ORIGINAL SUBMISSION

DYADIC®

DYADIC INTERNATIONAL (USA), INC.
140 INTRACOASTAL POINTE DRIVE, SUITE 404
JUPITER, FLORIDA 33477
(561) 743-8333 tel.
(561) 743-8343 fax
http://www.dyadic.com

RECEIVED
MAY 4 2009
OFFICE OF
FOOD ADDITIVE SAFETY

5/1/09

BY OVERNIGHT COURIER

Mr. Robert Martin
Food and Drug Administration
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Dyadic International (USA), Inc. ("Dyadic"); Submission of GRAS Notice for a Cellulase Enzyme Preparation Derived From a Genetically Modified *Myceliophthora thermophila*

Dear Mr. Martin:

Pursuant to the submission requirements set forth in 62 FR 18937, enclosed please find Dyadic's GRAS Notice presented in triplicate, together with an electronic copy on CD for your convenience.

The cellulase enzyme preparation that is the subject of the enclosed GRAS Notice is derived from a genetically modified, non-pathogenic and non-toxigenic fungal mircroorganism, *Myceliophthora thermophila*. Additional copies of the *M. thermophila* cellulase gene were incorporated into the chromosome of the *M. thermophila* recipient, resulting in a hyper-producing cellulase strain. The cellulase enzyme preparation is produced using current good manufacturing processes (cGMP) via a pure culture submerged fermentation of source microorganism using methods and ingredients that are standard to the industry. The characterizing activity of the preparation is a cellulase (E.C. 3.2.1.4; systematic name: 1,4-(1,3:1,4)-β-D-glucan 4-glucanohydrolase), an enzyme responsible for the endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans.. Additionally, cellulases will hydrolyze the 1,4 linkages in β-D-glucans also containing 1,3-linkages. The cellulase enzyme preparation can be used to breakdown cellulose in a wide variety of cellulose-containing food products.

DYADIC INTERNATIONAL (USA), INC.
140 INTRACOASTAL POINTE DRIVE, SUITE 404
JUPITER, FLORIDA 33477
(561) 743-8333 tel.
(561) 743-8343 fax
http://www.dyadic.com

5/1/09

BY OVERNIGHT COURIER

Mr. Robert Martin
Food and Drug Administration
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Dyadic International (USA), Inc. ("Dyadic"); Submission of GRAS Notice for a Cellulase Enzyme Preparation Derived From a Genetically Modified *Myceliophthora thermophila*

Dear Mr. Martin:

Pursuant to the submission requirements set forth in 62 FR 18937, enclosed please find Dyadic's GRAS Notice presented in triplicate, together with an electronic copy on CD for your convenience.

The cellulase enzyme preparation that is the subject of the enclosed GRAS Notice is derived from a genetically modified, non-pathogenic and non-toxigenic fungal mircroorganism, *Myceliophthora thermophila*. Additional copies of the *M. thermophila* cellulase gene were incorporated into the chromosome of the *M. thermophila* recipient, resulting in a hyper-producing cellulase strain. The cellulase enzyme preparation is produced using current good manufacturing processes (cGMP) via a pure culture submerged fermentation of source microorganism using methods and ingredients that are standard to the industry. The characterizing activity of the preparation is a cellulase (E.C. 3.2.1.4; systematic name: 1,4-(1,3:1,4)-β-D-glucan 4-glucanohydrolase), an enzyme responsible for the endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans.. Additionally, cellulases will hydrolyze the 1,4 linkages in β-D-glucans also containing 1,3-linkages. The cellulase enzyme preparation can be used to breakdown cellulose in a wide variety of cellulose-containing food products.

This cellulase enzyme preparation meets the general and additional requirements for enzyme preparations as outlined in the Food Chemical Codex (National Academy of Sciences, 2003) and by the Joint WHO/FAO Expert Committee on Food Additives (JECFA, 2001). In accordance with 21 CFR 184.1(b)(1), the preparation will be used as an enzyme in the production of wine, beer and fruit juices as defined in 21 CFR 170.3 (o)(9) in food with no limitations other than current GMP conditions.

This Notice summarizes the basis of Dyadic's determination of the general recognition of safety of the cellulase preparation derived from a genetically modified strain of *M. thermophila* expressing additional copies of the *M. thermophila* cellulase gene. Information on the source mircroorganism, the enzyme's identity, the manufacturing procedures, specifications, potential dietary exposure, and enzyme food applications is included. The safety evaluation of these component parts includes a review of both published and unpublished data.

A number of safety studies, both with the organism and the cellulase enzyme test product were performed. There was no evidence of a safety concern demonstrated in any of these studies. In addition, a scientific panel of experts was assembled to review the documented safety information. Based on the accumulated safety data evidence and scientific procedures, Dyadic has concluded that cellulase derived from *M. thermophila* expressing additional copies of the *M. thermophila* cellulase gene meets the regulatory definition (21 CFR 170.30) of generally recognized as safe (GRAS).

Respectfully submitted,

(b)(6)

Richard H. Jundzil
Director of Development & Quality

Enclosures

A Generally Recognized As Safe (GRAS) Notice for a Cellulase Enzyme Preparation Derived From a Genetically-Modified *Myceliophthora thermophila*

Dyadic International (USA), Inc.

March, 2009

INTRODUCTION 4

SOURCE 5

A. Recipient Strain 5

B. Introduced DNA Sequences 5

C. Production Strain 5

D. Stability of the Introduced DNA Sequences 5

ENZYME IDENTITY 6

A. Identity 6
1. Systematic Name: 6
2. EC Classification: 6
3. Common or Usual Name: 6
4. Other Names: 6
5. CAS Registry Number: 6
6. Reaction Catalyzed: 6

B. Sequence Comparison to Other Cellulases 7

C. Enzyme Preparation Composition 7
Table 1—Enzyme Preparation Composition 7

MANUFACTURING PROCESS 8

A. Raw Materials 8

B. Production Microorganism 8

C. Fermentation Process 8

D. Recovery Process 8

E. Formulation 8

SPECIFICATIONS 9

A. FCC and JECFA Specifications 9
Table 2-- Cellulase Product Specifications 9

B. Absence of Production Microorganism in Final Product 9

C. Analytical Methodology 9

APPLICATION 10

A. Foods in Which Used/Purpose of Use 10

B. Mode of Action 10

C. Level of Use 10

SAFETY EVALUATION 11

A. Food Processing Enzymes 11

B. Cellulases 11

C. Production organism 11
1. Taxonomy 11
2. Literature Search 11
3. Pathogenicity/Toxigenicity Study 12

D. Enzyme Product 12
1. Subchronic toxicology studies 12
2. Genotoxicity Studies 14

E. Allergenicity 15

F. Manufacturing Process 15

G. Potential Dietary Exposure/Safety Margin Calculations 16

H. Evaluation by the Pariza and Johnson Decision Tree 17

SCIENTIFIC EXPERT PANEL 19

CONCLUSIONS 19

REFERENCES 21

APPENDICES 22
1. Scientific Expert Panel Comments 22

INTRODUCTION

Dyadic International (USA), Inc. has developed a cellulase enzyme preparation derived from a genetically modified *Myceliophthora thermophila* strain designated strain Eg5#27. Additional copies of the *M. thermophila* cellulase gene were incorporated into the chromosome of the *M. thermophila* recipient, resulting in a hyperproducing cellulase strain. The cellulase enzyme preparation is produced using current good manufacturing practices (cGMP) via a pure culture submerged fermentation of the nonpathogenic and nontoxigenic source microorganism using methods and ingredients that are standard to the industry. The characterizing activity of the preparation is a cellulase (E.C. 3.2.1.4; systematic name: 1,4-(1,3:1,4)-β-D-glucan 4-glucanohydrolase), an enzyme responsible for the endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. Additionally, cellulases will hydrolyze the 1,4-linkages in β-D-glucans also containing 1,3-linkages. The enzyme preparation can be used to breakdown cellulose in a wide variety of cellulose-containing food products.

The cellulase enzyme preparation meets the general and additional requirements for enzyme preparations as outlined in the *Food Chemicals Codex* (National Academy of Sciences, 2003) and by the Joint WHO/FAO Expert Committee on Food Additives (JECFA, 2001). In accordance with §21 C.F.R. 184.1 (b)(1), the preparation will be used as an enzyme in the production of wine, beer, and fruit juices as defined in §21 C.F.R. 170.3 (o)(9) in food with no limitations other than current GMP conditions.

The information contained in this notification summarizes the basis of Dyadic's determination of the general recognition of safety of the cellulase preparation derived from a genetically modified strain of *M. thermophila* expressing additional copies of a *M. thermophila* cellulase gene. Information on the source microorganism, the enzyme's identity, the manufacturing procedures, specifications, potential dietary exposure, and enzyme food applications is provided. The safety evaluation of these component parts includes a review of both published and unpublished data.

A number of safety studies, both with the organism and the cellulase enzyme test product, were performed. In none of these studies did the results show evidence of a safety concern. In addition, a scientific panel of experts was assembled to review the documented safety information. Based on the accumulated safety data evidence and scientific procedures, Dyadic International (USA), Inc. has concluded that cellulase derived from *M. thermophila* expressing additional copies of the *M. thermophila* cellulase gene meets the regulatory definition (§21 C.F.R. 170.30) of generally recognized as safe (GRAS).

SOURCE

A. Recipient Strain

The recipient strain used in construction of the production strain *Myceliophthora thermophila* Eg5#27 is a *M. thermophila* strain designated UV18pyr5. Strain UV18pyr5 is a derivative of the wild type strain C1, an isolate obtained in the early 1990s from alkaline soil in Eastern Russia. The parent strain was deposited with the [redacted] (b)(4) [redacted] on August 29, 1996, as a deposit according to the Budapest Treaty and was assigned Accession Number VKM F-3500D. Its classification is described in several patents, including US 6,573,086.

The C1 strain was originally identified as *Chrysosporium lucknowense*, but recent work performed at [redacted] (b)(4) [redacted] shows the classification of the strain to be *Myceliophthora thermophila*. The recipient strain is distinguished from the ancestral strain C1 by its decreased sporulation capability, mycelial fragmentation, and high-level protein synthesis and secretion. Strain UVpyr5 is also pyrimidine requiring, i.e., this strain has a defect in the *pyr5* gene encoding orotate phosphoribosyl transferase and requires uracil and uridine for growth.

B. Introduced DNA Sequences

To construct the production strain, UV18pyr5 protoplasts were incubated with two DNA fragments, each of which contained genomic DNA originating from strain C1 and only from strain C1. The first fragment was a 3.6 kb fragment containing the strain C1 *pyr5* gene and associated flanking sequences. The second fragment was a 3459 base pair *Kpn*I fragment containing the strain C1 *eg5* gene, encoding an endoglucanase (or cellulase), fused to the promoter of the strain C1 *cbh1*gene, which encodes a cellobiohydrolase. The gene was then followed by the strain C1 *cbh1* terminator. Transformants were selected for restoration of the *pyr5* function, *i.e.*, lack of requirement for uracil and uridine, and screened for the presence of the p*cbh1-eg5* fusion construct.

C. Production Strain

One resulting strain, designated Eg5#27, was shown to produce an approximately twenty-fold elevated level of the endoglucanase (cellulase) encoded by *eg5* and was chosen as the production microorganism for the enzyme of this notification.

D. Stability of the Introduced DNA Sequences

Southern hybridization studies indicate that strain Eg5#27 contains approximately six additional copies of the endoglucanase (cellulase) gene

and does not contain sequences that hybridize to the cloning vectors from which the strain C1 genomic DNA fragments were isolated. Because neither of the DNA fragments introduced to the UV18pyr5 recipient strain is capable of autonomous replication, their presence in the strains must be as a result of random integration into the genome. No attempt has been made to target integration to a particular site or sites. Because the transforming DNA is stably integrated into the *M. thermophilia* chromosome, it is poorly mobilizable for genetic transfer to other microorganisms and is mitotically stable.

ENZYME IDENTITY

A. Identity

The following information was obtained from the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) website (www.chem.qmul.ac.uk/iubmb/enzyme/).

1. Systematic Name:
   1,4-(1,3:1,4)-β-D-glucan 4-glucanohydrolase

2. EC Classification:
   EC 3.2.1.4

3. Common or Usual Name:
   Cellulase

4. Other Names:
   Endo-1,4-β-D-glucanase; β-1,4-glucanase; β-1,4-endoglucan hydrolase; cellulase A; cellulosin AP; endoglucanase D; alkali cellulase; cellulase A 3; celludextrinase; 9.5 cellulase; avicelase; pancellase SS

5. CAS Registry Number:
   9012-54-8

6. Reaction Catalyzed:
   This enzyme is responsible for the endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. Additionally, it will hydrolyze 1,4-linkages in β-D-glucans also containing 1,3-linkages.

B. Sequence Comparison to Other Cellulases

A BLAST search, obtained by searching the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/), using the default parameters, indicated similarities of the endoglucanase (cellulase) protein to several known and hypothetical proteins from filamentous fungi. The *M. thermophila* endoglucanase had a 92%, 83%, and 80% relatedness similarity to cellulase genes from *Humicola grisea*, *Scopulariosis brevicaulis*, and *Magnaporthe grisea*, respectively. In addition to these cellulases, an endoglucanase from the nematode *Bursaphelenchus xylophilus* showed 92% similarity.

C. Enzyme Preparation Composition

Like most other enzyme preparations used for food use, the enzyme preparation of this GRAS Notice is a mixture. It contains other active enzymes, including β-glucanase and xylanase; the final product is standardized to the cellulase (glucanohydrolase) activity. The cellulase enzyme preparation derived from *M. thermophila* strain Eg5#27 has the following typical composition (with the difference being water):

Table 1—Enzyme Preparation Composition

Substance	**Amount** (w/w%)
Enzyme preparation solids (as TOS)	19.5
Sorbitol	45
Sodium chloride	5
Sodium benzoate	0.3
Potassium sorbate	0.1
Caramel coloring	0.3

MANUFACTURING PROCESS

A. Raw Materials

The cellulase enzyme preparation from *M. thermophila* strain Eg5#27 is manufactured according to procedures outlined by Pariza and Foster (1983) using standard microbial enzyme production technology (Aunstrup, 1979; Aunstrup *et al*., 1979; Enzyme Applications, 1994) and according to current good manufacturing practice (cGMP). The fermentation ingredients used in the manufacture of cellulase enzyme preparations are substances that are acceptable for general use in food..

B. Production Microorganism

Stock cultures of the *M. thermophila* Eg5#27 production strain are maintained at -80°C as frozen stocks containing glycerol as a cryopreservant. The production microorganism is stable under these storage conditions. Each stock culture batch is examined for strain identity, purity, and enzyme-producing capability before use in manufacturing.

C. Fermentation Process

The large-scale growth of *M. thermophila* Eg5#27 for the production of cellulase is performed using a liquid medium in a submerged fermentation. All fermentations begin with a pure culture of the production microorganism. Generally accepted microbiological techniques are used to exclude contaminating organisms and to avoid development of substrains from the production culture itself. Factors such as pH, temperature, and aeration are controlled. During growth, fermentors are routinely sampled and tested for possible contamination.

D. Recovery Process

The recovery process is a multi-step operation that begins immediately following the fermentation step and involves both the purification and concentration of the product. After fermentation, the culture broth is subjected to a rotary drum filtration system whereby the source organism is separated from the liquid enzyme, removed and disposed according to local, state and federal regulatory requirements. The enzyme remaining in the filtrate is concentrated by ultrafiltration and polish filtered prior to formulation as appropriate for the end-use application.

E. Formulation

Stabilizers, diluents, and/or preservatives that are suitable for general use in food may be added to the cellulase enzyme preparation from *M. thermophila* Eg5#27. The cellulase enzyme preparation is formulated as a liquid product. The product is standardized according to the product specifications.

SPECIFICATIONS

A. FCC and JECFA Specifications

The *M. thermophila* Eg5#27 derived cellulase preparations meet the requirements for enzyme preparations as set forth in the *Foods Chemical Codex* (National Academy of Sciences, 2003) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001).

In total, the cellulase products conform to the following specifications:

Table 2-- Cellulase Product Specifications

Cellulase Activity, U/g*	According to declaration
Total viable count/g	Not more than 50,000 CFU
Total coliforms/g	Not more than 30
Escherichia coli/25 g	Absent
Salmonella spp./25 g	Absent
Production strain	Negative by test
Antibiotic activity	Negative by test
Lead	Not more than 5mg/kg
Mycotoxin	Negative by test

* One unit is defined as the quantity of enzyme that liberates 1 micromole of reducing sugar (expressed as glucose equivalents) in one minute under conditions of the test (50 ˚C, pH 4.8, 10 minutes).

B. Absence of Production Microorganism in Final Product

The absence of the production microorganism in the commercial cellulase product is an established specification.

C. Analytical Methodology

The cellulase assay can be performed using the method contained in the *Food Chemicals Codex* (FCC) (2003) or by any appropriate validated method. The FCC assay measures the release of reducing sugars by the action of the enzyme on a cellulase substrate. One unit of activity liberates 1 micromole of reducing sugar (expressed as glucose equivalents) in one minute under conditions described (50 ˚C, pH 4.8, 10 minutes). The sample activity is then related to a standard with a stated carboxymethylcellulase activity.

APPLICATION

A. Foods in Which Used/Purpose of Use

Enzymes, such as the cellulase of this GRAS Notice, are used in small quantities in food to accomplish their intended technical effects. Cellulase enzymes have a long history of safe use in the food industry and are typically used for the following purposes: breakdown of cellulose in citrus products or other fruits used in juice and wine production, removal of fiber from edible oil press cakes, increase in starch recovery from potatoes and other starch sources, extraction of proteins from leaves and grasses, tenderizing fruits and vegetables prior to cooking, extraction of essential oils and flavoring material from plant materials, treatment of distiller's mash, extraction of green tea components, and other uses.

B. Mode of Action

This enzyme is responsible for the endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. Additionally, it will hydrolyze 1,4-linkages in β-D-glucans also containing 1,3-linkages.

C. Level of Use

The cellulase enzyme preparation is used in food at minimum levels necessary to achieve the desired effect and according to cGMP. The optimum dosage levels of the cellulase enzyme product will vary with different substrates and operating conditions (including pH, temperature, substrate concentration, and reaction time).

The recommended dosage range for cellulase for use in beer, wine and fruit juice production is 0.004-0.02% (w/w).

Many food processes that use cellulase include a step that removes and/or inactivates most, if not all, of the added enzyme preparation.

SAFETY EVALUATION

A. Food Processing Enzymes

Pariza and Foster (1983) and Pariza and Johnson (2001) have published papers that address the issue of food processing enzyme safety. They conclude that enzymes *per se* that are used or likely to be used in food processing are inherently nontoxic, thus the safety evaluation should focus on possible contaminants which could be present in the final enzyme preparation. The fact that enzymes are a biological necessity for all living organisms offers a high degree of safety assurance for these proteins. Enzymes have always been present as natural components of food, thus the history of human exposure to enzymes is as old as human life itself. The application of enzymes to food processing has developed from traditional recipes such as those for fermented foods whose preparation is older than recorded history.

B. Cellulases

Cellulases have a long history of safe use in the food industry (Hjortkjaer *et al*., 1986). A search of relevant literature shows that cellulase derived from *Aspergillus niger* has been used for a variety of food purposes since at least 1952 (Beckhorn, 1965). In addition, several cellulase enzyme preparations from numerous sources have been determined to be Generally Recognized As Safe (GRAS), including those from: *Aspergillus aculeatus, Aspergillus niger, Aspergillus oryzae, Trichoderma longibrachiatum* (*reesei*) (FDA, 1999), and *Trichoderma viride* (Pariza and Johnson, 2001).

C. Production organism

1. Taxonomy

The *M. thermophila* C1 strain was isolated in the early 1990's from alkaline soil in Eastern Russia. At that time, it was classified by its morphological characteristics as a strain of *Chrysosporium lucknowense*. More recently, classification of the organism by **(b)(4)** using genetic-based testing showed that the C1 strain was a strain of *M. thermophila.*

2. Literature Search

A literature search on the production microorganism keyed to toxicity and pathogenic potential was performed. No articles were uncovered that would indicate that the use of *M. thermophila* as a source organism for food enzyme production could be a potential health hazard to humans. In addition, no reports of mycotoxin production by this microorganism were found.

M. thermophila strains have been reported to attack plant-based materials and may be opportunistic plant pathogens under some

circumstances, which is very common among fungi. Three reports of *M. thermophila* as an opportunistic pathogen were uncovered Bourbeau, 1992; Tekkok, I. H., 1996; Farina, C. et al. 1998). In each case, the patient was compromised by other medical conditions, thus *M. thermophila* appears to be an opportunistic pathogen capable of infecting severely compromised individuals, but not likely to be of concern to healthy individuals.

Myceliophthora thermophila has been reported as the donor microorganism for a GRAS enzyme, laccase, produced by a recombinant *Aspergillus oryzae* strain (GRASN 122).

3. Pathogenicity/Toxigenicity Study

The safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food (Pariza and Foster, 1983; Pariza and Johnson, 2001). In order to assess the pathogenicity of the production microorganism, an infectivity/pathogenicity/toxigenicity study with *M. thermophila* Eg5#27 was conducted.

The results of the study demonstrate that *M. thermophila* Eg5#27 is non-infective, non-pathogenic, and non-toxigenic, and support the safety of this fungal strain for use in the production of a cellulase product for use in the food processing industry. Further, the study shows that, in mammals, conditions within the abdominal cavity do not favor the growth of the fungus. Intraperitoneal injection into mice elicited defense mechanisms (*i.e.,* inflammation and abscess formation) common to the isolation and clearance of foreign proteins from host tissues.

D. Enzyme Product

The enzyme test preparation that was used in the feeding studies contained 2410 u/g and had a Total Organic Solids (TOS) content of 14.59 % (w/w). The enzyme test material was manufactured similarly to the commercial enzyme preparation with the exception of the preservatives and stabilizers which were not added.

1. Subchronic toxicology studies

a) 14-Day Range Finding Study

The objective of this range-finding study was to provide data on the acceptability of the cellulase test substance in rats upon administration via the diet for 14 consecutive days. Clinical signs, growth, food consumption, food conversion

efficiency, organ weights, and gross examination at necropsy were used as criteria for assessment of palatability and/or disclosing possible adverse effects. The results of the study showed that treatment with the cellulase enzyme test material via the diet up to a level of 5% (w/w) for a period of 14 days was well tolerated and did not result in any adverse effects.

b) 13-Week Oral Toxicity Study

The oral toxicity of the cellulase test preparation was examined in a 13-week subchronic oral toxicity study with four groups of 20 male and 20 female rats. The test substance was incorporated in the feed at levels of 0% (control), 1%, 2% and 4%.

Results of the study indicate that there were no treatment-related clinical signs. The results of neurobehavioral observations and motor activity assessments did not indicate any neurotoxic potential of the test substance. Ophthalmoscopic examination did not reveal any treatment-related changes. Growth, overall food intake and food conversion efficiency were not affected by treatment. Red blood cell variables, total white blood cell count and clotting potential were not affected by the treatment.

There were no noticeable changes in clinical chemistry parameters as determined in 10 rats/sex/group at necropsy. Urinalysis, conducted in 10 rats/sex/group in week 13 of the study, did not reveal any treatment-related changes in renal concentrating ability, semi-quantitative urinary measurements or in microscopy of the urinary sediment. There were no significant changes in absolute or relative organ weights among the groups. Macroscopic examination at necropsy and microscopic examination did not reveal adverse histopathological changes.

The no-observed-adverse-effect level (NOAEL) in this study was placed at the highest dose level applied, 4% in the diet (equivalent to 2.8 g/kg/body weight/day).

2. Genotoxicity Studies

a) Bacterial Reverse Mutation (Ames) Test

The cellulase enzyme test product was examined for mutagenic activity in the bacterial reverse mutation test using histidine-requiring *Salmonella typhimurium* strains TA1535, TA 1537, TA 98 and TA 100, the tryptophan-requiring *Escherichia coli* strain WP2 *uvrA* , and a liver fraction of Aroclor 1254-induced rats for metabolic activation (S9-mix). In both the absence and presence of the S9-mix and in all strains, the cellulase did not cause a more than two-fold increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative control. It was concluded that the cellulase preparation was not mutagenic under the conditions employed in this study.

b) Chromosomal Aberration Test

The cellulase enzyme test product was examined for its potential to induce structural chromosomal aberrations in human lymphocytes, in both the absence and presence of a metabolic activation system (S9-mix). In two independent chromosomal aberration tests, in both the absence and presence of S9-mix, the test substance did not induce a statistically significant increase in the number of cells with structural chromosomal aberrations at any of the dose levels and time points analyzed, when compared to the negative controls. The data support the conclusion that, under the conditions used in both tests, the test substance was not clastogenic for cultured human lymphocytes.

c) Gene Mutation Test at the TK-locus of L5178Y Cells

The cellulase enzyme test product was examined for its potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells, in both the absence and presence of a metabolic activation system (S9-mix). The test product (highest level tested was 5000 µg/ml) was not cytotoxic to the L5178Y cells, nor was an increase of the mutant frequency observed at any dose level in either the absence or presence of S9-mix. It was concluded that, under the conditions used in this study, the cellulase was not mutagenic at the TK-locus of mouse lymphoma L5178Y cells.

E. Allergenicity

The allergenicity of foods, particularly food proteins, is receiving increasing attention as food allergic responses are on the rise in the general population. While the cellulase enzyme is a protein that may have the potential to illicit an allergic response, it is important to note that, to date, there have only been a few rare cases where an enzyme used as a processing aid in food and ingested has been reported to cause an allergic response. Dauvin *et al.* (1998) report that there are a few papers describing cases of allergy symptoms elicited by the ingestion of enzymes in the people, but only if they had a pre-exisiting occupational allergy to an enzyme.

In a recent study by C. Bindslev-Jansen *et al.* (2006), a wide variety of enzymes from various classes and sources (including a cellulase) were investigated for their ability to elicit an allergic response when ingested. The authors reported there were no allergic findings of clinical relevance and concluded that ingestion of food enzymes, in general, is not considered to be a concern with regard to food allergy.

Food processing enzymes have a long history of safe consumption, and since enzymes occur naturally as a component of many foods, enzymes have been consumed since the beginning of human existence. The enzyme preparation of this GRAS Notice will be used as a food processing aid where consumer exposure will be very small, thus the likelihood of allergic sensitization and reactions is extremely low.

F. Manufacturing Process

The cellulase enzyme preparation meets the general and additional requirements for enzyme preparations as outlined in the monograph on Enzyme Preparations in the *Food Chemicals Codex* (2003) and by JECFA (2001). The cellulase preparation is produced in accordance with current good manufacturing practices, using ingredients that are acceptable for general use in foods, and under conditions that ensure a controlled fermentation. These methods are based on generally available and accepted methods used for production of microbial enzymes (Aunstrup, 1979; Aunstrup *et al*., 1979, Enzyme Applications, 1994).

G. Potential Dietary Exposure/Safety Margin Calculations

The cellulase of this notification has similar uses to, and thus can be a replacement for, the currently marketed GRAS cellulase enzyme preparations that are in use today. While most uses of cellulase will include a processing step where the cellulase would be removed from the food or inactivted, the following calculation assumes no enzyme removal or inactivation occurs.

The maximum recommended dosage for the cellulase of this GRAS Notice in beverage production is 0.02% (w/w).

The following table shows the reported mean consumption of beer, wine and fruit juices in the United States for the year 2003 (data from http://www.ers.usda.gov/data/foodconsumption/). In total, the mean consumption per capita of these three beverages was 32.2 gallons.

Table 3—Annual U. S. Wine, Beer, and Fruit Juice Consumption-2003

Food Product	**Mean Beverage Consumption** (gallons/capita/year)
Wine	2.2
Beer	21.6
Fruit juice	8.4
Total	**32.2**

The maximum daily consumption of cellulase TOS from use in the beverage industry for a person weighing 60 kg calculates is calculated below:

A dosage of 0.02% w/w calculates to 2176 cellulase u/gal of beverage. The enzyme preparation contains approximately 16,518 units/g of TOS, thus:

2176 u/gal. x 1 g TOS/16, 518 units = 0.1318 g TOS/gal.

The average consumption of beverages for a person weighing 60 kg is:

(32.2 gal./365 day per year)/60 kg = 0.00147 gal/kg of body weight (kgbw)/day

The TOS consumption is determined to be:

0.00147 gal./kgbw/day x 0.1318 TOS/gal. x 1000 mg TOS/1 g TOS = 0.194 mg TOS/kgbw/day

The no-observed-adverse-effect level (NOAEL) in the 13-week study was placed at the highest dose level applied, 4% in the diet, an equivalent of 2,800 mg of enzyme preparation/kg/body weight/day.

The safety margin would therefore be:

2800 mg/kgbw/day x 0.1459 mg TOS/mg of enzyme preparation = 409 mg TOS/kgbw/day

The safety margin would therefore be:

NOAEL/EDI=409/0.194 or 2108

H. Evaluation by the Pariza and Johnson Decision Tree

The decision tree outlined by Pariza and Johnson (2001) is commonly used by the enzyme industry today to evaluate the safety of microbial enzyme preparations intended for use as processing aids. The decision tree begins by asking whether or not the production strain is genetically modified. Since the production strain of this Notification has been genetically modified using rDNA techniques, one must answer a number of questions relating to the introduced DNA. The decision tree questions if the expressed enzyme product(s), which are encoded by the introduced DNA, have a history of safe use in food. While many cellulases have a long history of safe use, the specific cellulase from *M. thermophilia* does not, thus one must determine whether the NOAEL for the test article in appropriate short-term oral studies is sufficiently high to ensure safety. The 14-day range finding study and the 13-week oral toxicity studies described above both are sufficient to demonstrate at least a 100-fold safety factor.

The test article is free of transferable antibiotic resistance DNA; no antibiotic resistance markers were used during the genetic modification steps used to obtain the *M. thermophila* production microorganism. All other introduced DNA is well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products.

Question 4 of the decision tree asks if the introduced DNA is randomly integrated into the chromosome, and if so, whether the production strain is sufficiently characterized so that one make reasonable assumptions about unintended pleiotropic effects. Data described above show that about six additional copies of the gene are randomly inserted into the chromosome of the *M. thermophila* production strain. The five toxicity studies performed using the cellulase test preparations derived from the *M. thermophila* production microorganism did not uncover any treatment related toxicity, or

induction of gene mutation or chromosomal aberrations, thus it is reasonable to assume that no unintended pleiotropic effects which could result in the synthesis of toxins or other unsafe metabolites have occurred due to the genetic modification that was employed.

The production strain is not from a known safe strain lineage, thus one must test the production microorganism for pathogenicity, antibiotic production, and for oral toxicity. The pathogenicity study conducted with the production microorganism found that the strain was nonpathogenic, the test for presence of antibiotics in enzyme product (JECFA, 2001) was negative, and the results of the 13-week dietary study showed that the NOAEL for cellulase test article was sufficiently high to ensure safety.

With these questions answered, the decision tree has been navigated to the point where the product can be accepted.

SCIENTIFIC EXPERT PANEL

A panel of scientific experts with training and experience in microbiology, food enzymology, molecular biology, and toxicology reviewed the scientific data available on the cellulase enzyme preparation from *M. thermophila* Eg5#27 and concluded that the enzyme preparation was GRAS, based on scientific procedures, for its intended use. Appendix 1 contains a list of the Scientific Expert Panel members and the conclusions of the panel.

CONCLUSIONS

The subject of this GRAS Notification is a cellulase enzyme preparation derived from a genetically modified strain, designated *Myceliophthora thermophila* Eg5#27, which expresses additional copies of the *M. thermophila* cellulase gene. Dyadic International (USA), Inc. has determined the cellulase enzyme preparation to be GRAS, through scientific procedures, for use as a processing aid in the food industry. The enzyme is used to hydrolyze cellulose in a variety of cellulose-containing food products, specifically wine, beer and fruit juices, and at minimum levels necessary to achieve the intended technical effects. Cellulases have a long history of safe use in food processing; published literature provides evidence that cellulases have been used in food since at least 1952. Many GRAS cellulases are commercially available today from several different microbial sources.

The safety of the cellulase enzyme preparation derived from *M. thermophila* has been evaluated using the safety scheme of Pariza and Johnson (2001) and others (IFBC, 1990; JECFA, 2001). Published and unpublished information is provided which assesses the safety of the following: recipient strain, introduced genetic material, production microorganism, cellulases and their use in food, manufacturing process, the final cellulase enzyme preparation, and potential residues in food.

The safety of the production organism is the prime consideration when assessing the probable degree of safety of an enzyme preparation intended for use in food. If the enzyme production organism is nonpathogenic and nontoxigenic, and the enzyme is made according to current GMP, then one can assume the food ingredient made from the production microorganism is safe to consume. Published and unpublished testing data, including a pathogenicity test with the production strain, demonstrate that *M. thermophila* is nonpathogenic and nontoxigenic, and is a suitable source for production of a food enzyme.

The DNA introduced into the *M. thermophila* recipient strain is well characterized and shown to be safe. The DNA sequences of the inserted DNA are known and are minimal such that no unnecessary DNA was incorporated into the production microorganism during the genetic modification. Additionally, no antibiotic resistances markers were used during the genetic manipulations. The toxicity

studies performed using cellulase test product further show the introduced DNA is free of attributes that would render it unsafe for use in the production of a food enzyme. The introduced DNA is integrated into the chromosome, is non-mobilizable, and is stable in the production strain during storage and fermentation.

In assessing the safety of the cellulase preparation, Dyadic conducted the following safety studies: a 14-day range finding gavage in rats; a 13-week subchronic gavage in rats; a bacterial reverse mutation (Ames) test, a gene mutation test at the TK-locus of L5178Y cells, and a chromosomal aberration test. These studies did not find any treatment related toxicity, or induction of gene mutation or chromosomal aberrations in tests using the cellulase test preparations derived from the *M. thermophila* production microorganism.

The manufacturing process used to make the cellulase enzyme preparation employs a pure culture submerged fermentation of the *M. thermophila* production strain. Good Manufacturing Practices are used throughout the process which utilizes generally accepted, published methods for enzyme manufacture and formulation. All raw materials used in the fermentation and recovery processes are food grade and standard materials used in the enzyme industry. The final cellulase enzyme preparations meet the general and additional requirements for enzyme preparations as outlined in *Food Chemicals Codex* and by JECFA.

A panel of scientists with training and expertise in the fields of food enzymology, molecular biology, and toxicology was assembled to review the safety information compiled on the cellulase from the genetically modified *M. thermophila* source. The conclusion of the scientific panel was that the cellulase of this Notification derived from *M. thermophila* was safe for its intended use in food.

Based on the information provided in this GRAS Notification and using scientific procedures, Dyadic concludes that the cellulase preparation derived from *M. thermophila* expressing additional copies of the *M. thermophila* cellulase gene is GRAS under the intended conditions of use.

REFERENCES

Aunstrup, K. Production, Isolation, and Economics of Extracellular Enzymes in Applied Biochemistry and Bioengineering, Vol. 2, Enzyme Technology, *eds*. L. B. Wingard, E. Katchalski-Katzir, and L. Goldstein, pp. 28-68, 1979.

Aunstrup, K. O. Anderson, E. A. Falch, and T. K. Nielsen. 1979. Production of Microbial Enzymes *in* Microbial Technology, 2nd ed., Vol. 1, *eds* H.J. Peppler and D. Perlman. Ch. 9, pp. 282-309.

Beckhorn, E.J., M. D. Labbe, and L. A. Underkofler. 1965. Production and Use of Microbial Enzymes for Food Processing. J. Agr. Food Chemistry 13:30-34.

Bindslev-Jensen, C., Stahl Skov, P., Roggen, E. L, Hvass, P., Sidelmann, Brinch, D. Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. *Food and Chemical Toxicology*, 44:1909-1915, 2006.

Bourbeau, P. *et al*. Fatal disseminated infection caused by *Myceliophthora thermophila*, a new agent of mycosos: case history and labority charactistics. J. Clin. Microbiol. 30(11):3019-3023, 1992.

Dauvin T., G. Groot, K. H. Maurer, D. Derijke, H. Ryssov-Nielson, M. Simonsen, T. B. Sorensen. Consumer allergy risk from enzyme residues in food. Amfep.1998.

Enzyme Applications *in* Encyclopedia of Chemical Technology, 4th ed., *ed*. J. I. Kroschwitz, Vol. 9, pp. 597-620, 1994.

Farina, C. et al. Fatal aortic *Myceliophthora thermophila* infection in a patient affected by cystic medial necrosis. Med. Mycol. 36(2):113-118, 1998.

Food and Drug Administration (FDA). 1999. Direct food substances affirmed as Generally Recognized as Safe: Cellulase enzyme preparation derived from *Trichoderma longibrachiatum* for use in food processing. Fed. Regist. 64:28358-228362.

Hjortkjaer, R. K., V. Bille-Hansen, K. P. Hazelden, M. McConville, D. B. McGregor, E. Chapman, J. R. Gardner, and R. Ashby. 1986. Safety evaluation of Celluclast ®, an acid cellulase derived from *Trichoderma reesei*. Fd. Chem. Toxic. 24(1):55-63.

International Food Biotechnology Council (IFBC). 1990. Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms in Biotechnologies and Food: Assuring the Safety of Foods Produced by

Genetic Modification. Regulatory Toxicology and Pharmacology 12:S1-S196.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), General Specifications and Considerations for Enzyme Preparations Used in Food Processing, Compendium of Food Additive Specifications, FAO FNP (Food and Nutrition Paper) 52, Add.9, FAO, Rome, 2001.

National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemicals Codex. 2003. Food Chemicals Codex, 5th Edition, National Academy Press, Washington, D.C., p. 133.

Pariza, M. W. and E. M. Foster. 1983. Determining the safety of enzymes used in food processing. J. Food Protect. 46:453-468.

Pariza, M. W. and E. A. Johnson. 2001. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Reg. Toxicol. Pharmacol. 33: 173-186.

Tekkok, I. H. et al. Posttraumatic gas-containing brain abscess caused by *Clostridium perfringes* with unique simultaneous fungal suppuration by *Myceliophthora thermophila*: case report. Neurosurgery 39(2):1247-1251, 1996.

APPENDICES

1. Scientific Expert Panel Comments

APPENDIX 1—SCIENTIFIC EXPERT PANEL COMMENTS

The Generally Recognized As Safe (GRAS) Status of a Cellulase Enzyme Preparation Derived from a Genetically-Modified strain of *Myceliophthora thermophila*

Opinion of an Expert Panel

Dyadic International (USA), Inc. ("Dyadic"), convened a panel of independent, internationally recognized scientific experts ("the Expert Panel") who are qualified by their scientific expertise and experience to evaluate the safety of food enzyme ingredients derived from microorganisms. Dyadic provided the Expert Panel members with relevant documents and materials related to the safety of a cellulase enzyme preparation derived from a genetically-modified strain of *Myceliophthora thermophila* (designated as strain Eg5#27), and asked them to independently and collectively critically evaluate Dyadic's conclusion that the enzyme preparation is GRAS for use in the manufacture of wine, beer and fruit juices as defined in 21 C.F.R. 170.3 (o)(9) with no limitations other than those that are consistent with current Good Manufacturing Practice (cGMP).

The Expert Panel's evaluation included consideration of the biology of *Mycelipohthora thermophila*, information available in the peer-reviewed scientific literature including consideration of the questions specified in the Pariza-Johnson Safety Decision Tree, information provided by Dyadic regarding the cloning methodology that was utilized to produce *M. thermophila* Eg5#27, and data supporting the lack of pathogenicity (i.e. infectious or toxigenic potential) of this enzyme production organism in mice.

Dyadic provided data and information to the Expert Panel from *in vivo* and *in vitro* safety evaluations that were conducted on the cellulase enzyme product. The studies included two subchronic feeding trials (14 day and 13 week) in rats, and three *in vitro* genotoxicity studies (Ames bacterial mutagenesis test, Chromosome Abberration test, and a Gene Mutation test at the TK-locus of L5178Y cells). There was no indication of any toxicity or adverse effects in the the results from any of these *in vivo* or *in vitro* safety evaluation studies. Dyadic also provided the Expert Panel with information relating the manufacturing process that is used to produce the cellulose enzyme indicating that the enzyme is being manufactured according to cGMP.

Based in its independent and collective critical evaluation of the data and information available in the scientific literature and provided by Dyadic, the members of the Expert Panel concur in Dyadic's conclusion that the cellulase enzyme preparation derived from a *M. thermophila* Eg5#27 is GRAS for use in the manufacture of wine, beer and fruit juices as defined in 21 C.F.R. 170.3 (o)(9) with no limitations other than those that are consistent with current Good Manufacturing Practice (cGMP).

(b)(6) 16 May 2007

Michael W. Pariza, Ph.D.
Wisconsin Distinguished Professor of Food Microbiology
and Toxicology GRAS Panel Chair

(b)(6) May 28, 2007

Ian C Munro, Ph.D., F.A.T.S., FRCPath
Executive Vice President
Senior Scientific Consultant

(b)(6)

Eric A. Johnson, Sc. D.
Professor of Bacteriology
University of Wisconsin - Madison

Ramos-Valle, Moraima

From: Ramos-Valle, Moraima
Sent: Thursday, May 14, 2009 12:01 PM
To: 'Richard Jundzil'
Cc: Ramos-Valle, Moraima; Martin, Robert L
Subject: RE: test email

Dear Mr. Jundzil,

This email is to follow up our earlier conversation. As I mentioned we have received your GRAS submission for the use of Cellulase enzyme preparation derived from genetically modified *Myceliophora thermophila*, but we can not file it as a GRAS notice because we could not locate the GRAS exemption claim.

Below are some helpful links that provide information on what to include in a GRAS exemption claim.

Also as I mentioned since this organism is new in uses for food applications and it is genetically modified, please provide as much information as possible about the microorganism.

The link to the GRAS Notification Program website: http://www.cfsan.fda.gov/~dms/opa-noti.html
The link to "How to submit a GRAS notice": http://www.cfsan.fda.gov/~dms/opa-frgr.html

This next link has been recently added to our website and here you can access a list of previous GRAS notices and you can see the entire document, so it is helpful for notifiers because they can use them as a guide to prepare their packages: http://www.cfsan.fda.gov/~prise/prisesum.html

You can go through the list of GRN's but I suggest you look at the most recent ones maybe GRN 240 and higher.

Please let me know if you have any questions before submitting the GRAS exemption claim that will complete your submission.

Sincerely,
Moraima

Moraima J. Ramos Valle
Consumer Safety Officer
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Phone: 301-436-1248

5/14/2009

Email: Moraima.Ramos-Valle@fda.hhs.gov

From: Richard Jundzil [mailto:rjundzil@dyadic.com]
Sent: Thursday, May 14, 2009 11:28 AM
To: Ramos-Valle, Moraima
Subject: test email

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

DYADIC®

DYADIC INTERNATIONAL (USA), INC.
140 INTRACOASTAL POINTE DRIVE, SUITE 404
JUPITER, FLORIDA 33477
(561) 743-8333 tel.
(561) 743-8343 fax
http://www.dyadic.com

May 15, 2009

BY ELECTRONIC MAIL

Moraima J. Ramos Valle
Consumer Safety Officer
Food and Drug Administration
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Dyadic International (USA), Inc. (the "Notifier"); Submission of GRAS Notice for a Cellulase Enzyme Preparation Derived From a Genetically Modified *Myceliophthora thermophila* (the "Notice"); GRAS Exemption Claim

Dear Ms.Valle:

Responsive to your request, outlined below is the GRAS Exemption Claim pertaining to the above-referenced Notice for a cellulase enzyme preparation derived from a genetically modified *Myceliophthora thermophila* (the "Notified Substance") in accordance with 21C.F.R. §170.36(c)(l). The Notifier respectfully submits that the Notified Substance is exempt from premarket approval requirements as set forth under the Federal Food, Drug and Cosmetic Act because the Notifier has determined through scientific procedures that the use of the Notified Substance in beer, wine and fruit juice is GRAS.

GRAS Exemption Claim

In accordance with proposed §170.36(c)(l)(i), the name and address of the Notifier is as follows: Dyadic International (USA), Inc., 140 Intracoastal Pointe Drive, Suite 404, Jupiter, FL 33477.

In accordance with proposed §170.36(c)(l)(ii), the common or usual name of notified substance is a cellulase enzyme preparation derived from a genetically modified *Myceliophthora thermophila.*

In accordance with proposed §170.36(c)(l)(iii) the Notified Substance is used in the processing of beer, wine and fruit juice at minimum levels necessary to achieve the desired effect and according to cGMP. The optimum dosage levels of the Notified Substance will vary with different substrates and operating conditions (including pH, temperature, substrate concentration, and reaction time). The recommended dosage range for the Notified Substance for use in beer, wine and fruit juice production is 0.004-0.02% (w/w). Many food processes that use cellulase include a step that removes and/or inactivates most, if not all, of the added enzyme preparation. Nevertheless, the Notice contains detailed calculations based on reported human consumption levels of beer, wine and fruit juice assuming no enzyme removal or inactivation occurs.

In accordance with proposed §170.36(c)(l)(iv), the Notifier has determined that the Notified Substance is GRAS based on scientific procedures.

In accordance with proposed §170.36(c)(l)(v), the supporting data and information that were reviewed by the Notifier's GRAS Expert Panel and that are the basis for the Notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and will be sent to FDA upon request.

Respectfully submitted,

(b)(6)

Mark A. Emalfarb
Chief Executive Officer

DYADIC INTERNATIONAL (USA) INC. CORPORATE SEAL FLORIDA

Ramos-Valle, Moraima

From: Richard Jundzil [rjundzil@dyadic.com]
Sent: Friday, May 15, 2009 3:15 PM
To: Ramos-Valle, Moraima
Cc: Martin, Robert L
Subject: RE: GRAS exemption claim for Dyadic
Attachments: GRAS exemption claim.pdf

Dear Mrs. Moraima,

Please find attached the requested GRAS exemption claim.
Please do not hesitate to contact me if your require anything further.

I will be traveling next week, but should have email access.

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: Thursday, May 14, 2009 12:01 PM
To: Richard Jundzil
Cc: Ramos-Valle, Moraima; Martin, Robert L
Subject: RE: test email

Dear Mr. Jundzil,

This email is to follow up our earlier conversation. As I mentioned we have received your GRAS submission for the use of Cellulase enzyme preparation derived from genetically modified *Myceliophora thermophila*, but we can not file it as a GRAS notice because we could not locate the GRAS exemption claim.

Below are some helpful links that provide information on what to include in a GRAS exemption claim.

Also as I mentioned since this organism is new in uses for food applications and it is genetically modified, please provide as much information as possible about the microorganism.

The link to the GRAS Notification Program website: http://www.cfsan.fda.gov/~dms/opa-noti.html
The link to "How to submit a GRAS notice": http://www.cfsan.fda.gov/~dms/opa-frgr.html

This next link has been recently added to our website and here you can access a list of previous GRAS notices and you can see the entire document, so it is helpful for notifiers because they can use them as a guide to prepare their packages: http://www.cfsan.fda.gov/~prise/prisesum.html

You can go through the list of GRN's but I suggest you look at the most recent ones maybe GRN 240 and higher.

Please let me know if you have any questions before submitting the GRAS exemption claim that will complete your submission.

Sincerely,
Moraima

Moraima J. Ramos Valle
Consumer Safety Officer
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Phone: 301-436-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

From: Richard Jundzil [mailto:rjundzil@dyadic.com]
Sent: Thursday, May 14, 2009 11:28 AM
To: Ramos-Valle, Moraima
Subject: test email

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

5/18/2009

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

Ramos-Valle, Moraima

From: Ramos-Valle, Moraima
Sent: Monday, May 18, 2009 1:12 PM
To: 'Richard Jundzil'
Subject: RE: GRAS exemption claim for Dyadic

Dear Mr. Jundzil,

I appreciate your fast response. Just two minor things: first I will appreciate if in the first paragraph instead of saying "the Notifier" you replace it with the name of the company in this case "Dyadic International Inc." and second we will need 3 hard copies of the GRAS exemption claim (at least one of the three should be an original signature and company seal). This is important as this will become part the official record together with the original submission received May 4th, 2009.

Thank you so much and please feel free to contact me if you have any questions.

Moraima J. Ramos Valle
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Phone: 301-436-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

From: Richard Jundzil [mailto:rjundzil@dyadic.com]
Sent: Friday, May 15, 2009 3:15 PM
To: Ramos-Valle, Moraima
Cc: Martin, Robert L
Subject: RE: GRAS exemption claim for Dyadic

Dear Mrs. Moraima,

Please find attached the requested GRAS exemption claim.
Please do not hesitate to contact me if your require anything further.

I will be traveling next week, but should have email access.

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

5/18/2009

Ramos-Valle, Moraima

From: Richard Jundzil [rjundzil@dyadic.com]
Sent: Wednesday, May 27, 2009 1:20 PM
To: Ramos-Valle, Moraima
Subject: RE: GRAS exemption claim for Dyadic
Attachments: GRAS Exemption Claim 05.27.09.pdf

Dear Mrs. Moraima,

Please find attached a scan of the revised GRAS exemption claim.
Three originals with Dyadic's company seal will be sent via Fed Ex to your attention this evening.

Please accept my apologies for the delay, I was traveling in Europe last week.

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: Monday, May 18, 2009 1:12 PM
To: Richard Jundzil
Subject: RE: GRAS exemption claim for Dyadic

Dear Mr. Jundzil,

I appreciate your fast response. Just two minor things: first I will appreciate if in the first paragraph instead of saying "the Notifier" you replace it with the name of the company in this case "Dyadic International Inc." and second we will need 3 hard copies of the GRAS exemption claim (at least one of the three should be an original signature and company seal). This is important as this will become part the official record together with the original submission received May 4th, 2009.

Thank you so much and please feel free to contact me if you have any questions.

Moraima J. Ramos Valle
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

Phone: 301-436-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

From: Richard Jundzil [mailto:rjundzil@dyadic.com]
Sent: Friday, May 15, 2009 3:15 PM
To: Ramos-Valle, Moraima
Cc: Martin, Robert L
Subject: RE: GRAS exemption claim for Dyadic

Dear Mrs. Moraima,

Please find attached the requested GRAS exemption claim.
Please do not hesitate to contact me if your require anything further.

I will be traveling next week, but should have email access.

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: Thursday, May 14, 2009 12:01 PM
To: Richard Jundzil
Cc: Ramos-Valle, Moraima; Martin, Robert L
Subject: RE: test email

Dear Mr. Jundzil,

This email is to follow up our earlier conversation. As I mentioned we have received your GRAS submission for the use of Cellulase enzyme preparation derived from genetically modified *Myceliophora thermophila*, but we can not file it as a GRAS notice because we could not locate the GRAS exemption claim.

Below are some helpful links that provide information on what to include in a GRAS exemption claim.

Also as I mentioned since this organism is new in uses for food applications and it is genetically modified, please provide as much information as possible about the microorganism.

The link to the GRAS Notification Program website: http://www.cfsan.fda.gov/~dms/opa-noti.html
The link to "How to submit a GRAS notice": http://www.cfsan.fda.gov/~dms/opa-frgr.html

This next link has been recently added to our website and here you can access a list of previous GRAS notices and you can see the entire document, so it is helpful for notifiers because they can use them as a guide to prepare their packages: http://www.cfsan.fda.gov/~prise/prisesum.html

You can go through the list of GRN's but I suggest you look at the most recent ones maybe GRN 240 and higher.

Please let me know if you have any questions before submitting the GRAS exemption claim that will complete your submission.

Sincerely,
Moraima

Moraima J. Ramos Valle
Consumer Safety Officer
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Phone: 301-436-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

From: Richard Jundzil [mailto:rjundzil@dyadic.com]
Sent: Thursday, May 14, 2009 11:28 AM
To: Ramos-Valle, Moraima
Subject: test email

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

DYADIC®
DYADIC INTERNATIONAL, INC.
140 INTRACOASTAL POINTE DRIVE, SUITE 404
JUPITER, FLORIDA 33477-5094 USA
561.743.8333 TEL
561.743.8343 FAX
http://www.dyadic-group.com

Transmittal

TO: Moraima J. Ramos Valle, Consumer Safety Officer

COMPANY: Food and Drug Administration

FROM: Richard Jundzil / Mark Emalfarb

RE: GRAS Exemption Claim

DATE: May 27, 2009

Dear Ms. Valle:

Please see enclosed three (3) original signed copies of the GRAS Exemption Claim for Dyadic. Again, please accept my apologies for the delay in getting these to you as I was traveling abroad last week.

Please feel free to contact me with any questions.

Thanks and best regards,

(b)(6)

Richard H. Jundzil
Director of Development & Quality
rjundzil@dyadic.com

THE INFORMATION ON THIS PAGE AND ANY ENCLOSURES IS CONFIDENTIAL.
IF YOU HAVE RECEIVED THESE DOCUMENTS IN ERROR, PLEASE CALL (561) 743-8333.

DYADIC®

DYADIC INTERNATIONAL (USA), INC.
140 INTRACOASTAL POINTE DRIVE, SUITE 404
JUPITER, FLORIDA 33477
(561) 743-8333 tel.
(561) 743-8343 fax
http://www.dyadic.com

RECEIVED
MAY 28 2009
BY: MPV

May 27, 2009

BY FEDERAL EXPRESS

Moraima J. Ramos Valle
Consumer Safety Officer
Food and Drug Administration
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Dyadic International (USA), Inc. (the "Notifier"); Submission of GRAS Notice for a Cellulase Enzyme Preparation Derived From a Genetically Modified *Myceliophthora thermophila* (the "Notice"); GRAS Exemption Claim

Dear Ms.Valle:

Responsive to your request, outlined below is the GRAS Exemption Claim pertaining to the above-referenced Notice for a cellulase enzyme preparation derived from a genetically modified *Myceliophthora thermophila* (the "Notified Substance") in accordance with 21C.F.R. §170.36(c)(l). Dyadic International (USA), Inc. respectfully submits that the Notified Substance is exempt from premarket approval requirements as set forth under the Federal Food, Drug and Cosmetic Act because Dyadic International (USA), Inc. has determined through scientific procedures that the use of the Notified Substance in beer, wine and fruit juice is GRAS.

GRAS Exemption Claim

In accordance with proposed §170.36(c)(l)(i), the name and address of the Notifier is as follows: Dyadic International (USA), Inc., 140 Intracoastal Pointe Drive, Suite 404, Jupiter, FL 33477.

In accordance with proposed §170.36(c)(l)(ii), the common or usual name of notified substance is a cellulase enzyme preparation derived from a genetically modified *Myceliophthora thermophila.*

In accordance with proposed §170.36(c)(l)(iii) the Notified Substance is used in the processing of beer, wine and fruit juice at minimum levels necessary to achieve the desired effect and according to cGMP. The optimum dosage levels of the Notified Substance will vary with different substrates and operating conditions (including pH, temperature, substrate concentration, and reaction time). The recommended dosage range for the Notified Substance for use in beer, wine and fruit juice production is 0.004-0.02% (w/w). Many food processes that use cellulase include a step that removes and/or inactivates most, if not all, of the added enzyme preparation. Nevertheless, the Notice contains detailed calculations based on reported human consumption levels of beer, wine and fruit juice assuming no enzyme removal or inactivation occurs.

In accordance with proposed §170.36(c)(l)(iv), the Notifier has determined that the Notified Substance is GRAS based on scientific procedures.

In accordance with proposed §170.36(c)(l)(v), the supporting data and information that were reviewed by the Notifier's GRAS Expert Panel and that are the basis for the Notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and will be sent to FDA upon request.

Respectfully submitted,

(b)(6)

Mark A. Emalfarb
Chief Executive Officer

DYADIC INTERNATIONAL (USA) INC.
CORPORATE
SEAL
FLORIDA

Ramos-Valle, Moraima

From: Richard Jundzil [rjundzil@dyadic.com]
Sent: Thursday, May 28, 2009 11:38 AM
To: Ramos-Valle, Moraima
Subject: FW: FedEx Shipment 796640971312 Delivered

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

From: TrackingUpdates@fedex.com [mailto:TrackingUpdates@fedex.com]
Sent: Thursday, May 28, 2009 10:21 AM
To: Richard Jundzil
Subject: FedEx Shipment 796640971312 Delivered

This tracking update has been requested by:

Company Name:	DYADIC INTERNATIONAL, INC
Name:	Julie Hyatt
E-mail:	rjundzil@dyadic.com

Our records indicate that the following shipment has been delivered:

Reference:	GRAS Notice
Ship (P/U) date:	May 27, 2009
Delivery date:	May 28, 2009 10:13 AM
Sign for by:	.JOHNSON
Delivered to:	Shipping/Receiving
Service type:	FedEx Standard Overnight
Packaging type:	FedEx Envelope
Number of pieces:	1
Weight:	0.50 lb.
Special handling/Services:	Deliver Weekday

Tracking number: 796640971312

Shipper Information	Recipient Information
Julie Hyatt	Moraima J. Ramos Valle, CSO
DYADIC INTERNATIONAL, INC	FOOD AND DRUG ADMINISTRATION
140 INTRACOASTAL POINTE DR; #404	5100 PAINT BRANCH PKWY; CENTER FOR FOOD SAFETY&APPLIED NUTR
JUPITER	COLLEGE PARK
FL	

US	MD
33477	US
	20740

Please do not respond to this message. This email was sent from an unattended mailbox. This report was generated at approximately 9:20 AM CDT on 05/28/2009.

Learn more about new ways to track with FedEx.

All weights are estimated.

To track the latest status of your shipment, click on the tracking number above, or visit us at fedex.com.

This tracking update has been sent to you by FedEx on the behalf of the Requestor noted above. FedEx does not validate the authenticity of the requestor and does not validate, guarantee or warrant the authenticity of the request, the requestor's message, or the accuracy of this tracking update. For tracking results and fedex.com's terms of use, go to fedex.com.

Thank you for your business.

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

Ramos-Valle, Moraima

From: Richard Jundzil [rjundzil@dyadic.com]
Sent: Thursday, May 28, 2009 4:17 PM
To: Ramos-Valle, Moraima
Cc: Martin, Robert L
Subject: RE: FedEx Shipment 796640971312 Delivered

Yes, that is a standard transmittal sheet provided should the package not be properly delivered to its intended recipient.

It is not confidential for the purposes sent to you. Please discard the transmittal page.

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: Thursday, May 28, 2009 4:09 PM
To: Richard Jundzil
Cc: Martin, Robert L
Subject: RE: FedEx Shipment 796640971312 Delivered

Dear Mr. Jundzil,

I received Fed Ex envelop that contained the GRAS exemption claim for the cellulose enzyme preparation derived from a genetically modified *Myceliophthora thermophila*. On the transmittal page there is a footnote that states: "the information on this page and any enclosures is confidential. If you have received these documents in error, please call (561) 743-8333."

I understand that this might be a "standard" transmittal page but I anticipate this will be an issue and based on that I will not be able to file it. Can you please send me a clarification note saying that the information is not intended to be confidential and I will add to the file?

Thanks,

Moraima

From: Richard Jundzil [mailto:rjundzil@dyadic.com]
Sent: Thursday, May 28, 2009 11:38 AM
To: Ramos-Valle, Moraima
Subject: FW: FedEx Shipment 796640971312 Delivered

Best Regards,

Richard H. Jundzil
Director of Development & Quality
Dyadic International (USA), Inc.
140 Intracoastal Pointe Drive, Suite 404
Jupiter Florida 33477-5094
(561) 743-8333 x35

From: TrackingUpdates@fedex.com [mailto:TrackingUpdates@fedex.com]
Sent: Thursday, May 28, 2009 10:21 AM
To: Richard Jundzil
Subject: FedEx Shipment 796640971312 Delivered

This tracking update has been requested by:

Company Name:	DYADIC INTERNATIONAL, INC
Name:	Julie Hyatt
E-mail:	rjundzil@dyadic.com

Our records indicate that the following shipment has been delivered:

Reference:	GRAS Notice
Ship (P/U) date:	May 27, 2009
Delivery date:	May 28, 2009 10:13 AM
Sign for by:	.JOHNSON
Delivered to:	Shipping/Receiving
Service type:	FedEx Standard Overnight
Packaging type:	FedEx Envelope
Number of pieces:	1
Weight:	0.50 lb.
Special handling/Services:	Deliver Weekday
Tracking number:	796640971312

Shipper Information	Recipient Information
Julie Hyatt	Moraima J. Ramos Valle, CSO
DYADIC INTERNATIONAL, INC	FOOD AND DRUG ADMINISTRATION
140 INTRACOASTAL POINTE DR; #404	5100 PAINT BRANCH PKWY; CENTER FOR FOOD SAFETY&APPLIED NUTR
JUPITER	COLLEGE PARK
FL	MD
US	US
33477	20740

Please do not respond to this message. This email was sent from an unattended mailbox. This report was generated at approximately 9:20 AM CDT on 05/28/2009.
Learn more about new ways to track with FedEx.

All weights are estimated.

To track the latest status of your shipment, click on the tracking number above, or visit us at fedex.com.
This tracking update has been sent to you by FedEx on the behalf of the Requestor noted above. FedEx does not validate the authenticity of the requestor and does not validate, guarantee or warrant the authenticity of the request, the requestor's message, or the accuracy of this tracking update. For tracking results and fedex.com's terms of use, go to fedex.com.

Thank you for your business.

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please notify the sender and delete the material from your computer.

Disclaimer added by **CodeTwo Exchange Rules**
www.codetwo.com

SUBMISSION END