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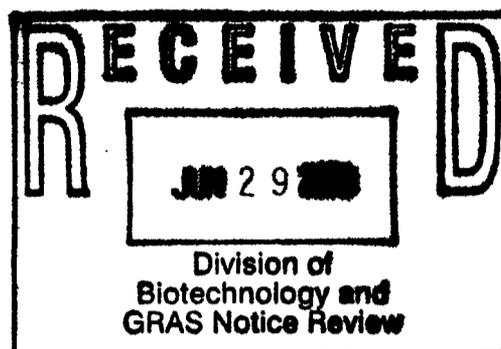
KOHJIN Co., Ltd.

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SENT VIA FEDEX

June 25, 2009

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835



Re: Re-Submission of GRAS Notice for Glutathione (Original GRN 000244)

Dear Dr. Martin:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [Kohjin Co., Ltd, 1-21, Nihombashi Muromachi 4 Chome, Chou-ku, Tokyo, 103-0022, Japan], a Notice of the determination, on the basis of scientific procedures, that glutathione, produced by Kohjin Co., Ltd (KOHJIN), as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. The enclosed Notice replaces the original Notice for glutathione, which was submitted on January 31, 2008 and subsequently withdrawn on May 7, 2008 (GRN 000244). Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance, information on self-limiting levels of use, and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of the glutathione ingredient of KOHJIN under the intended conditions of use, also are enclosed for review by the agency.

I trust that the enclosed Notice is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

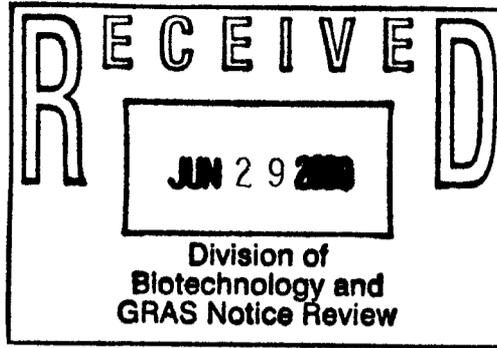
Sincerely,

(b)(6)

Yosuke Uchida
General Manger, Sales Department, Bio-Chemicals Division
y_uchida@kohjin.co.jp

Encl.

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GRAS NOTICE FOR GLUTATHIONE

Prepared for:

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by:

Kohjin Co., Ltd
1-21, Nihombashi Muromachi 4 Chome.
Chou-ku, Tokyo, 103-0022
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June 25, 2009

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GRAS NOTICE FOR GLUTATHIONE

Table of Contents

	Page
I. GRAS EXEMPTION CLAIM	1
Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]	1
I.A Name and Address of Notifier	1
I.B Common Name of the Notified Substance	1
I.C Conditions of Intended Use in Food	2
I.D Basis for the GRAS Determination	3
I.E Availability of Information	4
II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE	5
II.A Identity	5
II.B Method of Manufacture	5
II.C Specifications for Food-Grade Material	9
II.C.1 Product Specifications and Analysis	9
II.C.2 Additional Analyses	9
II.C.3 Stability of L-Glutathione	10
III. SELF-LIMITING LEVELS OF USE	13
IV. BASIS FOR GRAS DETERMINATION	14
IV.A Documentation to Support the Safety of L-Glutathione	14
IV.B Probable Consumption of L-Glutathione	14
IV.B.1 Estimated Intake of L-Glutathione Under the Intended Conditions of Use in Foods	14
IV.B.2 Occurrence of GSH in the Diet and Background Dietary Intakes	16
IV.B.3 Cumulative Consumption of GSH in the Diet	17
IV.C Endogenous Presence of Glutathione	17
IV.D Metabolic Fate of L-Glutathione	20
IV.E Preclinical Studies Pertaining to the Safe Consumption of L-Glutathione	21
IV.E.1 Acute and Short-Term Studies	21
IV.E.2 Subchronic Toxicity Studies	21
IV.E.3 Genotoxicity and Mutagenicity Studies of GSH	23
IV.E.4 Carcinogenicity Studies	23
IV.E.5 Developmental Toxicity Studies	24
IV.F Studies in Humans	24
IV.G Summary and Basis for GRAS Conclusion	25
REFERENCES	28

List of Figures and Tables

Figure II.B-1	Schematic Overview of the Manufacturing of L-Glutathione	8
Figure II.C.3.3-1	Degradation Products of L-Glutathione	12
Figure IV.C-1	Outline of the Biochemistry of Glutathione	19
Table I.C-1	Summary of the Individual Intended Food Uses and Use Levels for L-Glutathione in the United States	2
Table II.C.1-1	Product Specifications for L-Glutathione	9
Table IV.B.1-1	Summary of the Estimated Daily Intake of L-Glutathione from Intended Food Uses in the U.S. by Population Group (2003-2004 NHANES Data)	15
Table IV.B.1-2	Summary of the Estimated Daily per Kilogram Body Weight Intake of L-Glutathione from Intended Food Uses in the U.S. by Population Group (2003-2004 NHANES Data)	15

List of Appendices

Appendix A	Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of L-Glutathione as a Food Ingredient Following Changes In Uses and Use Levels
Appendix B	Literature Search Strategy for Torula Yeast
Appendix C	Methods of Analysis
Appendix D	Batch Analyses of L-Glutathione
Appendix E	Translated Pre-Clinical Studies
Appendix F	Tathion® Monograph

I. GRAS EXEMPTION CLAIM

Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

As defined herein, glutathione from torula yeast (*Candida utilis*) has been determined by Kohjin Co., Ltd. (KOHJIN) to be Generally Recognized as Safe (GRAS) consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures, as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of glutathione under the conditions of its intended use in food. Therefore, the use of glutathione derived from torula yeast in food as described below is exempt from the requirement of premarket approval.

Signed,

(b)(6)

June 25, 2009

Date

Yosuke Uchida
General Manger,
Sales Department,
Bio-Chemicals Division
Kohjin Co., Ltd.
yosuke.uchida@kohjin.co.jp

I.A Name and Address of Notifier

Yosuke Uchida
Kohjin Co., Ltd
1-21, Nihombashi Muromachi 4 Chome
Chou-ku, Tokyo, 103-0022, Japan

I.B Common Name of the Notified Substance

L-Glutathione¹

¹ L-Glutathione is the current trade name for KOHJIN's ingredient; however, a different trade name may be selected in the future.

I.C Conditions of Intended Use in Food

KOHJIN intends to market L-Glutathione as a food ingredient in the United States in a variety of food, beverage, and confectionary products at levels between 0.004 and 6.667%. L-Glutathione is not intended to be used in any meat or meat-containing products. A summary of the foods in which L-Glutathione is intended to be used as a food ingredient and the levels of use of L-Glutathione in such foods is presented in Table I.C-1.

Table I.C-1 Summary of the Individual Intended Food Uses and Use Levels for L-Glutathione in the United States				
Food Category	Proposed Food Use	Level of L-Glutathione (mg/serving)	RACC* (g or mL)	Use Level for L-Glutathione (%)
Baked Goods and Baking Mixes	Cookies	100 to 133.2	30 to 40	0.250 to 0.333
	Crackers	100	30	0.333
Beverages and Beverage Bases	Ice Teas (Powdered)	300	15	2.000
	Sports and Isotonic Beverages	360	240	0.150
Breakfast Cereals	Instant and Regular Hot Cereals	50 to 68.7	40 to 55	0.091 to 0.125
	Ready-to-Eat Cereals	50 to 183.1	15 to 55	0.091 to 0.333
Cheeses	Cottage Cheese	50	110	0.045
	Cream Cheese	50	30	0.167
	Imitation Cheese	50	30	0.167
	Natural Cheese	50	30	0.167
	Processed Cheese	50	30	0.167
Chewing Gum	Chewing Gum	200	3	6.667
Coffee and Tea	Instant Coffee (Powdered)	100	15	0.667
Condiments and Relishes	Soy Sauce	10	15	0.067
Dairy Product Analogs	Soy-Based Meal Replacements (Powdered)	100	15	0.667
	Soy Milk	50	240	0.021
Gelatin, Puddings, and Fillings	Gelatin, Jams, and Jelly	50 to 399.6	15 to 120	0.042 to 0.333
	Gelatin Drinks	100	240	0.042
	Puddings	30	120	0.025
Grain Products and Pastas	Gratin	30	30	0.100
	Pizza (Crust)	30	140	0.021
	Ready-Made Noodles and Canned Pasta	30	245	0.012
Gravies and Sauces	Barbecue Sauces	10	30	0.033
	Gravy Sauces	10	60	0.017
Hard Candy	Hard Candy	300	15	2.000
	Mints	100	2	5.000
Milk Products	Cocoa Powder Mixtures	10	15	0.067

Table I.C-1 Summary of the Individual Intended Food Uses and Use Levels for L-Glutathione in the United States				
Food Category	Proposed Food Use	Level of L-Glutathione (mg/serving)	RACC* (g or mL)	Use Level for L-Glutathione (%)
	Milk-Based Meal Replacements (Powdered)	100	15	0.667
	Milk (Dry and Powdered Mixtures)	100	15	0.667
	Yoghurt (Includes Frozen)	396 to 742.5	120 to 225	0.330
	Yoghurt Drinks and Fruit Smoothies	216	240	0.090
Plant Protein Products	Plant-Protein-Based Meal Replacements (Powdered)	100	15	0.667
	Protein Bars	100	40	0.250
Processed Fruits and Fruit Juices	Fruit Flavored Drinks (Powdered)	225	15	1.500
	Fruit Flavored Drinks (Frozen)	200	240	0.833
Processed Vegetables and Vegetable Juices	Vegetable Juices	100	240	0.042
Salty Snacks	Potato and Corn Based Chips	100	30	0.333%
Soft Candy	Chocolate Confectionary	400	40	1.000
	Soft Candy	100	40	0.250
Soups and Soup Mixes	Canned Soups	100	245	0.041
	Consommé	10	245	0.004
	Dehydrated and Powdered Soup Mixes	100	30	0.333
Sugar Substitutes	Sugar Substitutes	5	4	0.125

*Reference Amounts Customarily Consumed (RACC) per Eating Occasion (21 CFR §101.12 [U.S. FDA, 2008]. When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.

I.D Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, L-Glutathione has been determined by KOHJIN to be GRAS on the basis of scientific procedures (U.S. FDA, 2008). This GRAS determination is based on scientific data generally available in the public domain pertaining to the safety of L-Glutathione, as discussed herein, and on a consensus of opinion among a panel of experts qualified by scientific training and experience to evaluate the safety of L-Glutathione as a component of food² [see GRAS Notice No. 244 and Appendix A].

² The panel of experts consisted of Prof. Jack Bend, Ph.D. (University of Western Ontario), Prof. Joseph F. Borzelleca, Ph.D. (Medical College of Virginia), and Prof. Gary M. Williams, M.D. (New York Medical College).

GRAS NOTICE FOR GLUTATHIONE

I.E Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Mr. Tetsuo Kato
Kohjin Co., Ltd
1-21, Nihombashi Muromachi 4 Chome
Chou-ku, Tokyo, 103-0022, Japan

Should the FDA have any questions or additional information requests regarding this notification, KOHJIN will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

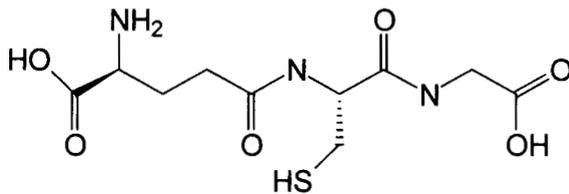
L-Glutathione is a white crystalline powder that is freely soluble in water, diluted alcohol, liquid ammonia, and dimethylformamide (Merck, 2006). The ingredient is isolated from torula yeast (*Candida utilis*) and occurs as the reduced form of glutathione.

Common or Usual Name: Glutathione
Chemical Name: Glutathione reduced; Glutathione, Reduced Form; 5-L-Glutamyl-L-cysteinylglycine; Glycine, N-(N-L-gamma-glutamyl-L-cysteinyl)-; GSH; L-Glutathione reduced; Reduced glutathione; gamma-L-Glutamyl-L-cysteinylglycine

Chemical Abstracts

Service (CAS) Number: 70-18-8
Empirical Formula: C₁₀H₁₇N₃O₆S
Molecular weight: 307.33 g/mol

Structural Formula:



II.B Method of Manufacture

As mentioned, L-Glutathione is manufactured *via* a fermentation process using torula yeast (*C. utilis*). Dried torula yeast is permitted for direct addition to food without limitation (21 CFR 172.896) (U.S. FDA, 2008). It also is permitted for use in enriched macaroni products (21 CFR 139.115), enriched nonfat milk macaroni products (21 CFR 139.122), and enriched noodle products (21 CFR 139.155) as a source of a prescribed quantity of specific vitamins and/or minerals (U.S. FDA, 2008). The strain of yeast used in the manufacturing process of L-Glutathione is non-genetically modified torula yeast strain IAM4264.

GRAS NOTICE FOR GLUTATHIONE

Prior to intake into the fermenter, the process water used in the fermenter, as well as the media composition, is sterilized by a heat-process-sterilizer. The fermentation process parameters (*i.e.*, aeration, temperature, pressure, and pH) are controlled through a computerized centrally-processed unit. Samples of seed culture and main culture are collected aseptically and assessed by microscopic observation and microbiological tests to ensure that no contamination has occurred during the fermentation process. Following fermentation, the yeast culture is washed with water, and the resulting yeast/water suspension is heated (to less than boiling point (*i.e.*, 100°C) to extract L-Glutathione from the yeast by breaking the cell membrane. Rapid cooling (4 to 60°C) and centrifugation separates L-Glutathione from the yeast cell body. Copper oxide is then added to form an L-Glutathione-copper complex. The L-Glutathione-copper salt is separated from other components using an ultrafiltration membrane (21 CFR 177.2910) and is cleansed with water (U.S. FDA, 2008). Hydrogen sulfide is infused to form a slurry, resulting in the decomposition of the GSH-copper salt to GSH and copper sulfide. The water-insoluble copper sulfide is removed through centrifugation, leaving a partially purified GSH solution. Ion exchange (using ion exchange resins permitted under 21 CFR 173.25) removes contaminating ions, including copper, and L-Glutathione is eluted from the resin with the use of a solvent (21 CFR 184.1005) and pH-adjusting agent (21 CFR 184.1095) (U.S. FDA, 2008). The purified L-Glutathione solution is filtered through an ultrafiltration membrane (21 CFR 177.2910) (U.S. FDA, 2008). Following filtration, the GSH solution is concentrated under reduced pressure at 4 to 60°C. Ethanol (21 CFR 184.1293) is then added to crystallize L-Glutathione (U.S. FDA, 2008). The crystals are collected by centrifugation, further washed with ethanol, and dried under reduced pressure at 40 to 70°C to evaporate any remaining ethanol and acetic acid. Several batches of L-Glutathione are blended to form 200 kg lots, which are weighed and packaged.

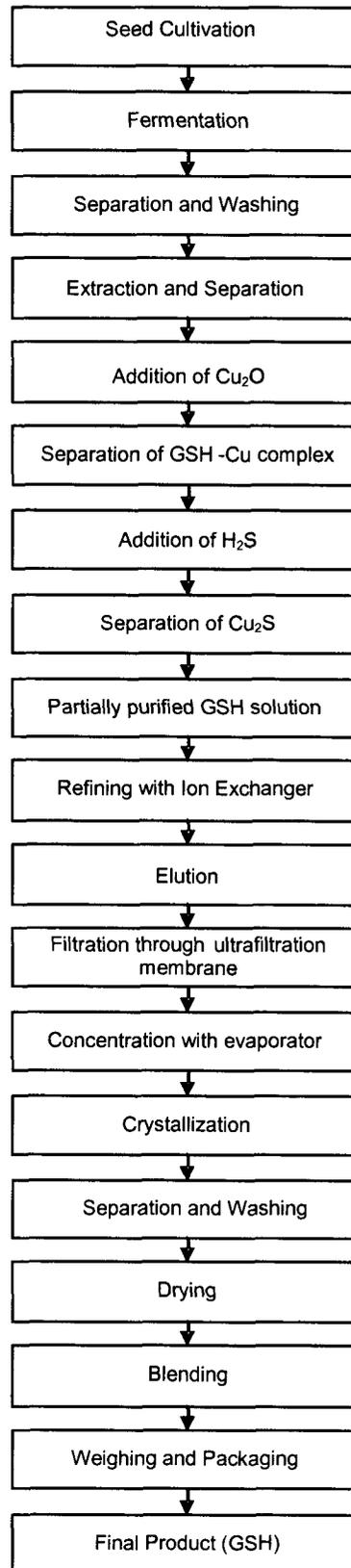
The torula yeast used in the manufacture of L-Glutathione is produced under similar conditions (*i.e.*, growth media, temperature, and pH) as the dried torula yeast used as a food ingredient (Boze *et al.*, 1992). For the manufacture of L-Glutathione, in order to extract glutathione from the yeast cell, the yeast, suspended in water, is heated to <100°C. As a food ingredient, dried torula yeast is permitted for use in enriched macaroni and noodle products, which are heated to boiling temperatures during cooking; therefore, the torula yeast used to produce L-Glutathione is not subjected to conditions different from those already incorporated in the manufacture of torula yeast as a food ingredient and/or in the preparation of yeast-containing foods. A literature search did not reveal additional published information pertaining to the safety of torula yeast. The strategy used to identify literature pertaining to the safety of torula yeast is presented in Appendix B.

All of the processing aids used in the processing steps are permitted for use in food in the U.S., with the exception of copper (I) oxide and hydrogen sulfide. These substances, along with all other processing aids and solvents, are removed during the extensive purification processes, as evidenced by the high purity of the final product (>98% GSH) and the low levels of residual

GRAS NOTICE FOR GLUTATHIONE

ethanol and lack of residual copper (see Section II.C.1). Therefore, the processing aids employed in the manufacturing of L-Glutathione are considered suitable safe and suitable for their respective uses. A schematic overview of the manufacturing process for L-Glutathione is presented in Figure II.B-1.

Figure II.B-1 Schematic Overview of the Manufacturing of L-Glutathione



II.C Specifications for Food-Grade Material

II.C.1 Product Specifications and Analysis

L-Glutathione is produced in accordance with current Good Manufacturing Practices (cGMP) and in order to ensure a consistent, safe product, KOHJIN has established food-grade specification parameters for the final preparation. These parameters comprise physical, chemical, and microbiological specifications, and are presented in Table II.C.1-1. Methods of analysis for the specification parameters also are presented below. Methods of analysis for glutathione purity and microbiological parameters are provided in Appendix C.

Specification Parameter	Specification	Method of Analysis
Appearance	White crystals or crystalline powder	Visual inspection
Glutathione (GSH)	Not less than 98.0%	JP, Glutathione assay method
Loss on drying	Not more than 0.5%	JP Method <2.41>
Residue on ignition	Not more than 0.1%	JP Method <2.44>
Lead	Not more than 1 ppm	JP Method <1.07>
Arsenic	Not more than 1 ppm	JP Method <1.11>
Total plate count	Not more than 3,000 CFU/g	JFSA (with modification)
Yeast and Mold (CFU)	Not more than 100 CFU/g	JFSA (with modification)
Coliforms	Negative per 2 g	JFSA (with modification)
<i>Salmonella</i> sp.	Negative per 25 g	JFSA (with modification)

CFU = colony forming units; JFSA = Japan Food Sanitation Act; JP = Japanese Pharmacopeia, 15th Ed.
 Note: Remainder of components (1.4%) consists of oxidized glutathione (GSSG) and other impurities such as cystenyl-glycine and glutamyl-cysteine, as measured by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE).

Several lots of the manufactured product were analyzed to confirm that the manufacturing process produced a consistent product within the physical, chemical, and microbiological parameters of the product specifications. A summary of lot analysis results for 3 non-consecutive lots of L-Glutathione are presented in Appendix D-1, along with the certificates of analysis.

II.C.2 Additional Analyses

Ethanol is used in the manufacturing process, and therefore the levels of residual ethanol, acetic acid (a metabolite of ethanol), and ethyl acetate (the ester from ethanol and acetic acid) in 5 non-consecutive lots of L-Glutathione were analyzed to demonstrate that these substances are present at low levels [similar to levels in a control substance, which was glutathione purchased from Wako Pure Chemical Industries (Osaka, Japan)] or absent (detection limit of 10 ppm). Levels of residual ethanol were below the maximum residual ethanol levels

GRAS NOTICE FOR GLUTATHIONE

determined to be acceptable for pharmaceuticals (5,000 ppm) by the International Conference of Harmonisation (ICH, 2005). A summary of the analyses for solvent residues in L-Glutathione is presented in Appendix D-2, along with a certificate of analysis.

Additionally, as copper is used as a complexation agent in the manufacturing process of L-Glutathione, the levels of residual copper were analyzed in 3 non-consecutive lots of the ingredient, with a limit of detection of 0.1 ppm, to confirm the removal of copper in the purification steps included in the production of L-Glutathione. A summary of the complete analyses for these lots and the original certificates of analysis are presented in Appendix D-3.

II.C.3 Stability of L-Glutathione

The stability of L-Glutathione has been evaluated under bulk storage conditions (*i.e.*, in dry powder form) and in solution under numerous conditions that represent the processing conditions of the intended food uses.

II.C.3.1 Bulk Stability

The stability of L-Glutathione was evaluated under various conditions, including high temperature and relative humidity, UV light, day light (lamp), and direct sunlight. The results of these analyses indicated that L-Glutathione is stable when stored in an airtight container at room temperature and normal relative humidity levels for 39 months (approximately 1% decrease in purity). L-Glutathione should not be exposed to strong light or high humidity levels.

II.C.3.2 Stability in Solution

Glutathione is an endogenous molecule comprising the 3 amino acids, cysteine, glycine, and glutamate. When dissolved in water and stored under conditions of variable pH for 7 days at room temperature, L-Glutathione is more stable at pH values of 3 to 6 (approximately 80% of the original amount of L-Glutathione remaining in the solution) than at lower or higher values (approximately 65% remaining in the solution at pH 2 and 7). Extended testing (30 days) revealed that 80% of L-Glutathione remains in solution at pH 4, 4.5, 5, and 6 (steady-state occurs by 30 days of storage), 75% remains at pH 7, 70% remains at pH 3, 60% remains at pH 2.5, and 50% remains at pH 2.

When stored at various temperatures for 7 days at a constant pH of 3, L-Glutathione dissolved in water is stable at lower temperatures (more than 85% L-Glutathione remaining at 4 to 25°C, with up to 10% CG formed), but is less stable at higher temperatures. For example, when dissolved in water and stored at 60°C, approximately 20% L-Glutathione remains. Following storage for 30 days (dissolved in water), it was determined that 90% of L-Glutathione remains at 4°C, 40% remains at 40°C, and 0% remains at 60°C. It should be noted that L-Glutathione will not be stored under conditions of higher temperatures for prolonged periods of time.

GRAS NOTICE FOR GLUTATHIONE

L-Glutathione dissolved in water also has been tested under high temperature (90 and 110°C) conditions for short time periods (10, 30, and 60 minutes), which reflect the processing conditions for some of the proposed food uses such as crackers, etc. When heated to 90°C, 94% of L-Glutathione remained after 10 minutes, 86% remained after 30 minutes, and 80% remained after 60 minutes. At a higher temperature (110°C), 87% remained after 10 minutes, 71% remained after 30 minutes, and 57% remained after 60 minutes.

II.C.3.3 Degradation Products of L-Glutathione Identified in Stability Studies

The results of the stability studies indicate that L-Glutathione is stable in dry powder form at high temperatures (70°C) and in solution at temperatures of 4 to 25°C and under moderately acidic conditions (pH 3 to 6) which supports the use of L-Glutathione in many of the proposed food uses. Under conditions outside of these ranges, representing the remaining food uses, L-Glutathione is less stable; however, the major degradation products have been identified as components of the endogenous GSH metabolic cycle, as further outlined in Section IV.C, and have been identified as occurring naturally in food products.

The major degradation products formed at lower pH values are cysteinylglycine (CG) (up to 20% of the original amount of L-Glutathione is degraded to CG) and pyroglutamic acid (PA) (up to 10%), while at higher pH values, L-Glutathione is primarily oxidized to glutathione disulfide (GSSG) (up to 30%, with 5% CG formed as well).

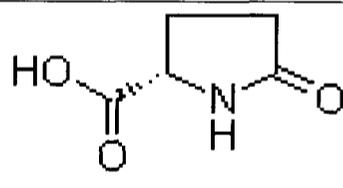
When L-Glutathione is dissolved in water and stored at 60°C, the major degradation products are CG (40%), PA (35%), and oxidized glutathione (5%). Minor degradation products include oxidized CG (occurring at 0.5 to 2%) and glutathione-cysteinylglycine mixed disulfide (occurring at 0.5 to 5%).

PA occurs in various foods, including cheese (16 to 600 mg/100 g) and soy sauce (3 to 5 mg/mL) (Nishimura *et al.*, 2001; Mucchetti *et al.*, 2002). GSSG, the oxidized form of glutathione, occurs naturally in various foods at levels 2 to 3-fold higher than does GSH (Wierzbicka *et al.*, 1989), and there is evidence of a mechanism in the small intestine of rats that reduces GSSG (Hagen *et al.*, 1990a). Cysteinylglycine disulfide, or oxidized CG, occurs in the human plasma at higher levels than the reduced form (Mansoor *et al.*, 1992). Although glutathione-cysteinylglycine mixed disulfide has not been measured in humans, it is likely present as mixed disulfides are commonly occurring compounds. Furthermore, if ingested glutathione-cysteinylglycine mixed disulfide were to be reduced, the resulting products would be GSH and CG, which occur endogenously. Also, both oxidized CG and glutathione-cysteinylglycine mixed disulfide are minor degradation products of GSH, occurring at levels up to 2 and 5%, respectively. Therefore, the formation of these products during storage of L-Glutathione in solution at various pH levels and temperature is of no safety concern.

GRAS NOTICE FOR GLUTATHIONE

The chemical structures of the degradation products of L-Glutathione are presented in Figure II.C.3.3-1.

Figure II.C.3.3-1 Degradation Products of L-Glutathione

L-glutathione (GSH)	$\begin{array}{c} \text{NH}_2\text{-CH-CH}_2\text{-CH}_2\text{-CO-NH-CH-CO-NH-CH}_2\text{-COOH} \\ \qquad \qquad \qquad \\ \text{COOH} \qquad \qquad \text{CH}_2\text{SH} \\ \text{Glutamic acid} \qquad \text{Cysteine} \qquad \text{Glycine} \end{array}$
Oxidized glutathione or GSSG (S-S bonding between 2 GSH Molecule)	$\begin{array}{c} \text{NH}_2\text{-CH-CH}_2\text{-CH}_2\text{-CO-NH-CH-CO-NH-CH}_2\text{-COOH} \\ \qquad \qquad \qquad \\ \text{COOH} \qquad \qquad \text{CH}_2\text{-S} \\ \qquad \qquad \qquad \\ \qquad \qquad \qquad \text{CH}_2\text{-S} \\ \text{NH}_2\text{-CH-CH}_2\text{-CH}_2\text{-CO-NH-CH-CO-NH-CH}_2\text{-COOH} \\ \\ \text{COOH} \end{array}$
Cysteinyl glycine (CG)	$\begin{array}{c} \text{NH}_2\text{-CH-CO-NH-CH}_2\text{-COOH} \\ \\ \text{CH}_2\text{SH} \end{array}$
Oxidized Cysteinyl glycine (S-S bonding between 2 CG Molecule)	$\begin{array}{c} \text{NH}_2\text{-CH-CO-NH-CH}_2\text{-COOH} \\ \\ \text{CH}_2\text{-S} \\ \\ \text{CH}_2\text{-S} \\ \\ \text{NH}_2\text{-CH-CO-NH-CH}_2\text{-COOH} \end{array}$
GSH-CG (S-S bonding between Cysteinyl glycine and Glutathione Molecule)	$\begin{array}{c} \text{NH}_2\text{-CH-CO-NH-CH}_2\text{-COOH} \\ \\ \text{CH}_2\text{-S} \\ \\ \text{CH}_2\text{-S} \\ \\ \text{NH}_2\text{-CH-CH}_2\text{-CH}_2\text{-CO-NH-CH-CO-NH-CH}_2\text{-COOH} \\ \\ \text{COOH} \end{array}$
Pyroglutamic acid (5-oxopyrrolidine-2-carboxylic acid)/ (Pyrrolidone carboxylic acid)	 <p style="text-align: right;">(C₅H₇NO₃)</p>

III. SELF-LIMITING LEVELS OF USE

The use of L-Glutathione in food and beverage products is self-limiting due to its sour taste and sulfur odor. The level of use of L-Glutathione will be 0.004 to 6.667%.

IV. BASIS FOR GRAS DETERMINATION

IV.A Documentation to Support the Safety of L-Glutathione

The determination that L-Glutathione is GRAS is on the basis of scientific procedures. The safety of L-Glutathione under the intended conditions is based on the natural background occurrence of GSH in the diet and an estimate of the probable consumption of the ingredient as calculated using the most recent publicly-available survey of U.S. food consumption, as well as, an extensive amount of published scientific data demonstrating the endogenous presence of GSH in humans and publicly available pharmacokinetic studies, toxicity studies, and studies in humans of GSH. Studies conducted using orally-administered GSH were considered most relevant to the determination of the GRAS status of GSH; however, there is evidence that GSH may be absorbed intact and therefore data in the subchronic intravenous administration of GSH were considered.

These data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of L-Glutathione as a component of food, who concluded that the proposed uses of L-Glutathione are safe and suitable and would be GRAS based on scientific procedures [see GRAS Notice No. 244 and Appendix A, entitled, "**EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-GLUTATHIONE AS A FOOD INGREDIENT FOLLOWING CHANGES IN USES AND USE LEVELS**"] and that other qualified experts would concur with these conclusion. It also is KOHJIN's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. A summary of these data is presented herein.

IV.B Probable Consumption of L-Glutathione

IV.B.1 Estimated Intake of L-Glutathione Under the Intended Conditions of Use in Foods

As mentioned, L-Glutathione is intended for use in a variety of food products, including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, cheeses, chewing gum, coffee and tea, condiments and relishes, dairy product analogs, gelatins, puddings and fillings, grain products and pastas, gravies and sauces, hard candy, milk products, plant protein products, processed fruits and fruit juices, processed vegetables and vegetable juices, salty snacks, soft candy, soups and soup mixes, and sugar substitutes, at levels of 0.004 to 6.667% (see Section I.C).

The consumption of L-Glutathione from all intended uses and use levels was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). Under the intended food uses,

GRAS NOTICE FOR GLUTATHIONE

96.9% of the total U.S. population was identified as potential consumers of L-Glutathione (8,009 actual users identified). On an all-user basis, the mean intake of L-Glutathione by the total U.S. population from all intended food uses was estimated to be 448 mg/person/day or 8.4 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of L-Glutathione by the total U.S. population from all intended food uses was estimated to be 961 mg/person/day or 20.1 mg/kg body weight/day. Under the intended conditions of uses the estimated intakes of L-Glutathione for all population groups are presented in Tables IV.B.1-1 and IV.B.1-2 on a per person and per kilogram body weight basis, respectively.

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg)	90 th Percentile (mg)	Mean (mg)	90 th Percentile (mg)
Infants	0 to 2	76.0	707	257	604	308	634
Children	3 to 11	99.9	1,286	490	897	491	897
Female Teenagers	12 to 19	99.7	989	437	949	438	949
Male Teenagers	12 to 19	99.4	993	561	1,161	563	1,167
Female Adults	20 and Up	99.6	2,121	403	899	404	902
Male Adults	20 and Up	99.2	1,913	474	1,095	477	1,095
Total Population	All Ages	96.9	8,009	443	953	448	961

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg/kg bw)	90 th Percentile (mg/kg bw)	Mean (mg/kg bw)	90 th Percentile (mg/kg bw)
Infants	0 to 2	76.0	707	21.3	48.8	25.6	52.3
Children	3 to 11	99.9	1,286	18.6	36.1	18.7	36.1
Female Teenagers	12 to 19	99.7	989	7.6	16.7	7.6	16.7
Male Teenagers	12 to 19	99.4	993	8.9	19.8	8.9	19.8
Female Adults	20 and Up	99.6	2,121	5.8	12.8	5.8	12.8
Male Adults	20 and Up	99.2	1,913	5.6	12.7	5.6	12.7
Total Population	All Ages	96.9	8,009	8.3	20.0	8.4	20.1

As mentioned, the estimated intakes of L-Glutathione under the intended conditions of use were calculated using recently published U.S. dietary consumption surveys (*i.e.*, 2003-2004

NHANES) (CDC, 2006; USDA, 2009), which include two 24-hour dietary recall 24-hour dietary recalls administered on 2 non-consecutive days. The surveys provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States; however, it is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys overestimate consumption over longer time periods (Anderson, 1988). Moreover, the calculations assume that all food products within a food category contain the ingredient at the maximum specified level of use. Therefore, the estimated intakes of L-Glutathione are over-estimates of anticipated actual consumption.

IV.B.2 Occurrence of GSH in the Diet and Background Dietary Intakes

Glutathione is present in foods, generally in the reduced form (GSH), which is the form that is absorbed in the small intestine (Hagen and Jones, 1989), but also in some foods in all oxidized or disulfide forms, including GSSG (Wierzbicka *et al.*, 1989; Jones *et al.*, 1992), with small amounts available for absorption by the small intestine (Hagen *et al.*, 1990a).

GSH has been reported to occur at a level of 5 to 20 mg/100 g in fresh meats, fish, and poultry (mean 9.7 mg/100 g), and at 4 to 15 mg/100 g in fruits (mean 3.2 mg/100 g) and vegetables (mean 4.7 mg/100 g) (Wierzbicka *et al.*, 1989; Jones *et al.*, 1992). GSH content is generally low in breads, cereals, legumes and nuts, oils and fats, sweets and snacks, and beverages (excluding fruit and dairy beverages). Fresh foods contain higher levels of GSH compared to frozen, canned, and processed foods, and cooking tends to decrease the natural GSH content of foods (Wierzbicka *et al.*, 1989; Jones *et al.*, 1992). GSH also has been identified in breast milk at levels ranging from approximately 164 to 253 $\mu\text{mol/L}$, resulting in GSH intakes of approximately 40 mg/day³ in infants (Ankrah *et al.*, 2000).

Estimates of dietary GSH intake may vary substantially due to differences in GSH content among foods and variations in consumption frequency; however, Wierzbicka *et al.* (1989) reported that the estimated dietary intake of GSH in the American population ranges from 2.9 to 131 mg/day, and Flagg *et al.* (1994) estimated daily intake levels of 13 to 110 mg/day. Additionally, in its purified form, GSH is currently sold by a number of manufacturers as a dietary ingredient in supplement products and is commonly manufactured *via* fermentation of yeast, similar to KOHJIN's L-Glutathione. As a nutritional supplement, GSH is usually supplied in capsule, powder, or tablet form with recommended doses ranging from 50 to 600 mg daily (PDRHealth, 2009).

³ Assuming that an infant consumes up to 0.75 L breast milk/day.

IV.B.3 Cumulative Consumption of GSH in the Diet

Based on the estimated background dietary GSH intake of up to 131 mg/day (Wierzbicka *et al.*, 1989) and the mean and 90th percentile total population estimated intake of L-Glutathione from the proposed food uses of 448 and 960 mg/person/day, respectively, the cumulative consumption of GSH in the diet is estimated to be approximately 600 to 1,000 mg/person/day. This estimate of cumulative consumption of GSH is within range of what is recommended for use as a dietary supplement product, and is likely an over-estimate due to over-estimates of consumption of L-Glutathione under the intended conditions of use due to the limitations in the methodology of calculating intakes (see Section IV.B.1).

IV.C Endogenous Presence of Glutathione

GSH is synthesized endogenously from the amino acids L-cysteine, L-glutamate, and glycine via γ -glutamylcysteine synthetase. The liver is the major site for the production and export of GSH, although virtually all cell types have the capacity to synthesize GSH.

Knowledge regarding the daily synthesis of GSH is limited due to complex compartmentalization of substrates and their metabolism at both the subcellular and organ levels (Wu *et al.*, 2004). Furthermore, GSH synthesis is affected by numerous factors, including oxidative stress and insult (Griffith, 1999). Lyons *et al.* (2000) reported the mean absolute synthesis rate of GSH in healthy adult males as 748 $\mu\text{mol/L/day}$ in whole blood. Assuming a blood volume of 5 L for the average adult, this value is equivalent to the endogenous synthesis of 1.15 g GSH/day. According to the authors, whole blood GSH synthesis may account for approximately 10% of whole body synthesis; therefore, it is estimated that 11.5 g GSH is synthesized in the body on a daily basis.

Glutathione occurs ubiquitously in human tissues, predominantly in its reduced form (GSH). Reported GSH levels in whole blood range from 684 to 2,525 $\mu\text{mol/L}$, which would be equivalent to total blood amounts of approximately 1 to 4 g⁴ (Pastore *et al.*, 2003). GSH is present in animal cells at levels of 0.5 to 10 mmol/L (Wu *et al.*, 2004). Extracellular GSH content is orders of magnitude lower, with typical plasma levels of 5 to 50 $\mu\text{mol/L}$ (Griffith, 1999). In neonatal infants, glutathione content in erythrocytes was reported in several studies, with levels of approximately 7 to 9 $\mu\text{mol/g}$ hemoglobin (Jean-Baptiste and Rudolph, 2003; Lee and Chou,

⁴ These values were calculated based on the assumption that the human body contains 5 L of blood. Sample calculation: 684 $\mu\text{mol/L}$ x 307.33 g/mol x 5 L blood = ~1 g GSH.

GRAS NOTICE FOR GLUTATHIONE

2005), corresponding to 0.08 to 0.13 g total glutathione in the blood⁵ (primarily GSH with a small fraction as GSSG).

Although GSH levels in the gut lumen of humans have not been measured, luminal GSH levels in the gastrointestinal tract of fasting rats range from 6 μ M in the stomach to 0.5 mM in the duodenum (Hagen *et al.*, 1990a), indicating that even in the absence of a dietary source, GSH is present in the small intestine. It also was demonstrated that GSH in the rat duodenum is derived from the bile. The authors suggested that luminal GSH may serve to detoxify xenobiotics present in the bile or food or may be absorbed for participation in intracellular detoxification reactions.

GSH is oxidized non-enzymatically to GSSG by reaction with electrophilic substances, including reactive oxygen/nitrogen species and free radicals. Under normal conditions, GSH levels are maintained by GSH reductase; however, GSSG may accumulate under conditions of oxidative stress, and may be secreted from the cell and degraded extracellularly contributing to a net loss of intracellular GSH levels. Because of the high concentration gradient between intracellular and extracellular GSH, influx of GSH or GSSG back into cells is thermodynamically unfavorable; however, exogenous GSH can be useful for increasing plasma and tissue GSH concentrations as, depending on the tissue, orally administered GSH may increase tissue GSH concentrations directly *via* uptake of intact GSH by transporters (as observed in the rat jejunum, lung and brain), indirectly through degradation of GSH and subsequent intracellular re-synthesis (liver), or by a combination of both mechanisms (heart) (Favilli *et al.*, 1997).

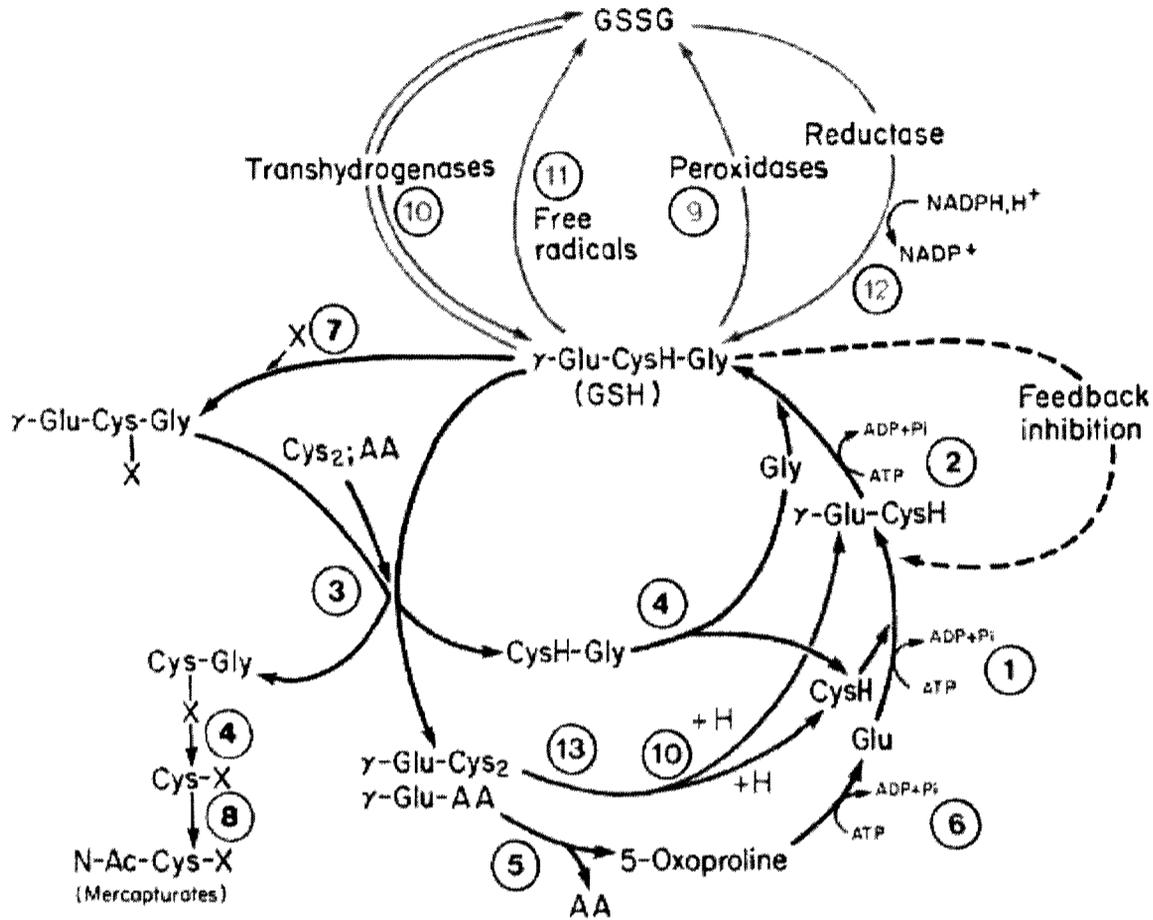
The endogenous catabolism of GSH occurs extracellularly by membrane-bound metabolic enzymes and results in the formation of CG and PA metabolites *via* the γ -glutamyl cycle. Within this metabolic pathway, the catabolism of GSH is initiated by γ -glutamyl transpeptidase, which catalyzes the transfer of the γ -glutamyl group of GSH to an acceptor amino acid. Direct products of this transpeptidase reaction are γ -glutamyl amino acids with CG formed as a by-product. γ -Glutamyl amino acids are further metabolized by γ -glutamyl cyclotransferase, with the resultant products being PA and the respective amino acids. PA is subsequently converted to the amino acid glutamine by the action of 5-oxoprolinase. CG also is broken down by dipeptidases into its constituent amino acids, cysteine and glycine. Thus, the final products of GSH catabolism are the amino acids glutamine, cysteine, and glycine, which are absorbed into the cell and recycled back into the γ -glutamyl cycle in which they are utilized for the biosynthesis of GSH. The biosynthesis of GSH requires two-enzyme catalyzed reactions and occurs intracellularly in all mammalian cells. First, the dipeptide γ -glutamylcysteine is

⁵ These values were calculated based on the assumption that the concentration of hemoglobin (Hb) in the blood is 150 g/L and that the neonatal body contains 0.25 to 0.32 L of blood (70 to 90 ml/kg body weight x 3.5 kg body weight). Sample calculation: 7 μ mol/g Hb x 150 g Hb/L blood x 307.33 g/mol x 0.25 L blood = ~0.08 g total glutathione.

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synthesized from the formation of an amide linkage between cysteine and glutamine, which is carried out by γ -glutamylcysteine synthetase. Subsequently, GSH synthetase catalyzes the reaction between γ -glutamylcysteine and glycine to produce GSH (Meister, 1988; Wang and Balatori, 1998; Ristoff and Larsson, 2007). Therefore, ingested CG and PA are expected to be broken down into their constituent amino acids, which will then be absorbed into cells and incorporated in the γ -glutamyl cycle and utilized directly in the biosynthesis of GSH. The pathways involved in GSH synthesis and catabolism are presented in Figure IV.C-1.

Figure IV.C-1 Outline of the Biochemistry of Glutathione



Adapted from Meister (1988)
 AA, amino acids, X, compounds that react with GSH to form conjugates. 1, γ -glutamylcysteine synthetase; 2, GSH synthetase; 3, γ -glutamyltranspeptidase; 4, dipeptidases; 5, γ -glutamylcyclotransferase; 6, 5-oxoprolinase; 7, GSH S-transferases; 8, N-acetyltransferase; 9, GSH peroxidases; 10, GSH thiol transferases; 11, reaction of free radicals with GSH, 12, glutathione disulfide (GSSG) reductase; 13, transport of γ -Glu-(Cys)₂.

IV.D Metabolic Fate of L-Glutathione

The results of studies involving oral administration of GSH to laboratory animals indicated that GSH is absorbed from the gastrointestinal tract intact, and following transport across the epithelial cell, is released into the blood and is taken up by various organs and tissues as needed. Specifically, the administration of GSH by gavage or *via* the diet increased plasma and tissue (*i.e.*, jejunum, lung, heart, liver, and brain) GSH concentrations in rats, but administration of the amino acid constituents of GSH did not affect plasma GSH concentrations, indicating that the increase in GSH concentration resulted from absorption of intact GSH and not from its metabolism and re-synthesis (Hagen *et al.*, 1990b; Favilli *et al.*, 1997). In mice, gavage administration of GSH increased plasma but not tissue GSH concentrations, demonstrating that changes in plasma GSH concentrations did not affect tissue levels and that cellular GSH homeostasis was tightly controlled under GSH-sufficient conditions (Aw *et al.*, 1991). Pre-treatment with a GSH synthesis inhibitor produced decreased tissue GSH levels in the rat, and subsequent oral GSH administration significantly increased GSH levels in all tissues measured (*i.e.*, the kidney, heart, brain, small intestine, and skin) except the liver, likely because this organ does not take up exogenous GSH (Hahn *et al.*, 1978). The principal site of GSH absorption in the rat is the upper jejunum (Hagen *et al.*, 1990a), which contains a sodium-dependent uptake system (Linder *et al.*, 1984; Hunjan and Evered, 1985; Hagen and Jones, 1987; Vincenzini *et al.*, 1987). Circulating GSH in rats is primarily cleared by the kidney (Hahn *et al.*, 1978).

The effects of oral GSH administration on plasma GSH levels in humans also have been investigated, with results indicating some similarities in its pharmacokinetic profile in humans and in laboratory animals. Witschi *et al.* (1992) provided a single oral dose of 0.15 mmol GSH/kg body weight (approximately 2.7 g GSH in a 60 kg individual) dissolved in water to 7 healthy male and female volunteers and reported no significant increases in plasma GSH levels when measured at 30-minute intervals for up to 270 minutes, although a transient increase was observed in 2 women and a slight (less than 2-fold) elevation was reported for the 4-hour time-point in 1 man. The authors suggested that the interspecies differences in plasma GSH levels following oral administration may be attributed to higher hepatic gamma-glutamyltransferase (γ -GT) activity in humans compared to rats, resulting in increased hydrolysis of GSH. Metabolism by intestinal γ -GT also may have contributed to the lack of increase in circulating GSH levels in humans. Alternatively, Hagen and Jones (1989) reported an increase in plasma GSH levels in 4 of 5 subjects provided a single dose of 15 mg GSH/kg body weight orally (0.9 g in a 60 kg individual). Plasma GSH concentrations peaked at 1 hour after administration to 300% of basal levels, and decreased to approximately 200% of baseline values by 3 hours post-GSH ingestion. Administration of the constituent amino acids of GSH to humans did not result in the same increase in plasma GSH as did administration of GSH (Hagen and Jones, 1989), demonstrating that GSH is absorbed intact.

IV.E Preclinical Studies Pertaining to the Safe Consumption of L-Glutathione

Few studies of the oral administration of GSH to animals were identified *via* a comprehensive search of the publicly available scientific literature. It is expected that few studies have been published due to the fact that GSH is an endogenous compound and is present in the background diet, and thus is not expected to pose concern for adverse effects in humans when consumed. The studies involving oral administration of GSH to animals are presented below. Due to the paucity of identified oral toxicity studies of GSH, studies involving intravenous administration were included in the safety assessment to support the safety of GSH. Intravenous administration results in 100% bioavailability, providing greater bioavailability of GSH compared to that achieved by oral administration, and therefore, provide a conservative margin of safety when translating to the safety of GSH from oral exposure. Studies that were available publicly in Japanese only have been translated and are provided in Appendix E.

IV.E.1 Acute and Short-Term Studies

In an acute toxicity study, male ICR-JCL mice, aged 7 to 8 weeks, were administered single doses of GSH sodium *via* oral, intravenous, or subcutaneous administration and were observed for 7 days, and the median lethal dose (LD₅₀) values were reported to be >10,000 mg/kg body weight (oral and subcutaneous) and >5,000 mg/kg body weight (intravenous), which were the highest doses tested (Nozaki *et al.*, 1972).

The efficacy of short-term oral GSH administration (up to 24 hours) for the treatment of toxicity caused by exposure to acetaminophen, methylmercury, and 95% O₂ has been investigated in several studies in laboratory animals at doses up to 1,200 mg/kg body weight (Ogawa *et al.*, 1972; Viña *et al.*, 1989; Brown *et al.*, 1996; Sugimura and Yamamoto, 1998). No adverse effects due to GSH administration were reported in these studies, and GSH was shown to be efficacious in the management of these toxicities.

IV.E.2 Subchronic Toxicity Studies

In an attempt to define the potential protective effects of oral GSH on the toxicity of inhaled sulfur dioxide (SO₂), mice (dd strain; 3 groups of 10 mice/group or 2 groups of 15 mice/group) and rats (hybrid; 3 groups of 5 rats/group) were administered a diet comprising 0.5% TATHION[®] (a product containing 0.1% GSH), providing approximately 150 and 50 mg GSH/kg body weight/day for mice and rats, respectively (U.S. FDA, 1993), while housed in an inhalation chamber providing air with up to 0.4 ppm SO₂ for 6 weeks or 3 months (Oshima and Imai, 1970). The authors reported that the mice exposed to the TATHION[®] diet and SO₂ had increased body weights in comparison to the mice that did not consume the TATHION[®] diet. These results were not observed in rats. Furthermore, the number of mice and rats who died during the experimental period was decreased in the animals administered the TATHION[®] diet. Upon histopathological examination of the lungs, heart, liver, spleen, and kidneys using

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hematoxylin and eosin staining techniques, there were no differences observed in the mice fed the different diets, but the rats fed TATHION® did not have as severe tissue damage as the rats that were not administered the TATHION® diet. The authors concluded that TATHION® had a positive effect on SO₂-induced toxicity. The monograph for TATHION® is provided in Appendix F.

Beagle dogs (number not reported) were given a single intravenous dose of 500 or 1,000 mg/kg body weight of GSH sodium (in a saline solution) as a preliminary phase of the chronic toxicity study (no control group was reported). Dogs injected with 500 mg/kg body weight vomited and were less active, and dogs given 1,000 mg/kg body weight exhibited vomiting, salivation, cramping in the 4 extremities and ataxia for approximately 1 hour. Consequently, 300 mg/kg body weight was selected as the maximum daily dose for the chronic study. Six dogs (3 males and 3 females) per treatment group were intravenously administered 0 (control), 30, 100, or 300 mg/kg body weight of GSH sodium per day for 26 weeks. General symptoms and food consumption were measured every day. Body weight was measured once every 2 weeks. Blood samples were taken at 3 time points before administration and at 3, 5, 10, 15, 20, and 25 weeks for measurement of hematology and clinical chemistry parameters. A bromosulfonphthalein and phenolsulfonphthalein excretion test was conducted during weeks 5, 15, and 25 before daily GSH administration. The day after the last injection, the animals were anesthetized and exsanguinated for autopsy. Organ weights were recorded and tissues were stained and subjected to microscopic examination. The results obtained from this study were not reported to be statistically analyzed (Suzuki *et al.*, 1972).

No signs of toxicity were observed at intravenous levels of up to 100 mg GSH/kg body weight/day; however, 4 of 6 dogs in the 300 mg/kg body weight/day group vomited several times during the treatment period. Serum glutamic oxaloacetic acid transaminase (GOT) was elevated compared to baseline values and the control animals in 1 male in the 30 mg/kg body weight/day dose group at Week 25 and 1 male in the 300 mg/kg body weight/day dose group at Week 20. The authors concluded that the elevation of GOT was coincidental as only 2 dogs displayed the elevated levels throughout the study. The ovary weights of 1 to 2 females in each of the control, 100, and 300 mg/kg body weight/day dose groups were reported to be heavier than the others in the groups. The authors reported that this was due to the formation of luteal bodies on the ovaries and as the incidence was similar in the control and treatment groups, was considered not to be related to GSH administration. The authors reported that no other abnormalities in the parameters tested were associated with GSH administration. No adverse effects were reported at the highest dose tested in the study (300 mg/kg body weight/day), and therefore, this dose could be considered to be the no-observed-adverse-effect level (NOAEL).

IV.E.3 Genotoxicity and Mutagenicity Studies of GSH

The potential mutagenic activity of GSH was assessed in an Ames assay using *Salmonella typhimurium* (*S. typhimurium*) strains TA100, TA1537, TA1538, TA98 and TA1535 (Glatt *et al.*, 1983). The authors noted that the concentrations of GSH used in this study were similar to intracellular GSH levels in mammals. No mutagenic activity was reported in any of the bacterial strains tested in the presence or absence of a metabolic activating system [post-mitochondrial supernatant (S9) fraction from the liver or kidney of male Sprague-Dawley rats] with the exception of significant increases in the number of revertants observed in TA100 when 5, 10, or 20 mM GSH was incubated with kidney S9 and with kidney microsomal fraction but not cytosol.

To investigate the mechanism of *in vitro* GSH mutagenicity in *S. typhimurium* TA100, Ross *et al.* (1986) incubated GSH with various subcellular fractions from the kidneys of male Sprague-Dawley rats, including the post-microsomal supernatant, S9, microsomes, and partially-purified plasma membrane. The highest number of revertants was reported in cells incubated with the kidney plasma membrane fraction, which is rich in the enzymes γ -GT and glutathione oxidase. Incubation with inhibitors of these enzymes (anthglutin to inhibit γ -GT and various metal-chelating agents to inhibit glutathione oxidase) completely inhibited the mutagenic activity of GSH with the plasma membrane fraction and inhibited to varying degrees the mutagenicity of GSH with the S9 fraction. The authors concluded that the mechanism of GSH mutagenicity when incubated with kidney subcellular fractions involved the cleavage of GSH to cysteinyl glycine catalyzed by γ -GT and the activity of free transition metals or enzymes that are dependent on transition metals for their activity, such as glutathione oxidase. The authors also hypothesized that the *in vitro* mutagenic activity of GSH is unlikely to occur *in vivo* because γ -GT and glutathione oxidase are located on the outer surface of kidney cell membranes and are not exposed to high levels of GSH (since the majority of GSH is present intracellularly), and because levels of free metals are tightly controlled by metal-binding proteins.

IV.E.4 Carcinogenicity Studies

No traditional carcinogenicity studies of GSH were identified in the literature. A number of studies were identified that involved investigation of the potential of GSH supplementation for the prevention and treatment of chemically-induced carcinogenesis in rats and hamsters, at doses ranging from 8 to 1,600 mg/kg body weight/day for up to 11 months, with beneficial results (Novi, 1981; Wagner *et al.*, 1985; Trickler *et al.*, 1993; Schwartz and Shklar, 1996). Neal and Legg (1983) reported that gavage treatment with 100 mg GSH/day (corresponding to 1,000 mg/kg body weight/day) for 10 weeks did not result in the development of lesions in liver sections of male Fischer 344 rats.

IV.E.5 Developmental Toxicity Studies

GSH has been tested in developmental toxicity studies conducted in mice and rabbits (Suzuki *et al.*, 1972). In mice, more than 20 pregnant ICR-RLC mice/treatment group (exact number not reported) were administered GSH sodium saline solution intravenously in doses providing 0 (control), 30, 300, or 1,000 mg/kg body weight/day of GSH from gestational days (GD) 7 to 13, and were sacrificed on GD 18. In the second developmental toxicity experiment, pregnant New Zealand white rabbits (more than 8 animals/treatment group, exact number not reported) were given GSH sodium saline solution intravenously in doses providing 0 (control), 80, or 300 mg/kg body weight/day of GSH from GD 8 to 15, and were sacrificed on GD 30 (Suzuki *et al.*, 1972). The rabbits were exsanguinated on day 30 of gestation.

In both studies, general condition, body weight, autopsy findings, and organ weights were normal in the dams of all test groups. The numbers of implantations and dead fetuses, mean body weight of fetuses, and incidences of external anomalies and skeletal variations in the offspring were similar among all groups. Because no adverse effects were reported at the highest doses tested in the studies (1,000 mg/kg body weight/day in mice and 300 mg/kg body weight/day in rabbits), these doses could be considered to be the NOAEL. The value in the rabbit study supports the NOAEL determined in the dog chronic toxicity study.

IV.F Studies in Humans

Details of various human studies in patients treated for drug poisoning, acetonemic vomiting (auto-intoxication, periodic vomiting), and pesticide and metal poisoning were provided in a monograph of TATHION[®] prepared by Yamanouchi Pharmaceutical Co., Ltd. (KOHJIN, personal communication, 2007). Additionally, details of the use of oral glutathione for ameliorating gestational toxicosis were included in the monograph, although the original study article was not identified. Of the 6,522 patients included in these studies, of which 1,750 patients were provided the ingredient orally, side effects were reported for 24 patients (~0.4%; further information on patient population not specified), and included anorexia, nausea and vomiting, without any observed changes in clinical laboratory test values. The doses and duration of use were not specified in the monograph; however, the recommended dose is 50 to 100 mg, 1 to 3 times per day.

Published studies designed to determine the effects of intravenous GSH administration in subjects with male infertility (Lenzi *et al.*, 1992, 1993), diabetes (Paolisso *et al.*, 1992), or lead poisoning (Nakao *et al.*, 1968) were identified; however, only one study was identified in which oral administration of GSH was utilized (Dalhoff *et al.*, 1992). In this study, 8 hepatocellular carcinoma patients were given 5,000 mg GSH (dissolved in orange juice) daily beginning shortly after diagnosis. Two patients withdrew from the study because of intolerable side effects, which were gastrointestinal irritation and sulfur odor. Of the 6 remaining patients, 5 died within a year

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of diagnosis of pre-existing hepatocellular carcinoma, but tumors regressed or stabilized in 2 of these patients. The tumor did not progress in the surviving patient.

Murao *et al.* (1974) investigated the efficacy of TATHION® in relieving morning sickness (hyperemesis) during pregnancy. Subjects between the first day of the fifth week of pregnancy and the sixth day of the sixteenth week of pregnancy with mild (113 patients), moderate (150 patients), or advanced (81 patients) morning sickness were provided 3 tablets of TATHION® containing 100 mg GSH/tablet or placebo twice daily between meals for 14 days (providing a daily dose of 200 mg GSH). Symptoms of morning sickness were evaluated during the first consultation and at 1 and 2 weeks after study commencement. Blood samples were collected before and after the study period for measurement of blood total protein, albumin/globulin ratio, total bilirubin, aspartate aminotransferase, alanine aminotransferase, zinc sulfate turbidity test, alkaline phosphatase, total cholesterol, ketone bodies, red and white blood cells, and urine samples were collected during the same visits for assessment of urinary occult blood, ketone bodies, sugar protein, pH, and urobilinogen. Blood pressure also was measured at these visits. A follow-up survey was conducted to determine if abnormalities occurred in neonates. TATHION® administration resulted in improvement of major hyperemesis symptoms in moderate cases compared to placebo (providing 200 mg GSH orally/day). No differences in blood and urinalysis measurements between TATHION® and placebo groups were reported. No adverse reactions attributable to TATHION® were reported, but the authors stated that it was not possible to distinguish compound-related adverse reactions from hyperemesis symptoms, as they are similar. The results from neonatal follow-up were not reported by the authors.

Kudo (1972) reported the results of 5 subjects diagnosed with organophosphorus pesticide (Parathion) poisoning who were orally administered 300 mg GSH/day for 4 weeks. Administration of GSH, an endogenous cellular detoxicant, improved serum cholinesterase activity. No adverse event or tolerance reporting were included in the published article.

IV.G Summary and Basis for GRAS Conclusion

The GRAS determination for L-Glutathione based on scientific procedures. L-Glutathione is manufactured in accordance with cGMP and meets appropriate food-grade specifications. The production process for L-Glutathione involves fermentation using torula yeast, which, in its dried form, is permitted for use as an ingredient in enriched macaroni and noodle products in the U.S. The fermentation process for L-Glutathione involves conditions similar to the conditions that torula yeast used as a food ingredient is produced and to conditions under which torula yeast-containing foods are prepared.

The processing aids used in the processing steps for L-Glutathione are permitted for use in food in the U.S., and/or are removed during the extensive purification processes, resulting in a final product of high purity (>98% GSH). KOHJIN has established chemical and microbiological

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specifications for L-Glutathione consistent with other food-grade materials. Lot samples are routinely assayed to verify compliance with the specifications, including tests for the presence of residual processing aids and solvents.

GSH is present naturally in many foods, including fresh meat products and fruits and vegetables, with the reported dietary intake of GSH ranging from approximately 3 to 130 mg/day and therefore, humans are already exposed to GSH in the diet. Under the intended conditions of use, the mean all-user intake of L-Glutathione by the total U.S. population was estimated to be 448 mg/person/day or 8.4 mg/kg body weight/day. The 90th percentile all-user intake of L-Glutathione by the total U.S. population from all intended food uses was estimated to be 961 mg/person/day or 20.1 mg/kg body weight/day. The cumulative consumption of GSH from the background diet and the intended food uses is estimated to be up to approximately 1 g/person/day.

GSH is an abundantly-occurring endogenous tripeptide in humans and in animal species that serves an important cellular protective function. It is present in all cells, with levels in human blood of approximately 1 to 4 g. Following oral administration, GSH is absorbed intact, resulting in increased plasma and tissue levels.

The results of the identified published animal and human studies of GSH have been determined by KOHJIN to indicate that there is reasonable certainty that L-Glutathione is not harmful under the intended conditions of use. As GSH is reported to be absorbed intact, data from studies involving oral and intravenous administration of GSH were reviewed for the purpose of assessing the safety and GRAS status of L-Glutathione. No histopathological effects attributable to GSH were reported in a study in which mice and rats received 150 and 50 mg GSH/kg body weight/day, respectively, *via* the diet for up to 3 months (Oshima and Imai, 1970). Similarly, no adverse effects were reported in dogs intravenously administered up to 300 mg GSH/kg body weight/day for 26 weeks (Suzuki *et al.*, 1972). In developmental toxicity studies conducted in mice and rabbits, intravenous injection of up to 1,000 mg GSH/kg body weight/day in mice and 300 mg GSH/kg body weight/day in rabbits during gestation did not result in any significant differences in developmental parameters compared to the control groups (Suzuki *et al.*, 1972).

In human studies involving an oral GSH product used for resolving drug poisoning, acetonemic vomiting (autointoxication, periodic vomiting), pesticide and metal poisoning, hyperemesis, and gestational toxicosis (doses not reported), no changes in clinical laboratory test values were observed, although side effects were reported for approximately 0.4% of patients (24 of 6,522 patients) and included anorexia, nausea, and vomiting (Yamanouchi Pharmaceutical Co., Ltd., Undated). Murao *et al.* (1974) reported no differences in clinical chemistry, hematology, or urinalysis measurements between women who received oral doses of 600 mg GSH/day for 14 days during pregnancy and those who received placebo. No adverse effects attributed to GSH

GRAS NOTICE FOR GLUTATHIONE

administration were reported in a study in which 8 hepatocellular carcinoma patients were given oral doses of 5 g GSH/day beginning shortly after diagnosis, although 2 patients withdrew due to intolerable side effects, which were reported as gastrointestinal irritation and sulfur odor (Dalhoff *et al.*, 1992).

The Expert Panel convened by KOHJIN, independently and collectively, critically evaluated the data and information summarized above and concluded that the proposed use of L-Glutathione as an ingredient in foods and beverages, produced consistently with cGMP and meeting appropriate food grade specifications described herein, is safe. They further concluded that the proposed use of L-Glutathione as a food ingredient is GRAS based on scientific procedures. It is also KOHJIN's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

Based on scientific procedures, L-Glutathione is GRAS under the intended conditions of use as a food ingredient, and therefore, L-Glutathione is exempt from the definition of a food additive and thus may be marketed and sold for the uses designated above in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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List of U.S. FDA (2008) CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
101—Food labelling	101.12	Reference amounts customarily consumed per eating occasion
139—Macaroni and noodle products	139.115	Enriched macaroni products
	139.122	Enriched nonfat milk macaroni products
	139.155	Enriched noodle products
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)

GRAS NOTICE FOR GLUTATHIONE

List of U.S. FDA (2008) CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
172—Food additives permitted for direct addition to food for human consumption	172.896	Dried yeasts
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
177—Indirect food additives: Polymers	177.2910	Ultra-filtration membranes
184—Direct food substances affirmed as generally recognized as safe	184.1005	Acetic acid
	184.1095	Sulfuric acid
	184.1293	Ethyl alcohol

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Appendix A

**Expert Panel Consensus Statement Concerning the Generally
Recognized as Safe (GRAS) Status of L-Glutathione as a Food
Ingredient Following Changes In Uses and Use Levels**

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EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF L-GLUTATHIONE AS A FOOD INGREDIENT FOLLOWING CHANGES IN USES AND USE LEVELS

December 22, 2008

As independent experts qualified by relevant national and international experience and scientific training to evaluate the safety of food ingredients, we, the undersigned, Prof. Jack Bend, Ph.D. (University of Western Ontario), Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), and Prof. Gary M. Williams, M.D. (New York Medical College), were requested by Kohjin Co., Ltd. (KOHJIN), as an Expert Panel (hereinafter referred to as the Panel) to evaluate the impact of changes to the uses and use levels on the Generally Recognized as Safe (GRAS) status of L-Glutathione¹, derived from torula yeast (*Candida utilis*), under the conditions of intended use in conventional foods.

Previously, the safety and GRAS status of L-Glutathione for various intended food uses was critically evaluated by the Panel. The Panel concluded that the use of L-Glutathione at specified levels in the intended foods was safe and GRAS based on scientific procedures. The mean and 90th percentile all-user intake of L-Glutathione by the total population from all previously intended food uses was estimated to be 0.34 g/person/day (7.9 mg/kg body weight/day) and 0.69 g/person/day or (14.9 mg/kg body weight/day), respectively. KOHJIN have since modified the intended food uses and/or use levels for their ingredient, with changes including the removal or addition of certain food categories and slight increases in the use levels. A complete summary of the new intended food uses and use levels for L-Glutathione is presented in Table 1.

In the course of assessing the impact of changes in the uses and use levels of L-Glutathione, the Panel reviewed intake estimates for the previous GRAS uses alongside the new estimated exposures from the changes in uses and use levels, information present in the original GRAS dossier (*i.e.*, data pertaining to the method of manufacture and product specifications of L-Glutathione, supporting analytical data, and a comprehensive assessment of the available scientific literature pertaining to the safety of L-Glutathione), and any additional relevant information.

¹ "L-Glutathione" is the trade name of KOHJIN's ingredient, which occurs as the reduced form. L-Glutathione is the current trade name for KOHJIN's ingredient; however, a different trade name may be selected in the future. In this document, "glutathione" refers to reduced and oxidized forms of glutathione, GSH refers to the reduced form, and GSSG refers to the oxidized form.

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Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the modified conditions of intended uses and use levels in foods described herein, L-Glutathione, derived from torula yeast, meeting appropriate food-grade specifications and manufactured in accordance with current good manufacturing practices, is safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

INTENDED USES AND ESTIMATED EXPOSURE OF L-GLUTATHIONE

The intended food uses and use levels for L-Glutathione are detailed in Table 1. Following consultation with the United States Food and Drug Administration (FDA), all infant-related food categories have been removed. Additionally, the intended food uses pertaining to fats and oils, meat products, and vinegar were removed for technical reasons. Two food uses were added to the intended food list (frozen fruit juices and potato based salty snacks), and use levels in several categories were increased. For example, the proposed use levels of L-Glutathione in powdered fruit flavored drinks increased from 0.667% to 1.5%

Table 1 Summary of the Individual Intended Food Uses and Use Levels for L-Glutathione in the United States				
Food Category	Proposed Food Use	Level of L-Glutathione (mg/serving)	RACC* (g or mL)	Use Level for L-Glutathione (%)
Baked Goods and Baking Mixes	Cookies	100 to 133.2	30 to 40	0.250 to 0.333
	Crackers	100	30	0.333
Beverages and Beverage Bases	Ice Teas (Powdered)	300	15	2.000
	Sports and Isotonic Beverages	360	240	0.150
Breakfast Cereals	Instant and Regular Hot Cereals	50 to 68.7	40 to 55	0.091 to 0.125
	Ready-to-Eat Cereals	50 to 183.1	15 to 55	0.091 to 0.333
Cheeses	Cottage Cheese	50	110	0.045
	Cream Cheese	50	30	0.167
	Imitation Cheese	50	30	0.167
	Natural Cheese	50	30	0.167
	Processed Cheese	50	30	0.167
Chewing Gum	Chewing Gum	200	3	6.667
Coffee and Tea	Instant Coffee (Powdered)	100	15	0.667
Condiments and Relishes	Soy Sauce	10	15	0.067
Dairy Product Analogs	Soy-Based Meal Replacements (Powdered)	100	15	0.667
	Soy Milk	50	240	0.021
Gelatins, Puddings, and Fillings	Gelatin, Jams, and Jelly	50 to 399.6	15 to 120	0.042 to 0.333
	Gelatin Drinks	100	240	0.042
	Puddings	30	120	0.025

Table 1 Summary of the Individual Intended Food Uses and Use Levels for L-Glutathione in the United States				
Food Category	Proposed Food Use	Level of L-Glutathione (mg/serving)	RACC* (g or mL)	Use Level for L-Glutathione (%)
Grain Products and Pastas	Gratin	30	30	0.100
	Pizza (Crust)	30	140	0.021
	Ready-Made Noodles and Canned Pasta	30	245	0.012
Gravies and Sauces	Barbecue Sauces	10	30	0.033
	Gravy Sauces	10	60	0.017
Hard Candy	Hard Candy	300	15	2.000
	Mints	100	2	5.000
Milk Products	Cocoa Powder Mixtures	10	15	0.067
	Milk-Based Meal Replacements (Powdered)	100	15	0.667
	Milk (Dry and Powdered Mixtures)	100	15	0.667
	Yoghurt (Includes Frozen)	396 to 742.5	120 to 225	0.330
	Yoghurt Drinks and Fruit Smoothies	216	240	0.090
Plant Protein Products	Plant-Protein-Based Meal Replacements (Powdered)	100	15	0.667
	Protein Bars	100	40	0.250
Processed Fruits and Fruit Juices	Fruit Flavored Drinks (Powdered)	225	15	1.500
	Fruit Flavored Drinks (Frozen)	200	240	0.833
Processed Vegetables and Vegetable Juices	Vegetable Juices	100	240	0.042
Salty Snacks	Potato and Corn Based Chips	100	30	0.333%
Soft Candy	Chocolate Confectionary	400	40	1.000
	Soft Candy	100	40	0.250
Soups and Soup Mixes	Canned Soups	100	245	0.041
	Consommé	10	245	0.004
	Dehydrated and Powdered Soup Mixes	100	30	0.333
Sugar Substitutes	Sugar Substitutes	5	4	0.125

*Reference Amounts Customarily Consumed (RACC) per Eating Occasion (21 CFR §101.12 [U.S. FDA, 2008]). When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.

The consumption of L-Glutathione from all original intended uses and use levels was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2008). Under the previous intended food uses, 99.4% of the total U.S. population was identified as potential consumers of L-Glutathione (8,213 actual users identified). On an all-user basis, the mean intake of L-Glutathione by the total U.S. population from all proposed food uses was estimated to be 0.34 g/person/day or 7.9 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of L-Glutathione by the total U.S. population from all proposed food uses was estimated to be 0.69 g/person/day or 14.9 mg/kg body weight/day.

Using the same method for estimating the intake of L-Glutathione for the amended list of food uses and use levels, the change in intended food uses and use levels for L-Glutathione resulted in a small increase in exposure. Under the previous proposed food uses, 96.9% of the total U.S. population was identified as potential consumers of L-Glutathione (8,009 actual users identified). On an all-user basis, the mean intake of L-Glutathione by the total U.S. population from all proposed food uses was estimated to be 0.45 g/person/day or 8.4 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of L-Glutathione by the total U.S. population from all proposed food uses was estimated to be 0.96 g/person/day or 20.1 mg/kg body weight/day. The revised intakes of L-Glutathione for all population groups are presented in Tables 2 and 3 on a per person and per kilogram body weight basis, respectively.

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (g)	90 th Percentile (g)	Mean (g)	90 th Percentile (g)
Infants	0 to 2	76.0	707	0.26	0.60	0.31	0.63
Children	3 to 11	99.9	1,286	0.49	0.90	0.49	0.90
Female Teenagers	12 to 19	99.7	989	0.44	0.95	0.44	0.95
Male Teenagers	12 to 19	99.4	993	0.56	1.16	0.56	1.17
Female Adults	20 and Up	99.6	2,121	0.40	0.90	0.40	0.90
Male Adults	20 and Up	99.2	1,913	0.47	1.10	0.48	1.10
Total Population	All Ages	96.9	8,009	0.44	0.95	0.45	0.96

Table 3 Summary of the Estimated Daily per Kilogram Body Weight Intake of L-Glutathione from All Intended and Amended Food Categories in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg/kg bw)	90 th Percentile (mg/kg bw)	Mean (mg/kg bw)	90 th Percentile (mg/kg bw)
Infants	0 to 2	76.0	707	21.3	48.8	25.6	52.3
Children	3 to 11	99.9	1,286	18.6	36.1	18.7	36.1
Female Teenagers	12 to 19	99.7	989	7.6	16.7	7.6	16.7
Male Teenagers	12 to 19	99.4	993	8.9	19.8	8.9	19.8
Female Adults	20 and Up	99.6	2,121	5.8	12.8	5.8	12.8
Male Adults	20 and Up	99.2	1,913	5.6	12.7	5.6	12.7
Total Population	All Ages	96.9	8,009	8.3	20.0	8.4	20.1

Therefore, the new intended food uses and use levels for L-Glutathione resulted in an increase in estimated mean and 90th percentile all-user intakes of L-Glutathione by the total U.S. population from 0.34 and 0.69 g/person/day, respectively, to 0.45 and 0.96 g/person/day, respectively.

DATA SUPPORTING THE SAFETY OF L-GLUTATHIONE

The determination of the safety of L-Glutathione under the conditions of intended use is based on the results of published toxicological and human studies of glutathione (GSH), information on the background dietary consumption of GSH and its metabolic fate, as well as its presence endogenously in humans.

GSH is synthesized endogenously from the amino acids L-cysteine, L-glutamate, and glycine via γ -glutamylcysteine synthetase. The liver is the major site for the production and export of GSH, although virtually all cell types have the capacity to synthesize GSH. Assuming a blood volume of 5 L for the average adult, this value is equivalent to 1.15 g GSH/day (Lyons *et al.*, 2000). Whole blood GSH synthesis may account for approximately 10% of whole body synthesis; therefore, it is estimated that 11.5 g GSH is synthesized in the body on a daily basis. Glutathione occurs ubiquitously in human tissues, predominantly in its reduced form (GSH), and reported GSH levels in whole blood range from 684 to 2,525 $\mu\text{mol/L}$, which would be equivalent to total blood amounts of approximately 1 to 4 g² (Pastore *et al.*, 2003).

² These values were calculated based on the assumption that the human body contains 5 L of blood. Sample calculation: 684 $\mu\text{mol/L}$ x 307.33 g/mol x 5 L blood = ~1 g GSH.

GSH also is present naturally in many foods, including fresh meats, fish, and poultry, as well as fruits and vegetables. Dietary intake of GSH from its natural occurrence is estimated to range from 3 to 130 mg/day (Wierzbicka *et al.*, 1989; Flagg *et al.*, 1994). Following oral administration, GSH is absorbed intact (Hagen *et al.*, 1990; Favilli *et al.*, 1997), resulting in increased plasma and tissue levels (Hagen and Jones, 1989; Witschi *et al.*, 1992).

The data from the available toxicological studies indicate that short- and long-term administration of GSH in animals does not result in compound-related adverse effects. As GSH is reported to be absorbed intact, data from studies involving oral and intravenous administration of GSH were reviewed for the purpose of assessing the safety and GRAS status of L-Glutathione. No histopathological effects attributable to GSH were reported in a study in which mice and rats received 150 and 50 mg GSH/kg body weight/day, respectively, *via* the diet for up to 3 months (Oshima and Imai, 1970). Similarly, no adverse effects were reported in dogs intravenously administered up to 300 mg GSH/kg body weight/day for 26 weeks (Suzuki *et al.*, 1972). In developmental toxicity studies conducted in mice and rabbits, intravenous injection of up to 1,000 mg GSH/kg body weight/day in mice and 300 mg GSH/kg body weight/day in rabbits during gestation did not result in any significant differences in developmental parameters compared to the control groups (Suzuki *et al.*, 1972).

The safety of GSH also is supported by human studies that demonstrated its tolerability following oral administration. In studies involving an oral GSH product used for treating drug poisoning, acetonemic vomiting (autointoxication, periodic vomiting), pesticide and metal poisoning, hyperemesis, and gestational toxicosis (doses not reported), no changes in clinical laboratory test values were observed, although side effects were reported for approximately 0.4% of patients (24 of 6,522 patients) and included anorexia, nausea, and vomiting (Yamanouchi Pharmaceutical Co., Ltd.). Murao *et al.* (1974) reported no differences in clinical chemistry, hematology, or urinalysis measurements between women who received oral doses of 600 mg GSH/day for 14 days during pregnancy and those who received placebo. No adverse effects attributed to GSH administration were reported in a study in which 8 hepatocellular carcinoma patients were given oral doses of 5 g GSH/day beginning shortly after diagnosis, although 2 patients withdrew due to intolerable side effects, which were reported as gastrointestinal irritation and sulfur odor (Dalhoff *et al.*, 1992).

Following a search in December 2008 by Cantox Health Sciences International of the online database, PubMed, no new scientific information pertaining to the oral administration of GSH in humans and animals that would raise any concerns of the safety of the intended conditions of use of L-Glutathione was identified.

Based on the information supporting the safety of L-Glutathione that was reviewed previously and the fact that the changes in the intended uses and use levels of L-Glutathione resulted in only slightly increased estimated consumption levels, the changes in the proposed food uses and use levels do not impact the safety or GRAS status of L-Glutathione.

December 22, 2008

000046

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CONCLUSION

We, the Expert Panel, have independently critically evaluated the data and information summarized above and conclude that the proposed uses of L-Glutathione derived from torula yeast, *Candida utilis*, meeting food-grade specifications and produced in accordance with current good manufacturing practices, are safe. We further conclude that L-Glutathione is Generally Recognized as Safe (GRAS) by scientific procedures for use in conventional foods under the conditions of intended use described herein. It is our opinion that other qualified experts would concur with these conclusions.

(b)(6)

Prof. Jack Bend, Ph.D.
University of Western Ontario

Jan. 16, 2009
Date

(b)(6)

Prof. Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

19 January 2009
Date

(b)(6)

Prof. Gary M. Williams, M.D.
New York Medical College

22 Jan 09
Date

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USDA. 2008. What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2003-2004. U.S. Department of Agriculture (USDA); Riverdale, Maryland. Available from: <http://www.ars.usda.gov/Services/docs.htm?docid=13793#release>.

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APPENDIX B

LITERATURE SEARCH STRATEGY FOR TORULA YEAST

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Literature Search Strategy for Torula Yeast

Abbreviations used in the literature searches are defined as follows: “()” = with; “?” = words beginning with the preceding text; “2n” = within 2 words of the preceding text.

The following search terms were used to identify published studies pertaining to Torula yeast: candida()utilis or torula()(yeast or utilis) or torulopsis()utilis or pichia()jadinii. Within these search records, the following terms were searched to identify studies conducted to assess the safety of torula yeast:

- toxic? or mortal? or lethal? or adverse? or safe? or risk?;
- tumor? or tumour? or carcino? or neoplas? or cancer?;
- teratol? or teratogen? or (reproduct? or developmental?)(toxic? or effect?) or pregnan? or fetus or fetal or litter or reproduct? or developmental?(toxic? or foetus? or malform? or birth()defect? or fetotoxic? or prenatal or perinatal);
- human or humans or subject or subjects or patient? or clinical? or volunteer? or man or double()blind or epidemiol? or case()(stud? or control?) or cohort? or occupat? or workplace or industr?);
- chronic? or acute? or short()term or subchronic? or sub()chronic or long()term or repeat()dose? or 28()day? or twenty()eight()day? or 90()day? or ninety(day)? or 2()year? or two()year?; and
- antibiotic()resistance or pathogenicity or pathogenic or antibiotic or infect or infection or infect? or infection? or secondary()metabolite or antibiotic()product?

Of the search records identified, only records in which torula yeast or its synonyms appeared in the title were selected. Within these search records, the following terms were searched to identify studies in which oral administration was used: oral? or feeding or diet or diets or dietary or supplement? or dose? or admin?. Additionally, within the search records pertaining to torula yeast, the following terms were searched: food()manufact? or food()product? or food()process or product?()organism? Duplicate titles were removed. No published information pertaining to the safety of torula yeast was identified in the literature search.

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APPENDIX C

METHODS OF ANALYSIS

APPENDIX C

METHODS OF ANALYSIS

Pages 000055 - 000056 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Microbiological Test Methods for Glutathione

A. Total Plate Count

Materials:

Soybean Casein Digest (SCD) Agar & Peptone-saline diluent were from Nissui Pharmaceuticals Co., Ltd., Japan. Membrane filter (KP-47S) was from Advantec.

Working Condition, Instruments, Solution:

Aseptic / sterilized.

Procedure:

Glutathione sample 5 g mixed well with peptone-saline solution. Then sample solution passed through membrane filter equipped with filtration unit. Dispensed 100 ml of peptone-saline solution trice (total 300 ml) through the filter. Removed the membrane filter from filtration unit very carefully and plated onto the surface of a pre-poured SCD agar Petri-dish. Kept the Petri-dish (invert position) in a incubator at 30. C for 5 days. After 5 days colony formation was observed. Calculation was done colony/g

B. Mold and Yeast

Ref.: Japan Food Sanitation Act (with modification)

Materials:

Potato Dextrose Agar (PDA) from Nissui Pharmaceuticals Co., Ltd., Japan. Sodium hydrogen carbonate injection ampoule (8.4%) 20 ml from Otsuka pharmaceutical, Japan.

Working Condition, Instruments, Solution:

Aseptic / sterilized.

Procedure:

Glutathione sample 2 g was dissolved well in 20 ml Sodium hydrogen carbonate solution. Sample solution 0.2 ml each was added in 5 PDA plate. Kept the plates in a incubator at 27. C for 120 h. Colony formation was observed after 120 h. Colony was then calculated.

C. Coliform

Ref.: Japan Food Sanitation Act (with modification)

Materials:

Lactic Bouillon (specific for Coliform test) from Nissui Pharmaceuticals Co., Ltd., Japan. Sodium hydrogen carbonate injection ampoule (8.4%) 20 ml from Otsuka pharmaceutical, Japan.

000057

Working Condition, Instruments, Solution:

Aseptic / sterilized.

Procedure:

Glutathione sample 2 g dissolved well in 20 ml Sodium hydrogen carbonate solution. Sample solution 5 ml was added to Lactic Bouillon 10 ml (half of standard protocol). Three preparations were prepared and kept at 37. C for 48 h. Lactic bouillon was observed for color, formation of gas in drum. Formation of color and gas in any preparation indicates presence of Coliform.

D. Salmonella

Ref.: Japan Food Sanitation Act (with modification)

pH of glutathione is low and therefore pH was befitted with the protocol.

Materials:

Sodium hydrogen carbonate injection ampoule (8.4%) 20 ml from Otsuka pharmaceutical, Japan. MLCB Salmonella selected media, Hajna tetrathionate and peptone water

Working Condition, Instruments, Solution:

Aseptic / sterilized.

Procedure:

Peptone solution concentration was doubled from standard protocol. Peptone solution 112 ml was taken in 500 ml conical flash and Glutathione sample 25 g was then added and mixed well. Sodium hydrogen carbonate solution 100 ml (20mlX5) was added to sample solution. pH was checked (pH range 5-8) by handy pH meter withdrawing 2 ml sample. Sample solution was incubated (cultured) for 18h±2 at 35. C±2. Cultured broth 1 ml was then added to 10 cc test tube contained Hajna tetrathionate and then incubated for 18h at 43. C±2. After incubation loopful of broth was picked-up and streaked on the surface of MLCB media and then kept at 35. C±1 for 24h±2. No black colony formation indicates absence of *Salmonella*.

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APPENDIX D

BATCH ANALYSES OF L-GLUTATHIONE

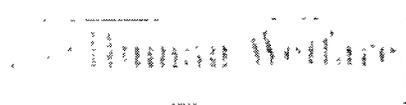
000060

GRAS NOTICE FOR GLUTATHIONE

Table D-1 Summary of Batch Analyses of L-Glutathione				
Specification Parameter	Specification	Lot Number		
		PF07G45901	PF07G46001	PF07G46101
Appearance	White crystals or crystalline powder	Conforms	Conforms	Conforms
Glutathione (%)	Not less than 98.0	99.0	99.3	99.1
Loss on drying (%)	Not more than 0.5	0.1	0.1	0.1
Residue on ignition (%)	Not more than 0.1	0.02	0.02	0.02
Lead (Pb) (ppm)	Not more than 1	≤1	≤1	≤1
Arsenic (ppm)	Not more than 1	≤1	≤1	≤1
Total plate count (CFU/g)	Not more than 3,000	Conforms	Conforms	Conforms
Yeast and Mold (CFU/g)	Not more than 100	Conforms	Conforms	Conforms
Coliforms	Negative per 2 g	Conforms	Conforms	Conforms
<i>Salmonella</i> sp.	Negative per 25 g	Negative	Negative	Negative

CFU = colony-forming units;

000061



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Tokyo, 103-0022 Japan
Phone +81 3-3242-3011 Fax +81 3-3242-3054
E-mail: www.kohjin.co.jp

CERTIFICATE OF ANALYSIS

Product Name **L-GLUTATHIONE (For Health Food)**

Lot No.	PF07G45901	Quantity	Date	DEC. 20, 2007
Analysis item	Specification	Analysis data		
Appearance	White crystals or crystalline powder	Corresponds		
Total Chloride	Not less than 98.0%	99.0 %		
Iron (as Fe)	Not more than 0.5%	0.1 %		
2,2'-bipyridine	Not more than 0.1%	0.02 %		
Lead	Not more than 1ppm	Not more than 1ppm		
Asbestos	Not more than 1ppm	Not more than 1ppm		
Microbial count	Not more than 3000CFU/g	Corresponds		
Yeast and Mold,CFU/g	Not more than 100CFU/g	Corresponds		
Salmonella	Negative	Negative		
Staphylococcus	Negative	Negative		
Conclusion	Corresponds			

Manufactured on JUL 12, 2007

Expire date JUL 11, 2009

Storage: Store at room temperature without moisture

Approved by _____
Name of Issuer
Manager
Quality Assurance Dept
SAIKI FACTORY

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000062



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CERTIFICATE OF ANALYSIS

Product Name **L-GLUTATHIONE (For Health Food)**

Lot No.	PF07G46001	Quantity	Date	DEC. 20, 2007
---------	------------	----------	------	---------------

Analysis Item	Specification	Analysis data
Appearance	White crystals or crystalline powder	Corresponds
Identification	Not less than 98.0%	99.3 %
Loss on drying	Not more than 0.5%	0.1 %
Residue on ignition	Not more than 0.1%	0.02 %
Lead	Not more than 1ppm	Not more than 1ppm
Cadmium	Not more than 1ppm	Not more than 1ppm
Total coliform count	Not more than 3000CFU/g	Corresponds
Total acid fast bac	Not more than 100CFU/g	Corresponds
Salmonella	Negative	Negative
Staphylococcus	Negative	Negative
Microbial count		Corresponds

Manufacture Date: JUL. 22, 2007
 Expiry Date: JUL. 21, 2009
 Storage: Store in a cool, dry place without moisture

Approved By: _____

Osamu Nakata
 Manager
 Quality Assurance Dept
 ANALYSIS DIV

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000063

Shimizu Kasei



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CERTIFICATE OF ANALYSIS

Product Name **L-GLUTATHIONE (For Health Food)**

Lot No.	PF07G46101	Quantity		Date	DEC. 20, 2007
Inspected Item		Specification		Analysis data	
Appearance		White crystals or crystalline powder		Corresponds	
Assay		Not less than 98.0%		99.1%	
Water content		Not more than 0.5%		0.1%	
Residue on ignition		Not more than 0.1%		0.02%	
Iron		Not more than 10ppm		Not more than 10ppm	
Copper		Not more than 1ppm		Not more than 1ppm	
Microbial count		Not more than 3000CFU/g		Corresponds	
Coliform (Method B)		Not more than 100CFU/g		Corresponds	
Salmonella		Negative		Negative	
Shigella		Negative		Negative	

Result

Corresponds

Manufactured on JUL. 22, 2007

Expire on JUL. 21, 2009

Storage: Store in a cool, dry place without moisture

Approved by _____

Syoko Nishida
Manager
Quality Assurance Dept
KOHJIN FACTORY

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000064

GRAS NOTICE FOR GLUTATHIONE

Table D-2 Solvent Residue Analysis of L-Glutathione						
Residue	Lot Number					
	Control ^a	C10-333	C10-338	C10-342	C10-353	C10-361
Ethanol (ppm)	1,370	1,510	1,570	1,650	1,490	1,490
Ethyl acetate (ppm)	ND	ND	ND	ND	ND	ND
Acetate (ppm)	560	740	590	680	500	740

ND = not detected

^a Glutathione purchased from Wako Pure Chemical Industries (Osaka, Japan)

000065

Product analysis report

Ref No : 05-01-2-8
To be distributed to : Quality assurance dept.
(b)(6)

Approve	Check	PIC

Product	Glutathione	Production No : C-10 series
---------	-------------	-----------------------------

(Sample) Glutathione (C-10 series)

(Items) ①Residual ethanol →Gas chromatograph
 ②Residual ethylacetate →Gas chromatograph
 ③Residual acetate →Ion chromatograph

(Measurement)

	Residual ethanol	Residual ethylacetate	Residual acetate
C10-333	1510	not detected	740
C10-338①	1570	not detected	590
C10-342②	1650	not detected	680
C10-353①	1490	not detected	500
C10-361②	1490	not detected	740
Highest value	1650	-	740
Lowest value	1490	-	500
Average	1542	-	650
Fluctuation	160	-	240
Standard deviation	61	-	93

Control(C-9-301)	1370	not detected	560
*Previous result	1400	not detected	550

(Conclusion)
 Residual ethylacetate weren't be found in this analysis.

000066

"Original"

製品分析報告書

報告No. : 佐品 05 - 01 - 208

配布先

品質保証室 : 行本様

2006年 1月24日
品質保証室

承認者	確認者	担当

製品名: YP 生産No.: C-10 シリーズ

(サンプル) YP工程製品 (C-10 シリーズ)

※ 各シリーズ中の5、20、35、50、61Batch目

- (測定操作) ①、残存エタノール → ガスクロ分析 *gas chromatograph*
 ②、残存酢酸エチル → ガスクロ分析
 ③、残存酢酸 → イオンクロマト分析 *ion*

(測定値)

	<i>ethanol</i> 残存エタノール	<i>ethyl acetate</i> 残存酢エチ (単位: ppm)	<i>acetic acid</i> 残存酢酸
C10-333	1510	検出せず	740
C10-338①	1570	検出せず	590
C10-342②	1650	検出せず	680
C10-353①	1490	検出せず	500
C10-361②	1490	検出せず	740
最高値	1650	---	740
最低値	1490	---	500
平均値	1542	---	650
ふれ幅	160	---	240
標準偏差	61	---	93

コントロール(C-9-301)	1370	検出せず	560
※ 前回測定値	1400	検出せず	550

(結果及び考察)

※ 本シリーズではメタノール及び酢酸エチルを検出しなかった。

以上

000067

GRAS NOTICE FOR GLUTATHIONE

Table D-3 Residue Analysis of Copper in L-Glutathione			
Residue	Lot Number		
	PF 07E45001	PF 07E45101	PF 07E45201
Copper (ppm)	ND	ND	ND

ND = not detected using a detection limit of 0.1 ppm

000068

C

C

C

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APPENDIX E

TRANSLATED PRE-CLINICAL STUDIES

000070

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Pages 000150 - 000174 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

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APPENDIX F

TATHION® MONOGRAPH

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SUBMISSION END

000180

Reference List for Industry Submission, GRN 000293

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000055 - 000056	NA	Glutathione	NA	Official Monographs	Volume XV, pgs 701 - 702
000071 - 000092	Kudo, Naoyoshi	A study on the Pathological Physiology of Intoxication Caused by Agricultural Chemicals The Clinical Investigation About Chronic Intoxication of Alkylphosphates	1972	J Jpn SOC Agric Med	Volume 21, Number 340, pgs 1 - 21
000093 - 000098	Kudo, Naoyoshi	A study on the Pathological Physiology of Intoxication Caused by Agricultural Chemicals The Clinical Investigation About Chronic Intoxication of Alkylphosphates	1972	J Jpn SOC Agric Med	Volume 21, Number 340, pgs 1 - 21
000099 - 000111	Murao, Akitoshi; Imamura, Hiroshi; Miyazaki, Yoshinobu; Katsuno, Kazuo; Noda, Takashi; Ohtsuka, Akira; Ishii, Giichil; Abe, Hiroshi; Morita, Takashi; Shindo, Masaharu; Ozara, Shuichi; Asano, Sanichiro; Goto, Shiro; Bando, Shigeru; Oda, Kinichi; Kuriya, Norimori	Effect of Tahion against hyperemesis during the pregnancy	1974	The World of Obstetrics and Gynecology	Volume 26, Number 1153, pgs 1-13
000112 - 000128	Nozaki, J.; Ida, E.; Kotani, Y.	General Pharmacological Actions of Glutathione	1972	Clinical Reports	Volume 6, Number 2384
000129 - 000138	Ogawa, Eiichi; Suzuki, Shiro; Tsuzuki, Hiroshi; tobe, Masuo; Kobayashi, Kazuo; Hojo, Masatsune	Experimental studies on tissue distribution and excretion of methylmercury chloride 1	1972	Accident Medicine	Volume 15, Number 222
000139 - 000149	Oshima, Hidehiko; Imai Masayuki	Effect of Tathion on the chronic exposure to low concentration of sulfur dioxide SO ₂	1970	Treatment and New Drug	Volume 7, Number 8, pgs 1 - 11
000150 - 000174	Suzuki, H.; Miki, S.; Oshima, M.; Sado, T.;	Chronic Toxicity and Teratogenicity Studies of Glutathione Sodium Salt	1972	Clinical Reports	Volume 6, Number 2393

NA- Not applicable

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000177 - 000179	NA	Reduce Glutahione	NA	NA	pgs 779 -781

NA- Not applicable