September 20, 2021

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
CPK-2 Building, Room 2092
5001 Campus Drive, HFS-225
College Park, MD 20740

Dear GRAS Filing Team:

Enclosed please find a CD containing "GRAS Notification for Lactase Enzyme Derived from *Aspergillus Oryzae*", Form 3667, and all corresponding references. The data and information that serve as the basis for this GRAS notification is available for review and copying at reasonable times at the office of Claire Kruger, PhD, DABT, Managing Partner, Spherix Consulting Group, Inc., 751 Rockville Pike, Unit 30-B, Rockville, MD 20852, Telephone: 301-775-9476; Email: ckruger@spherixgroup.com, or will be sent to FDA upon request.

It is our opinion that the enclosed GRAS determination constitutes a new GRAS Notice because the subject is a novel food ingredient. This Notice also addresses the concerns raised by the FDA during the prefiling review of the Notice as described in the decline to file opinion issued in December 2020.

We thank you for taking the time to review this GRAS notification. Should you have additional questions, please let us know.

Sincerely,

[redacted]

Claire L. Kruger, PhD, DABT, CFS
Managing Partner

Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B, Rockville, MD 20852
301-557-0375; info@spherixgroup.com

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION FOR LACTASE ENZYME DERIVED FROM *ASPERGILLUS ORYZAE*

Prepared for:

Godo Shusei Co., Ltd.
Enzymes & Pharmaceuticals Division
250, Aza-Nakahara, Kamihongo, Matsudo, Chiba 271-0064 Japan

Prepared by:

Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

August 31, 2021

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I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

Godo Shusei Co., Ltd. is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

Godo Shusei Co., Ltd.
Enzymes & Pharmaceuticals Division
250, Aza-Nakahara, Kamihongo, Matsudo, Chiba 271-0064 Japan
Tel: +81-47-705-7795
Fax: +81-47-705-7798

C. COMMON OR USUAL NAME

Lactase, β-galactosidase (IUB Number: 3.2.1.23)

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secret or confidential information.

E. INTENDED USE

The lactase is intended to be used in the hydrolysis of lactose in milk and whey products.

F. BASIS FOR GRAS DETERMINATION

The lactase preparation derived from *Aspergillus oryzae* strain GD-FAL (GODO-FAL) for the intended use has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intended conditions of use of GODO-FAL has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of the substances directly added to food and is based on generally available and accepted information.

The intended use of GODO-FAL is as an enzyme in the processing of milk, milk powder, fermented milk products and yogurt, fresh cheese, milk-based desserts, whey, baked goods,

confectionary, cereal bars, soft drinks, and in the processing of milk for non-exempt infant formulas, and has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

- GODO-FAL is a lactase, a hydrolase that can transfer non-reducing β-D-galactose residues from β-D-galactosides, produced by *Aspergillus oryzae* strain GD-FAL. *A. oryzae* strain GD-FAL has not been subjected to genetic modifications.
- The amino acid sequence of GODO-FAL is 100% identical to the amino acid sequence of the enzyme that was the subject of GRN 510 (2015), acid lactase from *A. oryzae* expressed in *A. niger*.
- There is no evidence in the available information on GODO-FAL that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public if GODO-FAL is used at levels that might reasonably be expected from the proposed applications.
    - *A. oryzae* has a long history of safe use in the production of food ingredients and has been used to produce numerous food ingredients that are GRAS (GRN 8, 1999; GRN 10, 1999; GRN 34, 2000; GRN 43, 2000; GRN 75, 2001; GRN 90, 2002; GRN 103, 2002; GRN 106, 2002; GRN 113, 2003; GRN 122, 2003; GRN 142, 2004; and GRN 201, 2006).
    - The strain of *A. oryzae* used in the production of GODO-FAL, *A. oryzae* strain GD-FAL, lacks the genes necessary to produce aflatoxins.
    - Unlike some members of the genus *Aspergillus,* there is no record of *A. oryzae* producing mycotoxins, and three lots of the finished product, GODO-FAL, had non-detectable levels of the following mycotoxins: T-2 toxin, zearalenone, ochratoxin A, sterigmatocystin, and aflatoxins B1, B2, G1, and G2.
    - Three lots of GODO-FAL did not contain detectable levels of the secondary metabolites kojic acid, cyclopiazonic acid, or 3-nitropropionic acid.
- All steps in the GODO-FAL manufacturing process follow current good manufacturing practices (cGMP), using food grade processing aids and food contact materials.
    - GODO-FAL is produced using an industry-standard production process that is also used to produce the subjects of GRNs 743 (2018), 649 (2016), 579 (2015), 572 (2015), 510 (2014), and 132 (2003).

- Appropriate specifications and quality control parameters assure the production of a food grade product.

- Published toxicology studies demonstrate the safety of GODO-FAL:

  - Genotoxicology assays of GODO-FAL include a bacterial reverse mutation assay, an in vivo micronucleus assay, and a chromosome aberration assay. GODO-FAL was not genotoxic in these three assays.

  - The safety of GODO-FAL was assessed in toxicology studies including an acute toxicity study, a 28-day study in rats, and a 90-day subchronic toxicity study in rats. The results of the 28-day study and the 90-day subchronic toxicity study were published by Symonds et al. (2020). The LD_{50} of GODO-FAL was greater than 2000 mg/kg and there were no test article related adverse effects noted in the 28-day range-finding study at doses up to 2000 mg/kg/day. The subchronic toxicity study was performed in male and female rats administered 0 or 2000 mg/kg/day (total organic solids (TOS) 206 mg/kg/day). No test article related adverse effects were noted; the no observed adverse effect level (NOAEL) was determined to be at least 2000 mg/kg/day.

  - Because GODO-FAL is essentially equivalent to other lactases that are GRAS, the toxicology studies conducted using the other sources of lactase as the test article also support the safety of GODO-FAL. These studies established NOAELs of at least 4000 mg/kg/day, 1646 mg/kg/day, and 2000 mg/kg/day (Flood and Kondo 2004; Zou et al., 2014; Ke et al., 2018;), the highest doses tested.

  - Based on the fact that none of the safety studies showed signs of toxicity, the long history of use of lactase, and that GODO-FAL is essentially equivalent to other lactases that are GRAS, it can be concluded that the use of GODO-FAL for the intended purpose is safe.

- The intended use of GODO-FAL is as an enzyme in the processing of milk, milk powder, fermented milk products and yogurt, fresh cheese, milk-based desserts, whey, backed goods, confectionary, cereal bars, soft drinks, and in the processing of milk for non-exempt cow's milk-based infant formula. The enzyme will be used at the minimum level necessary to achieve the desired effect and according to requirements for normal production following cGMP.

- GODO-FAL will be used as a processing aid and will have no function in the finished food. The enzyme is either denatured or inactivated during production.

- Because GODO-FAL is intended to be used as a substitute for other lactases that are GRAS, the intended use and estimated intake of GODO-FAL will be the same as described for the lactase that is the subject of GRN 825 (which received a "no questions" letter from the FDA in 2018 regarding its GRAS status). As stated in GRN 825, the estimated daily intake of lactase for users 2 years of age and older is 3.7 mg TOS/kg body weight/day. For infant formula applications, the estimated daily intake is 9.6 mg TOS/kg body weight/day, assuming a maximum amount of lactase as 36 mg TOS/kg milk raw material and the maximum consumption of 267 g infant formula/kg body weight/day.

Therefore, GODO-FAL is safe and GRAS for the proposed use as an enzyme in the processing of milk, milk powder, fermented milk products and yogurt, fresh cheese, milk-based desserts, whey, baked goods, confectionary, cereal bars, soft drinks, and in the processing of milk for non-exempt infant formulas, and is, therefore, excluded from the definition of a food additive and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR. Godo Shusei Co., Ltd., therefore, concludes that GODO-FAL is GRAS for its intended uses and use levels.

Determination of the GRAS status of GODO-FAL, an *A. oryzae*-derived lactase preparation, under the intended conditions of use has been made through the deliberations of Roger Clemens, Dr PH, CNS, FACN, FIFT, A. Wallace Hayes, Ph.D. DABT, CNS, FACN, and Thomas Sox, Ph.D. J.D. These individuals are qualified by scientific training and experience to evaluate the safety of ingredients added to food. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety to humans from its intended use as a processing aid, and have concluded that it is GRAS.

G. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, Managing Partner, Spherix Consulting Group, Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852. Telephone: 301-775-9476; Email: ckruger@spherixgroup.com, or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative, and balanced. It contains both favorable and unfavorable information, known to Godo Shusei Co. Ltd., and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

[redacted]
Signature of Authorized Representative of
Godo Shusei Co., Ltd.

September 8, 2021
Date

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

Lactase, β-galactosidase (IUB Number: 3.2.1.23)

B. TRADE NAME

GODO-FAL

C. DESCRIPTION OF GODO-FAL

GODO-FAL is a lactase-containing enzyme preparation, which is purified from a non-genetically modified strain of *Aspergillus oryzae*, *Aspergillus oryzae* strain GD-FAL and diluted in glycerin. Lactase hydrolyzes lactose to a mixture of glucose and galactose (Juers et al., 2012).

1. Amino Acid Sequence of GODO-FAL

Godo Shusei's lactase (GODO-FAL) has the following amino acid sequence, described in Figure 1, consisting of 1005 amino acids. GODO-FAL shares 100% amino acid sequence identity with the lactase that was the subject of GRN 510, a lactase from *A. oryzae* expressed in *A. niger*.

```
GRN510      MKLLSVAAVALLAAQAAGASIKHRLNGFTILEHPDPAKRDLLQDIVTWDDKSLFINGERI    60
GODO-FAL    MKLLSVAAVALLAAQAAGASIKHRLNGFTILEHPDPAKRDLLQDIVTWDDKSLFINGERI    60
            ************************************************************

GRN510      MLFSGEVHPFRLPVPSLWLDIFHKIRALGFNCVSFYIDWALLEGKPGDYRAEGIFALEPF    120
GODO-FAL    MLFSGEVHPFRLPVPSLWLDIFHKIRALGFNCVSFYIDWALLEGKPGDYRAEGIFALEPF    120
            ************************************************************

GRN510      FDAAKEAGIYLIARPGSYINAEVSGGGFPGWLQRVNGTLRSSDEPFLKATDNYIANAAAA    180
GODO-FAL    FDAAKEAGIYLIARPGSYINAEVSGGGFPGWLQRVNGTLRSSDEPFLKATDNYIANAAAA    180
            ************************************************************

GRN510      VAKAQITNGGPVILYQPENEYSGGCCGVKYPDADYMQYVMDQARKADIVVPFISNDASPS    240
GODO-FAL    VAKAQITNGGPVILYQPENEYSGGCCGVKYPDADYMQYVMDQARKADIVVPFISNDASPS    240
            ************************************************************

GRN510      GHNAPGSGTGAVDIYGHDSYPLGFDCANPSVWPEGKLPDNFRTLHLEQSPSTPYSLLEFQ    300
GODO-FAL    GHNAPGSGTGAVDIYGHDSYPLGFDCANPSVWPEGKLPDNFRTLHLEQSPSTPYSLLEFQ    300
            ************************************************************

GRN510      AGAFDPWGGPGFEKCYALVNHEFSRVFYRNDLSFGVSTFNLYMTFGGTNWGNLGHPGGYT    360
GODO-FAL    AGAFDPWGGPGFEKCYALVNHEFSRVFYRNDLSFGVSTFNLYMTFGGTNWGNLGHPGGYT    360
            ************************************************************

GRN510      SYDYGSPITETRNVTREKYSDIKLLANFVKASPSYLTATPRNLTTGVYTDTSDLAVTPLI    420
GODO-FAL    SYDYGSPITETRNVTREKYSDIKLLANFVKASPSYLTATPRNLTTGVYTDTSDLAVTPLI    420
            ************************************************************

GRN510      GDSPGSFFVVRHTDYSSQESTSYKLKLPTSAGNLTIPQLEGTLSLNGRDSKIHVVDYNVS    480
GODO-FAL    GDSPGSFFVVRHTDYSSQESTSYKLKLPTSAGNLTIPQLEGTLSLNGRDSKIHVVDYNVS    480
            ************************************************************

GRN510      GTNIIYSTAEVFTWKKFDGNKVLVLYGGPKEHHELAIASKSNVTIIEGSDSGIVSTRKGS    540
GODO-FAL    GTNIIYSTAEVFTWKKFDGNKVLVLYGGPKEHHELAIASKSNVTIIEGSDSGIVSTRKGS    540
            ************************************************************

GRN510      SVIIGWDVSSTRRIVQVGDLRVFLLDRNSAYNYWVPELPTEGTSPGFSTSKTTASSIIVK    600
GODO-FAL    SVIIGWDVSSTRRIVQVGDLRVFLLDRNSAYNYWVPELPTEGTSPGFSTSKTTASSIIVK    600
            ************************************************************

GRN510      AGYLLRGAHLDGADLHLTADFNATTPIEVIGAPTGAKNLFVNGEKASHTVDKNGIWSSEV    660
GODO-FAL    AGYLLRGAHLDGADLHLTADFNATTPIEVIGAPTGAKNLFVNGEKASHTVDKNGIWSSEV    660
            ************************************************************

GRN510      KYAAPEIKLPGLKDLDWKYLDTLPEIKSSYDDSAWVSADLPKTKNTHRPLDTPTSLYSSD    720
GODO-FAL    KYAAPEIKLPGLKDLDWKYLDTLPEIKSSYDDSAWVSADLPKTKNTHRPLDTPTSLYSSD    720
            ************************************************************

GRN510      YGFHTGYLIYRGHFVANGKESEFFIRTQGGSAFGSSVWLNETYLGSWTGADYAMDGNSTY    780
GODO-FAL    YGFHTGYLIYRGHFVANGKESEFFIRTQGGSAFGSSVWLNETYLGSWTGADYAMDGNSTY    780
            ************************************************************

GRN510      KLSQLESGKNYVITVVIDNLGLDENWTVGEETMKNPRGILSYKLSGQDASAITWKLTGNL    840
GODO-FAL    KLSQLESGKNYVITVVIDNLGLDENWTVGEETMKNPRGILSYKLSGQDASAITWKLTGNL    840
            ************************************************************

GRN510      GGEDYQDKVRGPLNEGGLYAERQGFHQPQPPSESWESGSPLEGLSKPGIGFYTAQFDLDL    900
GODO-FAL    GGEDYQDKVRGPLNEGGLYAERQGFHQPQPPSESWESGSPLEGLSKPGIGFYTAQFDLDL    900
            ************************************************************

GRN510      PKGWDVPLYFNPGNNTQAARAQLYVNGYQYGKFTGNVGPQTSFPVPEGILNYRGTNYVAL    960
GODO-FAL    PKGWDVPLYFNPGNNTQAARAQLYVNGYQYGKFTGNVGPQTSFPVPEGILNYRGTNYVAL    960
            ************************************************************

GRN510      SLWALESDGAKLGSFELSYTTPVLTGYGNVESPEQPKYEQRKGAY     1005
GODO-FAL    SLWALESDGAKLGSFELSYTTPVLTGYGNVESPEQPKYEQRKGAY     1005
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Figure 1. GODO-FAL Amino Acid Sequence and Its Alignment with the Amino Acid Sequence of the Lactase in GRN 510

2. Identification of the Production Organism

Accurate identification of *Aspergillus* sp. is essential to evaluating the safety of a production organism, as some members of the genus, such as *A. parasiticus* and *A. flavus*, can produce mycotoxins that are hazardous to human health, while others, like *A. oryzae* and the domesticated *A. flavus*, do not produce mycotoxins (Tominaga et al., 2006). Identification within the *Aspergillus* genus has historically relied on phenotypic characteristics, such as morphologies of colonies grown in agar and microscopic morphology of spore producing structures like conidiophores. Advances in genotypic analysis have further refined the identification and characterization of *Aspergillus* sp. through sequencing internal transcribed spacers (ITS) of noncoding DNA (Samson et al., 2014). Through a combination of phenotypic and genotypic techniques, the production strain used to generate GODO-FAL, *A. oryzae* strain GD-FAL, has been identified as a strain of *A. oryzae*.

a. Phenotypic Characterization of A. oryzae strain GD-FAL

Members of the *Aspergillus* genus are characterized by a distinct conidiophore structure consisting of a round vesicle producing many phialides, called an aspergillum. Accordingly, this characteristic also gave rise to the name of the genus. Further phylogenetic analysis of molds found that while not all *Aspergillus* sp. form aspergillum, the *Flavi* section, including *A. oryzae* and *A. flavus*, do form these structures (Samson et al., 2014).

The microscopic morphology described the following produced from one cell: a vegetative hypha with conidiophore at the tip, forming an aspergillum typical to the *Flavi* section of the *Aspergillus* genus. (Figure 2, top left). The conidiophore is surrounded by globose or subglobose phialides (Figure 2, top right) producing conidia (spores) (Figure 2, bottom). These morphological features are typical of the *Flavi* section of *Aspergillus*.

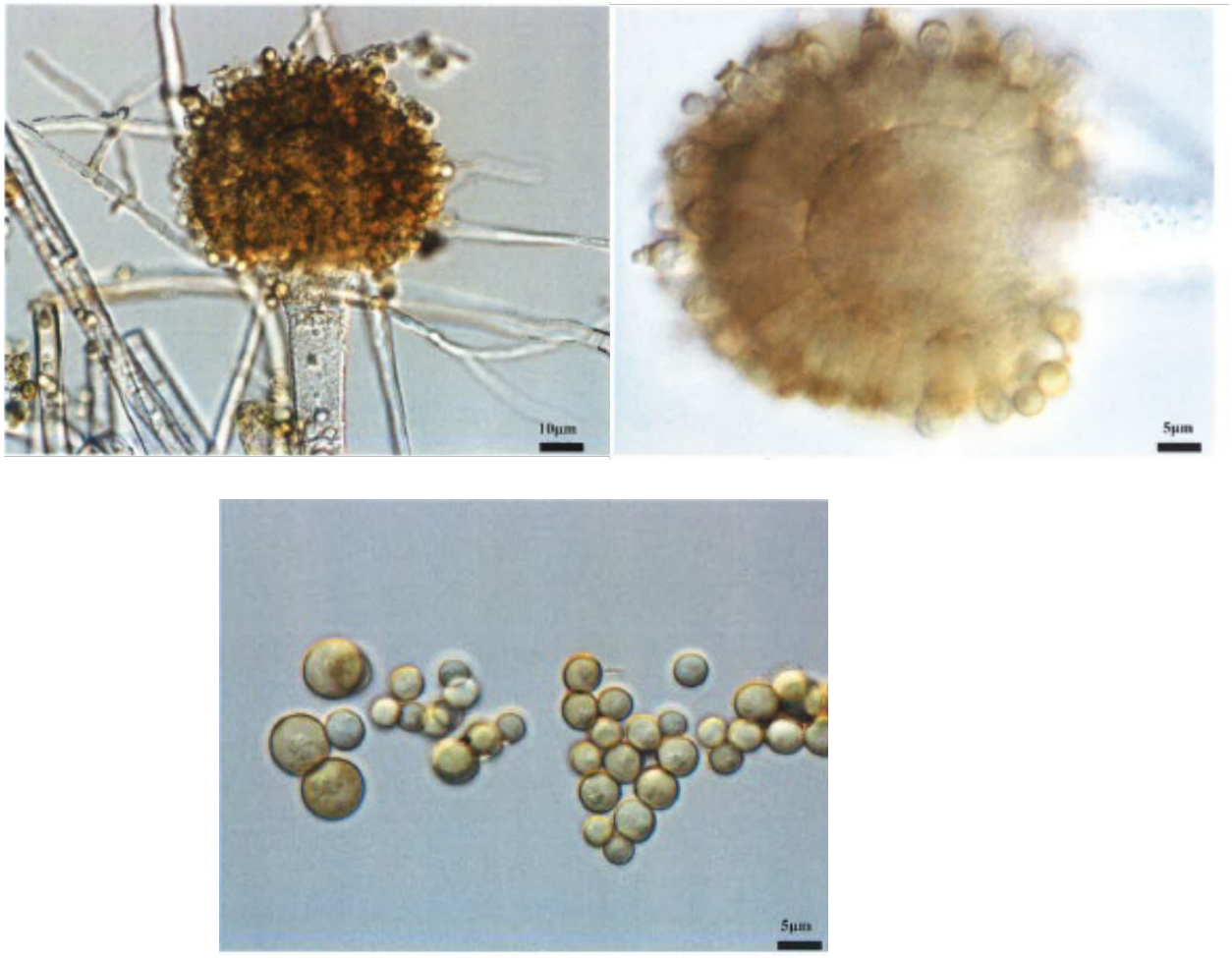


Figure 2. Microscopic Morphology of *A. oryzae* strain GD-FAL Used in the Production of GODO-FAL

Top left, vegetative hypha with conidiophore at the tip, scale bar is 10 μm. Top right, the conidiophore with phialides present, producing conidia (spores), scale bar is 5 μm. Bottom right, *A. oryzae* strain GD-FAL conidia, scale bar is 5 μm.

The colony morphology of *Aspergillus* on agar plates with different growth media CYA (Czapek Yeast Autolysate agar) and MEA (malt extract agar) plates can be used to distinguish between *Aspergillus* species. *A. oryzae* can be distinguished from *A. flavus* by its floccose (fluffy) colony texture and pale brown color and the absence of sclerotia after three weeks of culture. Unlike *A. flavus, A. oryzae* does not form sclerotia, a compact mass of hardened fungal mycelium (Frisvad et al., 2019).

To determine the morphological characteristics of *A. oryzae* strain GD-FAL, the strain was grown under different culture conditions for 7 days as indicated in the upper left corner of the images (Figure 3). Culturing the production organism on the indicated agar plates demonstrated floccose colonies yellow-green to ocher or yellow-brown in color, which is typical for *A. oryzae* (Figure 3). Culturing the production organism in CYA (Czapek Yeast Autolysate agar) medium at 25ºC produced floccose, radially-corrugate sulcus colonies greyish yellow to white in color, and 50-54 mm in diameter. When cultured in CYA medium at 5ºC, no colonies were produced, serving as a control. When cultured in CYA medium at 37ºC, *A. oryzae* GD-FAL produced velvety, radially sulcus colonies greyish yellow to white in color, and 47-52 mm in diameter. Growing the production organism in MEA (malt extract agar) medium produced velvety colonies yellow green to yellowish white in color and 40-44 mm in diameter. When grown in CY20S (Czapek yeast autolysate agar with 20% sucrose) medium, the production organism produced velvety, radially corrugate sulcus colonies olive yellow to white in color that were 65-70 mm in diameter. No sclerotia were observed in the colonies after three weeks of culture. These colony morphology characteristics are consistent with *A. oryzae* (Frisvad et al., 2019).

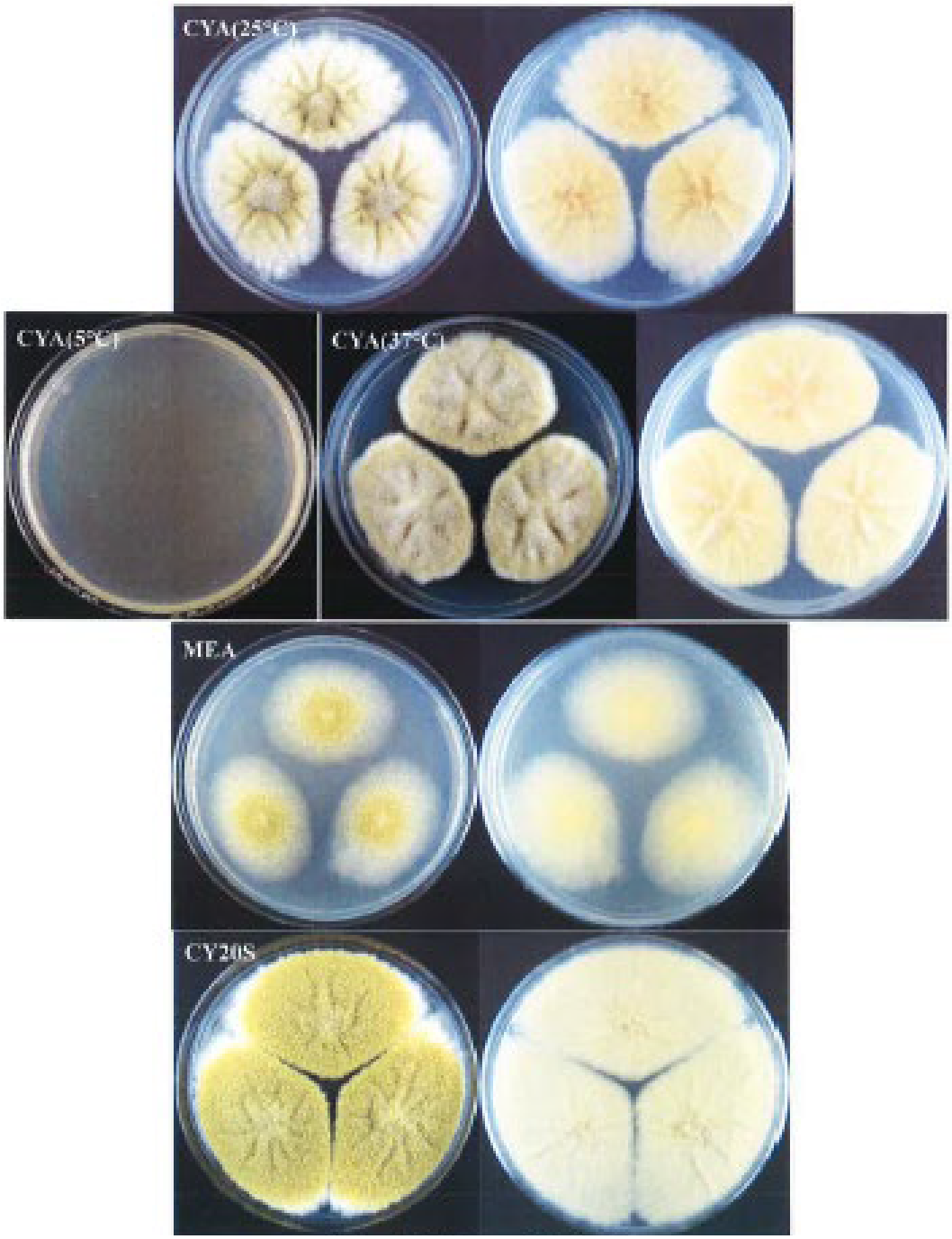


Figure 3. Colony Morphology of *A. oryzae* strain GD-FAL Used in the Production of GODO-FAL

A. oryzae strain GD-FAL was cultured under different culture conditions for 7 days, as indicated in the upper left corner of the images: CYA (25ºC): CYA (Czapek Yeast Autolysate agar) medium, 25ºC; CYA (5ºC): CYA medium cultured at 5ºC, negative control; CYA (37ºC): CYA medium cultured at 37ºC; MEA: MEA (malt extract agar) medium; CY20S: Czapek yeast autolysate agar with 20% sucrose. The colony morphology characteristics are consistent with *A. oryzae*, as discussed in the above text.

b. Genotypic Characterization of A. oryzae strain GD-FAL

To confirm the results of the phenotypic characterization, gene sequencing was performed to verify the production organism is *A. oryzae*. A BLAST (Basic Local Alignment Sequencing Tool) search was performed on the ITS sequence of *A. oryzae* strain GD-FAL to identify homologous sequences in the NCBI (National Center for Biotechnology Information) database. This search found that the ITS sequence of *A. oryzae* strain GD-FAL demonstrated 100% identity to other ITS sequences from *A. oryzae*.

c. Aflatoxin Biosynthetic Gene Homologous Cluster Analysis

i. Introduction

The *Aspergillus* genus consists of many highly related species, some of which can produce alfatoxins. The aflatoxin genes are located in the aflatoxin (AFL) gene cluster, which includes *aflT, nor1, aflR, norA, avnA, verB*, and *vbs*. The *aflR* and *aflT* genes are transcription factors thought to regulate the transcription of the synthesis genes *nor1, norA, avnA, verB*, and *vbs.* Strains of *A. oryzae* have previously been characterized into three groups determined by which genes in the AFL cluster can be detected by polymerase chain reaction (PCR). Although the AFL genes are present in varying degrees among the three groups, none are known to make aflatoxins (Tominaga et al., 2006).

Aspergillus oryzae group 1: AFL gene cluster is nearly intact, but many of the genes are mutated compared to aflatoxin producing *Aspergillus sp*., and there is no documented aflatoxin production. Group 1 strains have the following PCR amplification pattern: *aflT, nor-1, aflR, norA, avnA, verB,* and *vbs*.

Aspergillus oryzae group 2: The AFL gene cluster has many deletions. Importantly, Group 2 strains lack the *aflR* gene, the major transcriptional regulator for the AFL cluster genes. Group 2 strains have the following PCR amplification pattern: *avnA, verB*, and *vbs.*

Aspergillus oryzae group 3: The AFL gene cluster has more deletions than Group 2 strains. Group 3 strains have the following PCR amplification pattern: *verB* or *vbs*.

To ensure that the *A. oryzae* cultured by Godo Shusei Co. does not produce aflatoxins, the presence of genes associated with aflatoxin production in the AFL gene cluster was assessed by PCR amplification.

ii. Methods

A. oryzae strain GD-FAL cultured by Godo Shusei Co. Ltd. was cultivated for 1 week on potato dextrose agar at 27ºC. DNA was extracted and used as the template DNA for PCR amplification analysis of the following genes: *aflT, nor1, aflR, norA, avnA, verB*, *vbs*, and ITS5/ITS4 (loading control, internal transcribed spacer region 5/4). The same analysis was performed on *A. oryzae* RIB40, a group 1 *A. oryzae* strain known to amplify these genes in the AFL cluster. One PCR reaction was performed with ITS5/ITS4 using water as a template instead of DNA as a negative control. The products of these PCR reactions were then separated by agarose gel electrophoresis and imaged.

iii. Results

Only the *vbs* gene was amplified from *A. oryzae* strain GD-FAL cultured by Godo Shusei Co. Ltd. (Figure 4a), while all seven genes assessed were amplified in the *A. oryzae* RIB40 positive control (Figure 4b). The negative control lane shows that there were no contaminating sources of DNA in the reactions.

iv. Discussion

One gene from the AFL cluster, *vbs*, was amplified from *A. oryzae* strain GD-FAL, indicating that this strain belongs to *Aspergillus oryzae* Group 3 and lacks the genes necessary to produce aflatoxins.

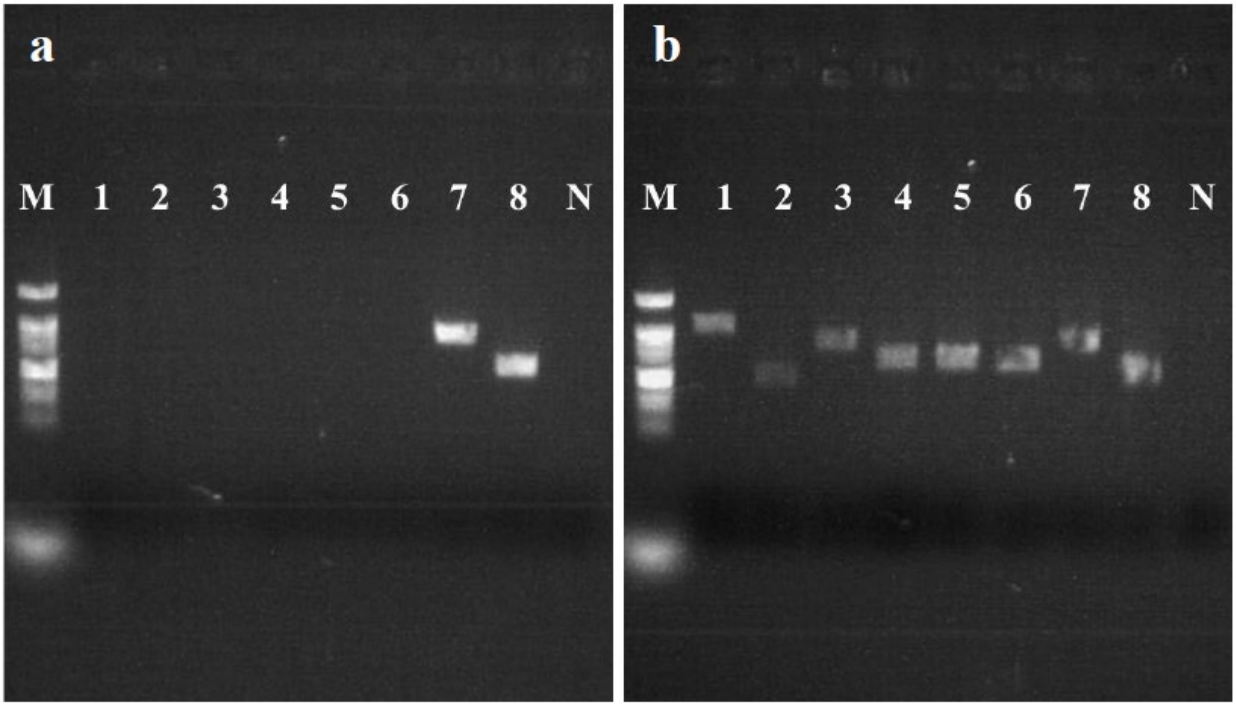


Figure 4. Gel Electrophoresis Results of PCR Amplified AFL Cluster Genes in *A. oryzae* strain GD-FAL Used in the Production of GODO-FAL

a.) *A. oryzae* strain GD-FAL used in the production of GODO-FAL, and b.) *A. oryzae* RIB40, a group 1 strain of *A. oryzae*. Lanes: M: 100 bp marker, 1: *aflT*, 2: *nor-1*, 3: *aflR*, 4: *norA*, 5: *avnA*, 6: *verB* 7: *vbs,* 8: ITS5/ITS4 (loading control), N: negative control, ITS5/ITS4 with water instead of template DNA.

D. PRODUCTION PROCESS

GODO-FAL is produced using an industry-standard production process which is also used to produce the subjects of GRNs 743 (2018), 649 (2016), 579 (2015), 572 (2015), 510 (2014), and 132 (2003). All production occurs at Godo Shusei Co. Ltd., which is FSSC 22000 Food Safety System Certification certified. The subject of this notice is therefore manufactured according to Good Manufacturing Practice (cGMP).

The original strain of *A. oryzae* strain GD-FAL was purchased from Kawauchi Genichiro Shoten in 2007 and is maintained in frozen stocks at -80ºC. Frozen stocks are used as needed.

The production process of GODO-FAL consists of fermentation of *A. oryzae* strain GD-FAL and a series of concentration and purification steps to yield a concentrated lactase in glycerin, GODO-FAL. Due to the multiple filtration steps including a final filtration step with a pore size ~10 times smaller than an *A. oryzae* strain GD-FAL conidium (spore), environmental

controls in the process, and product specifications to control the presence of fungi in the final product (see Table 2), no *A. oryzae* strain GD-FAL cells are expected to be present in the finished product.

All processing aids used in the concentration and purification steps are compliant with United States rules and regulations and are food grade.

1. Production of GODO-FAL

a. A. oryzae strain GD-FAL Fermentation

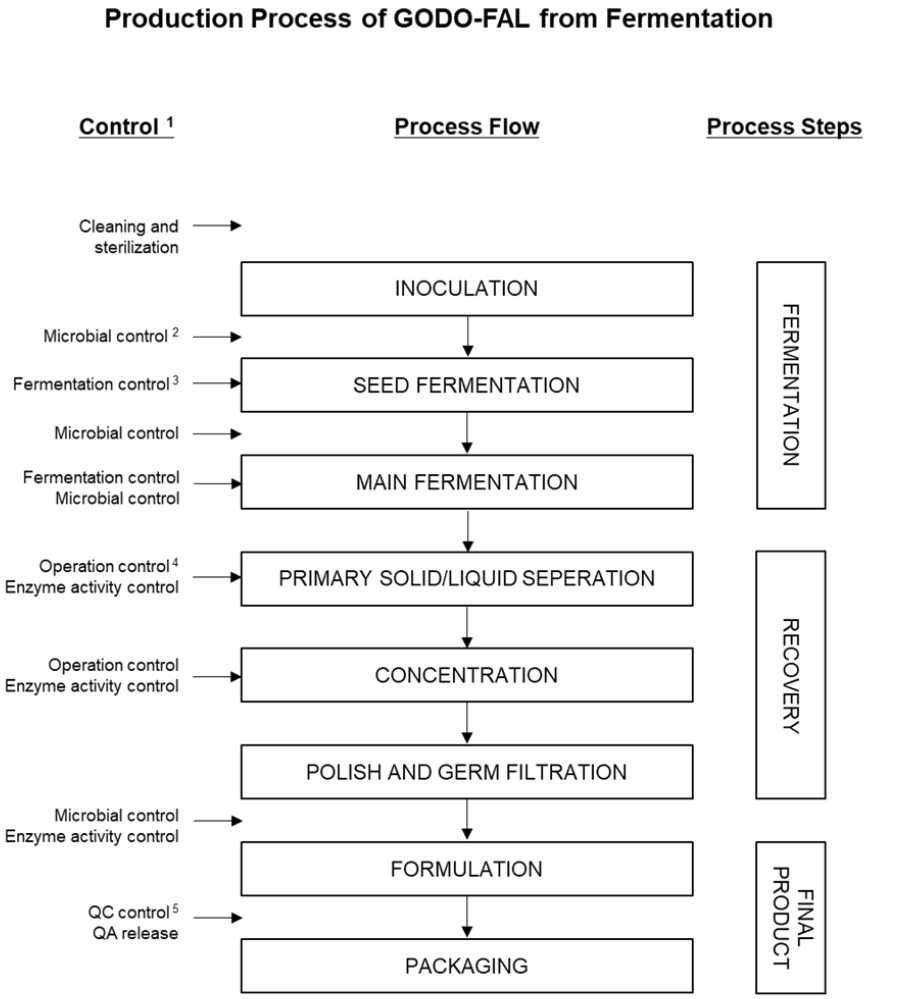
All culture medium is sterilized and cooled prior to inoculation. All culture steps take place under controlled aeration, pressure, temperature, pH, and stirring. The quality of the *A. oryzae* strain GD-FAL cells is assessed at internally specified timepoints during the fermentation process according to a strict set of quality control parameters to ensure healthy cultures. The absence of microbial contamination is assessed via light microscopy or by culturing samples of biomass and performing plate counts.

To begin the fermentation process, a frozen stock is thawed and cultured to establish a healthy *A. oryzae* strain GD-FAL culture. The thawed cells are then used to inoculate a flask containing culture medium and grown until it reaches internal quality control parameters. A portion of the biomass (*A. oryzae* strain GD-FAL cells in culture medium) is used to inoculate the seed fermentation vessel. The process of expanding the culture into larger seed fermentation vessels is repeated until the biomass is expanded to the main fermentation vessel. The fermentation process is complete when the biomass in the main fermentation vessel demonstrates sufficient lactase activity to begin the GODO-FAL recovery steps. The biomass is then cooled and enters the GODO-FAL concentration and purification process (Figure 5).

b. GODO-FAL Concentration and Purification

The cooled biomass from the main culture is separated into solid and liquid components. The solids are discarded, and the filtrate is then submitted to concentration steps including ultrafiltration and precipitation of the lactase. The precipitated lactase is submitted to polishing and microfiltration steps to remove residual solids and any potential microbial contaminants. The pH of the solution for this step is controlled. It is mixed with activated carbon and the solids are removed by filtration. The resulting filtrate is cooled before beginning an additional ultrafiltration step to remove excess salts remaining from the earlier precipitation. The critical control points for the second ultrafiltration step are assessing the turbidity (an indicator of microbial contamination), lactase activity, and conductivity to ensure sufficient polishing. The retentate from the second ultrafiltration step is then pH adjusted and clarified by filtration. The

critical control point for this step is that lactase activity must meet internal specifications. The filtrate is then submitted to the formulation step. It is cooled and glycerin is added until the resulting mixture meets the product specification for lactase activity. The glycerin-concentrated protein mix is filter sterilized. The resulting sterile glycerin-concentrated protein mix is the finished product, GODO-FAL. Product specifications are assessed in the final product before packaging and storage at < 10ºC (Figure 5).



[1] The controls shown on the flow chart may vary depending on the production set-up. Controls are conducted at various steps throughout production process as relevant.
[2] Microbial control: Absence of significant microbial contamination is analyzed by microscope or plate counts.
[3] During fermentation parameters like e.g. pH, temperature, Oxygen, CO_2, sterile air overflow are monitored / controlled.
[4] Operation control in downstream processes cover monitoring and control of parameters like e.g. pH, temperature.
[5] Final QC control will check that product does live up to specifications like e.g. enzyme activity as well as chemical and microbial specification.

Figure 5. GODO-FAL Production Process

2. Raw Materials, Processing Aids, and Food Contact Materials

The raw materials used in the production of GODO-FAL are the fermentation medium ingredients and drinking water. The water used is municipal water and complies with the quality standards of the Japanese Water Supply Act. Due to the extensive filtering steps used in the production process, fermentation medium ingredients are not present in the final product. The fermentation vessels and tubing are stainless steel. The filters used in the production process are stainless steel or magnesium-aluminum alloy. The ultrafilter membranes used in both ultrafiltration steps comply with 21 CFR, see Table 1.

Table 1. Compliance of Processing Aids and Packaging Materials with US Regulations

Role in Production	Processing Aid/Raw Material	Compliance
pH	85% Phosphoric acid	§182.1073
pH	Sodium hydroxide	§184.1763 FCC Monograph (FCC 11 3S)
Purification Process	Ammonium Sulfate	FCC Monograph (FCC 11 3S)
Purification Process	Radiolite #100 (diatomaceous earth)	FCC Monograph (FCC 11 3S)
Purification Process	Silica	FCC Monograph (FCC 11 3S)
Purification Process	Rokahelp 4109 (perlite)	FCC Monograph (FCC 11 3S)
Filter	Durapore (ultrafilter)	Complies with the following regulations/ monographs: 21 CFR §210.3(b)(6), §177 -182 USP <88>
Filter	UF (polyacrylonitrile)	§180.22
Purification	Activated Carbon	FCC Monograph (FCC 11)
Dilution	Glycerin	§182.1320
Final Packaging	High-density polyethylene	Complies with 21 CFR, see Appendix

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications

To ensure a food grade product, Godo Shusei Co. Ltd. tests each lot of GODO-FAL for compliance with a defined set of product specifications (Table 2). These parameters are assessed using validated methods and are fit for purpose. Data from three lots of GODO-FAL demonstrate control of the production process and compliance with the product specifications.

Table 2. Product Specifications and Compliance of Three Lots of GODO-FAL

Parameter	Methods	LOQ	Specifications	Lot No.		
				2801	2803	3001
Lactase Activity (U/g)	FCC IV method		NLT 10000	10800	12800	11000
Appearance	Visual Inspection	-	Light yellow to light green liquid	Light green liquid	Light green liquid	Light green liquid
Specific Gravity	Measurement using a pycnometer	-	1.1-1.2	1.14	1.15	1.14
pH	Glass electrode method	-	4.5-6.5	6.22	6.22	6.17
Heavy Metals (as Pb)	Sodium Sulfide colorimetric method[a]	5 ppm	NMT 10 ppm	N.D.	N.D.	N.D.
Lead	Atomic Absorption Spectrometry [b]	0.05 ppm	NMT 0.2 ppm	N.D.	N.D.	N.D.
Arsenic (as As_2O_3)	Atomic Absorption Spectrometry[b]	0.1 ppm	NMT 0.1 ppm	N.D.	N.D.	N.D.
Glycerol	HPLC	-	NLT 40%	43.6%	43.4%	42.8%
Microbials						
Total Plate Count	Standard Agar Plating Method[b]	1/g	NMT 100/g	Negative	Negative	Negative
Escherichia coli	LST Broth Enrichment Culture Method[c]	1/25 g	Negative/25g	Negative	Negative	Negative
Coliform bacteria	BGLB Broth Inoculating Method[b]	1/2.22 g	Negative/g	Negative	Negative	Negative
Pseudomonas aeruginosa	Enrichment Culture Method[b]	1/1.1 g	Negative/g	Negative	Negative	Negative
Salmonella	Enrichment Culture Method[d]	1/25 g	Negative/25g	Negative	Negative	Negative
Staphylococcus aureus	Surface Spread Plating Method[b]	1/0.01 g	Negative/0.01 g	Negative	Negative	Negative
Fungi	Potato Dextrose (10%) Agar Plating Method[b]	1/g	Negative/g	Negative	Negative	Negative
Yeast	Potato Dextrose (10%) Agar Plating Method[b]	1/g	Negative/g	Negative	Negative	Negative
Listeria monocytogenes	FDA Method[c]	1/25 g	Negative/25g	Negative	Negative	Negative
Bacillus cereus	Surface Spread Plating Method[b]	1/0.01 g	Negative/0.01g	Negative	Negative	Negative

N.D.: not detected, NLT: not less than, NMT: not more than
[a]Japan's Specifications and Standards for Food Additives
[b]Japan Food Hygiene Association: standard methods of analysis in food safety regulation
[c]FDA: bacteriological analytical manual
[d]NIHSJ method

2. Other Quality Attributes

To further characterize the quality of GODO-FAL, Godo Shusei Co., Ltd. analyzed three lots of GODO-FAL for secondary metabolites produced by *A. oryzae*, including aflatoxins and other mycotoxins, and quantified the total organic solids. Godo Shusei Co., Ltd. also analyzed three lots of GODO-FAL for additional toxins known to be in the food supply. None of the toxin screens reported any detectable levels of these metabolites or toxins.

a. Secondary Metabolites Screened in GODO-FAL

A. oryzae is known to have the potential to produce secondary metabolites of toxicological concern to humans such as kojic acid, cyclopiazonic acid, and 3-nitropropionic acid (Burdock et al., 2001a; Burdock et al., 2001b; Burdock and Flamm 2000). Godo Shusei Co. Ltd. has analyzed three lots of GODO-FAL and none had detectable levels of these secondary metabolites (Table 3).

Table 3. Absence of Secondary Metabolites Produced by *A. oryzae* in GODO-FAL

Secondary Metabolite	Method	LOQ	GODO FAL Lot Number		
			2801	2803	3001
Kojic acid	HPLC	5 ppm	N.D.	N.D.	N.D.
Cyclopiazonic acid	LCMS	0.05 ppm	N.D.	N.D.	N.D.
3-Nitropropionic acid	LCMS	1 ppm	N.D.	N.D.	N.D.

Abbreviations used:
HPLC: High performance liquid chromatography
LCMS: Liquid chromatography-mass spectrometry
N.D.: not detected
ppm: parts per million

b. Mycotoxins Screened in GODO-FAL

This screen was performed to verify the absence of mycotoxins, including aflatoxins, in GODO-FAL, generated by *A. oryzae*. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus. A. oryzae* is not known to produce aflatoxins, although one report suggests certain strains of *A. oryzae* have some, but not all, of the genes necessary for aflatoxin synthesis (Kim et al., 2014). GODO-FAL had no detectable amounts of the following mycotoxins: T-2 toxin, zearalenone, ochratoxin A, sterigmatocystin, and aflatoxins B1, B2, G1, and G2 (Table 4).

Table 4. Absence of Mycotoxins Screened in Three Lots of GODO-FAL				
Mycotoxin	**LOD**	**GODO-FAL Lot Number**		
		2801	**2803**	**3001**
T-2 toxin[1]	0.01 ppm	N.S.	N.D.	N.D.
Zearalenone[1]	0.05 ppm	N.S.	N.D.	N.D.
Ochratoxin A[2]	5 ppb	N.S.	N.D.	N.D.
Sterigmatocystin[2]	0.05 ppm	N.S.	N.D.	N.D.
Aflatoxin B_1[2]	1 ppb	N.D.	N.D.	N.D.
Aflatoxin B_2[2]	1 ppb	N.D.	N.D.	N.D.
Aflatoxin G_1[2]	1 ppb	N.D.	N.D.	N.D.
Aflatoxin G_2[2]	1 ppb	N.D.	N.D.	N.D.
LOD: limit of detection N.S.: not screened N.D.: not detected ppm: parts per million ppb: parts per billion Methods used: [1]Liquid chromatography-mass spectrometry [2]High performance liquid chromatography				

c. Total Organic Solids

Total organic solids (TOS) were calculated for three lots of GODO-FAL to determine the proportion of the lactase preparation that was derived from *A. oryzae* strain GD-FAL to diluents and other additives and ingredients (Table 5). The three lots have an average TOS of 9.6%, and the lot used in the toxicology studies described in Chapter VI: Narrative on the Conclusion of GRAS Status (lot 2803) has a TOS of 10.3%.

Table 5. Total Organic Solids (TOS) in Three lots of GODO-FAL			
Parameters to assess TOS	**GODO-FAL Lot Number**		
	2801	**2803**	**3001**
Ash (%)	0	0	0
Water (%)	47.9	46.3	47.3
Glycerin (%)	43.6	43.4	42.8
TOS (%)	8.5	10.3	9.9

F. STABILITY OF GODO-FAL

GODO-FAL is stable up to 24 months when stored at 11°C. Three nonconsecutive lots of GODO-FAL were stored at 11°C in high density polyethylene bottles and lactase activity was assessed at the beginning of the study and at the following months: 1, 3, 6, 9, 12, 18, and 24. The stability studies for lot 3001 are currently underway (Table 6). All three lots of GODO-FAL complied with the product specification of not less than 10,000 U/g up to 18 months, two of the three lots complied with the product specifications for up to 24 months.

Table 6. Lactase activity (U/g) in GODO FAL is Stable Up to 24 months

GODO-FAL Lot Number	Month							
	0	**1**	**3**	**6**	**9**	**12**	**18**	**24**
2801	11400	11100	10700	10900	11200	11600	11700	11300
2803	12900	12800	12600	12700	12500	12600	12800	12800
3001	11300	11700	11200	10900	11000	10400	10300	-

Stored at 11ºC
-: indicates stability studies currently underway

III. DIETARY EXPOSURE

The lactase derived from *A. oryzae* strain GD-FAL catalyzes the hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides, such as lactose. GODO-FAL is intended for use as an enzyme in the processing of milk, milk powder, fermented milk products and yogurt, fresh cheese, milk-based desserts, whey, baked goods, confectionary, cereal bars, soft drinks, and in the processing of milk for non-exempt infant formulas.

A. HISTORY OF USE

Lactase, or β-galactosidase, has been used in food manufacturing for over 50 years and utilized as a dietary supplement for over 40 years (https://www.lactaid.com/about-lactaid). Active β-galactosidases of microbial and human origin are naturally present in human gastrointestinal tracts; however, in the USA about 33% of the population, mainly of non-European descent, experience lactose intolerance as they get older and have difficulty digesting lactose or lactose-containing foods (Johnson et al., 1993). Therefore, there is increased need to use lactase in milk manufacturing to produce lactose-free or lactose-reduced dairy products. As a food enzyme, lactase can also sweeten dairy products such as ice cream since glucose and galactose are sweeter than lactose, reduce "sandiness" in ice cream due to the limitation of lactose crystallization, and is included in yogurt production by aiding the ability of cultures to hydrolyze lactose. Lactase can also be used to hydrolyze lactose in whey, which can subsequently be used in bakery products, confectionaries, dairy desserts, and as an ingredient in protein concentrate supplements. The enzymatic hydrolysis of lactose by lactase has also been used to produce GOS for use in infant formula, milk drinks, and yogurt (GRNs 620 and 721).

Lactase enzyme preparations have been isolated from a variety of microorganisms including *Aspergillus niger, Aspergillus oryzae*, *Bifidobacterium bifidum*, *Bacillus circulans*, *Kluyveromyces lactis*, and *Pedilochilus terrestris*. Lactase, or β-galactosidase has a long history of safe use in the United States and has been extensively reviewed for safety. In the US, there have been a total of nine GRAS notifications (GRN 88, 132, 485, 510, 572, 579, 649, 743, and 825), all of which have received "no questions" letters from the FDA.

B. INTENDED USE

The lactase derived from *A. oryzae* strain GD-FAL is intended to be used as a substitute for the subject of GRN 825 with the same uses and use levels. This includes the processing of milk, milk powder, fermented milk products and yogurt, fresh cheese, milk-based desserts, whey, baked goods, confectionary, cereal bars, soft drinks, and in the processing of milk for non-

exempt infant formulas. Importantly, the enzyme will be either denatured or inactivated during production of the final food product to render it non-functional.

C. ESTIMATED DAILY INTAKE

Because GODO-FAL is intended to be used as a substitute for the lactase that is the subject of GRN 825, which received a "no questions" letter from the FDA, the dietary exposure of GODO-FAL will be the same as the subject of GRN 825. Therefore, the estimated daily intakes calculated in GRN 825 are incorporated by reference (see pages 18-22 of GRN 825). From the use of the enzyme preparation in the preparation of non-exempt infant formulas for use from birth to 12 months and milk-based products for children 12 to 36 months of age at a maximum level of 36 mg TOS/kg in the final formula, the estimated daily exposure to the enzyme preparation is 9.6 mg TOS/kg bw/d. From the use of the enzyme preparation in dairy products, baked goods, and beverages, the dietary exposure to the enzyme preparation is 3.7 mg TOS/kg bw/day.

IV. SELF-LIMITING LEVELS OF USE

The use of GODO-FAL is not self-limiting. Due to the cost of the product, the amount of enzyme used is not expected to be higher than the minimum level required for optimal digestion of lactose.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

Active β-galactosidases of microbial and human origin are naturally present in the gastrointestinal tract. Pariza and Foster (1983) have noted that the results of exhaustive literature reviews, conducted by FDA, relating to the safety of microbial and non-microbial enzymes used in food production, support the position that enzymes from non-toxicogenic, non-pathogenic organisms, such as GODO-FAL, are safe to consume. Additionally, an identical lactase (β-galactosidase) enzyme preparation from *A. oryzae,* but expressed in *A. niger,* is GRAS for use in the hydrolysis of lactose in milk and whey (GRN 510, 2014) and lactase enzyme preparations from other sources, such as *Candida pseudotropicalis* and *Kluyveromyces lactis*, are GRAS for use in the production of food (21 CFR §184.1387; 21 CFR §184.1388)).

The production organism for GODO-FAL, *A. oryzae* strain GD-FAL, a mold, also known as *koji,* is used to ferment soybeans and rice to make soy sauce, miso, sake, and other foods (Shurtleff and Aoyagi, 2012). *A. oryzae* has also been used to produce many enzymes that are GRAS (GRN 8, 1999; GRN 10, 1999; GRN 34, 2000; GRN 43, 2000; GRN 75, 2001; GRN 90, 2002; GRN 103, 2002; GRN 106, 2002; GRN 113, 2003; GRN 122, 2003; GRN 142, 2004; GRN 201, 2006; GRN 811, 2019). During production, the enzyme is secreted into the culture medium, viable cells are then removed, and the enzyme is concentrated using multiple filtration and ultrafiltration steps, including a final sterilizing filtration step. Additionally, product specifications control the activity of the final enzyme preparation as well as the presence of microbes in the final product, including fungi, which would include *A. oryzae* (see Table 2).

To support the safety of GODO-FAL for the intended uses, Godo Shusei Co., Ltd. conducted a series of toxicology studies with GODO-FAL. These toxicology studies include a genotoxicity battery, an acute study, a 28-day toxicology study in rats, and a subchronic toxicity study in rats. GODO-FAL is not genotoxic, the LD50 was greater than 2000 mg/kg and the no observed adverse effect level (NOAEL) is at least 2000 mg/kg/day. Additional studies performed on lactases derived from different source organisms established NOAELs of at least 4000 mg/kg/day (TOS not reported), 1646 mg/kg/day (TOS not reported), and 2000 mg/kg/day (TOS 1800 mg/kg/day) (Flood and Kondo 2004; Zou et al., 2014; Ke et al., 2018), the highest doses tested in each of the studies, indicating that lactases are not toxigenic.

Because none of the safety studies showed signs of toxicity, the long history of use of lactase, and that GODO-FAL is essentially equivalent to other lactases that are GRAS, it can be concluded that the use of GODO-FAL for the intended purpose is safe. Therefore, there is reasonable certainty that the use of GODO-FAL per the intended uses and use levels is of no harm to consumers, and Godo Shusei Co., Ltd. concludes that GODO-FAL is GRAS for its intended uses and use levels.

A. PUBLISHED GENOTOXICITY STUDIES

The genotoxicity of GODO-FAL has been assessed by published studies including an chromosome aberration test, an in vivo micronucleus test, and a bacterial reverse mutation test (Ames). None of these studies found GODO-FAL to be genotoxic (Symonds et al. 2020).

1. Chromosome Aberration Test in Cultured Chinese Hamster Cells (Symonds et al. 2020)

a. Methods

A mammalian chromosome aberration test was performed in CHL/IU cells derived from the lung of a female Chinese hamster in accordance with OECD 473 and in compliance with Good Laboratory Practice (GLP). The CHL/IU cells were purchased from DS Pharma Biomedical Co., Ltd. and used at passage 14-22. The cells were negative for mycoplasma. The cells were cultivated in a 60 mm culture plate in a CO_2 humidified incubator set at 5% CO_2 and 37ºC. The culture medium for this assay was Eagle's MEM liquid medium (Lot No. DSG7016, Wako Pure Chemical Industries, Ltd.) with 10% inactivated Fetal Bovine Serum (Lot No. AZM197211, Hyclone) and 1% Penicillin/Streptomycin (Lot No. 1786393, GIBCO).

A cell growth inhibition test was conducted according to OECD 474, with the following doses: 0, 125, 250, 500, 1000, and 2000 µg/mL to determine the dosages used for the main study. Cell growth inhibition was measured by measurement of cell proliferation rate (relative population doubling, RPD). RPD was calculated by the following formula.

$$RPD = \frac{Population\ Doubling\ in\ test\ substance\ treated\ cultures}{Population\ doubling\ in\ negative\ control\ cultures} X100$$

$$Population\ Doubling = \frac{Log\ (number\ of\ cells\ post\ treatment/number\ of\ cells\ pre\ treatment)}{\log 2}$$

For both the cell proliferation assay and the main chromosome aberration assay, 5 mL of cell suspension at $4x10^3$ cells/mL was seeded to a plate and cultured for 3 days.

Results of the cell growth inhibition test are shown in Table 7. No cell growth inhibition exceeding 50% was observed in any test substance treatment groups; therefore, 2000 µg/mL was selected as the highest dose and a total of 4 dose levels were prepared by using a common dilution ratio of 2.

Table 7. Cell Growth Inhibition Test for Chromosome Aberration Test in GODO-FAL

GODO-FAL (µg/mL)	Short term (6 h) treatment				24 h continuous treatment	
	S9 mix (-)		S9 mix (+)			
	Increase in number of cells (10^4)	RPD (%)	Increase in number of cells (10^4)	RPD (%)	Increase in number of cells (10^4)	RPD (%)
0	235.0	100	136.3	100	201.3	100
125	197.5	90.1	125.0	94.3	180.0	93.4
250	227.5	98.1	157.5	110.1	183.8	94.6
500	208.8	98.1	138.8	101.2	158.8	86.2
1000	193.8	89.1	140.0	101.9	131.3	75.9
2000	160.0	79.0	140.0	101.9	105.0	65.0
RPD: relative population doubling						

Short term tests with and without S9 metabolic activation were conducted after 6 hours of treatment with GODO-FAL. Rat liver S9 was produced by Oriental Yeast Co., Ltd., and stored at -80ºC until use. After 6 hours of treatment, the cells were washed with Dulbecco's phosphate buffer saline (pH 7.1), and 5 mL of fresh culture medium was added to the plate. The cells were further cultured for 18 hours. Continuous treatment tests without S9 metabolic activation were conducted after 24 hours of treatment with GODO-FAL. Sterile water was used as the negative control. The positive control without metabolic activation was 0.05 µg/mL mitomycin C (MMC, Lot no. 577AEE, Kyowa Hakko Kirin Co., Ltd.). The positive control with metabolic activation was 5.0 µg/mL cyclophosphamide (CP, Lot No. MKBS0021V, Sigma-Aldrich Inc.) added to the culture medium at 1% volume. Two hours prior to the preparation of specimens, the cultures were treated with 0.2 µg/mL colcemid (GIBCO). The cells were separated with trypsin solution and then centrifuged. The collected cells were re-suspended in hypotonic solution (0.075 M potassium chloride), and then Carnoy's fixative (methanol:acetic acid, 3:1) was added. Fixing procedures were repeated three times. The fixed cells were dropped onto a slide and air-dried and stained with 2% Giemsa solution. Duplicate slides were prepared for each plate.

Analysis for chromosome aberration was performed in 3 test substance treatment doses from the highest dose. For structural chromosome aberrations, 300 well-spread metaphase cells in total per dose (150 metaphase cells per plate) were observed under a microscope at a magnification of 1000. For numerical aberrations, 400 well-spread metaphase cells in total per dose (200 metaphase cells per plate) were observed under a microscope at a magnification of 200. The chromosome aberrations were classified as shown below. The cells with structural aberrations excluding gap (-gap) and including gap (+gap) were separately totaled. The frequency of the cells with structure aberrations excluding gap (-gap) was used for the evaluation of chromosome aberrations.

Results would be considered positive for chromosomal aberrations if the test substance-treated samples were statistically significantly increased (the χ^2 test with Yates's correction with a 5%, one-tailed, level of significance) compared to the negative control.

b. Results

Exposure to GODO-FAL did not inhibit cell growth of CHL/IU cells at doses up to 2000 µg/mL (TOS 206 µg/mL), the highest dose used (Table 7). No statistical difference was observed between the negative control and any dose of GODO-FAL in the frequencies of cells with structural aberrations and numerical aberrations in the 6h or 24h treatments (Table 8). The frequencies of cells with structural aberrations in all positive controls were statistically increased compared with the negative controls, demonstrating the validity of the assay.

Table 8. Chromosome Aberration Test in Cultured Chinese Hamster Cells Exposed to GODO-FAL

Treatment Conditions	Dose (µg/mL)	RPD (%)	Observed	Cells showing structural aberrations							Cells showing numerical aberrations				
				Gap	CTB	CSB	CTC	CSC	others	Total (%)	Observed	Pol	End	Total	(%)
6hr S9 mix (-)	Water	100	150	0	1	0	0	0	0	1	200	0	0	0	
			150	1	2	0	0	0	0	2	200	0	0	0	
			Total 300	1	3	0	0	0	0	3 (1.0%)	Total 400	0	0	0	0.0
	500	99.2	150	0	0	1	0	0	0	1	200	1	0	1	
			150	1	0	0	0	0	0	0	200	1	0	1	
			Total 300	1	0	1	0	0	0	1 (0.3%)	Total 400	2	0	2	0.5
	1000	105.4	150	0	2	0	0	0	0	2	200	0	0	0	
			150	0	0	0	0	0	0	0	200	0	0	0	
			Total 300	0	2	0	0	0	0	2 (0.7%)	Total 400	0	0	0	0.0
	2000	104.9	150	0	1	0	0	0	0	1	200	0	0	0	
			150	0	2	0	0	0	0	2	200	1	0	1	
			Total 300	0	3	0	0	0	0	3 (1.0%)	Total 400	1	0	1	0.3
	MMC 0.05	-	150	1	9	1	0	0	0	19	200	1	0	1	
			150	0	5	1	0	0	0	16	200	0	0	0	
			Total 300	1	14	2	0	0	0	35 (11.7%*)	Total 400	1	0	1	0.3
6hr S9 mix (+)	Water	100	150	0	1	0	0	0	0	1	200	0	0	0	
			150	0	0	0	0	0	0	1	200	0	0	0	
			Total 300	0	1	0	0	0	0	2 (0.7%)	Total 400	0	0	0	0.0
	500	100.9	150	1	2	0	0	0	0	5	200	1	0	1	
			150	0	1	0	0	0	0	2	200	0	0	0	
			Total 300	1	3	0	0	0	0	7 (2.3%)	Total 400	1	0	1	0.3
	1000	101.3	150	0	0	0	0	0	0	1	200	2	0	2	
			150	0	2	0	0	0	0	2	200	1	0	1	
			Total 300	0	2	0	0	0	0	3 (1.0%)	Total 400	3	0	3	0.8
	2000	104.9	150	0	1	0	0	0	0	1	200	0	0	0	
			150	0	1	0	0	0	0	1	200	1	0	1	
			Total 300	0	2	0	0	0	0	2 (0.7%)	Total 400	1	0	1	0.3
	CP 5.0	-	150	0	10	0	0	0	0	27	200	0	0	0	
			150	2	11	3	0	0	0	29	200	0	0	0	
			Total 300	2	21	3	0	0	0	56 (18.7%*)	Total 400	0	0	0	0.0
24 hr S9 mix (-)	Water	100	150	0	1	0	0	0	0	1	200	2	0	2	
			150	2	2	0	0	0	0	2	200	0	0	0	
			Total 300	2	3	0	0	0	0	3 (1.0%)	Total 400	2	0	2	0.5
	500	97.0	150	0	1	0	0	0	0	1	200	0	0	0	
			150	0	1	0	0	0	0	1	200	1	0	1	
			Total 300	0	2	0	0	0	0	2 (0.7%)	Total 400	1	0	1	0.3

Table 8. Chromosome Aberration Test in Cultured Chinese Hamster Cells Exposed to GODO-FAL

Treatment Conditions	Dose (µg/mL)	RPD (%)	Observed	Cells showing structural aberrations							Cells showing numerical aberrations				
				Gap	CTB	CSB	CTC	CSC	others	Total (%)	Observed	Pol	End	Total	(%)
	1000	81.8	150	1	1	0	0	0	0	1	200	1	0	1	
			150	0	0	0	0	0	0	0	200	0	0	0	
			Total 300	1	1	0	0	0	0	1 (0.3%)	Total 400	1	0	1	0.3
	2000	80.9	150	1	4	1	0	0	0	5	200	0	0	0	
			150	0	3	0	1	0	0	4	200	1	0	1	
			Total 300	1	7	1	1	0	0	9 (3.0%)	Total 400	1	0	1	0.3
	MMC 0.05	-	150	1	15	1	26	0	0	38	200	1	0	1	
			150	2	8	0	26	0	0	31	200	0	0	0	
			Total 300	3	23	1	52	0	0	69 (23.0%*)	Total 400	1	0	1	0.3

Negative Control: Water for injection (Japanese Pharmacopoeia)
RPD: relative population doubling
MMC: Mitomycin C, CP: Cyclophosphamide, ctb: chromatid break, csb: chromosome break, cte: chromatid exchange, cse: chromosome exchange, Others: multiple aberration, pol: polyploids, end: endoreduplication
*$p<0.05$, statistically significantly different from negative control.

The main chromosome aberration test was performed with short-term treatments with and without metabolic activation, and 24 hours continuous treatment without metabolic activation with 0, 500, 1000, and 2000 µg/mL GODO-FAL. The frequencies of cells with structural aberrations and numerical aberrations in the GODO-FAL treated cells were not statistically significantly different than the negative control. In contrast, the positive controls for the 6 and 24 h treatments with and without S9 activation were statistically significantly increased compared to the negative control. Based on these results, GODO-FAL did not induce chromosomal aberrations under the study conditions.

2. Micronucleus Test (Symonds et al. 2020)

a. Methods

The micronucleus test was performed using an OECD-compliant protocol (OECD 474) in 8-week old Crl:CD(SD) male rats in compliance with GLP. Six animals each in the test substance groups were given 0 (negative control, water for injection) 500, 1000, or 2000 mg/kg daily of GODO-FAL for two days via oral gavage. The positive control (cyclophosphamide, Sigma-Aldrich Inc., Lot No. MKBS0021V, 20 mg/kg) was administered via one intraperitoneal injection on the second day. All animals were observed for clinical signs daily and body weights were measured on administration day and on the day of termination. Animals were terminated and specimens were collected 18-24 hours after the final administration of the test substance. Bone marrow cells in the femur were washed with fetal bovine serum. Serum was then removed from the bone marrow cells and the cells were smeared onto three slides/animal. The cell-smeared specimens were dried at room temperature, fixed with methanol for 4 min, and stained with 0.007% acridine orange stain. The slides were washed twice with phosphate buffer solution (1/15 M, pH 6.8) and allowed to dry. Two specimens per animal were observed with a fluorescence microscope at a magnification of 1000 at random. Four thousand immature erythrocytes/animal were examined and the frequency of micronucleated immature erythrocytes was calculated. One thousand erythrocytes/animal were observed, and the ratio of immature erythrocytes was also calculated.

The Kastenbaum and Bowman statistical analysis method was used to evaluate the frequency of micronuclei between the negative control group and test substance and positive control groups. Dunnett's test was used to evaluate differences in body weight and frequency of immature erythrocytes between the negative control group and other test substance groups.

b. Results

No abnormal clinical signs and no significant body weight changes were observed in any of the rats orally administered GODO-FAL for the in vivo micronucleus test. The frequency of micronuclei in GODO-FAL administered groups was not statistically different from the negative control (Table 9) and within the range of the background data of the negative control (Table 10). Conversely, the rats treated with the positive control had a statistically increased frequency of micronuclei. Thus, GODO-FAL did not induce micronucleus formation in rats up to 2000 mg/kg (TOS 206 mg/kg), the highest dose tested.

Table 9. In vivo Micronucleus Test of GODO-FAL in Rats

Test substance	Dose (mg/kg)	No. of rats	Number of observed micronucleated immature erythrocytes							Frequency of micronuclei (mean ± SD %)
			1	2	3	4	5	6	total	
Negative Control	0	6	3	6	10	7	12	3	41	0.17 ± 0.09
GODO-FAL	500	6	7	7	7	12	8	5	46	0.19 ± 0.06
	1000	6	9	1	7	3	4	7	31	0.13 ± 0.07
	2000	6	6	6	9	8	5	3	37	0.15 ± 0.0
Positive Control (CP)	20	6	132	156	97	133	66	77	661	2.75 ± 0.89*

Negative Control: Water for injection (Japanese Pharmacopeia)
Positive Control (CP): Cyclophosphamide
*p<0.01, significantly different than the control, as assessed by Kastenbaum and Bowman method

Table 10. Background Data for Micronucleus Test in Rats

Treatment	Number of tests	Min	Max	Mean	SD	Variation range (%)
Frequency of micronucleated immature erythrocytes (%)						
Negative Control	17	0.05	0.33	0.15	0.07	0.01-0.29
Positive Control CP 20 mg/kg	9	2.25	3.75	3.09	0.52	2.05-4.13
Ratio of immature erythrocyte to total erythrocytes (%)						
Negative Control	17	47.7	64.7	54.7	3.9	46.9-62.5
Positive Control CP 20 mg/kg	9	32.7	52.4	45.3	6.0	33.3-57.3

Standard of background data on negative control values and positive control values, from 2007 to March 2016.
Animal: Crl:SD rats, male, 8 weeks old.
Variation range: Mean ± standard deviation (SD)
If the calculated value is less than zero or equal to zero, the minimum value is regarded as the lower limit
Positive Control: CP, cyclophosphamide

3. Reverse Mutation (Ames Assay, Symonds et al. 2020)

a. Methods

The reverse mutation test (Ames assay) was performed in accordance with OECD 471 and compliance with GLP. AF-2 (2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, Wako Pure Chemical Industries, Ltd., Lot No: SAE0315), AZI (sodium azide, Wako Pure Chemical Industries, Ltd., Lot No: JPG7700), 9AA (9-aminoacridine, Sigma-Aldrich, Lot No: BCBK1177V), and 2AA (2-aminoanthracene, Wako Pure Chemical Industries, Ltd., Lot No: DCK3519) were used as positive controls. Each positive control was dissolved in DMSO. All strains of *Salmonella typhimurium* were supplied by the Japan Bioassay Research Center and *Escherichia coli* WP2 *uvrA* was supplied by the National Institute of Genetics (Japan).

The mutagenicity of GODO-FAL was determined using the preincubation method, with and without metabolic activation (S9, as described in the chromosome aberration test). GODO-FAL was diluted in water (Japanese Pharmacopeia) at 0, 313, 625, 1250, 2500, and 5000 µg/plate incubated with strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) or *E. coli* (WP2 uvrA). The strains were then cultured for 48 hours at 37°C, and then colonies were counted. Precipitation was checked macroscopically at colony counting. Growth inhibition was examined by the growth of the background lawn with a stereoscope at colony counting. The numbers of the colonies treated with the test substance in *S. typhimurium* TA100 and the positive control of all bacterial strains were counted using a colony analyzer CA-11D (System Science Co., Ltd) and counted manually for other strains and conditions.

Two statistical analyses of Dunnett's multiple comparison method (one-side test) and linear regression method were used.

The number of revertant colonies for each bacterial strain and dose in the dose-finding study and main study was compared with that of the negative control in both the presence and the absence of metabolic activation, and statistically significant difference in the number of revertant colonies between those two groups was analyzed by multiple comparison method ($p < 0.05$). The dose-reactivity was analyzed by the linear regression method ($p < 0.05$) when the statistically significant difference was detected by the multiple comparison method. The numbers of revertant colonies per plate and the mean values and standard deviation per dose of the test substance, negative and positive controls were tabulated for each strain.

b. Results

No precipitation or growth inhibition was observed in the GODO-FAL treated group. No statistically significant increase in the number of revertant colonies was observed in any of the GODO-FAL treatment groups compared to the negative control. The numbers of revertant colonies in the positive control were twice or more than those of the negative control in all bacterial strains in both the presence and absence of metabolic activation, demonstrating the validity of the assay (Table 11). Based on these results, GODO-FAL was not mutagenic at any dose, up to 5000 µg/plate (TOS 515 µg/plate).

Table 11. GODO-FAL Bacterial Reverse Mutation Test

S9 Activation	Treatment	Dose (µg/plate)	Revertants per plate (mean ± standard deviation)				
			Base-pair substitution type			Frameshift mutation type	
			TA100	TA1535	WP2 uvrA	TA98	TA1537
- S9	Negative Control	-	125 ± 6.7	10 ± 3.1	24 ± 7.5	21 ± 4.9	8.0 ± 1.2
	GODO-FAL	313	118 ± 5.8	11 ± 3.0	24 ± 6.1	19 ± 3.6	6.0 ± 0.6
		625	118 ± 14.4	11 ± 2.5	21 ± 5.3	25 ± 6.5	4.0 ± 0.6
		1250	124 ± 9.5	12 ± 1.5	22 ± 3.5	21 ± 3.6	7.0 ± 4.0
		2500	130 ± 5.6	12 ± 6.1	26 ± 5.5	24 ± 4.5	7.0 ± 3.1
		5000	121 ± 17.0	10 ± 1.7	17 ± 2.6	17 ± 3.1	6.0 ± 1.7
	Positive Control: AF-2	0.01	490 ± 31.2	-	118 ± 12.1	-	-
		0.1	-	-	-	311 ± 19.7	-
	Positive Control: AZI	0.5	-	564 ± 42.5	-	-	-
	Positive Control: 9AA	80.0	-	-	-	-	290 ± 73.7
+ S9	Negative Control	-	142 ± 3.1	12 ± 2.0	30 ± 5.9	30 ± 4.0	14 ± 1.5
	GODO-FAL	313	142 ± 5.5	10 ± 3.8	30 ± 2.5	28 ± 5.0	14 ± 1.5
		625	144 ± 2.6	10 ± 4.0	29 ± 3.1	26 ± 7.8	13 ± 1.5
		1250	142 ± 13.6	10 ± 2.3	29 ± 6.7	27 ± 5.9	14 ± 3.1
		2500	138 ± 16.7	10 ± 2.0	26 ± 4.7	30 ± 3.8	15 ± 0.0
		5000	142 ± 7.8	11 ± 3.5	26 ± 7.9	31 ± 2.6	13 ± 1.7
	Positive Control: 2AA	0.5	-	-	-	560 ± 41.4	-
		1.0	1251 ± 108.3	-	-	-	-
		2.0	-	451 ± 29.0	-	-	220 ± 20.3
		10.0	-	-	1012 ± 19.5	-	-

Negative Control: water for injection (Japanese Pharmacopeia)
AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide
AZI: sodium azide
9AA: 9-aminoacridine
2AA: 2-aminoanthracene

B. PUBLISHED TOXICOLOGY STUDIES

The toxicity of GODO-FAL has been assessed by published studies including an acute oral toxicity study, a 28-day oral toxicity study, and a 90-day subchronic oral toxicity study. No test article related adverse effects were noted in either the acute oral or the 28-day oral toxicity studies, with the highest dose of GODO-FAL being 2000 mg/kg/day (TOS 206 mg/kg/day) (Symonds et al. 2020). The results of the 90-day subchronic toxicity study determined the NOAEL to be at least 2000 mg/kg/day (TOS 206 mg/kg/day), the highest dose tested.

Furthermore, additional studies performed on lactases derived from different source organisms described NOAELs of 2000 mg/kg/day (TOS 1800 mg/kg/day) and 4000 mg/kg/day (Flood and Kondo 2004; Ke et al., 2018).

1. Acute Oral Toxicity Study in Sprague Dawley Rats (Symonds et al. 2020)

a. Methods

This test was performed in accordance with OECD 420 and compliance with GLP. Five, 5-week old female Sprague Dawley (SD) rats were acclimatized and monitored for abnormalities and clinical signs for three weeks. Each animal was housed individually. The rats were provided feed and water *ad libitum*, except for fasting the evening before administration of GODO-FAL. Two thousand mg/kg GODO-FAL was administered by oral gavage and the rats were then monitored for 14 days. Mortality and clinical signs were observed before administration, 30 min, 1, 2, 4, and 6 hours after administration, and once a day for the 14 days following administration. Body weight was monitored on days 0 (before administration), 1, 2, 4, 7, and 14. Animals were necropsied on day 14.

b. Results

No deaths were observed after single oral administration of GODO-FAL at 2000 mg/kg (TOS 206 mg/kg) in five female SD rats. No abnormal clinical signs were observed in any rats during the observation period. A decrease in body weight was observed in two rats on Day 2 and one rat on Day 7 (data not shown). These decreases were slight and may have been GODO-FAL administration related. No abnormalities were found during necropsy and gross pathology. Accordingly, the lethal dose of GODO-FAL in rats was determined to be over 2000 mg/kg.

2. 28 Day Oral Toxicity Study in Sprague Dawley Rats (Symonds et al. 2020)

a. Methods

This test was performed in accordance with OECD 407, with the following exceptions: detailed functional observations were not recorded, and the following organs were collected but not subjected to histopathology: spinal cord, eye, thyroid, trachea, gonads (testis and ovaries), accessory sex organs (uterus and cervix, epididymides, prostate + seminal vesicles with coagulating glands), vagina, urinary bladder, peripheral nerve, skeletal muscle, bone, and bone marrow. Twenty male and 20 female 6-week old SD rats were acclimatized and monitored for abnormalities and clinical signs for 10 days prior to GODO-FAL administration. An ophthalmologic examination was also performed during the acclimatization period. Each animal was housed individually. The rats were provided feed and water *ad libitum*.

The rats were divided into groups of 5 animals/sex for each dose of GODO-FAL: 0, 500, 1000, or 2000 mg/kg/day administered by oral gavage for 28 days. Mortality and clinical signs were observed twice daily (before and after administration of GODO-FAL) and before necropsy. Body weights were recorded on the first day of GODO-FAL administration and weekly during the administration period. Rats were weighed the day of necropsy and this body weight measurement was used for the calculation of the relative organ weight. Feed consumption was measured (feed intake per day) by the amount of feed given and feed remaining. Ophthalmologic examinations were performed during week 4 of dosing. Urine was collected using a urine funnel during week 4 of dosing and the following parameters were analyzed: pH, protein, glucose, ketone bodies, urobilinogen, bilirubin, occult blood, sediments, color, volume, specific gravity, sodium, potassium, and chloride.

All animals were fasted the night before necropsy. Blood was collected from the abdominal aorta of all animals under isoflurane anesthesia at necropsy after the measurement of body weight, and the following hematological parameters were analyzed: red blood cell count, white blood cell count, hematocrit value, hemoglobin content, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, reticulocyte count, platelet count, prothrombin time, activated partial thromboplastin time, and differential leukocyte count. The following clinical chemistry parameters were also analyzed from serum collected at necropsy: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transpeptidase, glucose, total cholesterol, triglyceride, phospholipid, total protein, albumin, albumin/globulin ratio, urea nitrogen, creatine, total bilirubin, sodium, potassium, chloride, inorganic phosphorus, and calcium. At necropsy, the organs were weighed and fixed as described below. The pituitary gland and thyroid gland were fixed in 10% neutral buffered formalin and then weighed on the day after necropsy. Paired organs were weighed together. The following organs were weighed: brain, heart, thymus, spleen, lung (including bronchus), submandibular glands, liver, kidneys, prostate, seminal vesicle, testes, epididymes, ovaries, uterus,

pituitary gland, thyroid glands (including parathyroid gland) and adrenal glands. The eyes, including the optic nerve, were fixed in Davidson's fixative, the testes were fixed in Bouin fixative and other organs and tissue were fixed in 10% neutral buffered formalin. Bone tissues were decalcified with 10% formic acid formalin. For all rats of the control and high dose groups, the following fixed organs were embedded, thin sectioned, stained with hematoxylin and eosin (H-E), and examined microscopically: brain, heart, thymus, spleen, lung, liver, kidney, adrenal gland, stomach, duodenum, jejunum, ileum, cecum, colon. Statistical analysis for homogeneity of variance was performed by Bartlett's test (significant level: 5%). Dunnett's multiple comparison test (significant level: 5%, two-tailed) was used for homogeneous data, and Steel's multiple comparison test (significant level: 5%, two-tailed) was used for heterogeneous data.

b. Results

In the 28-day dose-range finding study, 0 (control), 500, 1000, and 2000 mg/kg GODO-FAL/day was given to male and female SD rats for 28 days. No deaths or abnormalities were observed in any groups during the dosing period. A small, but statistically significant, decrease in feed consumption was observed in males fed GODO-FAL at 1000 mg/kg/day on days 27-28 of the study (23 g compared to 28 g in the control). This change was not considered treatment-related because there was no dose relationship and no decrease in feed consumption was observed in the female rats. Additionally, there were no differences in body weights in the 1000 mg/kg/day fed male rats compared to control or to other treatment groups (data not shown). No differences in body weight were observed in female rats during the study.

Decreases in total urine excretion of Na, K, and Cl were observed in males at 500 and 2000 mg/kg/day during the last week of treatment. Decreases in total urine excretion of K and Cl were observed in males at 1000 mg/kg/day. These decreases were very slight and since no histopathological abnormality was observed in the kidney at 2000 mg/kg/day, and similar decreases were not found in the female treatment groups, these changes were not considered to be related to treatment or toxicologically significant. No other differences were observed in urinalysis among groups.

Hematology parameters showed a decrease in neutrophils (8.4% compared to 16.3% in the control) and an increase in lymphocytes (88.8% compared to 80.1% in the control) in females administered 1000 mg/kg/day GODO-FAL. There was no dose-relationship between these findings and they were not considered treatment-related (data not shown). No significant differences were observed in any of the other hematology parameters.

Clinical chemistry results noted increases in γ-GTP (0.5 U/L compared to 0.3 U/L in the control) and Cl (112 mEq/L compared to 110 mEq/L in the control) in males administered 2000 mg/kg/day GODO-FAL. These changes were very slight and were therefore not considered to have toxicological significance. No changes were noted in females from any treatment group.

One male administered 2000 mg/kg/day of GODO-FAL had an enlarged spleen and a small prostate. One male administered 500 mg/kg/day of GODO-FAL had an enlarged right adrenal gland. These gross pathology findings were considered incidental. No gross pathology abnormalities were found in any of the female groups.

The absolute and relative weights of the thymuses in females fed 500 mg/kg/day GODO-FAL were increased compared to the controls (data not shown). This increase was not considered treatment related as there were no changes in organ weights observed in male rats fed GODO-FAL and no dose dependent relationship was observed.

Histopathological examination in male rats fed 2000 mg/kg/day GODO-FAL for 28 days noted focal mononuclear cell infiltration in the liver. This finding was very slight and was also observed in the control group. Therefore, it was not considered to be treatment related nor toxicologically significant. No other histopathological abnormalities were observed in either male or female rats.

No adverse test-article related effects were observed in the 28-day repeated oral toxicity study up to 2000 mg/kg/day (TOS 206 mg/kg/day), the highest dose tested.

3. Subchronic Toxicity Study in Sprague Dawley Rats (Symonds et al. 2020)

a. Methods

A 90-day subchronic toxicity study was performed in 6-week old male and female SD rats in compliance with GLP. The study was performed in compliance with OECD 408, with the exception that only one dose of GODO-FAL was used in addition to the control (water). Rats were housed individually during quarantine, acclimation, and during the study period. Prior to dosing, the rats were randomized by body weight into two groups (n=10/sex/group). During the 90-day treatment period, animals were treated with either the control (water) or 2000 mg/kg GODO-FAL/day via oral gavage. All animals were observed for clinical signs and mortality twice daily, before and after test substance administration, and before necropsy. Body weight was recorded on the first day of dosing, then once weekly during the dosing period, on the day before necropsy, and the day of necropsy. The body weight measured at necropsy day was used for the calculation of the relative organ weight. Feed consumption was measured once weekly during the dosing period. Feed consumption was determined (feed intake per day) by the amount of feed given and feed remaining. Fresh urine samples were collected for 2 h from five males and five females from each group during the last week of the study and analyzed for pH, protein, glucose, ketone bodies, urobilinogen, bilirubin, occult blood, sediments, color, volume, specific gravity, sodium, potassium, and chloride.

All animals were fasted the evening before necropsy. Blood was collected from the abdominal aorta of all animals on the day of necropsy under isoflurane anesthesia at necropsy

after the measurement of body weight, and the following parameters were examined: red blood cell count, white blood cell count, hematocrit value, hemoglobin, content, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, reticulocyte, platelet count, prothrombin time, activated partial thromboplastin time, and differential leukocyte count. Serum collected at necropsy was analyzed for the following clinical chemistry parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transpeptidase, glucose, total cholesterol, triglyceride, phospholipid, total protein, albumin, albumin/globulin ratio, urea nitrogen, creatine, total bilirubin, sodium, potassium, chloride, inorganic phosphorus, and calcium.

The following organs were weighed and fixed: brain, heart, thymus, spleen, lung (including bronchus), salivary gland (submandibular glands, sublingual gland), liver, pancreas, kidneys, testes, seminal vesicle, prostate, epididymides, ovaries, uterus, pituitary gland, thyroid glands (including parathyroid gland) and adrenal glands. The fixed organs were embedded, thin sectioned, stained with hematoxylin and eosin (H-E), and examined microscopically. Bone tissues were decalcified with 10% formic acid formalin. Other organs and tissues were preserved in 10% neutral buffered formalin: brain, heart, thymus, spleen, lung, liver, kidney, adrenal gland, stomach, duodenum, jejunum, ileum, cecum, colon.

Analysis for homogeneity of variance was performed by F test (significant level: 5%). Statistical analysis for homogeneous data was performed using Student's t-test (significant level: 5%, two-tailed). Statistical analysis for heterogeneous data was performed using Aspin-Welch's test (significant level: 5%, two-tailed).

b. Results

To evaluate the toxicity of GODO-FAL, doses of GODO-FAL at 0 (control) and 2000 mg/kg were given to SD rats via oral gavage for 90 days. No significant differences in body weights in males or females (Figure 6) or feed consumed (Table 12) between the GODO-FAL and control groups were found during the dosing period.

No differences in urinalysis parameters were observed between control and treated male or female rats.

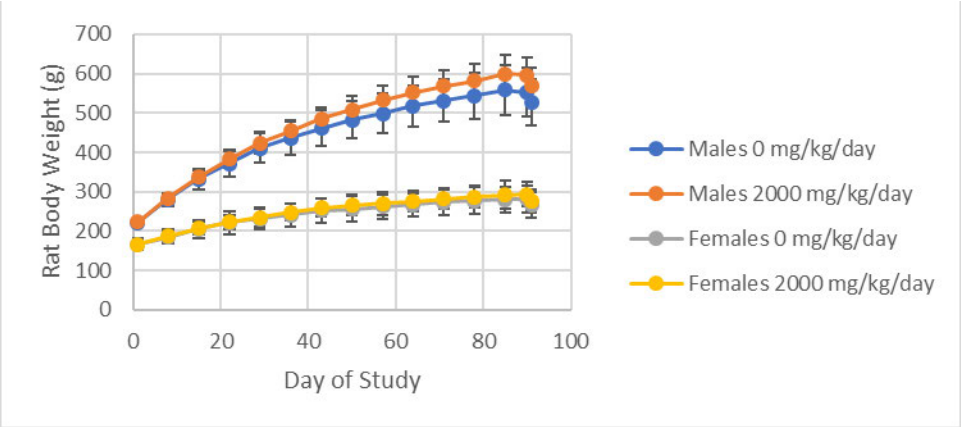


Figure 6. Sprague Dawley Rat Body Weight During Subchronic Toxicity Study, Treated with 0 or 2000 mg/kg/day GODO-FAL

Each point is the average of 10 rats/group with the standard deviations shown.

Table 12. Feed Consumption (g/day) in the 90 Day Subchronic Toxicity Study

GODO-FAL mg/kg	Day of treatment												
	5-6	**12-13**	**19-20**	**26-27**	**33-34**	**40-41**	**47-48**	**54-55**	**61-62**	**68-69**	**75-76**	**82-83**	**89-90**
Males, n=10/group													
0	25 ± 3	28 ± 3	27 ± 3	28 ± 3	26 ± 4	26 ± 4	26 ± 3	25 ± 3	25 ± 3	26 ± 3	25 ± 3	24 ± 4	25 ± 4
2000	25 ± 2	26 ± 2	28 ± 2	27 ± 2	27 ± 3	27 ± 3	27 ± 2	27 ± 3	26 ± 2	26 ± 3	27 ± 2	26 ± 2	27 ± 3
Females, n=10/group													
0	16 ± 2	17 ± 2	16 ± 2	18 ± 2	16 ± 2	16 ± 3	16 ± 2	17 ± 2	16 ± 2	15 ± 1	15 ± 2	16 ± 2	14 ± 2
2000	16 ± 2	16 ± 4	16 ± 2	18 ± 1	16 ± 2	17 ± 2	17 ± 2	17 ± 2	17 ± 2	15 ± 2	16 ± 2	17 ± 2	14 ± 2

Hematology analysis noted a decrease in prothrombin time (PT) in males, a decrease in mean corpuscular hemoglobin concentration (MCHC) and an increase in the absolute and relative numbers of reticulocytes (Ret) in females administered 2000 mg/kg/day GODO-FAL (Table 13). The PT in males fed GODO-FAL was statistically decreased when compared to the control (13.6 ± 0.7 vs. 14.5 ± 0.7 in the control) but was still within the historical control data range (mean: 14.4 ± 1.7, range 11.5-21.5). MCHC was statistically decreased in females fed GODO-FAL (37.5 ± 0.5 vs. 38.1 ± 0.5) but was within the normal historical control data range for SD rats (mean: 37.3 ± 0.8, range 35.4-39.1). Absolute and relative numbers of reticulocytes were statistically increased in females fed GODO-FAL (absolute reticulocytes in the test group were 26.68 ± 4.10 vs. 22.06 ± 5.11 in the control; relative reticulocytes in test group: 3.34 ± 0.56 vs. 38.1 ± 0.5 in the control) but were considered within normal historical control data ranges (mean absolute reticulocytes: 25.30 ± 4.33, range 15.94-39.55; mean relative reticulocytes: 3.00 ± 0.52, range 1.88-4.62) Although statistically different from control, these differences were very slight, not toxicologically significant and not considered test article related as the changes were within the historical control data for the testing facility.

Table 13. Hematology Results in Male and Female Rats from GODO-FAL 90-day Subchronic Toxicity

GODO-FAL	Males n= 10/group		Females n=10/group	
mg/kg/day	0 (Control)	2000	0 (Control)	2000
RBC (10^4/µL)	911 ± 20	876 ± 48	807 ± 35	801 ± 35
WBC (10^2/µL)	72.3 ± 20.0	80.1 ± 21.9	38.7 ± 12.4	39.2 ± 8.0
Ht (%)	42.0 ± 1.8	41.2 ± 1.4	40.2 ± 1.2	41.1 ± 0.9
Hb (g/dL)	16.1 ± 0.5	15.6 ± 0.6	15.3 ± 0.6	15.4 ± 0.4
MCH (pg)	17.7 ± 0.5	17.9 ± 0.6	19.0 ± 0.6	19.3 ± 0.4
MCV (fL)	46.1 ± 2.0	47.1 ± 2.0	49.8 ± 1.9	51.3 ± 1.6
MCHC (g/dL)	38.4 ± 0.8	38.0 ± 0.5	38.1 ± 0.5	37.5 ± 0.5*
Ret (%)	3.01 ± 0.54	3.37 ± 0.57	2.75 ± 0.67	3.34 ± 0.56*
Ret (10^4/µL)	27.45 ± 4.84	29.42 ± 4.75	22.06 ± 5.11	26.68 ± 4.10*
PLT (10^4/µL)	110.9 ± 10.6	112.6 ± 8.1	87.2 ± 19.2	96.3 ± 15.7
PT (sec)	14.5 ± 0.7	13.6 ± 0.7**	12.5 ± 17.2	12.4 ± 0.5
APTT (sec)	23.2 ± 2.4	21.7 ± 2.0	17.2 ± 1.1	17.7 ± 1.1
Lymphocyte (%)	66.6 ± 9.5	69.3 ± 6.3	75.4 ± 7.9	76.1 ± 6.9
Neutrophil (%)	27.4 ± 9.6	24.7 ± 6.4	19.9 ± 7.3	18.8 ± 6.1
Monocyte (%)	4.3 ± 0.9	4.1 ± 1.1	3.1 ± 0.9	3.1 ± 0.8
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Eosinophil (%)	1.8 ± 0.8	1.9 ± 0.6	1.6 ± 0.9	2.0 ± 1.0
Lymphocyte (10^2/µL)	47.6 ± 13.5	54.9 ± 12.9	29.3 ± 10.2	30.1 ± 7.6
Neutrophil (10^2/µL)	20.5 ± 10.0	20.3 ± 9.7	7.6 ± 3.8	7.2 ± 2.2
Monocyte (10^2/µL)	3.1 ± 1.1	3.3 ± 1.0	1.2 ± 0.4	1.2 ± 0.3
Basophil (10^2/µL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophil (10^2/µL)	1.2 ± 0.6	1.5 ± 0.7	0.6 ± 0.3	0.8 ± 0.3

Significantly different from control, *p<0.05, **p<0.01 (Dunnett's test)
Abbreviations: RBC: red blood cells, WBC: white blood cells, Ht: hematocrit value, Hb: hemoglobin content, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, Ret: reticulocytes, PLT: platelet count, PT: prothrombin time, APTT: activated partial thromboplastin time

Clinical chemistry results noted statistically significant increases in serum calcium (Ca) in males, albumin/globulin ratio (A/G), and total bilirubin (T.Bil.) in females fed 2000 mg/kg/day GODO-FAL (Table 14). The serum Ca in males fed GODO-FAL was statistically increased when compared to the control (10.1 ± 0.3 vs. 9.7 ± 0.3 in the control) but was still within the historical control data range (mean: 9.6 ± 0.4, range: 8.9-11.6). The A/G ratio in females fed GODO-FAL was statistically increased when compared to the control (0.78 ± 0.06 vs. 0.72 ± 0.04 in the control) but was still within the historical control data range (mean: 0.73 ± 0.07, range: 0.6-0.94). The females fed GODO-FAL also had a statistical increase in T. Bil compared to control animals (0.10 ± 0.02 vs. 0.07 ± 0.01 in the control). This result was within the historical control data range, mean: 0.08 ± 0.02, range: 0.04-0.17. These changes were considered not to be related to the GODO-FAL administration because these changes were within the historical data for control animals at the testing facility.

Table 14. Clinical Chemistry Results from the 90-Day Subchronic Toxicity Study of GODO-FAL in Rats

GODO-FAL	Males n = 10/group		Females n = 10/group	
	0 (Control)	2000 mg/kg/day	0 (Control)	2000 mg/kg/day
AST (U/L)	79.9 ± 19.1	76.1 ± 13.8	87.5 ± 19.6	87.9 ± 26.3
ALT (U/L)	26.5 ± 7.1	24.2 ± 4.7	24.1 ± 6.0	28.4 ± 9.2
ALP (U/L)	248.1 ± 47.1	238.6 ± 52.6	135.4 ± 24.8	125.0 ± 31.5
LDH (U/L)	82.2 ± 78.1	63.9 ± 16.7	59.3 ± 6.7	62.3 ± 16.9
γ-GTP (U/L)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
Glu. (mg/dL)	172 ± 38	167 ± 24	119 ± 17	134 ± 28
T. Chol (mg/dL)	61 ± 10	63 ± 13	63 ± 10	66 ± 14
TG (mg/dL)	48 ± 11	63 ± 28	16 ± 4	20 ± 10
PL (mg/dL)	98 ± 13	101 ± 19	118 ± 11	129 ± 21
TP (g/dL)	5.8 ± 0.2	5.9 ± 0.2	6.2 ± 0.2	6.3 ± 0.4
Alb. (g/dL)	2.2 ± 0.1	2.3 ± 0.1	2.6 ± 0.1	2.7 ± 0.2
A/G	0.6 ± 0.0	0.6 ± 0.0	0.72 ± 0.04	0.78 ± 0.06*
BUN (mg/dL)	14 ± 2	13 ± 2	18 ± 3	17 ± 2
Crea. (mg/dL)	0.27 ± 0.04	0.28 ± 0.07	0.41 ± 0.07	0.39 ± 0.05
T. Bil. (mg/dL)	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.10 ± 0.02**
Na (mEq/L)	143 ± 2	143 ± 1	143 ± 1	143 ± 1
K (mEq/L)	4.7 ± 0.2	4.7 ± 0.3	4.2 ± 0.4	4.1 ± 0.3
Cl (mEq/L)	111 ± 1	110 ± 2	114 ± 2	114 ± 1
P (mg/dL)	5.9 ± 0.5	5.7 ± 0.5	5.0 ± 0.6	5.0 ± 1.0
Ca (mg/dL)	9.7 ± 0.3	10.1 ± 0.3*	9.8 ± 0.3	10.0 ± 0.3

Significantly different from control, *p<0.05, **p<0.01 (Student's t-test)
Abbreviations: AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, γ-GTP: gamma-glutamyl transpeptidase, Glu: glucose, T. Chol: total cholesterol, TG: triglycerides, PL: phospholipid, TP: total protein, Alb: albumin, A/G: albumin/globulin ratio, BUN: urea nitrogen, Crea: creatine, T. Bil: total bilirubin, K: potassium, Cl: chloride, P: phosphorus, Ca: calcium.

A dilated pelvis in the right kidney and cyst cervix in the uterus were observed upon gross pathology in one female administered 2000 mg/kg/day GODO-FAL for 90 days. These findings were considered to be incidental, as it was only observed in one animal and was therefore not test substance related.

A decrease of relative weight in the brain, testes, epididymis, and seminal vesicle in males, and a decrease of relative weight in the kidney in females were observed in the GODO-FAL fed groups. The relative brain weights in males fed GODO-FAL were statistically decreased when compared to the control (0.38 ± 0.03 vs. 0.41 ± 0.04 in the control) but were still within the historical data range (mean: 0.41 ± 0.4, range: 0.3-0.52). The GODO-FAL fed males had a statistical decrease in relative testes weight (0.59 ± 0.04 vs. 0.64 ± 0.06 for controls), but this decrease was within the historical controls (mean: 0.64 ± 0.7, range: 0.45-0.80). Similarly, a statistical decrease in the relative weight of epididymides was observed between the GODO-FAL fed males and the controls males (0.21 ± 0.012 vs. 0.24 ± 0.019 in the control). This difference was within the range of expected epididymides relative weight (mean: 0.26 ± 0.034, range: 0.18-0.36). The relative weights of the seminal vesicles in the GODO-FAL fed males were statistically smaller than the control (0.31 ± 0.054 vs. 0.37 ± 0.040 in the control), but still within historical data (mean: 0.38 ± 0.061, range: 0.25-0.61).The kidney relative weights in females fed GODO-FAL were statistically decreased when compared to the control (0.60 ± 0.02 vs. 0.65 ± 0.05 in the control) but were still within the historical control data range (mean: 0.66 ± 0.5, range: 0.56-0.76). None of these observed changes in relative organ weights were considered to be test substance related as the changes were minimal and within the historical control data range (Table 15).

Table 15. Absolute and Relative Organ Weight Results in 90-Day Subchronic Toxicity Study of GODO-FAL in Rats

Organ	GODO-FAL mg/kg/day	Males		Females	
		0	2000	0	2000
Body weight (g)		527 ± 58	570 ± 44	266 ± 32	276 ± 30
Brain	g	2.14 ± 0.11	2.13 ± 0.09	1.94 ± 0.10	1.94 ± 0.09
	% Body Weight	0.41 ± 0.04	0.38 ± 0.03*	0.74 ± 0.08	0.71 ± 0.06
Heart	g	1.45 ± 0.12	1.50 ± 0.15	0.83 ± 0.0058	0.86 ± 0.0095
	% Body Weight	0.28 ± 0.022	0.26 ± 0.027	0.31 ± 0.022	0.31 ± 0.034
Thymus	g	0.29 ± 0.059	0.33 ± 0.069	0.22 ± 0.04	0.23 ± 0.046
	% Body Weight	0.054 ± 0.011	0.058 ± 0.012	0.082 ± 0.015	0.084 ± 0.017
Spleen	g	0.76 ± 0.093	0.76 ± 0.11	0.46 ± 0.063	0.49 ± 0.096
	% Body Weight	0.14 ± 0.018	0.13 ± 0.019	0.17 ± 0.024	0.18 ± 0.035
Lung	g	1.51 ± 0.10	1.55 ± 0.10	1.10 ± 0.10	1.13 ± 0.09
	% Body Weight	0.29 ± 0.02	0.27 ± 0.02	0.42 ± 0.03	0.41 ± 0.04
Submaxillary Salivary Glands	g	0.74 ± 0.084	0.78 ± 0.11	0.45 ± 0.047±	0.43 ± 0.07
	% Body Weight	0.14 ± 0.016	0.14 ± 0.019	0.170 ± 0.018	0.16 ± 0.025
Liver	g	13.68 ± 1.76	14.97 ± 1.97	6.40 ± 0.49	6.73 ± 1.08
	% Body Weight	2.60 ± 0.23	2.62 ± 0.25	2.42 ± 0.14	2.43 ± 0.20
Kidneys	g	3.15 ± 0.21	3.21 ± 0.38	1.71 ± 0.18	1.67 ± 0.18
	% Body Weight	0.60 ± 0.06	0.56 ± 0.05	0.65 ± 0.05	0.60 ± 0.02#
Pituitary Gland	g	0.015 ± 0.0014	0.016 ± 0.0019	0.021 ± 0.0026	0.021 ± 0.003
	% Body Weight	0.0029 ± 0.00027	0.0027 ± 0.00033	0.0078 ± 0.00098	0.0075 ± 0.0011
Thyroid Gland	g	0.027 ± 0.0053	0.028 ± 0.0048	0.019 ± 0.0028	0.021 ± 0.0033
	% Body Weight	0.0050 ± 0.0010	0.0048 ± 0.00084	0.0070 ± 0.0011	0.0075 ± 0.0012
Adrenal Gland	g	0.048 ± 0.008	0.055 ± 0.007	0.062 ± 0.009	0.057 ± 0.012
	% Body Weight	0.0091 ± 0.0015	0.0097 ± 0.0012	0.023 ± 0.0034	0.021 ± 0.0044
Testes	g	3.35 ± 0.19	3.37 ± 0.30	-	-
	% Body Weight	0.64 ± 0.06	0.59 ± 0.04*	-	-

Table 15. Absolute and Relative Organ Weight Results in 90-Day Subchronic Toxicity Study of GODO-FAL in Rats

Organ	GODO-FAL mg/kg/day	Males		Females	
		0	2000	0	2000
Epididymides	g	1.28 ± 0.1	1.21 ± 0.069	-	-
	% Body Weight	0.24 ± 0.019	0.21 ± 0.012**	-	-
Prostate	g	1.053 ± 0.18	1.1 ± 0.15	-	-
	% Body Weight	0.20 ± 0.034	0.19 ± 0.025	-	-
Seminal Vesicles	g	1.95 ± 0.21	1.74 ± 0.31	-	-
	% Body Weight	0.37 ± 0.040	0.31 ± 0.054**	-	-
Ovaries	g	-	-	0.11 ± 0.013	0.10 ± 0.014
	% Body Weight	-	-	0.042 ± 0.0049	0.037 ± 0.0051
Uterus	g	-	-	0.53 ± 0.11	0.55 ± 0.145
	% Body Weight	-	-	0.20 ± 0.042	0.20 ± 0.053
Significantly different from control, *p<0.05, **p<0.01 (Student's t-test), #p<0.05 (Aspin-Welch's t-test)					

Histopathological analysis found some slight (grade 1) pathological findings in the heart, liver, pancreas, and prostates in both treated and untreated males (Table 16). One female in the treated group had marked dilation of the right kidney, which was noted in the gross pathological findings (Table 16). These findings were considered incidental and not test-substance related.

Table 16. Histopathological Findings in the 90-Day Subchronic Toxicity Study of GODO-FAL in Rats

GODO-FAL mg/kg/day	Grade	Male: n=10/group		Female: n=10/group	
		0	2000	0	2000
Heart:					
Infiltration, mononuclear cell, focal, myocardium	Grade 1	3	2	0	0
Liver:					
Fatty change, hepatocyte	Grade 1	2	1	0	0
Necrosis, focal	Grade 1	0	0	1	0
Pancreas					
Fibrosis, islets	Grade 1	2	1	0	0
Yellow-brown pigmentation	Grade 1	2	3	0	0
Infiltration, eosinophil, focal	Grade 1	2	0	0	0
Infiltration, mononuclear cell, islet	Grade 1	0	0	1	0
Kidney					
Dilation, pelvis, right kidney	Grade 3	0	0	0	1
Urinary Bladder					
Infiltration, neutrophil, mucosa	Grade 1	0	0	0	1
Edema, mucosa	Grade 1	0	0	0	1
Prostate					
Infiltration, mononuclear cell, interstitium	Grade 1	5	5	-	-
Uterus					
Cyst, cervix	Grade 1	-	-	0	1
Vagina					
Mucinous degeneration	Grade 1	-	-	0	1
Pituitary gland					
Dilation, Rathke's cleft	Grade 1	0	0	0	1
Grade 1: slight, Grade 3: marked					

In conclusion, no significant treatment-related adverse effects were found in male or female rats administered 2000 mg/kg/day GODO-FAL. The NOAEL of GODO-FAL was determined to be at least 2000 mg/kg/day (TOS 206 mg/kg/day) under the present study conditions.

C. GENOTOXICOLOGY AND TOXICOLOGY STUDIES OF OTHER LACTASES

The safety of GODO-FAL is also supported by unpublished and published toxicology studies conducted with lactases derived from other source organisms (summarized in GRN 510, 2014; Flood and Kondo 2004; Zou et al., 2014; Ke et al., 2018). Because these studies have been extensively reviewed in GRNs 510 and 825, their summaries are incorporated by reference and are briefly summarized below. Additionally, a literature search using both PubMed and GoogleScholar was performed on August 8, 2021, to identify any new toxicology studies performed since 2018, the year that GRN 825 received a "no questions" letter from the FDA. The only new study that was identified was Symonds et al., 2020, which is described above. Collectively, these additional studies showed that lactases are not genotoxic and reported NOAELs the highest doses tested, indicating that lactases in general are non-toxigenic.

As described in pages 36-40 of GRN 510, an unpublished genotoxicology battery (bacterial reverse mutation test (OECD guideline no. 471), a chromosomal aberration test (OECD guideline no. 473), and an in vivo mouse micronucleus assay) and a subchronic oral toxicity study (OECD Guideline for the Testing of Chemicals No. 408) were performed using an enzyme preparation expressed in *A. niger* that contained an *A. oryzae* acid lactase and was identical in amino acid sequence to the lactase enzyme that is the subject of this GRAS Notice. The enzyme preparation was not mutagenic or clastogenic. In the subchonic toxicity study, the administration of the enzyme preparation in the diet up to 6452 mg/kg/day did not lead to any toxicologically relevant findings. The NOAEL was therefore determined to be at least 6452 mg/kg/day, which was the highest dose tested. This corresponds to 1000 mg TOS/kg body weight/day or 196,130 U/kg body weight/day.

As summarized on page 36 in GRN 825, Flood and Kondo (2004) administered a beta-galactosidase enzyme preparation produced by *Penicillium multicolor* to adult and juvenile rats for 35 days and 6 months, respectively. No adverse dose-related effects were observed in either study, which reported a NOAEL of at least 4000 mg/kg/day, the highest dose tested. In addition, a 30-day dog study was performed with no adverse dose-related effects observed at any dose, with 1000 mg/kg/day being the highest dose tested. Reproductive and developmental studies were also performed in rats and rabbits. These studies reported that the NOAELs were at least 4000 mg/kg/day and 1000 mg/kg/day, respectively (TOS not reported). In all animal studies, the NOAEL was the highest dose tested, and it was concluded that the beta-galactosidase preparation is safe.

Zou et al. (2014) investigated the safety of a recombinant beta-galactosidase derived from *Aspergillus oryzae* and expressed in *Pichia pastoris.* The beta-galactosidase showed no mutagenic activity in an Ames test or a mouse sperm abnormality test at levels of up to 5 mg/plate and 1250 mg/kg body weight, respectively. The recombinant beta-galactosidase also showed no genotoxic activity in a bone marrow cell micronucleus test at levels of up to 1250 mg/kg body weight. An acute oral toxicity study in rats found that the 50% lethal dose (LD_{50}) was greater than 30 mL/kg body weight. The test article had an activity of 10,000 U/mL. A 90-day subchronic repeated toxicity study via the diet with the recombinant beta-galactosidase used at levels up to 1646 mg/kg (TOS not reported) did not show any dose-related adverse effects on body weight, feed consumption, organ weights, hematological and clinical chemistry, or histopathology compared to the control group. This toxicological evaluation showed no genotoxic, acute, or sub-chronic toxicity for beta-galactosidase under the test conditions used.

Ke et al. (2018) evaluated the genotoxicity and subchronic toxicity of beta-galactosidase produced by *Papiliotrema terrestris* in a bacterial reverse mutation test (Ames test), a chromosomal aberration test in cultured Chinese hamster lung fibroblast (CHL/IU) cells, and a 13-week oral gavage study in Sprague-Dawley rats. The enzyme concentrate was not genotoxic, and no adverse effects were observed in any of the tested groups in the subchronic toxicology study. A NOAEL of 2000 mg/kg bw/day (total organic solids (TOS) 1800 mg/kg bw/day) was established, which was the highest dosage tested.

D. ALLERGENICITY

Enzymes have a long history of safe use in food processing, with no indication of adverse effects or reactions. Others have also published the potential low risk of allergenicity associated with enzymes used in the production of food. Bindslev-Jensen et al. (2006) concluded that food allergy is not likely to be a concern regarding the ingestion of food enzymes based on a study of enzymes produced by wild-type and genetically modified strains, as well as wild-type enzymes and engineered variants in 400 patients diagnosed with allergies to inhalation allergens, food allergens, bees, or wasps. An expert group convened by the Association of Manufacturers & Formulators of Enzyme Products (AMFEP), *i.e.*, the AMFEP Working Group on Consumer Allergy Risk from Enzyme Residues in Food, evaluated the existing scientific data and concluded that for exposure by ingestion, as opposed to exposure by inhalation, enzyme proteins are not potent allergens and sensitization to ingested enzymes is rare. Thus, the scientific data indicate that small amounts of enzymes in food are unlikely to sensitize or induce allergic reactions in consumers.

The allergenic potential of GODO-FAL is quite low. The allergenicity of GODO-FAL was assessed by performing ELISAs (enzyme-linked immunosorbent assay) for egg, milk, and soy allergens in two lots of GODO-FAL as a due diligence exercise. Each ELISA was performed

using two kits, one manufactured by Morinaga Institute of Biological Science, Inc. (Japan) and the other by Nipponham (Japan). No egg, milk, or soy allergens were detected using either kit (Table 17).

Table 17. Egg, Milk and Soy Allergens Screened in Two Lots of GODO-FAL

Allergen	LOQ	GODO-FAL Lot Number	
		2801	2803
Egg	1 µg/g	N.D.	N.D.
Milk	1 µg/g	N.D.	N.D.
Soy	1 µg/g	N.D.	N.D.

*Two ELISAs were performed for each allergen, using kits from Morinaga and Nipponham. The analysis was performed by Eurofins Food and Product Testing Japan.
LOQ: limit of quantitation
N.D.: not detected

The allergenic potential of GODO-FAL was further estimated by using the sequence for GODO-FAL as a query in the AllergenOnline Database v 19, a database of known allergenic protein sequences. Full-length alignments, 80 amino acid alignments, and 8 amino acid exact match searches were conducted using version 19 of the AllergenOnline Database maintained by the University of Nebraska - Lincoln and the amino acid sequence for GODO-FAL provided by GODO. No identity matches of greater than 50% were found in the full-length alignment search and no matches were found in either the 80 amino acid or 8 amino alignment searches. Therefore, the GODO-FAL amino acid sequence did not yield any hits in this screen. Taken together with the allergens screened in Table 18, these results further demonstrate that GODO-FAL is unlikely to be allergenic.

E. REGULATORY APPROVALS ACROSS THE WORLD

GODO-FAL is currently approved for use in Australia, New Zealand, Canada, and Japan.

VII. SUPPORTING DATA AND INFORMATION

A. REFERENCES

All information included in the following list of references is generally available.

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B. EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of *Aspergillus oryzae* (GODO-FAL) as a processing aid in the production of milk and whey products. The lactase preparation derived from GODO-FAL for the intended use has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intended conditions of use of GODO-FAL has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of the substances directly added to food and is based on generally available and accepted information.

The intended use of GODO-FAL as a processing aid for the hydrolysis of lactose in fluid milk, whey, cheese, yogurt, and other dairy products, and has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

- GODO-FAL is a lactase, a hydrolase that can transfer non-reducing β-D-galactose residues from β-D-galactosides, produced by *Aspergillus oryzae* strain GD-FAL. *A. oryzae* strain GD-FAL is not genetically modified.
- The amino acid sequence of GODO-FAL is 100% identical to the amino acid sequence of the enzyme that is the subject of GRN 510, acid lactase from *A. oryzae* expressed in *A. niger*.
- There is no evidence in the available information on GODO-FAL that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public if GODO-FAL is used at levels that might reasonably be expected from the proposed applications.
    - *A. oryzae* has a long history of human consumption and is the source organism for multiple "no questions" GRAS notices (GRNs 8, 10, 34, 43, 75, 90, 103, 106, 113, 122, 142 and 201).
    - Unlike other members of the genus *Aspergillus,* there is no record of *A. oryzae* producing mycotoxins, and three lots of the finished product, GODO-FAL, had non-detectable levels of the following mycotoxins: T-2 toxin, zearalenone, ochratoxin A, sterigmatocystin, and aflatoxins B1, B2, G1, and G2.
    - The strain of *A. oryzae* used in the production of GODO-FAL, *A. oryzae* strain GD-FAL, lacks the genes necessary to produce aflatoxins.

  - Three lots of GODO-FAL did not contain detectable levels of the secondary metabolites kojic acid, cyclopiazonic acid, or 3-nitropropionic acid.

- All steps in the GODO-FAL manufacturing process follow current good manufacturing practices (cGMP), using food grade processing aids and food contact materials.

  - GODO-FAL is produced using an industry standard production process which is also used to produce the subjects of GRNs 743, 649, 579, 572, 510, and 132.

- Appropriate specifications and quality control parameters assure the production of a food grade product.

- Published toxicology studies demonstrate the safety of GODO-FAL:

  - Genotoxicology assays of GODO-FAL include a bacterial reverse mutation assay, an in vivo micronucleus assay, and a chromosome aberration assay. GODO-FAL was not genotoxic in these three assays.

  - The safety of GODO-FAL was assessed in toxicology studies including an acute toxicity study, a 28-day oral range finding study in rats and a 90-day subchronic toxicity study in rats. The results of the 28-day oral range finding study and the 90 day subchronic toxicity study were published by Symonds et al. (2020). The LD_{50} of GODO-FAL was greater than 2000 mg/kg and there were no test article related adverse effects noted in the 28-day range-finding study at doses up to 2000 mg/kg/day. The subchronic toxicity study was performed in male and female rats administered 0 or 2000 mg/kg/day (total organic solids (TOS) 206 mg/kg/day). No test article related adverse effects were noted; the no observed adverse effect level (NOAEL) was determined to be at least 2000 mg/kg/day.

  - Because GODO-FAL is essentially equivalent to other lactases that are GRAS, toxicology studies performed using other sources of lactase as the test article support the safety of GODO-FAL. These studies establish NOAELs of at least 4000 mg/kg/day (TOS not reported), 1646 mg/kg/day(TOS not reported), and 2000 mg/kg/day (TOS 1800 mg/kg/day) (Flood and Kondo 2004; Zou et al., 2014; Ke et al., 2018;), the highest doses tested.

  - Based on the fact that none of the safety studies showed any signs of toxicity, the long history of use of lactase, and that GODO-FAL is essentially equivalent to other lactases that are GRAS, it can be concluded that the use of GODO-FAL for the intended purpose is safe.

- The intended use of GODO-FAL is to hydrolyze the lactose in fluid milk, whey, cheese, yogurt, and other dairy products. The enzyme will be used at the minimum level necessary to achieve the desired effect and according to requirements for normal production following cGMP.

- GODO-FAL will be used as a processing aid and will have no function in the finished food. The enzyme is either heat denatured or inactivated during production.

- The intended use and estimated intake of GODO-FAL will be the same as described for the lactase that is the subject of GRN 825, because GODO-FAL is intended to be used as a substitute for other lactases that are GRAS. In GRN 825, the estimated daily intake of lactase for users 2 years of age and older was calculated to be 3.7 mg TOS/kg body weight/day. For infant formula applications, the estimated daily intake was computed to be 9.6 mg TOS/kg body weight/day, assuming a maximum amount of lactase as 36 mg TOS/kg milk raw material and the maximum consumption of 267 g infant formula/kg body weight/day.

Therefore, GODO-FAL is safe and GRAS for the proposed use as a processing aid in the hydrolysis of lactose in fluid milk, whey, cheese, yogurt, and other dairy products and is, therefore, excluded from the definition of a food additive and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR. Godo Shusei Co., Ltd. therefore concludes that GODO-FAL is GRAS for its intended uses and use levels.

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature [redacted]

Date: August 31, 2021

A. Wallace Hayes, PhD, DABT, FATS, ERT
GRAS Expert Panel Member
University of South Florida College of Public Health

Signature: [redacted]

Date: August 31, 2021

Thomas E. Sox, PhD, JD
GRAS Expert Panel Member
Principal, Pondview Consulting LLC

Signature: [redacted]

Date: August 31, 2021

Claire Kruger, PhD, DABT
Scientific Advisor to the Panel

Signature: [redacted]

Date: August 31, 2021

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
GRN 001039	September 22, 2021
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person YoshihikoSuitani		Position or Title Quality Assurance Manager	
	Organization *(if applicable)* Godo Shusei Co., Ltd.			
	Mailing Address *(number and street)* 1-17-6, Higashikomagata			
City Sumida-ku	State or Province Tokyo	Zip Code/Postal Code 130-0005	Country Japan	
Telephone Number +81 3-3575-2611	Fax Number	E-Mail Address y-suitani@oenon.jp		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person Claire L. Kruger, PhD, DABT		Position or Title Managing Partner	
	Organization *(if applicable)* Spherix Consulting Group, Inc.			
	Mailing Address *(number and street)* 751 Rockville Pike, Unit 30-B			
City Rockville	State or Province Maryland	Zip Code/Postal Code 20852	Country United States of America	
Telephone Number 301-775-9476	Fax Number	E-Mail Address ckruger@spherixgroup.com		

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Lactase, β-galactosidase (IUB Number: 3.2.1.23) (GODO-FAL)

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 825 and 510

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

The lactase derived from A. oryzae strain GD-FAL is intended to be used as a substitute for the subject of GRN 825 with the same uses and use levels. This includes the processing of milk, milk powder, fermented milk products and yogurt, fresh cheese, milk-based desserts, whey, baked goods, confectionary, cereal bars, soft drinks, and in the processing of milk for non-exempt infant formulas. Importantly, the enzyme will be either denatured or inactivated during production of the final food product to render it non-functional.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Godo Shusei Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Lactase, β-galactosidase (IUB Number: 3.2.1.23) (GODO-FAL)
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Godo Shusei Co., Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

1-17-6, Higashikomagata, Sumida-ku, Tokyo 130-0005 Japan
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance. The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Claire L. Kruger, PhD Digitally signed by Claire L. Kruger, PhD Date 2021 09 08 16 21:11-04'00'	Claire L. Kruger, Managing Partner	09/08/2021

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert Clear Godo GRAS 9-8-21 - Signed	Submission
2	Insert Clear All References	Submission
	Insert Clear	
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