werman, 1998). Finally, the third enzyme of the fructose pathway, triokinase, catalyzes the phosphorylation of glyceraldehyde by ATP to form glyceraldehyde-3-phosphate (Hers, 1962). Glyceraldehyde-3-phosphate and dihydroxyacetone phosphate subsequently join the glycolytic pathway at the triose phosphate stage of metabolism and from this point on, the metabolism of glucose and fructose becomes qualitatively similar (Mayes, 1993). Therefore, glucose, glycogen, and lactate are the major end products of fructose, and consequently isomaltulose, metabolism in the liver, while carbon dioxide, ketone bodies, or triacylglycerol are the minor products (Extron and Park, 1967; Mayes and Laker, 1986; Levi and Werman, 1998). The pathways of metabolism for fructose and glucose in the liver are presented in Figure XIII.2.1-1.

Figure XIII.2.1-1 Metabolism of Glucose and Fructose in the Liver



Mayes, 1993
Pase – phosphatase; P – phosphate; VLDL – very low-density lipoprotein; NEFA – non-esterified fatty acids

Glucose is an essential substrate for the synthesis of ATP, which provides energy for cellular functions *via* the pentose phosphate shunt pathways and the Krebs cycle (Glinsmann *et al*.,

1986). The liver plays a major role in regulating blood glucose levels (Glinsmann *et al*., 1986). Excess glucose is converted to glycogen, which is stored in the liver and skeletal muscle tissues, or to fat in a process called lipogenesis (Glinsmann *et al*., 1986). Glycogen, as well as fat, provides a readily mobilizable source of energy for both liver and skeletal tissues during starvation or when increased energy needs arise (Glinsmann *et al*., 1986). Blood glucose levels also are regulated through insulin and glucagon secretion by the pancreas (Glinsmann *et al*., 1986). Similarly, the general disposition of fructose metabolism is altered by changes in nutritional and endocrine status (Van den Berghe, 1978). For instance, during instances of starvation and ethanol or glucagon administration, gluconeogenesis from fructose is increased (Van den Berghe, 1978).

XIII.2.2 Acute Studies

No data pertaining specifically to the acute toxicity of isomaltulose were available in the public literature. However, the tolerability demonstrated in human studies at bolus dose levels approximating 1 g isomaltulose/kg body weight (MacDonald and Daniel, 1983; Kawai *et al*., 1985, 1989; Spengler and Sommerauer, 1989) indicate that isomaltulose is not acutely toxic.

XIII.2.3 Subchronic Studies

Several oral feeding studies conducted in rats have consistently demonstrated the lack of toxicologically significant adverse effects attributable to the administration of isomaltulose under subchronic conditions at dose levels of up to 15,000 mg isomaltulose/kg body weight/day (Kashimura *et al*., 1990a, 1992; Jonker *et al*., 2002).

Groups of 40 Wistar rats (20/sex) received isomaltulose (Palatinose®; Südzucker's product of 97.8% purity) in the diet at concentrations of 0, 2.5, 5, or 10% for a period of 13 weeks (Jonker *et al*., 2002). Calculated intakes of isomaltulose were reported to be 0, 1,700, 3,500, and 7,000 mg/kg body weight/day for males, and 0, 2,000, 4,000, and 8,100 mg/kg body weight/day for females. The control group was provided with the basal diet supplemented with 10% sucrose. No early deaths, or compound-induced adverse effects, including neurotoxic or immunotoxic effects, were reported in the treatment groups. Growth, organ weights, including the liver, food and water consumption, as well as food conversion efficiency also were reported to remain unaffected by the treatment. No inter-group variability was reported with respect to haematological or clinical chemistry values, which were obtained at necropsy, and both ophthalmoscopic evaluations and urinalyses conducted in week 13, were unremarkable. Furthermore, the concentrating ability of the kidneys was unaffected as evidenced by a lack of variations reported in the urinary output and density. All animals were necropsied at the end of the study period, and with the exception of strain and age related lesions, neither gross nor microscopic examinations revealed compound-induced abnormalities in any of the rats

examined. No changes were reported in the liver. Consequently, under the conditions of this feeding study, isomaltulose was reported not to elicit any signs of toxicity in rats at dietary concentrations of up to 10% (approximately 7,000 and 8,100 mg isomaltulose/kg body weight/day, in males and females, respectively) determined therefore to be the NOAEL.

Two studies were conducted specifically to investigate the effect of isomaltulose on tissue mineral content in rats. Groups of 6 Wistar rats received diets containing 30% sucrose (control) or 30% isomaltulose (Palatinose®; provided by Shin Mitsui Sugar Co.) (approximately 15,000 mg/kg body weight/day) for a period of 8 weeks (Kashimura *et al*., 1990a). With the exception of a slight, but statistically significant decrease in absolute kidney weight, no other statistically significant variations in major organ weights and hemocratic values were reported between the control and the treatment groups. Compared to the control group, a non-significant decrease in average weight gain was reported in the group receiving isomaltulose. Concentrations of Ca, Mg, and P, in various tissues examined were unaffected by the treatment. In a subsequent feeding study, rats were provided with diets containing 30% sucrose (control), or 7.5, or 15% isomaltulose (provided by Shin Mitsui Sugar Co.) (approximately 15,000 mg sucrose/kg body weight/day, and 3,750, and 7,500 mg isomaltulose/kg body weight/day, respectively) for 13-week period (Kashimura *et al*., 1992). Average weight gain, hemocratic values, and tissue mineral contents, including that of Ca, Mg, P, Fe, Zn, Cu, and Mn, were unremarkable in all groups. Therefore, at doses of up to 15,000 mg/kg body weight/day, isomaltulose did not elicit any adverse effects, including no effects on mineral content status, in rats.

XIII.2.4 Chronic Studies

Two chronic toxicity studies have been published in the scientific literature (Yamaguchi *et al*., 1986, 1987). The identified studies include a 26-week study of isomaltulose (Palatinose®; provided by Shin Mitsui Sugar Co.) in Sprague-Dawley (SD) rats (Yamaguchi *et al*., 1986) and a second similar 26-week study of Palatinose® syrup (provided by Shin Mitsui Sugar Co.), also conducted in SD rats (Yamaguchi *et al*., 1987). These studies were reported in Japanese; however, English language summaries were included, and all figures and tables of data were presented in English. Additionally, a detailed description of the Yamaguchi *et al*. (1986) study is provided in a recently published review article (Lina *et al*., 2002).

In the first study, 15 male and 15 female SD rats/group were administered 0 (vehicle control), 1,500, 3,000, or 4,500 mg isomaltulose/kg body weight/day *via* oral gavage (Yamaguchi *et al*., 1986; Lina *et al*., 2002). Parameters monitored included clinical signs of toxicity, mortality, body weight, food and water consumption, and absolute and relative organ weights. Haematology, clinical chemistry, and urinalyses, as well as ophthalmology, and gross and

microscopic pathology also were performed. No compound-related adverse effects were reported with respect to clinical signs of toxicity, mortality, ophthalmoscopy, and body weight gain, and no variations in urinalysis and haematology values were reported compared to controls as evaluated at week 26 and at weeks 1, 4, 13, and 26 of the study period, respectively.

In males the administration of isomaltulose appeared to be related to a slight reduction in food consumption and a slight increase in water consumption; however, these parameters did not reach statistical significance. The results of the blood biochemistry analyses revealed, slight, but statistically significant, decreases in levels of uric acid (reported in males of the two highest-dose groups and in all groups of treated females), urea (reported in males of the two highest dose groups and in low-dose females) and creatinine (reported in both sexes in all treatment groups), as well as in levels of alkaline phosphatase, lactate dehydrogenase (LDH) and cystolic glutamic-oxaloacetic transaminase (S-GOT) (reported in high-dose females). Increases in levels of serum phosphorus and glucose were reported in high-dose males, whereas only elevated phosphorus levels were reported in high-dose females. These observations are likely physiological changes associated with the high sugar load imposed on the animals. It must therefore be reinforced that such variations on blood biochemistry are not necessarily representative of adverse effects, but rather may be indicative of physiological adaptations. Furthermore, the variations observed in the clinical chemistry values were within the ranges of historical control data, or otherwise did not demonstrate a dose-related response (Lina *et al*., 2002). Although relative liver weights were slightly increased in high-dose males, pathological evaluations of all organs examined were reported to be unremarkable. Specifically, macroscopic examinations conducted at necropsy and histopathology performed on organs and tissues of control and high-dose animals revealed no abnormalities (Lina *et al*., 2002). Additionally, it has been suggested that the non-random testing procedures employed in this study may have induced the apparent variations (Lina *et al*., 2002). As reported by Lina *et al*. (2002), the authors of the study concluded that no clear signs of toxicity were evident in rats receiving up to 4,500 mg isomaltulose/kg body weight/day orally. Therefore, in the absence of any pathological changes, the slight increase in liver weights limited to the males of the high-dose group are not considered to be of toxicological significance, and consequently, under the conditions of this study, a NOAEL of 4,500 mg isomaltulose/kg body weight/day was established in rats.

The second 26-week oral toxicity study followed a study protocol similar to that described in Yamaguchi *et al*. (1986) with the exception that rats received oral administrations of Palatinose® syrup (crystallisation mother liquor and co-products), instead of isolated isomaltulose. The sugar portion of the syrup (*i.e.,* 71.2% of the syrup) consisted of trehalulose, isomaltulose, fructose, glucose, maltose, sucrose, and other sugars, present in the syrup at concentrations of 32.5, 13.4, 10.3, 8.5, 2.6, 2.2, and 1.6%, respectively. The syrup was administered to rats at dose levels of

0, 1,500, 3,000, or 4,500 mg/kg body weight/day, such that rats were provided with daily doses of isomaltulose equivalent to approximately 0, 200, 400, and 600 mg isomaltulose/kg body weight (Yamaguchi *et al*. 1987). No compound-related adverse effects were observed with respect to survival rate and body weight gain, and no clinical signs of toxicity were apparent. A slight, but statistically significant, reduction in food consumption was reported in males of the mid- and high-dose groups (approximately 400 and 600 mg isomaltulose/kg body weight/day, respectively). Water consumption appeared to be significantly decreased only in high-dose males and only in the 4th week of treatment. Haematological evaluations and urinalyses were unremarkable. Blood chemistry revealed a decrease in total protein and albumin values in low- and mid-dose males, but not in high-dose males, and an increase in total bilirubin in mid- and high-dose females. Similar to the results noted in the study conducted with pure isomaltulose, plasma uric acid and creatinine concentrations also demonstrated significant decreases in mid- and high-dose males, and in females of all groups receiving Palatinose® syrup, respectively. With the exception of increased absolute lung and relative spleen weights noted in mid- and high-dose females, both absolute and relative organ weights were not reported to vary from controls. A summary of the histopathological findings, presented in an English language summary table, wherein results of the high-dose and control animals were presented exclusively, revealed no effects related to treatment with Palatinose® syrup. In the absence of any consistent dose-dependent adverse effects, a NOAEL of 4,500 mg Palatinose® syrup/kg body weight/day, equivalent to approximately 600 mg isomaltulose/kg body weight/day, the highest dose tested, can be specified under the conditions of this study.

Overall, given that the reported biochemical variations can be likely attributed to physiological adaptation rather than to a toxic effect *per se*, the chronic studies presented in Japanese are considered to strongly support the safety of isomaltulose.

XIII.2.5 Developmental Studies

Isomaltulose has been tested in a developmental toxicity study in rats (Lina *et al*., 1997). Isomaltulose (Südzucker's product of 98.5% purity) was administered at dietary concentrations of 0, 2.5, 5, or 10% (approximately 0, 1,250, 2,500, and 7,000 mg isomaltulose/kg body weight/day, respectively) to groups of 24 mated, female Wistar rats on days 0 to 21 of gestation. On day 21 of gestation the dams were killed and foetuses examined for abnormalities. For the maternal animals, body weight gain, food and water consumption, and ovarian and uterine weights were reported, and gross pathology of the major abdominal and thoracic organs was performed. Foetal and developmental toxicity parameters including number of corpora lutea, implantation sites, live and dead foetuses, early and late resorptions, foetal and placental weights, size of the foetuses, sex of the foetuses, and the presence of external and internal malformations

were assessed. No compound-related adverse effects were reported for any of the parameters evaluated. The only statistically significant effect reported was an increase in the number of foetuses in the low-dose group (1,250 mg/kg body weight/day) exhibiting incomplete ossification of the skull bones. In the absence of a dose-response, this finding was considered incidental and not related to treatment with the compound. Delayed ossification of the phalanges, which is a common finding of teratology studies and is completed after birth and considered a variation as opposed to a malformation, was observed with greater frequency in the treatment groups compared to the control group, but only in cases where more than 20 digits were not ossified. In fact, compared to controls, the incidence of incomplete or delayed ossification of 10 to 20 digits was decreased in the treatment groups. With respect to the hind phalanges, the delay in ossification of more than 20 digits was statistically significant at every dose-level, and similarly, delayed ossification of the front phalanges affecting more than 20 digits also reached levels of statistical significance in every treatment group, with the exception of the mid-dose group (2,500 mg/kg body weight/day); however, the increases were not dose-related. As concluded by the authors, under the conditions of this study, isomaltulose was reported not to induce any maternal, embryonic, or foetal adverse effects at dietary concentrations of up to 10% (approximately 7,000 mg isomaltulose/kg body weight/day); thus, the NOAEL for this study is approximately 7,000 mg/kg body weight/day, the highest dose tested.

Table XIII.2.5-1 Summary of Animal Toxicity Studies for Isomaltulose

Test material (source)	Species	No. of Animals per Group	Duration of Dosing	Dose/Concentration	Results/Comments	NOAEL (mg/kg bw/day)	Reference
Palatinose® (Shin Mitsui Sugar Co.)	Wistar rats	6	8 wks	30% sucrose or 30% isomaltulose (~15,000 mg/kg bw/day)	Slight ↓ absolute kidney weights.	15,000	Kashimura *et al*., 1990a
Palatinose® (Südzucker)	Wistar rats	40 (20/sex)	13 wks	0 (control), 2.5, 5, or 10% (M: 0, 1,700, 3,500, and 7,000 mg/kg bw/day F: 0, 2,000, 4,000, and 8,100 mg/kg bw/day, respectively)	No adverse effects.	M:7,000 F:8,100	Jonker *et al*., 2002
Palatinose® (Shin Mitsui Sugar Co.)	Wistar	6	13 wks	30% sucrose, or 7.5 or 15% isomaltulose (~15,000 mg sucrose/kg bw/day, and 3,750 and 7,500 mg isomaltulose/kg bw/day, respectively	No adverse effects.	7,500	Kashimura *et al*., 1992
Palatinose® (Shin Mitsui Sugar Co.)	Sprague-Dawley	30 (15/sex)	26 wks	0 (control), 1,500, 3,000, or 4,500 mg/kg bw/day	↓ food consumption; ↑ water consumption; ↑ relative liver weights (M: 4,500); Variations in biochemistry parameters; no pathological changes. In the absence of pathological changes, the slight increase in liver weights, limited to the males of the high-dose group, is not considered to be of toxicological significance	4,500	Yamaguchi *et al*., 1986

Table XIII.2.5-1 Summary of Animal Toxicity Studies for Isomaltulose

Test material (source)	Species	No. of Animals per Group	Duration of Dosing	Dose/Concentration	Results/Comments	NOAEL (mg/kg bw/day)	Reference
Isomaltulose (Südzucker)	Wistar rats	24 mated females	GD 0 to 21	0 (control), 2.5, 5, or 10% (0, 1,250, 2,500, and 7,000 mg/kg bw/day, respectively)	↑ number of foetuses with incomplete ossification of the skull bones (1,250); Delayed ossification of the phalanges. Findings were not dose dependent. No maternal, embryonic, or foetal adverse effects	7,000	Lina *et al*., 1997

NOAEL=No-observed adverse effect level; Wks=Weeks; M=Males; F=Females; GD=Gestation Day

XIII.2.6 Mutagenicity and Genotoxicity Studies

There were no published genotoxicity studies of isomaltulose available for review. The results of one unpublished reverse mutation assay were presented in a review article of isomaltulose (Lina *et al*., 1997). As demonstrated in the Ames assay performed in a standard battery of *Salmonella typhimurium* tester strains, isomaltulose at concentrations of up to 4,000 µg/plate did not exhibit any mutagenic activity in the absence and presence of metabolic activation (Asquith *et al*., 1986). Furthermore, given that isomaltulose is a simple disaccharide, and is hydrolyzed to fructose and glucose prior to absorption, mutagenic or other genotoxic activity would not be expected.

XIII.2.7 Human Studies

Human studies, which have assessed the safety of isomaltulose include an unpublished tolerance study (Spengler and Sommerauer, 1989), clinical studies to evaluate the effects of isomaltulose on the glucose and insulin response in normal (Kawai *et al*., 1985; Liao *et al*., 2001), diabetic (Kawai *et al*., 1989; Liao *et al*., 2001) and insulin-resistant subjects (Hespel *et al*., 2003); and a study to establish the bioavailability and digestibility of isomaltulose (MacDonald and Daniel, 1983). Three additional clinical trials have been performed using isomaltulose produced by Cerestar; to determine the intestinal absorption of isomaltulose in ileostomy patients (NutriScience, 2003), assess the glycaemic response following the ingestion of isomaltulose (NutriScience, 2002), and evaluate the oxidation of isomaltulose during periods of moderate-intensity exercise (Achten *et al*., 2003). None of these three studies, however, provided reports of any adverse effects, side effects, or lack thereof.

In the unpublished Spengler and Sommerauer (1989) study, cited by Lina *et al*. (2002), 60 subjects consumed isomaltulose (source not identified), either as a powder or provided in food, in increasing doses over a 12-week trial period. Dosing was started at 12 g isomaltulose/day and maintained at this level for the 1st 2 weeks. Subsequently, participants received 24 g isomaltulose/day in weeks 3 and 4, and finally 48 g isomaltulose/day over the course of the last 8 weeks of the trial period. The responses measured, including flatulence, diarrhoea, and stool frequency, were reported not to vary compared to results obtained following treatment with similar doses of sucrose.

No symptoms of gastrointestinal disturbances, including nausea and diarrhoea, were reported over the course of treatment in two studies evaluating the plasma glucose and insulin response following oral administration of a 50 g dose of isomaltulose (provided by Shin Mitsui Sugar Co.) to normal and diabetic subjects (Kawai *et al*., 1985, 1989). Solutions of isomaltulose and sucrose (50 g isomaltulose or sucrose dissolved in 150 mL water) were administered orally to 8

(4M, 4F) healthy individuals (Kawai *et al*., 1985). Following a gradual increase from basal levels (92.4±1.9 mg/dL), the plasma glucose levels were reported to plateau at approximately 110±4.9 mg/dL for the remainder of the study period (*i.e.,* 120 minutes). In contrast, peak glucose levels of 143±8.8 mg/dL were obtained within 30 minutes of sucrose administration, and were reported to decrease rapidly to near basal values after 90 minutes. Furthermore, in response to isomaltulose intake, the cumulative increase in plasma glucose was reported to be significantly lower than that observed with sucrose. In the companion study, 10 subjects diagnosed with non-insulin dependent diabetes mellitus (NIDDM) and 10 healthy subjects also were provided with oral administrations of 50 g isomaltulose or sucrose subsequent to an overnight fast (Kawai *et al*., 1989). Compared to the total increase in plasma glucose levels from basal levels obtained post-sucrose administration, relative increases in glucose levels measured following isomaltulose ingestion were reduced by 35 and 27% in healthy and diabetic individuals, respectively. In both studies, variations in plasma insulin levels were reported to closely resemble changes in plasma glucose levels. Therefore, results of both studies demonstrated in normal subjects as well as in NIDDM patients that the cumulative increases in plasma glucose and insulin following dosing with isomaltulose were significantly reduced compared to those observed in association with sucrose consumption. These data further support the conclusion that isomaltulose, at least at doses in the range of 1 g/kg body weight, is completely hydrolyzed and absorbed, although at a significantly slower rate than sucrose.

Similar results were demonstrated in the study conducted by Liao *et al*. (2001) involving 10 diabetic subjects (type II; also NIDDM) and 10 non-diabetic controls, provided with 75 g of isomaltulose (Palatinose®; source not identified) or sucrose on two separate days. Blood samples were obtained from each subject before, and at 30, 60, 120, and 180 minutes following dose administration. Parameters monitored included plasma glucose, as well as levels of serum insulin, C-peptide, and lipids. In non-diabetics, peak levels of glucose were attained at 60 and 30 minutes following administration of isomaltulose and sucrose, respectively. In diabetics, isomaltulose intake resulted in peak glucose levels after 120 minutes, whereas sucrose consumption yielded maximal glucose values after 60 minutes, following treatment. The serum insulin and C-peptide responses directly reflected the glucose response. Evaluated at 180 minutes post-sucrose and isomaltulose administration, no significant variations were observed in serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDLC), and high-density lipoprotein-cholesterol (HDLC) levels. Therefore, in both diabetic and non-diabetic subjects, the rate of absorption of isomaltulose hydrolysis products was reduced compared to that of sucrose.

In the bioavailability study conducted by MacDonald and Daniel (1983), bolus doses of isomaltulose (provided by Tate and Lyle Industries Limited) of up to 1 g/kg body weight,

administered in aqueous solutions, were reported not to cause "intestinal discomfort or hurry". Specifically, following an overnight fast, 10 healthy males (18 to 35 years of age) were provided with isomaltulose or sucrose at doses of 0.25, 0.50, 0.75, or 1 g/kg body weight dissolved in water, such that, in total, each subject ingested 8 samples, provided in random order. Serum glucose, fructose, and insulin concentrations were measured in blood drawn before, and at 15, 30, 60, and 90 minutes after ingestion of the sample. Urine samples were collected before as well as at the end of the study, for determination of urinary volume, and isomaltulose and sucrose levels. Mean urinary excretion of isomaltulose and sucrose was consistently reported at or below 0.03% of the dosed amounts. Serum insulin concentrations attained after isomaltulose ingestion were approximately half of those obtained following the intake of sucrose. The increase in serum fructose concentrations also was reduced compared to that after sucrose ingestion; however, while the insulin response increased linearly with the dose of either carbohydrate, the fructose response appeared to plateau at higher doses of isomaltulose. No significant differences, however, were noted with respect to glucose levels at any dose-level examined. Therefore, as in the Kawai *et al*. (1985, 1989) studies, the insulin response obtained following isomaltulose consumption appeared to be attenuated compared to similar treatments with sucrose. Moreover, the absence of any intestinal adverse effects may be attributable as suggested by the authors to almost complete absorption of isomaltulose hydrolysis products.

Eight (8) healthy subjects (4M, 4F) with an ileostomy, between 38 and 66 years of age, were recruited in order to investigate the intestinal absorption of isomaltulose (provided by Cerestar) (NutriScience, 2003). Isomaltulose was provided as a 75 g dose dissolved in 250 g of low fat strawberry yogurt. Patients fasted for 10 hours prior to trial administration, and continued to fast for 6 hours after receiving the test product. The ileostoma effluents were collected over a 24-hour period, at intervals of 2 hours for the first 12 hours, and every 6 hours thereafter, for a total of 9 samples, with the first sample collected immediately before consumption of the isomaltulose. The mean absorption of isomaltulose from the small intestine was reported to be 93.11±6.84%; however, the authors noted that individual results could be separated on the basis of the degree of absorption into two groups, one group where absorptions were in excess of 96% and another where absorption values of approximately 85% were attained. Specifically, a mean absorption of 97.8±0.8% was reported in 5 of the 8 subjects examined, in comparison to a mean absorption of 84.9±1.9% reported in the remaining 3 individuals.

Clinically healthy males (n=10) were enrolled in a comparative study in order to investigate the effects of dextrose (dextrose monohydrate 9%) and isomaltulose (provided by Cerestar) on the serum glucose and insulin response (NutriScience, 2002). Trial solutions provided to the individuals consisted of 81.75 and 78.75 g of dextrose and isomaltulose, respectively, dissolved in 250 mL of water. Each carbohydrate was examined in each candidate, allowing for at least

48-hours between subsequent trials. Following an overnight fast, subjects ingested the 250 mL solution, and blood samples were collected immediately prior to ingestion of solution, every 15 minutes for the 1st hour, and then every 30 minutes, providing for a total 4-hour sampling period. Glucose levels were obtained for every sample collected, whereas insulin levels were measured exclusively in the 1st 4 samples, and at the 120 and 240-minute collection times. The glucose index score (GI) and the insulin index score (II) were calculated on the basis of the incremental area under the curve (iAUC) determined in each candidate following the administration of either carbohydrate. The mean GI and II values were reported to be 56.37±36.97 and 47.97±34.60%, respectively. Both index scores were significantly different from a 'no difference in response' value of 100%, indicating a more pronounced effect of dextrose on both the glucose and insulin serum response. Moreover, both the mean glucose and insulin serum concentrations obtained following dextrose consumption were statistically greater than those obtained following isomaltulose ingestion. Therefore, isomaltulose exhibited a less pronounced effect on both the insulin and glucose response following ingestion of a 79 g dose as compared to the effects induced by 82 g of dextrose.

The effects of isomaltulose (provided by Cerestar) intake on blood glucose and insulin levels at rest and after exercise were determined in 9 obese female patients (mean age 41.5 years) with insulin resistance and compared to results obtained following fructose consumption (Hespel *et al*., 2003). Each subject participated in 2 separate experimental sessions during which either isomaltulose or fructose were provided for oral administration as a 50 g dose dissolved in 500 mL of water. Within the same session, blood samples were obtained in the morning, and then again in the afternoon following 20 minutes of exercise, for determinations of glucose and insulin concentrations. Results of the oral sugar tolerance tests at rest as well as following exercise revealed that although both blood and insulin concentrations were reported as generally low following intake of 50 g isomaltulose, compared to fructose values, the concentrations were significantly higher; however, consumption of isomaltulose was reported not to be associated with any gastrointestinal side effects, whereas ingestion of fructose was reported to elicit nausea, intestinal spasms, and diarrhoea.

Ten healthy male subjects were recruited to participate in a study designed specifically to investigate the exogenous oxidation rate of isomaltulose ingested during moderate-intensity exercise and compare to that following sucrose ingestion (Achten *et al*., 2003). Each individual participated in 3 trials, administered on separate occasions, during which water or a 150 mL drink containing 8.5% sucrose or isomaltulose, was provided every 15 minutes while subjects cycled for a period of 150 minutes at 50% of their maximal work rate. Based on the density of sucrose (*i.e*., 1.58 g/mL), an isomer of isomaltulose, a concentration of 8.5% sucrose or isomaltulose in 150 mL is equivalent to approximately 13 g of either carbohydrate. Additionally,

immediately before exercising, subjects received a bolus dose (600 mL) consisting of either water or 1 of the 2 8.5% carbohydrate solutions (approximately 51 g of sucrose or isomaltulose). Breath and blood samples were obtained prior to commencement of exercise, and at 15-minute intervals thereafter. Expired air gas analysis was performed for 4 minutes at the end of each 15-minute interval. Beginning at 60 minutes, a significant reduction in total carbohydrate oxidation was observed following intake of isomaltulose compared to water. Similarly, compared to sucrose, total carbohydrate oxidation observed with isomaltulose was reduced; however, levels of statistical significance were attained only at 60, 75, and 150 minutes. With respect to exogenous oxidation, sucrose oxidation was significantly higher compared to isomaltulose oxidation at 30 minutes and thereafter. A significant decrease in endogenous oxidation rates over course of the trials was observed in all cases. Specifically compared to sucrose, rates of endogenous carbohydrate oxidation were significantly higher at 90 and 105 minutes during the isomaltulose trial. In total, during the isomaltulose trial, 12, 44, and 43% of energy was derived from oxidation of isomaltulose, endogenous carbohydrate oxidation, and fat, respectively, compared to 38 and 24% obtained from exogenous and endogenous carbohydrate oxidation, respectively and the remainder from fat during the sucrose trial. Therefore, in comparison to isomaltulose, significantly less glycogen was utilized during the sucrose trials, while no variation was reported between isomaltulose and water. Overall, both mean and peak oxidation rates of isomaltulose were reduced by more than 45% compared to sucrose, indicating a 28% oxidation of ingested isomaltulose, compared to 63% oxidation of ingested sucrose. Consistent with other studies, intake of isomaltulose was associated with an attenuated glycaemic response. Insulin levels also were generally lower with isomaltulose consumption, compared to levels observed following ingestion of sucrose. A statistically significant difference was reported at 30 and 120 minutes. A significant increase in free fatty acid concentration was observed during isomaltulose administration compared to sucrose. The authors suggested that the apparent differences in the utilization of isomaltulose compared to that of sucrose during exercise are attributable possibly to a reduced rate of gastric emptying of isomaltulose but more likely to a slower rate of hydrolysis.

Consumption of 12 or 24 g isomaltulose/day for a period of 10 days by 6 healthy volunteers was reported by Kashimura *et al*. (1990b) to not induce any changes on faecal microflora populations, pH, or water content. A slight increase in total cholesterol was reported to occur with the isomaltulose intake. No significant effects were reported on LDL, very low-density lipid cholesterol (VLDLC), HDLC, or TC (Kashimura *et al*., 1989, 1990b, 1993).

In addition, results of liver function tests (parameters not specified) in an unpublished study revealed no significant variations after the oral administration of 50 g of isomaltulose to normal individuals (results reported in Kawai *et al*., 1985).

Overall based on the results of the studies described above, there exist clear data to support tolerability of isomaltulose at doses of up to 1 g/kg body weight (~70 g isomaltulose), administered under bolus dosing conditions. Additionally, unpublished Shin Mitsui Sugar Co.'s test reports indicate that 80 g of isomaltulose administered as an aqueous solution to healthy fasting volunteers did not induce any laxative effects, as evidenced by no reports of diarrhoea following consumption of the isomaltulose solution (Shin Mitsui Sugar Co., 2003). Thus, higher total daily intakes of isomaltulose are expected to be well tolerated, especially when isomaltulose is administered as smaller amounts throughout the day.

Table XIII.2.7-1 Summary of Human Trials for Isomaltulose					
Study Population	**Duration**	**Dose**	**End-points Measured**	**Results and Comments Related to Study**	**Reference**
8 healthy subjects with an ileostomy (4m; 4F)	Single dose	75 g isomaltulose in 250 mL of low fat strawberry yoghurt	Absorption	~93% mean absorption	NutriScience, 2003
10 healthy males	Single dose	0.25, 0.50, 0.75, or 1 g/kg bw of isomaltulose or sucrose (control) dissolved in water	Serum glucose, fructose and insulin levels, and urinary volume	≤0.03% of dosed amount excreted in the urine; Attenuated insulin and fructose response compared to control; similar glycaemic response; No intestinal discomfort.	MacDonald and Daniel, 1983
8 healthy subjects (4M, 4F)	Single dose	50 g isomaltulose or sucrose (control) dissolved in 150 mL water	Plasma insulin and glucose levels	Attenuated glycaemic and insulin response compared to control.	Kawai *et al*., 1985
10 diabetic (NIDDM) and 10 non-diabetic subjects	Single dose	50 g isomaltulose or sucrose (control) dissolved in 150 mL water	Plasma insulin and glucose levels	Attenuated glycaemic and insulin response in healthy and diabetic subjects compared to control.	Kawai *et al*., 1989
10 diabetic (NIDDM) and 10 non-diabetic subjects	Single dose	75 g isomaltulose or sucrose (control)	Plasma glucose, serum insulin, C-peptide, and lipid (TG, TC, LDLC, and HDLC) levels	Attenuated glycaemic, insulin, and C-peptide responses in healthy and diabetic subjects compared to sucrose; No variation in lipid levels.	Liao *et al*., 2001
10 healthy males	Single dose	81.75 g dextrose monohydrate 9% (control) or 78.75 g isomaltulose in 250 mL water	Plasma glucose and insulin levels	Attenuated glycaemic and insulin response compared to sucrose.	NutriScience, 2002
9 obese women with insulin resistance	Single dose	50 g isomaltulose or fructose in 500 mL water	Plasma glucose and insulin levels at rest and following exercise	Generally, plasma glucose and insulin levels were low levels; however, ↑ compared to fructose; No adverse GI effects with isomaltulose.	Hespel *et al*., 2003

Table XIII.2.7-1 Summary of Human Trials for Isomaltulose					
Study Population	**Duration**	**Dose**	**End-points Measured**	**Results and Comments Related to Study**	**Reference**
10 healthy males	Single dose	Beginning of trial: water, or 51 g isomaltulose or sucrose in 600 mL water; subsequently, water, or 13 g isomaltulose or sucrose in 150 mL water (every 15 min for a total of 10 administrations)	Endogenous and exogenous carbohydrate oxidation, fat utilization, glucose and insulin levels, and FFA levels, during moderate intensity exercise	↓ rate of isomaltulose oxidation during exercise; Attenuated glycaemic and insulin response	Achten *et al*., 2003
60 healthy subjects	12 wks	Wks 1 to 2: 12 g/day Wks 3 to 4: 24 g/day Wks 5 to 12: 48 g/day Isomaltulose or sucrose (control)	Body weight, blood pressure, pulse, frequency of flatulence, diarrhoea, and stool frequency	No variations compared to control.	Spengler and Sommerauer, 1989

M=Males; F=Females; NIDDM=Non-insulin dependent diabetes mellitus (type II); TG=Triglycerides; TC=Total cholesterol; LDL-C=Low density lipoprotein cholesterol; HDL-C=High density lipoprotein cholesterol; FFA=Free fatty acids; GI=gastrointestinal

XIII.2.8 Other Studies

The cariogenicity of isomaltulose has been evaluated in several *in vitro* and *in vivo* experiments; however, a clinical study to demonstrate the lack or reduced degree of cariogenicity over the studies of the course of long-term consumption does not appear to be publicly available. Although a detailed evaluation of the available data to support a "non-cariogenic" claim is beyond the scope of this evaluation of the safety data, a brief review of the available data does indicate isomaltulose to be non-cariogenic. Supporting data include evidence for the lack of utilization of isomaltulose by the caries-inducing *Streptococcus mutans* (Takazoe, 1985). Compared to sucrose feeding, a reduction in the incidence of caries was reported in *S. mutans*-infected rats fed isomaltulose (Sasaki *et al*., 1985). In human volunteers, consuming snacks made from isomaltulose, reduced plaque index scores and decreased counts of *S. mutans* in saliva were observed compared to results obtained from individuals consuming snacks containing sucrose (Ooshima *et al*., 1990). Additionally, compared to glucose, a reduction in acid production by human dental plaque, removed following 6 weeks of daily use of palatinose-containing mouth rinse, was demonstrated by Topitsoglou *et al*. (1984).

XIII.3 Potential Allergenicity Concerns

As described in Section II.3.3, isomaltulose is extensively purified following initial filtration intended to remove the biocatalyst, which minimizes the possibility of contamination of the final isomaltulose crystals with residual material from the microorganism (*P. rubrum*). The lack of a significant residual biomass presence is supported by the high level of purity (≥98%) of the final crystal product.

More importantly, however, *P. rubrum* cells are used in the production of isomalt, the manufacturing process of which is comparable to that of isomaltulose. No incidences of allergic reactions, related to the use of isomalt, have been reported since its approval for use in foods. Additionally, isomaltulose is currently marketed in Japan, where to the best of Cerestar's knowledge, no reported cases of allergic reactions as a result of isomaltulose consumption have been identified. Cumulatively, based on its history of use, there is no indication that trace residual protein, in either isomalt or isomaltulose, has been associated with allergenicity, in spite of the fact that isomalt and isomaltulose of Südzucker and Shin Mitsui Sugar Co. are manufactured by immobilization of the *P. rubrum* cells, rather than by filtration to discard the residual biomass once the enzymatic rearrangement is complete, as is practiced by Cerestar.

Therefore, the current exposure to the microorganism through the consumption of isomalt in the E.U. and isomaltulose in Japan, without incidence of allergic reactions, combined with the high

degree of purity of the final Cerestar isomaltulose product supports a limited, if any, potential for allergenicity.

XIII.4 Consideration of Possible Physiological Effects of the Isomaltulose Hydrolysis Products

As discussed in Section XIII.2.1, isomaltulose is hydrolyzed to equal parts fructose and glucose. Thus, in considering the safety of isomaltulose, clinical study data also have been provided for fructose. As the consumption of pure fructose has been associated with symptoms of malabsorption, the possibility of malabsorption resulting from the intake of isomaltulose was examined. It is important to note that gastrointestinal symptoms associated with malabsorption are considered physiological and reversible effects rather than toxicological effects (WHO, 1987); however, the possibility of malabsorption in relation to isomaltulose consumption is considered in the current report in the interest of completeness.

The second hydrolysis product of isomaltulose, glucose, which is the basic metabolic intermediate for carbohydrates and also an essential substrate for the synthesis of ATP (Glinsmann *et al*., 1986), is not of concern with respect to physiological or adverse effects (Truswell *et al*., 1988).

XIII.4.1 Supporting Studies on the Isomaltulose Hydrolysis Product, Fructose

In the past, consumption of certain sweeteners, especially in excessive amounts, has been associated with malabsorption, resulting in gastrointestinal symptoms such as diarrhoea, gassiness, and various other abdominal complaints. The potential to induce malabsorption, however, is not uniform among all sweeteners, and is dependent on the absorptive capacity demonstrated for any particular sugar.

More specifically, excessive consumption of fruit juices has been associated with diarrhoea, gassiness, stomachaches, and other abdominal complaints (Hyams *et al*., 1988; Rumessen and Gudmand-Høyer, 1988). Fruit juices are complex solutions containing various sugars of different concentrations (Hyams *et al*., 1988; Rumessen and Gudmand-Høyer, 1988). Sugars such as sucrose and glucose are well absorbed; fructose is not as well absorbed, while sorbitol is not absorbed at all (Hyams *et al*., 1988; Rumessen and Gudmand-Høyer, 1988). When the capacity for sugar absorption in the small intestine is exceeded, a substantial amount of the malabsorbed carbohydrate enters the large intestine where it acts as an osmotic laxative, resulting in the production of gas and organic acids (Hyams *et al*., 1988; Rumessen and Gudmand-Høyer, 1988). Therefore, typical symptoms of carbohydrate malabsorption include diarrhoea, flatulence, borborygmus, abdominal distention, and abdominal cramps (Lifshitz *et al*., 1992).

The absorptive capacity for fructose in humans has not been established conclusively (Riby *et al*., 1993); however, according to early perfusion studies conducted by Holdsworth and Dawson (1964), the total intestinal capacity for fructose absorption in adult males was estimated to be tremendous (approximately 4,800 g/day). There is a tremendous heterogeneity in the absorptive capacity and symptoms of fructose malabsorption within the general population. The ingestion of even a small quantity of foods or beverages with a high fructose content (*e.g.*, apples, apple juice, and pear juice) could result in symptoms of malabsorption among individuals whose absorptive capacity is at the lower end of the range (Hommes, 1993; Riby *et al*., 1993). However, fructose malabsorption can occur as frequently in healthy children and adults as in those with functional bowel disorders (Nelis *et al*., 1990). Additionally, the development of symptoms of malabsorption is influenced by an individual's ability to handle the colonic fermentation products (Hyams *et al*., 1988). Therefore, peak rises in hydrogen excretion may occur in individuals without the accompanying gastrointestinal effects.

Results of several studies in which healthy humans ingested fructose alone or in combination with glucose have showed that the absorptive capacity for fructose increases in direct proportion to the amount of glucose ingested (Ravich *et al*., 1983; Kneepkens *et al*., 1984; Rumessen and Gudmand-Høyer, 1986, 1988; Truswell *et al*., 1988). In the absence of glucose, a relatively large proportion of the subjects studied were unable to completely absorb fructose at doses ranging from 20 to 50 g, which are amounts commonly found in products sweetened with high-fructose syrup (HFS) or crystalline fructose (Riby *et al*., 1993). The addition of glucose to the oral dose of fructose significantly reduces the frequency and severity of fructose malabsorption in humans (Ravich *et al*., 1983; Kneepkens *et al*., 1984; Rumessen and Gudmand-Høyer, 1986, 1988; Truswell *et al*., 1988). Specifically, the presence of glucose facilitates the transport of fructose across the brush-border membrane of the small intestine in the glucose-dependent fructose co-transport mechanism, and thereby increases the rate at which fructose is absorbed (Rumessen and Gudmand-Høyer, 1986; Smith *et al*., 1995). The frequency of glucose, fructose, and sucrose malabsorption based on breath hydrogen levels in healthy adults and children is presented in Table XIII.4.1-1.

Table XIII.4.1-1 Frequency of Glucose, Fructose, and Sucrose Malabsorption in Healthy Adults and Children Following Bolus Dosing				
Carbohydrate	**Carbohydrate Dose (g)/ Volume Ingested (mL)**		**Proportion of Subjects Who Experienced Malabsorption[1] (%)**	**Reference**
Studies In Healthy Adults				
Fructose	25	250	52	Rumessen and Gudmand-Høyer, 1988
Fructose	25 50	250 500	19 58	Truswell *et al*., 1988
Fructose	25 37.5 50 50	250 370 250 500	0 14 37 71	Ravich *et al*., 1983
Fructose	12.5 20 25 37.5 50	250 200 250 370 500	10 40 50 70 50	Rumessen and Gudmand-Høyer, 1986
Fructose and glucose	50+12.5 50+25 50+50	500 500 500	70 30 0	Rumessen and Gudmand-Høyer, 1986
Fructose and glucose	25+25	250	0	Truswell *et al*., 1988
Glucose	50	500	0	Truswell *et al*., 1988
Sucrose	50	500	0	Truswell *et al*., 1988
Sucrose	50	500	0	Rumessen and Gudmand-Høyer, 1988
Sucrose	100	500	0	Rumessen and Gudmand-Høyer, 1986
Sucrose	50	500	0	Ravich *et al*., 1983
Studies in Healthy Children (1 month to 17 years of age; mean age: 8 years)				
Fructose	2 g/kg body weight		71	Kneepkens *et al*., 1984
Fructose and (glucose or galactose)	2+2 g/kg body weight		14	Kneepkens *et al*., 1984

Adapted from Riby *et al*. (1993)
[1]Estimates of malabsorption were based on breath hydrogen levels

In a study conducted by Rumessen and Gudmand-Høyer (1988), 13 of 25 patients with functional bowel disease experienced abdominal distress and exhibited >10 ppm rise in breath hydrogen following ingestion of 25 g fructose; 7 patients were reported to have such symptoms upon ingestion of <15 g fructose. Conversely, none of the subjects had symptoms of malabsorption after ingesting 50 g sucrose, which contains 25 g fructose and 25 g glucose.

Sixteen healthy adult volunteers (8/sex, 23 to 48 years of age) were subjected to a fructose-loading test with up to 50 g fructose as 10 or 20% solutions (Ravich *et al*., 1983). Incomplete absorption of fructose, as reflected by the breath-hydrogen test, was observed in 6 and 10 individuals who consumed solutions containing 10 and 20% fructose, respectively. Conversely, when the subjects ingested a 50 g sucrose load as a 10% solution, no signs of incomplete absorption could be detected. Additionally, abdominal symptoms (*i.e.*, gas, cramps, and diarrhoea) were reported in 5 individuals who consumed 50 g of fructose as a 10% solution. Similar symptoms were reported in those receiving the 20% solution. However, there was no correlation observed between the incidence of abdominal symptoms and breath hydrogen increases, since diarrhoea was reported in 1 individual subjected to the sucrose load.

Following a 12-hour fast, 293 male and female patients (aged 1 to 15 years) suffering from recurrent abdominal pains, gassiness in the abdomen or intestine (meteorism), or diarrhoea, were provided with 1 g/kg of a 10% fructose solution, up to a maximum dose of 25 g fructose (Götze and Mahdi, 1992). Breath hydrogen levels were measured before and at 60, 90, and 120 minutes after the fructose load ingestion. Breath hydrogen levels >20 ppm were reported in 108 patients, which included 79 patients who developed symptoms during the test and 29 who remained symptom-free. Clinical symptoms also were reported in 2 patients who exhibited normal breath hydrogen levels. In addition to the fructose-loading test, 19 patients who demonstrated extremely high levels of breath hydrogen and clinical symptoms during the initial trial were additionally provided with 1 g/kg body weight fructose and glucose solution (up to a maximum of 25 g fructose and 25 g glucose). None of these patients were reported to have clinical symptoms or abnormal levels of breath hydrogen following ingestion of the fructose-glucose mixture.

A fructose-loading test with 2 g fructose/kg body weight was administered to 31 children with abdominal symptoms or functional bowel disorders (Kneepkens *et al*., 1984). It was reported that 22 of 31 (71%) children had breath hydrogen levels of 103 ppm, including 4 children who complained of abdominal symptoms after the loading test. Seven children were re-challenged with a fructose solution to which equal amounts of glucose or galactose had been added. Breath hydrogen increases were 5 ppm after ingesting the solution of fructose plus glucose and 10 ppm after ingesting the solution of fructose plus galactose, indicating that malabsorption of carbohydrates did not occur.

In a later study by Kneepkens *et al*. (1989), 5 of 8 normal children showed fructose malabsorption following ingestion of apple juice, as evaluated by the breath-hydrogen test. However, the addition of glucose to apple juice consumed by children with chronic non-specific diarrhoea (CNSD) resulted in normalization of breath hydrogen output. Therefore, the authors

concluded that malabsorbed fructose, not sorbitol, was the cause of the increased breath hydrogen output in the children.

According to Smith *et al.* (1995), fructose absorption occurs more completely with white grape juice, which contains equal amounts of fructose and glucose than with apple juice, which contains fructose concentrations greater than that of glucose. In a randomized double-blind crossover study, 18 healthy infants and 10 toddlers (mean ages 6.3 and 18 months, respectively) were fed 4 oz. white grape juice or 8 oz. apple juice. The average consumption of fructose was approximately 0.78 and 1 g/kg body weight in the 6- and 18-month-old children, respectively. In general, approximately 19 and 54% of the children who consumed white grape juice and apple juice, respectively, exhibited carbohydrate malabsorption, as evidenced by the presence of ≥20 ppm breath hydrogen levels. Malabsorption was more common among toddlers than infants, with a significant difference occurring after ingestion of apple juice. Only one subject in the study developed loose stools after ingesting apple juice. According to the authors, the high glucose content of white grape juice may explain why it was better tolerated than the other fruit juices, while the higher frequency of malabsorption reported for apple juice was due to its high sorbitol content.

The breath-hydrogen method was performed in 20 children (aged 9 to 36 months), 13 of whom were healthy and 7 had chronic diarrhoea (Hyams *et al*., 1988). Following a 12-hour fast, the children were provided between 150 and 240 mL pear juice, apple juice, or grape juice, or a 2% solution of sorbitol. Each of the fruit juices contained 60 to 70 mg fructose/mL (approximately 9 to 17 g fructose/person); however, both pear and apple juices also contained 20 to 30 mg glucose/mL (approximately 3 to 7 g glucose/person), while grape juice contained 70 mg glucose/mL (approximately 10 to 17 g glucose/person). In addition, pear juice also contained 20 mg sorbitol/mL. Sugar malabsorption, as reflected by excess hydrogen excretion, was reported in 25, 50, and 100% of the subjects after ingestion of grape juice, apple juice, and pear juice or sorbitol, respectively. No significant difference in breath hydrogen levels was reported between healthy children and those with chronic diarrhoea. According to the authors, the high glucose content of grape juice (70 mg glucose/mL) may explain why it was better tolerated than the other fruit juices, while the high frequency of malabsorption reported for pear juice was due to the presence of sorbitol.

Overall, the results of the above studies demonstrate that the absorptive capacity for fructose increases in direct proportion to the amount of glucose ingested, such that when consumed in equal amounts no effects of malabsorption were reported in any of the individuals (Ravich *et al*., 1983; Kneepkens *et al*., 1984; Rumessen and Gudmand-Høyer, 1986, 1988; Truswell *et al*., 1988). Moreover, neither glucose nor sucrose provided alone, as single, oral, bolus doses of up

to 100 g were associated with any gastrointestinal discomforts (Ravich *et al*., 1983; Rumessen and Gudmand-Høyer, 1986, 1988; Truswell *et al*., 1988).

The reduction in the occurrence of symptoms of malabsorption observed with combined glucose/fructose bolus doses is particularly significant given that hydrolysis of isomaltulose yields equal parts glucose and fructose on a molar or weight basis. Consequently, effects observed with fructose in the absence of glucose would not be expected to occur with isomaltulose. Furthermore, due to the similarity in the pathways involved in sucrose and isomaltulose metabolism, the frequency of carbohydrate malabsorption resulting from isomaltulose ingestion could be considered equivalent to or less than that of sucrose, which as described above, did not elicit any symptoms of malabsorption in the clinical trials evaluating its tolerance.

Based on results of the tolerance studies, which demonstrate that subjects administered combined bolus doses of 50 g each of glucose and fructose, or single bolus doses of 100 g sucrose (Rumessen and Gudmand-Høyer, 1988) without evidence of malabsorption, it can be inferred that a daily intake of 100 g isomaltulose would likely be well tolerated, particularly if consumed in smaller amounts throughout the day. As described in Section IX.2.1, the highest 97.5th percentile isomaltulose consumption estimate from all proposed food uses for all-users is 97.8 g/day (male teenagers), which corresponds to 48.9 g/day of each glucose and fructose from isomaltulose. Both the estimated daily intake of isomaltulose, as well as the resulting intakes of glucose and fructose upon the hydrolysis of isomaltulose, therefore, are below the single bolus sucrose dose of 100 g and the combined bolus doses of 50 g each of glucose and fructose, respectively, which were consumed without eliciting any symptoms of malabsorption.

XIII.4.2 Estimates of Daily Intake of Total Sugars in the E.U.

Although individual estimates of the daily consumption of sucrose or fructose, which would facilitate a direct comparison to the estimated intakes of isomaltulose, were unavailable based on the food consumption data collected as part of the U.K. Food Standards Agency's DSP, data were provided for the combined intake of sugars in the U.K. (UKDA, 1991, 1995, 2001) (for further details pertaining to the study design see Section IX.2.1). Specifically, glucose, sucrose, fructose, lactose, maltose, other sugars, and non-milk extrinsic sugars[1] comprised the sugars category. Proportionally, of the sugar sources identified, it is expected that glucose, sucrose, and fructose would contribute most significantly to the overall intake of sugars.

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[1] Non-milk extrinsic sugar includes all sugars in fruit juices, table sugar, honey, and sucrose, glucose and glucose syrups added to food, as well as 50% of the sugars in canned, stewed, dried or preserved fruits.

Mean daily intakes of total sugars for individual age groups ranged from 87 g (approximately 6 g/kg body weight) for children to 125 g (approximately 2.4 g/kg body weight) for male teenagers. Similarly, for the 97.5th percentile, children and male teenagers also were identified as having the lowest and highest intakes of sugars on a daily basis, respectively (156 and 231.8 g/day, respectively or approximately 10.8 and 4.5 g/kg body weight/day). As in the case of isomaltulose, total sugar mean and 97.5th percentile intakes were determined to be the highest in male teenagers. Based on the data provided in Section IX.2.1, the estimated intake of isomaltulose for male teenagers is, therefore, 3.3- and 2.4-fold lower than that of combined sugars for the mean and 97.5th percentile, respectively. Accordingly, the estimated intake of fructose, as derived from the hydrolysis of isomaltulose, is expected to be 6.6- and 4.7-fold lower for the mean and 97.5th percentile, respectively, compared to the intake of sugars.

On a body weight basis, intake estimates of isomaltulose and of total sugars are highest for children. Mean and 97.5th percentile isomaltulose intake estimates for children are approximately 1.6 and 4.0 g/kg body weight/day, respectively, compared to 6 and 10.8 g/kg body weight/day, respectively, for total sugars. The estimated mean and 97.5th percentile intakes of fructose, as derived from the hydrolysis of isomaltulose are 0.8 and 2 g/kg body weight, respectively, which are 7.5- and 5.4-fold lower than that of combined sugars. Furthermore, it is expected, that isomaltulose, if approved for use, will be added to foods that will be used in place of other foods containing sucrose or high-fructose corn syrup (HFCS). This should not affect the overall intake of fructose.

Table XIII.4.2-1 below provides a general comparison of the estimated intakes of isomaltulose and total sugars for the specified population groups.

Table XIII.4.2-1 Comparison of Total Sugars and Isomaltulose Intake Estimates (g/day)

Population Group	Sugars		Isomaltulose[1]					
			Total		Fructose Component[2]		Glucose Component[2]	
	Mean	97.5th %ile	Mean	97.5th %ile	Mean	97.5th %ile	Mean	97.5th %ile
Children (1½ to 4½)	87.0	156	21.8	56.8	10.9	28.4	10.9	28.4
Female Teenagers (11 to 18)	95.7[3]	180.1[3]	28.0	71.5	14.0	35.8	14.0	35.8
Male Teenagers (11 to 18)	125.0[3]	231.5[3]	37.7	97.8	18.8	48.9	18.8	48.9

Table XIII.4.2-1 Comparison of Total Sugars and Isomaltulose Intake Estimates (g/day)								
Population Group	**Sugars**		**Isomaltulose[1]**					
			Total		**Fructose Component[2]**		**Glucose Component[2]**	
	Mean	**97.5th %ile**	**Mean**	**97.5th %ile**	**Mean**	**97.5th %ile**	**Mean**	**97.5th %ile**
Female Adults (16 to 64)	86.0	171.0	9.2	38.8	4.6	19.4	4.6	19.4
Male Adults (16 to 64)	115.0	224.0	10.2	46.1	5.1	23.1	5.1	23.1

[1] Intakes presented are based on all-person consumption as presented in detail in Section IX.b.1.
[2] Based on metabolism data indicating that isomaltulose is hydrolyzed in equal parts to fructose and glucose.
[3] A weighted average of estimated intakes provided for groups of 11 to 14 and 15 to 18 year old males and females.

XIII.4.3 Intake of High Single Doses of Isomaltulose in Tolerance Tests

Gastrointestinal symptoms such as diarrhoea, abdominal cramps, which may be associated with malabsorption, were not observed in any of the clinical trials in which isomaltulose was administered as a bolus dose, even at amounts as high as 1 g/kg body weight (~70 g) (MacDonald and Daniel, 1983; Kawai *et al.,* 1985, 1989) (see Section XIII.2.7). The consumption of isomaltulose at the intended use levels over the course of a day is not expected to be associated with physiological gastrointestinal effects attributable to malabsorption. Furthermore, the fructose component that would have been absorbed in the studies, following hydrolysis of isomaltulose, would be in the order of 25 to 35 g. The results of studies investigating the tolerance of fructose have indicated that 25 g bolus doses of fructose, when administered alone, were associated with malabsorption in up to 52% of subjects (Rumessen and Gudmand-Høyer, 1988; Truswell *et al.,* 1988); however, there were no reports of malabsorption when subjects were administered a combined bolus dose of 25 g (Truswell *et al.,* 1988; Götze and Mahdi, 1992) or 50 g (Rumessen and Gudmand-Høyer, 1988) each of glucose and fructose.

Although the estimated daily intakes in children and young people on a kilogram body weight basis from all proposed food categories at the 97.5th percentile (*i.e.*, 4 and 2.7 g isomaltulose/kg body weight, respectively) exceed the highest bolus dose of 1 g/kg body weight tested in the tolerance tests, results of the study conducted by Kneepkens *et al*. (1984) demonstrate absence of any gastrointestinal symptoms when fructose was consumed concurrently with glucose in equal amounts of 2 g/kg body weight, which given equal hydrolysis of isomaltulose to either carbohydrate would be derived from a dose of 4 g isomaltulose/kg body weight.

More specifically, the fructose-loading test with 2 g fructose/kg body weight was administered to 31 children aged between 1 month and 17 years with abdominal symptoms or functional bowel disorders (Kneepkens *et al*., 1984). It was reported that 22 of 31 (71%) children had breath hydrogen levels of 103 ppm, including 4 (13%) children who complained of abdominal symptoms after the loading test. In contrast, of 7 children re-challenged with a fructose solution to which equal amounts of glucose had been added, only 1 exhibited breath hydrogen excretion in excess of 10 ppm (*i.e*., 14%) and none were reported to suffer form any adverse gastrointestinal symptoms. Similar results were obtained with equal amounts of galactose and fructose. Overall, these results support the findings that malabsorption of carbohydrates does not occur when fructose is provided in combination with either glucose or galactose. Therefore, it can be expected that a dose of 2.7 or 4 g isomaltulose/kg body weight in young people and children, respectively, which will be hydrolysed to equal parts glucose and fructose, will be well tolerated, absorbed, and, consequently, not associated with gastrointestinal symptoms of malabsorption. Furthermore, the 97.5th percentile "worst-case" intake estimate for isomaltulose for children is 1.5 times lower than the mean intake estimate of total sugars, which includes fructose (4.0 g/kg body weight/day versus 6.0 g/kg body weight/day, respectively).

EVALUATIONS AND CONCLUSIONS

Isomaltulose is a naturally occurring reducing dissacharide, composed of a glucose and fructose molecule, linked by a 1,6-glycosidic bond. When viewed in its entirety, the scientific evidence presented indicates that under conditions of intended use in foods as a nutritive sweetener, isomaltulose, would not produce any adverse health effects. Isomaltulose is produced by enzymatic rearrangement of sucrose, using non-pathogenic, non-viable *P. rubrum* cells. Safety of the microorganism, administered intravenously to mice and rabbits, has been confirmed in a 14-day toxicity study.

Metabolic data indicate that prior to absorption isomaltulose is almost completely hydrolyzed to fructose and glucose, and both metabolites are subsequently utilized in well-characterized carbohydrate pathways. Nutritionally, the compound is, therefore, equivalent to sucrose, which has an extensive history of use in the European Community. The safety of isomaltulose is confirmed by a series of published animal toxicity and human clinical studies, including human trials performed specifically using Cerestar's isomaltulose, reporting no adverse toxicological effects relevant to the conditions of the intended uses in foods.

In conclusion, there is a substantial body of evidence to support the safety of isomaltulose, a novel food ingredient based on its lack of prior history of use in the European Community. On the basis of the available toxicology data, its nutritional equivalence to sucrose, and the established use of the microorganism *P. rubrum* in the production of isomalt (E953), it is concluded that isomaltulose does not present a significant risk for human health at the intake, which would result from its intended uses in food.

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