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ORIGINAL SUBMISSION

000001



# SRA International, Inc.

August 7, 2000

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Dr. Linda Kahl  
Office of Premarket Approval (HFS-206)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C Street, SW  
Washington, DC 20204

Re: GRAS Notification for Transglutaminase in Pasta and Grain Products

Dear Dr. Kahl:

The purpose of this letter is to inform the Agency of a recent GRAS determination using scientific procedures, for transglutaminase in pasta and specified grain products.

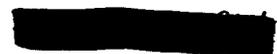
### **Background**

As you know, the use of the enzyme transglutaminase as a food additive in various human foods has been evaluated a number of times by the U.S. Food and Drug Administration (FDA).

The first of these evaluations occurred approximately three years ago at the request of the US Department of Agriculture (USDA). In a letter (Post to Pauli, August 8, 1997), the USDA requested the opinion of the FDA regarding a claim of Generally Recognized as Safe (GRAS) for use of transglutaminase (65 ppm) in processed meats. SRA International Inc. (SRA) on behalf of our client (Ajinomoto USA) had, using scientific procedures determined that this use was GRAS and had submitted this claim to the USDA. The FDA evaluated the safety evidence and responded to the USDA request stating, "Based on its review, FDA has determined that, at this time, the agency would not challenge Ajinomoto's conclusion that TGP [transglutaminase] is safe under the proposed conditions of use" (letter dated January 15, 1998, Rulis to Post). This was the first of three 'No-Objection Letters' issued by the FDA regarding the use of transglutaminase in human food.

The second evaluation occurred two years ago. It was precipitated by a letter from SRA to the FDA (dated March 3, 1998, Bernard to Kahl) that the use of transglutaminase in processed seafood at levels not to exceed 65 ppm, was exempt from statutory premarket approval requirements based on a GRAS determination performed through scientific procedures.

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Bernard to Kahl  
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In response to that submission, the FDA issued a second 'No Objection' letter consistent with the previously discussed Agency decision (letter dated June 22, 1998, Rulis to Bernard). The Agency identified this notification as FDA GRN No. 00004.

The third and most recent FDA evaluation occurred last year. In a letter to FDA, (Bernard to Kahl, 26 July 1999) SRA, on behalf of Ajinomoto USA, notified the Agency that the use of transglutaminase in Meat Substitutes and five specific categories of dairy products at specified use levels was exempt from statutory premarket approval requirements, based on a GRAS determination performed through scientific procedures. The FDA responded with its third 'No-Objection Letter' (Rulis to Bernard, December 29, 1999) and designated this notification as FDA GRN No. 000029.

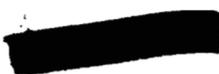
**Current Submission**

The purpose of this letter is to inform the Agency of a recent GRAS determination using scientific procedures, for transglutaminase in pasta and specified grain products. The specific categories and their respective not to exceed usage levels include:

<u>FOOD CATEGORY</u>	<u>USAGE LEVEL</u> <u>(PPM)</u>
Pasta products	25
Bread products	15
Pastry products (cakes, pies, doughnuts, etc.)	20
Ready to eat cereal products	45
Pizza dough	20
Grain mixtures (burritos, tacos, etc.)	25

Three (3) copies of this cover letter, the GRAS notification to FDA (including a summary of the data reviewed in support of this determination), and GRAS Certificate are attached. All data reviewed by the Expert Panel in conjunction with this GRAS determination is available for FDA to evaluate at the SRA offices listed below.

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Bernard to Kahl  
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If you have any questions regarding the process employed in this determination, the members of the expert panel or the underlying scientific data employed in the decision, we will be pleased to assist you.

Sincerely,

Bruce K. Bernard, Ph.D.  
President, SRA International  
Authorized Representative of  
Ajinomoto USA

BKB/km

ATT: GRAS Notification  
GRAS Certificate

CC: Ajinomoto USA

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# SRA International, Inc.

AJINOMOTO COMPANY INC.

GRAS SUMMARY/NOTIFICATION  
FOR THE USE OF TRANSGLUTAMINASE IN PASTA AND GRAIN PRODUCTS

August 7, 2000

I. DESCRIPTION OF THE SUBSTANCE

A. COMMON OR USUAL NAME

Transglutaminase (TG) is the common or usual name given to a principal acyl-transfer catalyzing enzyme (Folk and Chung, 1973; Folk and Finlayson, 1977; Folk 1980; Folk 1982). It forms crosslinks between the  $\gamma$ -carboxamide group of protein- or peptide-bound glutamine residues and the  $\epsilon$ -amino group of lysine residues, resulting in the  $\epsilon$ -( $\gamma$ -glutamyl) lysine bonds.

B. CHEMICAL NAME

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), transglutaminase has the following designation:

1. Formal Name  
Glutaminyl-peptide  $\gamma$ -glutaminyltransferase
2. Systematic Name  
Glutaminyl-peptide: amine  $\gamma$ -glutaminyl-transferase, EC 2.3.2.13
3. Synonyms  
There are various synonyms used for transglutaminase, whose names are based upon the organ(s) or tissue(s) of origin (e.g., Factor XIII in blood coagulating system).

C. CAS NUMBER

80146-85-6

D. EMPIRICAL FORMULA

Not applicable

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E. STRUCTURAL FORMULA

See prior FDA GRAS Notice #4

F. MOLECULAR WEIGHT

See prior FDA GRAS Notice #4

G. ENZYMATIC PROPERTIES

See prior FDA GRAS Notice #4

H. SPECIFICATIONS FOR FOOD GRADE MATERIALS

See prior FDA GRAS Notice #4

I. QUANTITATIVE COMPOSITION

See prior FDA GRAS Notice #4

J. MANUFACTURING

See prior FDA GRAS Notice #4

1. Source of the Enzyme

See prior FDA GRAS Notice #4

2. Manufacturing Process

See prior FDA GRAS Notice #4

II. USE OF THE SUBSTANCE

A. DATE OF FIRST USE

See prior FDA GRAS Notice #4

B. NATURAL OCCURRENCE/PAST USE IN FOODS

See prior FDA GRAS Notice #4

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C. TECHNICAL EFFECT

See prior FDA GRAS Notice #4

D. INTENDED USE AND USE LEVELS

The applicant seeks to use TG in the following the food categories and usage levels in the table below.

Table 1. Food Categories and Usage Levels

Food Category	Usage Level of TG* (PPM)
Pasta products	25
Bread products	15
Pastry products (cakes, pies, doughnuts, etc.)	20
Ready to eat cereal products	45
Pizza dough	20
Grain mixtures (burritos, tacos, etc.)	25

\* Expressed as ppm levels of active ingredient (TG) in a formulation of finished TG Enzyme Product or Preparation (TGP) containing approximately 90% dextrin (a GRAS substance, 21 CFR §184.1277). The dextrin is employed in the purification of TG and formulation of TGP. In the latter, it is employed to ensure a consistent level of enzyme activity across different batches of the enzyme. More detailed information is available in I.H. and I.I IN GRN nos. #4 and #29.

E. EXPOSURE

1. Calculation of Exposure Employing MRCA Data: Pasta and Grain Products Only

Detailed information on the procedures employed by MRCA in obtaining and analyzing consumption data can be found in GRN Nos. #29

a) Assumptions

MRCA provided frequency distributions of intake of TG from selected pasta and grain products. Intake frequency distribution were based upon several conservative and clearly unlike assumptions:

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- TG will be incorporated into every food category listed in Table 1.
- TG will be incorporated into 100% of the food in each of the categories listed in Table 1.
- TG will be incorporated into all foods at the maximum approved concentrations.

b) Anticipated Maximum Consumption in the U.S. from these Categories:

- For the total US population (all ages), the mean consumption of TG associated with these food categories would be 0.038 mg/kg/day (2.1 mg/p/day), with a range across age group of 0.029 – 0.12 mg/kg/day (1.0 – 2.6 mg/p/day).
- For the 90<sup>th</sup> percentile of the entire U.S. population, the mean is 0.090 mg/kg/day (5.3 mg/p/day), with a range of 0.069 – 0.30 mg/kg/day (3.2 – 6.4 mg/p/day).
- Of the total population (all ages), 82.8% are Eaters of these products. The distribution of Eaters across age groups was uniform (77.2%- 87.7%), except ages 0 - 23 months which had 54.3% Eaters.
- The mean consumption for Eaters Only of all ages was 0.045 mg/kg/day (2.6 mg/p/day). This measure has a range of 0.033 – 0.18 mg/kg/day (1.9 – 3.2 mg/p/day).
- The estimated consumption of TG for the 90th Percentile of Eaters Only is 0.098 mg/kg/day (5.6 mg/p/day) for these products and has a range of 0.073 – 0.350 mg/kg/day (3.7 – 7.0 mg/p/day).

2. Total Exposure to TG

a) Previous Approvals

- The use of TG in processed meats and seafood was declared to be Generally Recognized as Safe under Conditions of Intended Use (GRAS) by an Expert Panel on 11 February 1997. The USDA and FDA reviewed a notification summary submitted in June 1997 for processed meats and in February 1998 an approval was received from USDA.
- A GRAS Notification (GRN 000004) was submitted to FDA for the use of TG in processed seafood on June 1997 and on 22 June 1998 FDA issued a 'No-Objection Letter'.
- In May 1998, the use of TG in dairy products and meat substitutes was declared to be GRAS by an Expert Panel. A GRAS notification (GRN 000029) was submitted to FDA on 26 July 1999 and on 29 December 1999 FDA issued a 'No Objection Letter'.

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b) Exposure to TG from all Food Sources

The calculations presented below represent total consumption of TG regardless of the source (i.e., processed meat or seafood, processed cheeses, natural hard cheeses (domestic), cream cheese, refrigerated yogurt, frozen desserts, vegetable protein dishes/vegeburgers, meat substitutes, pasta and grain).

- For the total US population (all age groups), TG is consumed at the mean rate of 0.095 mg/kg/day (5.5 mg/p/day). The range for the measure is 0.072 – 0.32 mg/kg/day (2.5 – 6.6 mg/p/day).
- The 90% percentile TG intake for the entire US population is 0.21 mg/kg/day (12 mg/p/day). The range for this measure is 0.16 – 0.70 mg/kg/day (7.7 - 15 mg/p/day).
- Of the total US population (all ages), 89.0% are Eaters of these categories. The average consumption of TG by Eaters Only is 0.11 mg/kg/day (6.2 mg/p/day) and has a range of 0.079 – 0.40 mg/kg/day (4.3 – 7.4 mg/p/day). The 90<sup>th</sup> percentile for Eaters Only is 0.22 mg/kg/day (13 mg/p/day) and has a range of 0.17 – 0.89 mg/kg/day (9.6 – 15 mg/p/day).

III. DETECTION OF TG IN FOOD

See prior FDA GRAS Notice #4

IV. SAFETY INFORMATION

See prior FDA GRAS Notice #4

A. STREPTOVERTICILLIUM MOBARAENSE

1. Classification

See prior FDA GRAS Notice #4

2. General Microbiology of the Organism

See prior FDA GRAS Notice #4

3. Genetic stability, Cultural purity and Strain Integrity

See prior FDA GRAS Notice #4

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4. Non-Pathogenicity of the Organism
  - a) Search of the Literature for Pathogenicity  
See prior FDA GRAS Notice #4
  - b) Pathogenicity Test of the Organism  
See prior FDA GRAS Notice #4

B. TG ENZYME BROTH (following organism removal)

1. Lack of Antibiotic Production by the Organism  
See prior FDA GRAS Notice #4
2. Antimicrobial Activity  
See prior FDA GRAS Notice #4
3. β-Lactamase  
See prior FDA GRAS Notice #4
4. Phenomycin  
See prior FDA GRAS Notice #4

C. SAFETY TESTING OF THE WHOLE ENZYME PREPARATION (TG)

See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997

1. Chromosomal Aberration  
See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997
2. Reverse Mutation  
See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997
3. Mouse Micronucleus  
See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997

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4. Mouse Micronucleus

See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997

5. Dermal Sensitization

See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997

6. Dermal Maximization

See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997

7. Allergenicity (Amino Acid Sequence)

See prior FDA GRAS Notice #4

8. Acute Toxicity

See prior FDA GRAS Notice #4 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

9. 13-Week Toxicity

See toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997

10. Determination of the Presence of Aflatoxins

See prior FDA GRAS Notice #4

11. Antibiotics Evaluations

See prior FDA GRAS Notice #4

12. Antimicrobial Activity

See prior FDA GRAS Notice #4

13. Testing For Biologically Active Substances (BASs) Produced by the Genus *Streptoverticillium*

a) Literature Search:

See prior FDA GRAS Notice #4

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- b) Categorization of Biologically Active Substances (BASs):  
See prior FDA GRAS Notice #4
- c) Rationale for Selection of Hazardous Macromolecular BASs  
See prior FDA GRAS Notice #4
- d) Selection of the enzyme preparation  
See prior FDA GRAS Notice #4
- e) Trypsin Inhibitory Activity  
See prior FDA GRAS Notice #4
- f) β-Lactamase  
See prior FDA GRAS Notice #4
- g) Endotoxin  
See prior FDA GRAS Notice #4
- h) Phenomycin  
See prior FDA GRAS Notice #4
- i) Teleocidins  
See prior FDA GRAS Notice #4
  - (1) Extraction and Mass spectrometric analysis  
See prior FDA GRAS Notice #4
  - (2) Epstein-Barr Virus Early Antigen induction assay (EBV-EA)  
See prior FDA GRAS Notice #4

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# SRA International, Inc.

## SRA INTERNATIONAL, INC. GRAS DETERMINATION

A Generally Recognized as Safe (GRAS) Panel on a Transglutaminase Product (TGP) was presented information regarding the expansion of the use of TGP into pasta and grain products. The Panel membership consisted of Dr. Bernard Wagner, Dr. Jay Goodman, Dr. Philip Portoghese and Dr. Vernon Young. Dr. Bruce Bernard served as panel coordinator.

The Panel examined the materials presented in the application packet related to the TGP and considered the previous GRAS approvals for the use of TGP in processed seafood and meats, dairy products and meat substitutes. The intended condition of use is as a protein cross-linking agent in the following food categories and usage levels:

Food Category	Usage Level of TG (PPM)
Pasta products	25
Bread products	15
Pastry products (cakes, pies, doughnuts, etc.)	20
Ready to eat cereal products	45
Pizza dough	20
Grain mixtures (burritos, tacos, etc.)	25

Based upon these data and the Panel deliberations, it was concluded that TGP is GRAS for conditions of intended use. This decision was based in part on the following:

- extensive knowledge concerning the nature of the enzyme including the fact that the active site of microbial TG is homologous to the mammalian derived enzyme
- documentation of the abundant distribution of transglutaminase in nature including the occurrence as a normal human constituent
- evidence that transglutaminase, in both the active and inactive form, has been consumed by humans since ancient times

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Transglutaminase GRAS Certificate  
Expanded Use – Pasta and Grain Products  
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- knowledge of the producing organism; the Panel reviewed the data, agreed with the genus and species classification, and took note that there are no known pathogenic species in this genus
- previous GRAS approvals for the use of TGP in processed seafood and meats, dairy products and meat substitutes
- detailed evaluation of the manufacturing process
- regulatory approvals for use as an additive to human food and a history of human consumption in Japan and Europe
- regulatory review by the U.S. Food and Drug Administration for use as an additive to human food and resulting GRAS Notice No. GRN 000004, No Objection Letter issued on 22 June 1998
- an adequate package of safety studies (including exposure at high concentrations, prolonged exposure duration, and a wide variety of study types) sufficient to support the intended use in humans
- comparison of safety studies to estimations of human consumption

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Transglutaminase GRAS Certificate  
Expanded Use – Pasta and Grain Products  
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Panel Members

Dr. Jay Goodman

Dr. Philip Portoghese

Dr. Bernard Wagner

Dr. Vernon Young

Panel Coordinator

Dr. Bruce K. Bernard

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Transglutaminase GRAS Certificate  
Expanded Use – Pasta and Grain Products  
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Panel Members

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Dr. Jay Goodman

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Dr. Philip Portoghese

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Dr. Bernard Wagner

---

Dr. Vernon Young

Panel Coordinator

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Dr. Bruce K. Bernard

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Transglutaminase GRAS Certificate  
Expanded Use – Pasta and Grain Products  
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Panel Members

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Dr. Jay Goodman

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Dr. Philip Portoghese

---

Dr. Bernard Wagner

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Dr. Vernon Young

Panel Coordinator

---

Dr. Bruce K. Bernard

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Transglutaminase GRAS Certificate  
Expanded Use – Pasta and Grain Products  
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Panel Members

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Dr. Jay Goodman

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Dr. Philip Portoghese

---

Dr. Bernard Wagner

Dr. Vernon Young *VU*

Panel Coordinator

---

Dr. Bruce K. Bernard

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Transglutaminase GRAS Certificate  
Expanded Use – Pasta and Grain Products  
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Panel Members

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Dr. Jay Goodman

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Dr. Philip Portoghese

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Dr. Bernard Wagner

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Dr. Vernon Young

Panel Coordinator

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Dr. Bruce K. Bernard

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SUBMISSION END

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**SRA International, Inc.**

AM



10 January 2001

Dr. Martha Peiperl  
Office of Pre-market Approval, HFS-215  
FDA, 200 C Street, SW  
Washington, D.C. 20204

Re: GRAS Notice (GRN #000055)

Dear Dr. Peiperl:

As you requested during our recent phone discussion, SRA International Inc. (SRA) is providing the following clarification for inclusion in the GRN 0055 record.

The values for "TG" given in Food Master File (FMF) 612, GRAS Notice (GRN) 4, 29, and 55, for usage levels in various food categories and exposure estimates for new uses and for cumulative uses, refer to the TG enzyme preparation consisting of the enzyme plus the co-fermentation medium (i.e., the prediluted enzyme preparation).

Based upon the above, the "usage levels of TG" in parts per million (ppm) provided in GRN 0055 (pg. 3, Table I) refer to the prediluted enzyme preparation (as defined above). Thus, continuing with this example (i.e. Table 1), the prediluted enzyme preparation will be added to pasta products at a level of 25 ppm, but the actual enzyme product (i.e. 10% enzyme preparation + 90% dextrin) will be added to pasta products at a level of 250 ppm.

The above use of terms has been consistently employed by SRA throughout the entire series TG submission (i.e., FMF 612, GRN 0004, 0029, 0055). We trust this information proves useful to you. If you have any additional questions, we will be pleased to assist the Agency in any way possible.

Sincerely,

Bruce K. Bernard, Ph.D.  
Authorized Representative  
Ajinomoto USA

BKB/km

1920 L Street, NW, Suite 420, Washington, DC 20036  
Telephone: (202) 728 1400 Telefax: (202) 331-3393

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