ChromaDex
SPHERIX CONSULTING

#800

June 26, 2018

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

To Whom It May Concern:

Enclosed please find GRAS notification entitled "Generally Recognized As Safe (GRAS) Determination of γ-Oryzanol in Selected Foods". This GRAS notification has been prepared by ChromaDex Spherix Consulting on behalf of its client, Oryza Oil & Fat Chemical Co., Ltd.

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 L. Kruger, Ph.D., D.A.B.T., President, ChromaDex Spherix Consulting, 11821 Parklawn Drive, Suite 310, Rockville, MD 20852, Telephone: 301-230-2181; Email: clairek@chromadex.com, or will be sent to FDA upon request.

Should you have any questions or concerns, please contact me at the number listed above.

Sincerely,
(b) (6)

Claire L. Kruger, Ph.D., D.A.B.T.
President

Enclosures:

Signed Form 3667

Unsigned Form 3667 with All Files Embedded (please note that a persistent error message prevented us from adding an electronic signature to this file)

Generally Recognized As Safe (GRAS) Determination of γ-Oryzanol in Selected Foods

All References Cited in the Above-Referenced GRAS Notification

Flash Drive Containing All of the Above-Referenced Files

11821 Parklawn Drive, Suite 310
Rockville, MD 20852
T: (301) 230-2180; F: (301) 230-2188
https://chromadex.com/consulting-overview/

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 000800	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

RECEIVED
JUN 27 2018
OFFICE OF FOOD ADDITIVE SAFETY

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	Position or Title
Norihito Shimizu	Section Chief, Food Development Department

Organization *(if applicable)*: Oryza Oil & Fat Chemical Co., Ltd.

Mailing Address *(number and street)*: 1 Aza Numata Kitagata, Kitagata-cho,

City	State or Province	Zip Code/Postal Code	Country
Ichinomiya-city	Aichi-pref	493-8001	Japan

Telephone Number	Fax Number	E-Mail Address
+81-586-86-5141	+81-586-86-6191	kaihatsu@mri.biglobe.ne.jp

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Claire L. Kruger, PhD, DABT	President

Organization *(if applicable)*: ChromaDex Spherix Consulting

Mailing Address *(number and street)*: 11821 Parklawn Drive, Suite 310

City	State or Province	Zip Code/Postal Code	Country
Rockville	MD	20852	USA

Telephone Number	Fax Number	E-Mail Address
301-230-2181	301-230-2188	clairek@chromadex.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

γ-Oryzanol

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

Hard copy and a flash drive containing all files

3. For paper submissions only:

Number of volumes 1

Total number of pages 66 plus all references

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 _____

☐ 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.

Oryza intends to add ORYZA GAMMAX to selected foods and beverages in the U.S. food supply, including meat, poultry, and fish products, dried bean, pea, nut and seed products, grain products, fruit and vegetable products, oils and salad dressings, sugars, sweets, and beverages.

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 Oryza Oil & Fat Chemical Co., Ltd.
(name of notifier)

has concluded that the intended use(s) of γ-Oryzanol
(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. Oryza Oil & Fat Chemical Co., Ltd. *(name of notifier)* 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.

1 Aza Numata Kitagata, Kitagata-cho, Ichinomiya-city, Aichi-pref 493-8001 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)
(b) (6)	Claire L. Kruger, President, ChromaDex Spherix Consulting	06/26/2018

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)
	Gamma Oryazanol GRAS Notification to FDA 6-26-18.pdf	Submission
	Accinni 2006.pdf	Submission
	Berger 2005.pdf	Submission
	Bodner-Montville 2006.pdf	Submission
	CDC 2006.pdf	Submission
	CIREP 2006.pdf	Submission
	Endo 1968.pdf	Submission
	Eslami 2014.pdf	Submission
	Fry 1997.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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)
	Fujiwara 1980.pdf	Submission
	Fujiwara 1983.pdf	Submission
	Ghatak and Panchal 2012.pdf	Submission
	Gregory 1995.pdf	Submission
	Hasato 1974.pdf	Submission
	Kaneko 1954.pdf	Submission
	Kobayashi 2016.pdf	Submission
	Lerma-Garcia 2009.pdf	Submission
	Ling 1995.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Lubinus 2013.pdf	Submission
	MacDaniel 1993.pdf	Submission
	Mandak 2012.pdf	Submission
	Maruoka 1972.pdf	Submission
	Mattsson 1996.pdf	Submission
	Moon 2017.pdf	Submission
	Moon 2018 - Corrigendum.pdf	Submission
	Noda 1974.pdf	Submission
	Noda 1975.pdf	Submission

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

PART VIII – LIST OF ATTACHMENTS *(continued)*

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)
	Oka 2010.pdf	Submission
	Sasaki 1990.pdf	Submission
	SCOGS 1979.pdf	Submission
	Tamagawa 1992a.pdf	Submission
	Tamagawa 1992b.pdf	Submission
	Tsushimoto 1991.pdf	Submission
	USDA 2012.pdf	Submission
	Van den Berg 2006.pdf	Submission
	Wheeler 1991.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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)
	Yoshino 1989.pdf	Submission
	Zhu 2015.pdf	Submission

Generally Recognized As Safe (GRAS) Determination of γ-Oryzanol in Selected Foods

Prepared for:

Oryza Oil & Fat Chemical Co., Ltd.
1 Aza Numata Kitagata, Kitagata-cho,
Ichinomiya-city,
Aichi-pref 493-8001
Japan

Prepared by:

ChromaDex Spherix Consulting
A Business Unit of ChromaDex, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852
USA

June 5, 2018

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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

Oryza Oil & Fat Chemical Co., Ltd is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

Oryza Oil & Fat Chemical Co., Ltd.
1 Aza Numata Kitagata, Kitagata-cho,
Ichinomiya-city,
Aichi-pref 493-8001
Japan

Contact: Tadashi Okada
Chief, Legal Affairs Section
Phone number: +81-586-86-5141
Fax number: +81-586-86-6191

C. COMMON OR USUAL NAME

γ-Oryzanol

Trade Name: ORYZA GAMMAX

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

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

E. INTENDED USE

Oryza intends to add ORYZA GAMMAX (γ-oryzanol) to selected foods and beverages in the U.S. food supply.

F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of γ-oryzanol for the intended uses specified above 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 intake of

ORYZA GAMMAX 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 substances directly added to food, and is based on generally available and accepted information.

The proposed use of ORYZA GAMMAX as an ingredient for the intended uses in foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. γ-Oryzanol is a dried, refined, and bleached extract of *Oryza sativa Japonica* that contains a mixture of sterol ferulates, including cycloartenol ferulate, 24-methlene cycloartanol ferulate, campesterol ferulate, β-sitosteryl ferulate, cycloartanol ferulate, and cyclobranol ferulate, which account for not less than 85% of the finished product.

2. All *O. sativa* rice bran and germ used to manufacture ORYZA GAMMAX are grown by farmers that comply with Good Agricultural Practices (GAPs) and all applicable regulations for import of raw agricultural commodities into the United States, including compliance with pesticide tolerances.

3. ORYZA GAMMAX is manufactured from *Oryza sativa Japonica* rice bran in a series of stages according to Good Manufacturing Practices (GMPs).

4. All processing aids used in the production of ORYZA GAMMAX are Food Chemicals Codex (FCC) grade.

5. Product specifications and other quality testing (solvents, pesticides, mycotoxins, PCBs/dioxins/furans, allergens) are in place to control the levels of the predominating sterol ferulates, heavy metals, and microbes, and to ensure a consistent and food grade finished product.

6. Sterol ferulates are poorly absorbed from the intestinal tract, similar to other plant sterols, are distributed to a variety of tissues, including the adrenal glands, lungs, spleen, and liver, and are metabolized to ferulic acid, *m*-coumaric acid, *m*-hydroxy-hippuric acid, hippuric acid, *m*-hydroxyphenyl propionic acid, and dihydroferulic acid.

7. The safety of ORYZA GAMMAX was determined in a pivotal published 90-day toxicology study that identified a no observed adverse effect level (NOAEL) of 2000 mg γ-Oryzanol/kg/day (Moon et al., 2017).

8. Application of a 100-fold safety factor to the NOAEL determined in the pivotal 90-day toxicology study results in an acceptable daily intake for ORYZA GAMMAX of 20 mg/kg/day or 1.2 g/day for a 60 kg human.

9. The safety of ORYZA GAMMAX is corroborated by carcinogenicity studies conducted in rats and mice, a one-year chronic toxicity in rats, developmental toxicity in rats and mice, and genotoxicity studies using other γ-oryzanol-containing products.

10. Clinical studies have reported that γ-oryzanol-containing products are well tolerated up 3.4 g/day for up to six months.

11. The addition of ORYZA GAMMAX to the intended foods will result in a mean estimated daily intake (EDI) of 139 mg/day (2.0 mg/kg body weight/day) and a heavy consumer (90^{th} percentile) intake of 313 mg/day (4.6 mg/kg body weight/day). There are no known significant dietary sources of γ-oryzanol in the United States.

12. The EDI is substantially below the ADI, establishing the safety of ORYZA GAMMAX intake from the intended uses and use levels.

Determination of the GRAS status of ORYZA GAMMAX under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, CFS, FIFT, FASN, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, FACN, and Thomas Sox PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of ORYZA GAMMAX and the human exposure to ORYZA GAMMAX resulting from its intended use as an ingredient in selected foods:

> *There is no evidence in the available information on* ORYZA GAMMAX *that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when* ORYZA GAMMAX *is used at levels that might reasonably be expected from the proposed applications of* ORYZA GAMMAX *for use in selected food as proposed by Oryza Oil & Fat Chemical Co., Ltd.*

Therefore, ORYZA GAMMAX is safe and GRAS at the proposed levels of addition to the intended foods. ORYZA GAMMAX 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.

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, President, ChromaDex Spherix Consulting, A Business Unit of ChromaDex, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-230-2180; Email: clairek@chromadex.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 Oryza Oil & Fat Chemical Co., Ltd. and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

(b) (6)

Signature of Authorized Representative of
Oryza Oil & Fat Chemical Co., Ltd.

June 6, 2018
Date

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

A. COMMON OR USUAL NAME

γ-Oryzanol

B. TRADE NAME

ORYZA GAMMAX

C. DESCRIPTION OF ORYZA GAMMAX

ORYZA GAMMAX is a dried, refined, and bleached extract of *Oryza sativa Japonica* that contains a mixture of sterol ferulates. The major sterol ferulates in *Oryza sativa* include cycloartenol ferulate, 24-methylene cycloartanol ferulate, campesterol ferulate, β-sitosteryl ferulate, cycloartanol ferulate, and cyclobranol ferulate. Collectively, these sterol ferulates account for not less than 85% of ORYZA GAMMAX (Figure 1; Table 1). The remaining components of ORYZA GAMMAX include other less abundant sterol ferulates and lipids.

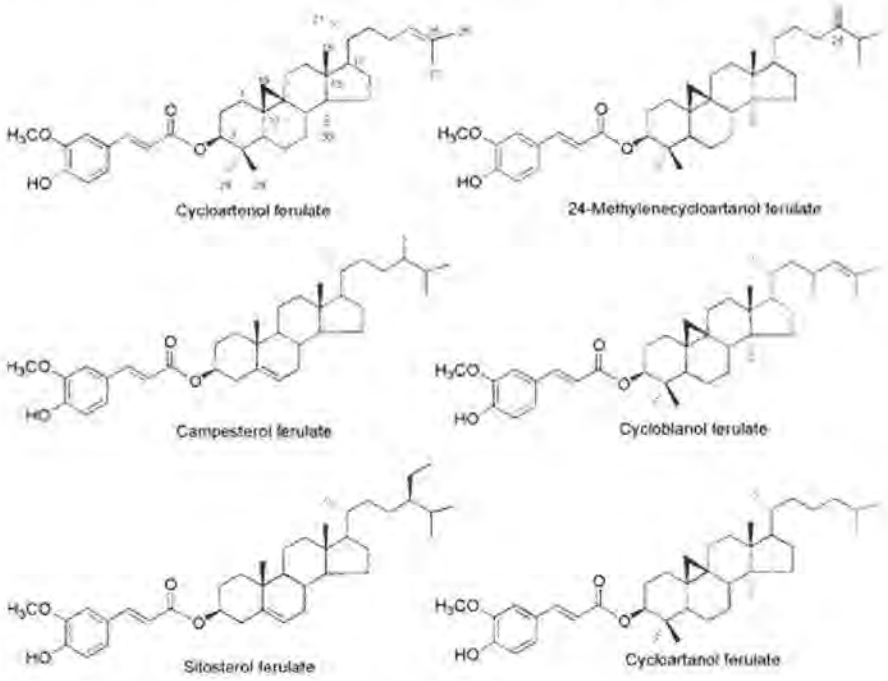

Figure 1. Structures of Sterol Ferulates:
cycloartenol ferulate, 24-methylene cycloartanol ferulate, campesterol ferulate, cycloartanol ferulate, β-sitosteryl ferulate, and cyclobranol ferulate (Moon et al., 2017)

Table 1. Relative Amount of Primary Sterol Ferulates in ORYZA GAMMAX	
Components[2]	**Amount (%w/w)[1]**
Cycloartenol ferulate	27.8 ± 1.3
24-methylene cycloartanol ferulate	38.3 ± 2.6
Campesterol ferulate	17.6 ± 0.9
β-Sitosterol ferulate and cycloartanol ferulate	6.2 ± 0.4
Cyclobranol ferulate	0.9 ± 1.0
[1]Amount = Average ± standard deviation (n=4). [2]Determined by liquid chromatography coupled with tandem mass spectrometry.	

D. PRODUCTION PROCESS

1. Production of ORYZA GAMMAX

ORYZA GAMMAX is manufactured using a combination of continuous and batch processes from *Oryza sativa Japonica* rice bran on a dedicated line for rice oil and γ-oryzanol at the Oryza Oil & Fat Chemical Co., Ltd production facility (Figure 2). The facility is compliant with good manufacturing practices (GMPs) and ISO 22000:2005. No raw materials or products containing soy, nuts or fish proteins are used in the production facility.

All incoming *O. sativa* rice bran and rice germ is stored in a silo dedicated for rice bran for not more than one day and tested for compliance to established acceptance criteria. The incoming material must contain more than 15% oil content, less than 14% moisture content and a 40-acid value. In a continuous process, the accepted material is mixed with hexane to extract rice bran/rice germ oil, which is then mixed with food-grade sodium hydroxide solution and centrifuged. The resulting supernatant fraction is saved and used as edible rice bran oil, and the lower layer, also known as the "foots" or soap, is dissolved in methanol. The mixture is neutralized with sulfuric acid, mixed with hexane, and allowed to settle into two phases. The upper phase is collected, checked for γ-oryzanol content, filtered using a stainless steel 5 μm filter, and washed with hexane to yield crude oryzanol.

Using a batch process, the crude oryzanol is dehydrated with hexane, bleached with activated charcoal and diatomaceous earth, filtered through a 0.5 μm cotton filter. The resulting material is then distilled to remove the volatile solvents, producing wet oryzanol. The distilled volatile solvents are collected, separated, and recycled whereas the wet oryzanol is vacuum dried, ground using a pin mill grinder, and sifted through a #40 mesh screen. The finished product is then packaged in food-grade polyethylene aluminum bags and tested for compliance

with the product specifications. Importantly, the quality of the finished product is ensured by monitoring: 1) oil content, moisture content, and acid value of the incoming rice bran and rice germ; 2) acid value, total moisture, and insoluble impurities in the rice bran and rice germ oil; 3) acid value of the soap; and 4) γ-oryzanol content of the upper layer during the production process.

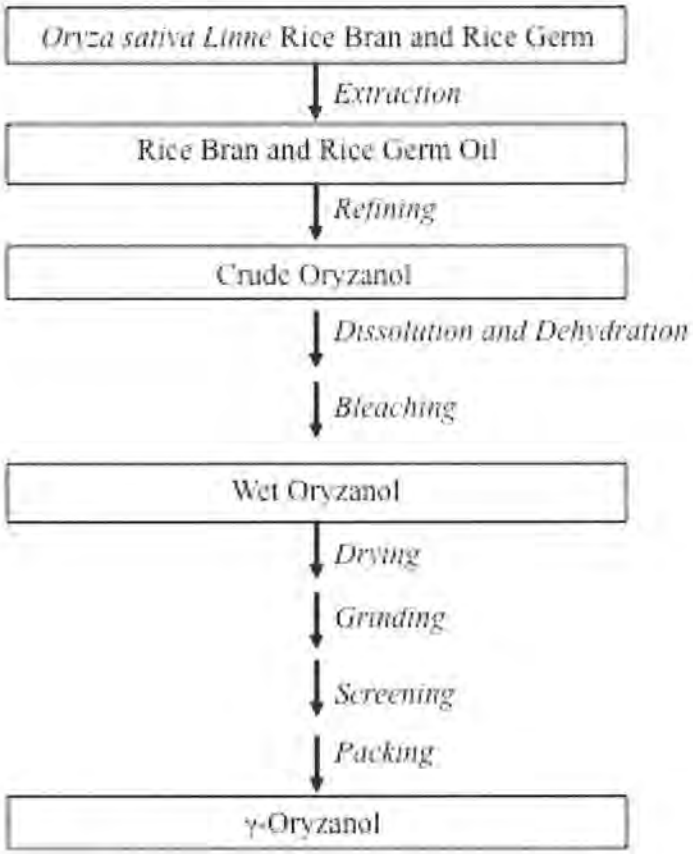

Figure 2. Production of γ-Oryzanol (ORYZA GAMMAX)

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

a. Food Contact Substances

The food contact substances used in the production of ORYZA GAMMAX include stainless steel, cotton, and food-grade polyethylene.

b. Raw Materials

The *O. sativa* rice bran and rice germ used to manufacture ORYZA GAMMAX is grown by farmers that comply with Japanese Good Agricultural Practices (GAPs) and all applicable regulations for import of raw agricultural commodities into the United States, including compliance with specified pesticide tolerances (21 CFR § 40.180). Additionally, the *O. sativa* rice bran and rice germ are milled in rice milling factories that comply with the Japanese Food Sanitation Law. All incoming raw material is tested and must contain more than 15% oil content, less than 14% moisture and a 40-acid value. If the material does not meet the Oryza Oil & Fat Chemical Co., Ltd acceptance criteria, it is returned to the supplier.

c. Processing Aids

The processing aids used in the production of ORYZA GAMMAX include hexane, methanol, sodium hydroxide, sulfuric acid, diatomaceous earth, and activated carbon (Table 2). All are Food Chemicals Codex (FCC) grade and either comply with the uses specified in Title 21 of the US Code of Federal Regulations or have been used in the production of other food products. Hexane, methanol, and activated charcoal, specifically, have been determined GRAS for use in the production of food oils (pre-1958) and triglycerides, and water purification (GRN 94; GRN 306; GRN 326; GRN 200; GRN 138; GRN 193; GRN 322; GRN 425; 21 CFR § 173.165; 21 CFR § 173.25). Additionally, all lots are monitored for solvent residues by gas chromatography or gas chromatography coupled with mass spectrometry with a limit of detection of 5 ppm.

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications

To ensure a consistent food-grade product, Oryza Oil and Fat Chemical Co., Ltd tests each lot of ORYZA GAMMAX for compliance with a defined set of product specifications (Table 3). Data from three lots of ORYZA GAMMAX demonstrate control of the production process and compliance with the product specifications.

Table 2. Compliance of Processing Aids and Food Contact Materials	
Processing Aid	**US Regulation**
N-Hexane	Although there are no US regulations stating that hexane can be used as a processing aid in the extraction of grain oils, hexane and similar paraffinic hydrocarbons were determined GRAS in the production of food oils (GRN 306). Hexane has also been determined GRAS for use as an extraction solvent for edible oils used in infant formulas (GRN 94 and 326).
Sodium Hydroxide	21 CFR § 184.1763
Sulfuric Acid	21 CFR § 184.1095
Methanol	There are no US regulations stating that methanol can be used as a processing aid in the extraction of grain oils. However, methanol has been determined GRAS for use in the production of tailored triglycerides in omega-3 fatty acids from fish oil (GRN 200).
Diatomaceous Earth	SCOGS report #61, 1979
Activated Carbon	Although there are no US regulations stating that activated carbon can be used as a processing aid in the extraction of grain oils, 21 CFR 173.165 and 173.25 states that activated carbon can be used in the purification of the water used in the production of enzyme preparations and micro-organisms or in the treatment of water used in the production of distilled alcoholic beverages, respectively. Activated carbon is also GRAS for use in the production of fish, refined pine nut, and canola oils intended for conventional foods and infant formulas (GRN 138, 193, 326, 332, and 425).
Polyethylene	21 CFR § 177.1520
Cotton	21 CFR § 177.2260
CFR = Code of Federal Regulations; GRAS = Generally Recognized As Safe; GRN= GRAS Notification; SCOGS = Scientific Community of GRAS Substances	

Table 3. Product Specifications and Lot Data for ORYZA GAMMAX

Parameter	Method	Specification	Lot Number			
			U-523	U-530	M-506*	Q-631
			Date of Manufacture			
			1-23-15	1-30-15	2-6-15	8-31-16
Physical/Chemical Characteristics						
Appearance	Visual inspection	White or light yellowish crystalline powder	Comply	Comply	Comply	Comply
Odor	Physical inspection	No or slight characteristic odor	Comply	Comply	Comply	Comply
γ-Oryzanol (%)[1]	HPLC	≥ 85 %	92.5	89.4	90.1	90.8
Melting point (°C)	The Japanese Standards for Food Additives	150 – 185°C	169	165	164	164
Loss on drying (%)	105°C for 2 hr	≤ 0.5%	0.12	0.05	0.08	0.08
Residue on ignition (%)	550°C for 3 hr	≤ 0.5%	0.04	0.01	0.05	0.05
Heavy Metals						
Arsenic (as As_2O_3)	Atomic absorption spectrophotometry[2]	≤ 1 ppm	≤ 1	≤ 1	≤ 1	≤ 1
Lead	Atomic absorption spectrophotometry[2]	≤ 1 ppm	0.3	0.7	0.3	0.2
Mercury	Cold vapor atomic absorption spectrophotometry[2]	≤ 0.1 ppm	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Cadmium	Atomic absorption spectrophotometry[2]	≤ 0.1 ppm	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Tungsten	ICP-OES (US EPA 3056)[3]	≤ 50 ppm	≤ 50	≤ 50	≤ 50	≤ 50
Microbes						
Standard Plate Count	Standard Plate Count Agar[4]	≤ 100 cfu/g	≤ 100	≤ 100	≤ 100	≤ 100
Molds and Yeasts	Potato Dextrose Agar[4]	≤ 100 cfu/g	≤ 100	≤ 100	≤ 100	≤ 100
Coliforms	Brilliant Green Lactate Bile Medium[4]	Negative/10 g	ND	ND	ND	ND

Neg. = Negative; HPLC = high performance liquid chromatography; AOAC = Association of Official Analytical Chemists; ICP-OES = inductively coupled plasma atomic emission spectroscopy; ND = not detected

[1]γ-Oryzanol represents the amount of cycloartenol ferulate, 24-methlene cycloartanol ferulate, campesterol ferulate, β-sitosteryl ferulate, and cyclobranol ferulate in the finished product.

[2]Performed by Hokuriku Institute of Environmental Science Co., Ltd; limit of detection for lead, mercury, cadmium, and arsenic are 0.2 ppm, 0.01 ppm, 0.02 ppm, and 0.05 ppm, respectively.

[3]Performed by SGS-CSTC Standards Technical Services (Shanghai) Co., Ltd; limit of detection is 50 ppm.

[4]These methods are consistent with the compendial methods specified in Japan's "Methods of Analysis in Health Science".

*Used in 90-day repeat oral dose toxicology study in rats.

2. Other Quality Attributes

To more fully understand the quality of ORYZA GAMMAX, Oryza Oil and Fat Chemical Co., Ltd quantified the levels of sterol ferulates, residual solvents, dioxins, PCBs, pesticides, mycotoxins, allergens, and pathogenic bacteria in the finished product.

a. Relative Quantity of Sterol Ferulates in γ-Oryzanol

To evaluate the variability in the levels of primary sterol ferulates in ORYZA GAMMAX, Oryza Oil and Fat Chemical Co. quantified the amount cycloartenol ferulate, 24-methylene cycloartenol ferulate, campesterol ferulate, cyclobranol ferulate, and β-sitosterol ferulate and cycloartenol ferulate in four lots of finished product (Table 4). Each lot of ORYZA GAMMAX contained similar amounts of the different primary sterol ferulates. Moreover, the lot used in the 90-day toxicology study was representative of other lot used for commercial purposes.

Table 4. Relative Amount of Sterol Ferulates in ORYZA GAMMAX

Components[1]	Lot Number				Average Amount ± St. Dev. (%w/w)
	U-523	U-530	M-506*	Q-631	
Cycloartenol ferulate	28.9	25.9	28.1	28.1	27.8 ± 1.3
24-methylene cycloartanol ferulate	41.7	39.0	36.0	36.6	38.3 ± 2.6
Campesterol ferulate	16.3	18.0	18.0	18.1	17.6 ± 0.9
β-Sitosterol ferulate and cycloartanol ferulate	5.6	6.5	6.2	6.3	6.2 ± 0.4
Cyclobranol ferulate	0	0	1.8	1.7	0.9 ± 1.0

*Used in 90-day repeat oral dose toxicology study in rats.
[1]Determined by liquid chromatography coupled with tandem mass spectrometry.

b. Solvent Residues in ORYZA GAMMAX

Because hexane and methanol are used during the production of ORYZA GAMMAX, Oryza monitors all lots of ORYZA GAMMAX for the presence of these and other solvents using gas chromatography or gas chromatography-mass spectrometry (Table 5). Analytical testing of three lots ORYZA GAMMAX show that residual solvents are undetectable with a limit of detection of 5 ppm.

Table 5. Potential Solvents Residues in ORYZA GAMMAX					
Solvents	Analytical Method	Limit of Detection	Lot Number		
			U-523	U-530	M-506*
Acetone	GC/MS	5 ppm	ND	ND	ND
Dichloromethane	GC/MS	5 ppm	ND	ND	ND
methyl tertiary butyl ether (MTBE)	GC/MS	5 ppm	ND	ND	ND
n-Hexane	GC/MS	5 ppm	ND	ND	ND
Acetic ether	GC/MS	5 ppm	ND	ND	ND
Cyclohexane	GC/MS	5 ppm	ND	ND	ND
Toluene	GC/MS	5 ppm	ND	ND	ND
Methanol	GC	5 ppm	ND	ND	ND
Isopropanol	GC	5 ppm	ND	ND	ND
Ethanol	GC	5 ppm	ND	ND	ND
GC-MS = Gas Chromatography – mass spectrometry; ppm = parts per million; ND = not detected *Used in 90-day repeat oral dose toxicology study in rats.					

c. Pathogenic Bacteria in ORYZA GAMMAX

In addition to quantifying the amount aerobic bacteria, yeasts and molds, and coliforms for batch release, Oryza monitors finished product for presence of *Staphylococcus aureus*, *Salmonella*, *Bacillus cereus*, *Cronobacter sakazakii*, *Listeria monocytogenes*, and *Escherichia coli* against quality specifications (Table 6) using methods that are either consistent with the compendial methods specified in Japan's "Methods of Analysis in Health Science" or the 16th edition of the Japanese Pharmacopeia. Data from three lots of ORYZA GAMMAX show that these bacteria were not detected. These parameters will be monitored in every seventh lot until a reduced testing frequency can be justified.

Table 6. Pathogenic Bacteria in ORYZA GAMMAX[1]

Bacteria	Analytical Method	Monitoring Specification	Lot Number		
			U-530	M-506*	Z1201
Staphylococcus aureus[2]	Mannitol Salt Egg-yolk Agar	Neg./25 g	ND	ND	ND
Salmonella[2]	Buffered Peptone Water, Rappaport-Vassiliadis Broth, DHL Agar	Neg./25 g	ND	ND	ND
Bacillus cereus[2]	NGKG Agar Base add Egg-yolk	Neg./25g	ND	ND	ND
Cronobacter sakazakii[3]	Enrichment Culture Method	Neg./10 g	ND	ND	ND
Listeria monocytogenes[4]	Immunochromatography	Neg./25 g	ND	ND	ND
Escherichia coli[2]	EC Broth	Neg./25 g	ND	ND	ND

ND = not detected
[1]Monitored once every three years.
[2]Methods are consistent with the compendial methods specified in Japan's "Methods of Analysis in Health Science".
[3]Methods is consistent with the compendial method specified in 16th edition of the Japanese Pharmacopeia.
[4]In-house validated method.
*Used in 90-day repeat oral dose toxicology study in rats.

d. Pesticides Residues in ORYZA GAMMAX

Although the maximum residue limits (MRLs) for pesticides specified in Title 40 of the United States Code of Federal Regulations apply to only raw agricultural products, Oryza performed a pesticide screen in four lots of ORYZA GAMMAX, including the lot used in a 90-day rat toxicology study, using LC-MS/MS or GC-MS/MS (Table 7). For all the pesticides analyzed, the limits of detection were below the MRLs for brown rice. All pesticides including aldrin (LOD = 1 ppb), dieldrin (LOD = 3 ppb), and endrin (LOD = 2 ppb), which have been banded from use in the United States, were not detected. Therefore, the production process for ORYZA GAMMAX does not appear to enrich for and/or concentrate pesticides.

Table 7. Pesticide Residues in ORYZA GAMMAX							
Compound	Analytical Method	Limit of Quantitation (ppm)	MRL (ppm)[1]	Lot Number			
				U-530	M-506*	Z1201	Q1302
Diflubenzuron	LC/MS/MS	0.01	1	ND	ND	ND	NA
Chlorpyrifos	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Chlorpyrifos-methyl	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Cyhalothrin	GC/MS/MS	0.01	0.5	ND	ND	ND	NA
Tebuconazole	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Cypermethrin	GC/MS/MS	0.01	0.9	ND	ND	ND	NA
Trifloxystrobin	LC/MS/MS	0.01	2	ND	ND	ND	NA
Paraquat	LC/MS	0.05	0.1	ND	ND	ND	NA
Diquat	LC/MS	0.05	1	ND	ND	ND	NA
α-BHC	GC/MS/MS	0.002	0.2	ND	ND	ND	NA
β-BHC	GC/MS/MS	0.002	0.2	ND	ND	ND	NA
γ-BHC	GC/MS/MS	0.001	0.3	ND	ND	ND	NA
δ-BHC	GC/MS/MS	0.002	0.2	ND	ND	ND	NA
Aldrin	GC/MS/MS	0.001	ND	ND	ND	ND	NA
Dieldrin	GC/MS/MS	0.003	ND	ND	ND	ND	NA
Endrin	GC/MS/MS	0.002	ND	ND	ND	ND	NA
Endosulfan-sulfate	GC/MS/MS	0.002	0.1	ND	ND	ND	NA
α-Endosulfan	GC/MS/MS	0.001	0.1	ND	ND	ND	NA
β-Endosulfan	GC/MS/MS	0.001	0.1	ND	ND	ND	NA
Chlordane	GC/MS/MS	0.002	0.02	ND	ND	ND	NA
Heptachlor	GC/MS/MS	0.002	0.02	ND	ND	ND	NA
Heptachlor epoxide	GC/MS/MS	0.002	0.02	ND	ND	ND	NA
Methoxychlor	GC/MS/MS	0.004	2	ND	ND	ND	NA
Mirex	GC/MS/MS	0.002	-	ND	ND	ND	NA
o,p'-DDD	GC/MS/MS	0.002	-	ND	ND	ND	NA
o,p'-DDE	GC/MS/MS	0.002	-	ND	ND	ND	NA
o,p'-DDT	GC/MS/MS	0.002	0.2	ND	ND	ND	NA
p,p'-DDD	GC/MS/MS	0.001	0.2	ND	ND	ND	NA
p,p'-DDE	GC/MS/MS	0.003	0.2	ND	ND	ND	NA
p,p'-DDT	GC/MS/MS	0.002	0.2	ND	ND	ND	NA
Azimsulfuron	LC/MS/MS	0.01	0.02	ND	ND	ND	NA
Azoxystrobin	LC/MS/MS	0.01	0.2	ND	ND	ND	NA
Anilofos	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Isoxathion	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Isotianil	LC/MS/MS	0.01	0.3	ND	ND	ND	NA
Isoprothiolane	GC/MS/MS	0.01	10	ND	ND	ND	NA
Ipconazol	LC/MS/MS	0.01	-	ND	ND	ND	NA
Ipfencarbazone	LC/MS/MS	0.01	0.05	ND	ND	NA	NA
Iprobenfos	GC/MS/MS	0.01	0.2	ND	ND	ND	NA

Table 7. Pesticide Residues in ORYZA GAMMAX							
Compound	Analytical Method	Limit of Quantitation (ppm)	MRL (ppm)[1]	Lot Number			
				U-530	M-506*	Z1201	Q1302
Imazosulfuron	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Imidacloprid	LC/MS/MS	0.01	1	ND	ND	ND	NA
Indanofan	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Uniconazol P	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Esprocarb	GC/MS/MS	0.01	0.02	ND	ND	ND	NA
Ethiprole	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Edifenphos	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Ethoxysulfuron	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Etofenprox	GC/MS/MS	0.01	0.5	ND	ND	ND	NA
Etobenzanid	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Oxadiazon	GC/MS/MS	0.01	0.02	ND	ND	ND	NA
Oxadiargyl	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Oxaziclomefone	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Orysastrobin	LC/MS/MS	0.01	0.2	ND	ND	ND	NA
Cafenstrole	LC/MS/MS	0.01	0.02	ND	ND	ND	NA
Carbaryl	GC/MS/MS	0.01	1.0	ND	ND	ND	NA
Carfentrazone-ethyl	GC/MS/MS	0.01	0.08	ND	ND	ND	NA
Carpropamid	LC/MS/MS	0.01	1	ND	ND	ND	NA
Carbendazim, Thiophanate-methyl	LC/MS/MS	0.01	1	ND	ND	ND	NA
Carbosulfan	LC/MS/MS	0.01	0.02	ND	ND	ND	NA
Quinoclamin	GC/MS/MS	0.01	0.03	ND	ND	ND	NA
Cumyluron	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Clothianidin	LC/MS/MS	0.01	0.7	ND	ND	ND	NA
Chromafenazide	LC/MS/MS	0.01	0.2	ND	ND	ND	NA
Clomeprop	GC/MS/MS	0.01	0.02	ND	ND	ND	NA
Chlorantraniliprole	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Chlorothalonil	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Chantraniliprole	LC/MS/MS	0.01	0.05	ND	ND	NA	NA
Diclocymet	LC/MS/MS	0.01	0.5	ND	ND	ND	NA
Cyclosulfamuron	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Diclomezine	GC/MS/MS	0.01	2	ND	ND	ND	NA
Dichlorvos	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Dinotefuran	LC/MS/MS	0.01	2	ND	ND	ND	NA
Cyhalofop-butyl	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Simeconazole	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Dimethametryn	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Simetryn	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Silafluofen	GC/MS/MS	0.01	0.3	ND	ND	ND	NA
Spinetoram	LC/MS/MS	0.01	0.1	ND	ND	ND	NA

Table 7. Pesticide Residues in ORYZA GAMMAX							
Compound	Analytical Method	Limit of Quantitation (ppm)	MRL (ppm)[1]	Lot Number			
				U-530	M-506*	Z1201	Q1302
Diazinon	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Daimuron	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Thiacloprid	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Tiadinil	LC/MS/MS	0.01	1	ND	ND	ND	NA
Thiamehtoxam	LC/MS/MS	0.01	0.3	ND	ND	ND	NA
Thiobencarb	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Thifluzamide	GC/MS/MS	0.01	0.5	ND	ND	ND	NA
Thenylchlor	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Tebufenozide	LC/MS/MS	0.01	0.3	ND	ND	ND	NA
Tefuryltrione	LC/MS/MS	0.01	0.02	ND	ND	ND	NA
Trichlorfon	LC/MS/MS	0.01	0.2	ND	ND	ND	NA
Tricyclazole	LC/MS/MS	0.01	3	ND	ND	ND	NA
Triflumizole	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Nitenpyram	LC/MS/MS	0.01	0.5	ND	ND	ND	NA
Paclobutrazol	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Halosulfuron methyl	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Pymetrozine	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Pyraclonil	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Pyrazoxyfen	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Pyrazosulfuron-ethyl	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Pyrazolynate	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Pyraflufen-ethyl	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Pyriftalid	LC/MS/MS	0.01	0.02	ND	ND	ND	NA
Pyributicarb	GC/MS/MS	0.01	0.03	ND	ND	ND	NA
Pyrimisulfan	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Pyriminobac-methyl	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Pyroquilon	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Fipronil	LC/MS/MS	0.01	0.01	ND	ND	ND	NA
Fenitrothion	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Fenoxasulfone	LC/MS/MS	0.01	0.05	ND	ND	NA	NA
Fenoxanil	GC/MS/MS	0.01	1	ND	ND	ND	NA
Fenobucarb	GC/MS/MS	0.01	1	ND	ND	ND	NA
Ferimzone	LC/MS/MS	0.01	2	ND	ND	ND	NA
Fenthion	GC/MS/MS	0.01	0.3	ND	ND	ND	NA
Phenthoate	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Fentrazamide	LC/MS/MS	0.01	0.02	ND	ND	ND	NA
Fthalide	GC/MS/MS	0.01	1	ND	ND	ND	NA
Butachlor	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Butamifos	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Buprofezin	GC/MS/MS	0.01	0.5	ND	ND	ND	NA
Furametpyr	LC/MS/MS	0.01	0.5	ND	ND	ND	NA

Table 7. Pesticide Residues in ORYZA GAMMAX

Compound	Analytical Method	Limit of Quantitation (ppm)	MRL (ppm)[1]	Lot Number			
				U-530	M-506*	Z1201	Q1302
Fludioxonil	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Flucetosulfuron	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Flutolanil	GC/MS/MS	0.01	2	ND	ND	ND	NA
Pretilachlor	GC/MS/MS	0.01	0.03	ND	ND	ND	NA
Prochloraz	LC/MS/MS	0.01	2	ND	ND	ND	NA
Propyrisulfuron	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Probenazol	GC/MS/MS	0.01	0.5	ND	ND	ND	NA
Prometryn	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Bromobutide	GC/MS/MS	0.01	0.7	ND	ND	ND	NA
Penoxsulam	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Pefurazoate	GC/MS/MS	0.01	-	ND	ND	ND	NA
Pencycuron	LC/MS/MS	0.01	0.3	ND	ND	ND	NA
Bensulfuron-methyl	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Benzobicyclon	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Benzofenap	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Bentazone	LC/MS/MS	0.01	0.2	ND	ND	ND	NA
Pentoxazone	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Benfuracarb	GC/MS/MS	0.01	0.2	ND	ND	ND	NA
Penflufen	LC/MS/MS	0.01	0.05	ND	ND	NA	NA
Benfuresate	GC/MS/MS	0.01	0.05	ND	ND	ND	NA
Malathion	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Mesotrione	LC/MS/MS	0.01	0.01	ND	ND	ND	NA
Metazosulfuron	LC/MS/MS	0.01	0.05	ND	ND	NA	NA
Metalaxyl	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Methoxyfenozide	LC/MS/MS	0.01	0.1	ND	ND	ND	NA
Metominostrobin	GC/MS/MS	0.01	0.5	ND	ND	ND	NA
Mefenacet	LC/MS/MS	0.01	0.05	ND	ND	ND	NA
Mepronil	GC/MS/MS	0.01	2	ND	ND	ND	NA
Molinate	GC/MS/MS	0.01	0.1	ND	ND	ND	NA
Endrin Aldehyde	GC/MS/MS	0.01	UA	ND	ND	NA	ND
Toxaphene	GC/MS/MS	0.01	UA	ND	ND	NA	ND

ND= not detected; NA = not analyzed; BHC=hexachlorocyclohexane; ppm = parts per million; "-"= provisional clarification (0.01 ppm); UA = unavailable
[1]MRLs are for brown rice.
*Used in 90-day repeat oral dose toxicology study in rats.

e. Polyaromatic Hydrocarbon (PAH) Residues in ORYZA GAMMAX

Oryza quantified the amount of polyaromatic hydrocarbons (PAHs) in three lots of ORYZA GAMMAX, including the lot used in a 90-day rat toxicology study, using GC-MS (Table 8). None of the PAHs tested was detected)

Table 8. Polyaromatic Hydrocarbon (PAH) Residues in ORYZA GAMMAX

Analysis items	Analytical Method	Limit of Detection (ppm)	Lot Number		
			U-530	M-506*	U-523
Naphthalene	GC-MS	0.1	ND	ND	ND
Acenaphthylene	GC-MS	0.1	ND	ND	ND
Acenaphthene	GC-MS	0.1	ND	ND	ND
Fluorene	GC-MS	0.1	ND	ND	ND
Phenanthrene	GC-MS	0.1	ND	ND	ND
Anthracene	GC-MS	0.1	ND	ND	ND
Fluoranthene	GC-MS	0.1	ND	ND	ND
Pyrene	GC-MS	0.1	ND	ND	ND
Benzo(a)anthracene	GC-MS	0.1	ND	ND	ND
Chrysene	GC-MS	0.1	ND	ND	ND
Benzo(k)fluoranthene	GC-MS	0.1	ND	ND	ND
Benzo(a)pyrene	GC-MS	0.1	ND	ND	ND
Benzo(e)pyrene	GC-MS	0.1	ND	ND	ND
Indeno(1,2,3-c,d)pyrene	GC-MS	0.1	ND	ND	ND
Dibenzo(a,h)anthracene	GC-MS	0.1	ND	ND	ND
Benzo(g,h,i)perylene	GC-MS	0.1	ND	ND	ND
Benzo(b)fluorathene	GC-MS	0.1	ND	ND	ND
Benzo(j)fluorathene	GC-MS	0.1	ND	ND	ND

ND = not detected; ppm = parts per million
*Used in 90-day repeat oral dose toxicology study in rats.

f. Polychlorinated Biphenyl in γ-Oryzanol

Polychlorinated biphenyls (PCBs) in ORYZA GAMMAX were quantitated by GC-MS per United States Environmental Protection Agency method 8082A:2007 (Table 9). No PCBs were detected.

Table 9. Polychlorinated Biphenyl (PCB) Residues in ORYZA GAMMAX					
Analysis items	Analytical Method	Limit of Detection (ppm)	Lot Number		
			U-530	M-506*	Q1302
2,4,4'-Trichloro-biphenyl (PCB 28)	GC-MS[1]	0.5	ND	ND	ND
2,2',5,5'-Tetrachloro-biphenyl (PCB 52)	GC-MS[1]	0.5	ND	ND	ND
2,2',4,5,5'-Pentachloro-biphenyl (PCB 101)	GC-MS[1]	0.5	ND	ND	ND
2,3',4,4',5-Pentachloro-biphenyl (PCB 118)	GC-MS[1]	0.5	ND	ND	ND
2,2',3,4,4',5'-Hexachloro-biphenyl (PCB 138)	GC-MS[1]	0.5	ND	ND	ND
2,2',4,4',5,5'-Hexachloro-biphenyl (PCB 153)	GC-MS[1]	0.5	ND	ND	ND
2,2',3,4,4',5,5'-Heptachloro-biphenyl (PCB 180)	GC-MS[1]	0.5	ND	ND	ND

ND= not detected.
[1]Determined according to the United States Environmental Protection Agency method 8082A:2007.
*Used in 90-day repeat oral dose toxicology study in rats.

g. Dioxin Residues in ORYZA GAMMAX

To quantify the amount of dioxin in ORYZA GAMMAX, Oryza determined the amount of each dioxin that is associated with a Toxic Equivalent Factor (TEF) by high resolution gas chromatography coupled with high resolution mass spectrometry per the methods specified in the Provision Manual for the Survey and Measurement of Dioxins in Food, published by the Ministry of Health, Labour, and Welfare of Japan (Table 10). A variety of dioxins/furans and PCBs were detected in ORYZA GAMMAX and reported as Toxic Equivalents (TEQs), ranging from 0 to 0.0001 and 0.000006 to 0.000042 pg TEQ/g, respectively. Toxic Equivalents are used for assessing the risk of developing adverse effects following the exposure to these compounds and calculated by multiplying the concentration of the specific compound by a Toxic Equivalency Factor (TEF), which is the "toxic equivalency" of the compound to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (Van den Berg et al., 2006). For reference, the no significant risk levels (NSRLs) for 2,3,7,8 tetrachlorodibenzo-*p*-dioxin and PCBs set by the Office of Environmental Health Hazard Assessment (OEHHA) in Proposition 65 are 0.0005 and 0.9 µg/day, respectively. The mean total dioxin/furan and PCB TEQs in the three lots of ORYZA GAMMAX were 0.00003 pg/g and 0.00004093 pg/g, respectively. Assuming an estimated daily intake of 0.3125 g ORYZA GAMMAX/day at the 90th percentile, the resulting daily exposures to dioxins and PCBs would be 0.000009 and 0.0000127 pg TEQ/day, respectively, which are orders of magnitude below the NSRLs of 2,3,7,8 tetrachlorodibenzo-*p*-dioxin and PCBs. Thus, there is reasonable certainty that the ingestion of dioxins/furans and PCBs with ORYZA GAMMAX would not result in adverse effects.

Table 10. Dioxin, Furan, and Coplanar Polychlorinated Biphenyl (PCB) Residues in ORYZA GAMMAX

Parameter	Method[1]	LOD	Toxic Equivalent Factor (TEF)[2]	Lot Number U530 Conc. (ppb)	U530 Toxic Equivalent Quantity (TEQ; pg-TEQ/g)[3]	M-506* Conc. (ppb)	M-506* Toxic Equivalent Quantity (TEQ; pg-TEQ/g)	Z1201 Conc. (ppb)	Z1201 Toxic Equivalent Quantity (TEQ; pg-TEQ/g)
Polychlorinated Dibenzo Para Dioxins (PCDDs)									
2,3,7,8-TeCDD	GC-MS	0.008	1	ND	0	ND	0	ND	0
1,2,3,7,8-PeCDD	GC-MS	0.008	1	ND	0	ND	0	ND	0
1,2,3,4,7,8-HxCDD	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
1,2,3,6,7,8-HxCDD	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
1,2,3,7,8,9-HxCDD	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
1,2,3,4,6,7,8-HpCDD	GC-MS	0.01	0.01	ND	0	ND	0	0.01	0.0001
OCDD	GC-MS	0.03	0.0003	ND	0	ND	0	ND	0
Polychlorinate Dibenzofurans (PCDFs; WHO2005-Toxic Equivalents (TEQ) (ng/kg))									
2,3,7,8-TeCDF	GC-MS	0.009	0.1	ND	0	ND	0	ND	0
1,2,3,7,8-PeCDF	GC-MS	0.009	0.03	ND	0	ND	0	ND	0
2,3,4,7,8-PeCDF	GC-MS	0.008	0.3	ND	0	ND	0	ND	0
1,2,3,4,7,8-HxCDF	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
1,2,3,6,7,8-HxCDF	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
1,2,3,7,8,9-HxCDF	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
2,3,4,6,7,8-HxCDF	GC-MS	0.02	0.1	ND	0	ND	0	ND	0
1,2,3,4,6,7,8-HpCDF	GC-MS	0.02	0.01	ND	0	ND	0	ND	0
1,2,3,4,7,8,9-HpCDF	GC-MS	0.02	0.01	ND	0	ND	0	ND	0
OCDF	GC-MS	0.03	0.0003	ND	0	ND	0	ND	0
			Total Dioxin TEF		0		0		0.0001
Coplanar Polychlorinated Biphenyls (WHO2005-Toxic Equivalents (TEQ) (ng/kg))									
3,4,4',5-TeCB (PCB-81)	GC-MS	0.01	0.0003	ND	0	ND	0	0.02	0.000006
3,3',4,4'-TeCB (PCB-77)	GC-MS	0.01	0.0001	0.027	0.0000027	0.042	0.0000042	0.36	0.000036
3,3',4,4',5-PeCB (PCB-126)	GC-MS	0.01	0.1	ND	0	ND	0	ND	0
3,3',4,4',5,5'-HxCB (PCB-169)	GC-MS	0.01	0.03	ND	0	ND	0	ND	0
2',3,4,4',5-PeCB (PCB-123)	GC-MS	0.02	0.00003	0.027	0	ND	0	0.03	0.0000009
2,3',4,4',5-PeCB (PCB-118)	GC-MS	0.01	0.00003	ND	0.0000018	0.1	0.0000030	1.4	0.000042
2,3,3',4,4'-PeCB (PCB-105)	GC-MS	0.01	0.00003	0.06	0.00000099	0.04	0.0000012	0.63	0.0000189

Table 10. Dioxin, Furan, and Coplanar Polychlorinated Biphenyl (PCB) Residues in ORYZA GAMMAX

Parameter	Method[1]	LOD	Toxic Equivalent Factor (TEF)[2]	Lot Number					
				U530		M-506*		Z1201	
				Conc. (ppb)	Toxic Equivalent Quantity (TEQ; pg-TEQ/g)[3]	Conc. (ppb)	Toxic Equivalent Quantity (TEQ; pg-TEQ/g)	Conc. (ppb)	Toxic Equivalent Quantity (TEQ; pg-TEQ/g)
2,3,4,4',5-PeCB (PCB-114)	GC-MS	0.01	0.00003	0.033	0.00000099	ND	0	0.07	0.0000021
2,3',4,4',5,5'-HxCB (PCB-167)	GC-MS	0.01	0.00003	ND	0	ND	0	0.02	0.0000006
2,3,3',4,4',5-HxCB (PCB-156)	GC-MS	0.02	0.00003	ND	0	ND	0	0.06	0.0000018
2,3,3',4,4',5'-HxCB (PCB-157)	GC-MS	0.01	0.00003	ND	0	ND	0	0.02	0.0000006
2,3,3',4,4',5,5'-HpCB (PCB-189)	GC-MS	0.02	0.00003	ND	0	ND	0	ND	0
	Total PCB TEQ	-	-	-	0.00000648	-	0.0000084	-	0.0001089

Abbreviations: CFR = Code of Federal Regulations; AOAC = Association of Official Analytical Chemists; EPA = Environmental Protection Agency; LOD= Limit of Detection; ND = Not Detected; TeCDF = tetrachloradibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TeCDD = tetrachlorodibenzodioxin; PeCDD = pentachlorodibenzo-p-dioxin; HxCDD = hexachlorodibenzo-p-dioxin; heptachlorodibenzo-p-dioxin; octachlorodibenzodioxin; TeCB = tetrachlorobiphenyl; PeCB = pentachlorobiphenyl; HxCB = hexachlorobiphenyl; HpCB = heptachlorobiphenyl; MCPA = 2-methyl-4-chlorophenoxyacetic acid ; MCPB = 4-(4-chloro-2-methylphenoxy) butanoic acid.
[1]Determined by high resolution gas chromatography coupled with high resolution mass spectrometry per the methods specified in the Provision Manual for the Survey and Measurement of Dioxins in Food, published by the Ministry of Health, Labour, and Welfare of Japan.
[2]Obtained from Van den Berg et al., 2006.
[3]TEQ = TEF x Concentration.

h. Mycotoxin Residues in ORYZA GAMMAX

Because all rice is grown according to Japanese GAPs, the presence of mycotoxins in the finished production is not expected. To confirm that mycotoxins are not detected in the finished product, three lots ORYZA GAMMAX, including the lot used in the 90-day rat toxicology study, were screened for mycotoxins using liquid chromatography coupled with two mass spectrometers. No mycotoxins were detected (Table 11). Additionally, mycotoxin residues will continue to be monitored by Oryza on a yearly basis.

Table 11. Mycotoxins Residues in ORYZA GAMMAX[1]

Analysis items	Analytical Method	Limit of Quantitation	Lot Number		
			U-530	M-506*	Z1201
Aflatoxin B1	LC/MS/MS	1.0 ppb	ND	ND	ND
Aflatoxin B2	LC/MS/MS	1.0 ppb	ND	ND	ND
Aflatoxin G1	LC/MS/MS	1.0 ppb	ND	ND	ND
Aflatoxin G2	LC/MS/MS	1.0 ppb	ND	ND	ND
Ochratoxin A	LC/MS/MS	0.5 ppb	ND	ND	ND
Fumonisin B1	LC/MS/MS	0.01 ppm	ND	ND	ND
Fumonisin B2	LC/MS/MS	0.01 ppm	ND	ND	ND
Fumonisin B3	LC/MS/MS	0.01 ppm	ND	ND	ND
Nivalenol	LC/MS/MS	0.1 ppm	ND	ND	ND
Patulin	LC/MS/MS	0.010 ppm	ND	ND	ND
Sterigmatocystin	LC/MS/MS	0.01 ppm	ND	ND	ND
Zearalenone	LC/MS/MS	0.01 ppm	ND	ND	ND

ND= not detected; ppm = parts per million; ppb = parts per billion.
[1]Monitored once a year.
*Used in 90-day repeat oral dose toxicology study in rats.

i. Allergen Residues in ORYZA GAMMAX

ORYZA GAMMAX is produced on dedicated production lines for rice oil and γ-oryzanol. No raw materials or products containing soy, nuts, or fish are used in the production facility and moreover, each lot of finished product is monitored for the presence of the allergens, milk, wheat, egg, peanut, buckwheat, shrimp and crab using an enzyme-linked immunosorbant assay (ELISA). To demonstrate that ORYZA GAMMAX does not contain residues of these allergens, ELISA data from three lots, including the lot used in the 90-day rat toxicology study, is provided in Table 12. The limit of detection of the assay is 1 ppm and none of the allergens were detected. Additionally, Oryza will confirm that allergen residues are not detected in the event of changes to the production process, such as in raw material or processing aid sourcing.

Table 12. Protein Allergens Residues in ORYZA GAMMAX					
Allergen	Analytical Method	Limit of Detection	Lot Number		
			U-523	U-530	M-506*
Milk	ELISA	1 ppm	ND	ND	ND
Wheat	ELISA	1 ppm	ND	ND	ND
Egg	ELISA	1 ppm	ND	ND	ND
Peanut	ELISA	1 ppm	ND	ND	ND
Buckwheat	ELISA	1 ppm	ND	ND	ND
Shrimp and crab	ELISA	1 ppm	ND	ND	ND
ND= not detected; ELISA = enzyme-linked immunosorbent assay *Used in 90-day repeat oral dose toxicology study in rats.					

F. STABILITY OF ORYZA GAMMAX

1. Stability of ORYZA GAMMAX

The stability of three lots of ORYZA GAMMAX stored in polyethylene-lined aluminum bags under ambient conditions (25°C) for one year (Table 13) was determined by evaluating the γ-Oryzanol and microbial content, and loss on drying using the same methods used for qualifying each lot for release. At the end of 12 months, all lots complied with the product specifications. Importantly, the stability of the finished product will continue to be monitored to support the intended shelf-life of the product.

Table 13. Stability of ORYZA GAMMAX										
Parameter	Specification	Lot B-612			Lot B-628			Lot G-623		
		Months			Months			Months		
		0	6	12	0	6	12	0	6	12
γ-Oryzanol	≥ 85 %	90.1	95.7	94.0	94.5	98.7	93.7	101.2	99.6	89.3
Loss on drying (%)	≤ 0. 5%	0.12	0.07	0.11	0.07	0.16	0.14	0.31	0.32	0.26
Standard Plate Counts	≤ 100 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND
Molds and Yeasts	≤ 100 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coliforms	Neg./10 g	ND	ND	ND	ND	ND	ND	ND	ND	ND
cfu – colony forming units; Neg. - negative										

2. Stability of ORYZA GAMMAX in Market Products

The stability of ORYZA GAMMAX in vegetable oil was determined over the course of 5 months under accelerated conditions (40°C). Glass bottles containing vegetable oil and 1% ORYZA GAMMAX were stored in the dark and γ-oryzanol content was quantified by HPLC-UV. γ-Oryzanol content over the course of the 5 months was similar to the amount detected at the beginning of the testing period (Figure 3). Importantly, the stability of the finished product will continue to be monitored to support the intended shelf-life of the product.

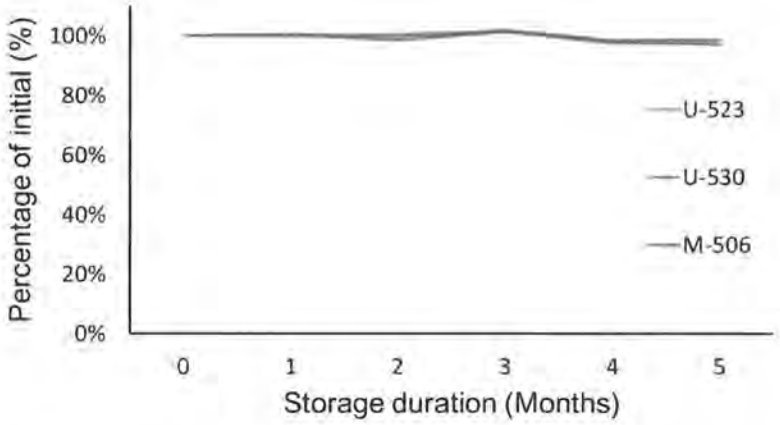

Figure 3. Stability of ORYZA GAMMAX in Vegetable Oil

Three lots of ORYZA GAMMAX (U-523, U-530, M-506) were added to vegetable oil and stored for increasing amounts of time at 40°C. Throughout the storage period, samples were collected and γ-Oryzanol content was determined using high performance liquid chromatography coupled with ultraviolet detector.

III. DIETARY EXPOSURE

A. INTENDED EFFECT

The intended effect of adding ORYZA GAMMAX to foods is to increase the ingestion of sterol ferulates.

B. HISTORY OF USE

γ-Oryzanol is found in and derived from rice bran or rice bran oil. Crude rice bran oil contains a minimum of 2% γ-oryzanol (Cosmetic Ingredient Review Expert Panel 2006). Rice bran oil is used extensively in cooking in Asian countries and the United States as well for the same uses as other vegetable oils. Thus, γ-oryzanol exposure in consumers residing in the United States is negligible. The global rice bran oil market size was estimated to be over 1.2 million tons in 2015 (https://www.gminsights.com/industry-analysis/rice-bran-oil-market). Rice bran oil has been concluded to be safe for use in cosmetics at the current practices of use and concentrations (Cosmetic Ingredient Review Expert Panel, 2006).

C. INTENDED USE

Oryza intends to add ORYZA GAMMAX to selected foods and beverages in the U.S. food supply, including meat, poultry, and fish products, dried bean, pea, nut and seed products, grain products, fruit and vegetable products, oils and salad dressings, sugars, sweets, and beverages.

D. ESTIMATED DAILY INTAKE

1. Introduction

A fixed concentration of ORYZA GAMMAX was used for each food and beverage, rather than a fixed mass to accommodate variations in serving size for the intended food products. The optimal fixed concentration was determined from the literature (Lerma-Garcia, 2009). In Lerma-Garcia et al., oxidation of whole milk powder during storage was reported to be reduced by adding 0.1% rice bran oil, and when compared with control milk powder, consumers could not detect any effect on the flavor of the reconstituted whole milk powder containing 0.1% rice bran oil. As a result, the total expected daily intake was calculated using this 0.1% concentration as a first approximation, and the effect of that concentration was also noted on the EDI for each of the following subcategories of food:

- meat, poultry, fish and mixtures,
- dry beans, peas, other legumes, nuts and seeds
- grain products
- fruits
- vegetables
- fats, oils and salad dressings
- sugars, sweets and beverages

ChromaDex Spherix Consulting has completed an assessment of the consumption of ORYZA GAMMAX by the U.S. population resulting from the proposed uses of ORYZA GAMMAX. Estimates for the intake were based on the proposed food uses and maximum use level in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2012; Bodner-Montville et al, 2006). Calculations for the mean and 90th percentile intakes were performed for all proposed food uses of ORYZA GAMMAX combined. The intakes were reported for the following population groups:

- children, ages 2 to 5 years,
- children, ages 6 to 12 years,
- teenagers, ages 13 to 19 years,
- adults, ages 20 years and up,
- total population (all age groups combined, excluding infants of 0-2 years),

2. Food Consumption Survey Data

a. Survey Description

The most recent National Health and Nutrition Examination Surveys (NHANES) for the years 2009-2010 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In 2009-2010, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is considered to be a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Gregory et al., 1995). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provides the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected *via* stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2.

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Among those who completed the food intake survey on both Day 1 and Day 2, 8301 respondents also provided physiological information including age, sex and weight; of these 7738 were 2 years and older.

Sample weights were incorporated with NHANES 2009-2010 to compensate for the potential under-representation of intakes from specific population groups because of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).

b. Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer using R to read and edit the SAS files, and R and Matlab to generate estimates for the intake of ORYZA GAMMAX by the U.S. population. The estimates for the daily intake represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. In the NHANES data, "All-person" intake refers to the estimated intake of ORYZA GAMMAX averaged over all individuals

surveyed, regardless of whether they consumed food products containing ORYZA GAMMAX, and therefore includes "zero" consumers (those who reported no intake of food products containing ORYZA GAMMAX during the 2 survey days). "All-user" intake refers to the estimated intake of ORYZA GAMMAX by those individuals consuming food products containing ORYZA GAMMAX, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing ORYZA GAMMAX on either Day 1 or Day 2 of the survey.

3. Food Usage Data

The individual proposed food uses for ORYZA GAMMAX employed in the current intake analysis and food codes representative of each proposed use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS). In FNDDS, the primary (usually generic) description of a given food is assigned a unique 8-digit food code (CDC, 2006; USDA, 2012).

4. Food Survey Results

The estimated "all-user" total intakes of ORYZA GAMMAX from all proposed food uses of ORYZA GAMMAX in the U.S. by population group is summarized in Table 14. Table 14 describes the "all-user" expected daily intake by age group in servings of 807 selected foods supplemented with 0.1% ORYZA GAMMAX in the United States.

Table 14. Estimated "All-user" Daily Intake (EDI) of ORYZA GAMMAX from All 807 Proposed Uses in the U.S. by Population Group (2009-2010 NHANES Data): 0.1% w/w per serving

Population	N_u/N_p [A]	Percent users	Absolute EDI (g/day)		Weight-based EDI (g/kg/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children, 2-5 years	699/707	98.87	0.1188	0.2440	0.006907	0.01419
Children, 6-12 years	1076/1097	98.09	0.1280	0.2633	0.003405	0.007002
Teenagers, 13-19 years	915/935	97.86	0.1506	0.3522	0.002186	0.005114
Adults, 20+ years	4840/4999	96.82	0.1416	0.3418	0.001726	0.004164
Total population (2+ years)	7530/7738	97.31	0.1387	0.3125	0.002036	0.004588

[A] Number of people consuming one or more foods containing γ-oryzanol on either Day 1 or Day 2 of the survey over the number of people surveyed.

5. Conclusions

In summary, the mean intakes of ORYZA GAMMAX by the all ORYZA GAMMAX consumers ("all-user") from all proposed food uses were estimated to be 139 mg/person/day or 2.0 mg/kg body weight/day from the added ORYZA GAMMAX of 0.1% w/w per serving/day. The heavy consumer (90^{th} percentile all-user) intakes of ORYZA GAMMAX from all proposed food-uses were estimated to be 313 mg/person/day or 4.6 mg/kg body weight/day from the maximum added ORYZA GAMMAX of 0.1% w/w per serving/day.

IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

A. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The absorption, distribution, metabolism and excretion of γ-oryzanol have been evaluated in *in vitro*, animal, and clinical studies (Kaneko and Tsuchiya, 1954; Endo et al., 1968). Although a limited amount of data exists, it is generally accepted that sterol ferulates and saturated sterols are poorly absorbed from the intestinal tract, similar to other plant sterols (for review see Wheeler et al., 1991; Ling and Jones, 1995). Specifically, *in vitro* studies have shown that less than 5% of cycloartenol ferulate, 24-methylenecycloartenol ferulate, campesteryl ferulate, sitosteryl ferulate, campestanyl ferulate, sitostanyl ferulate, stigmasteryl ferulate, and cholesteryl ferulate can be transported across monolayers of human colon carcinoma Caco-2 cells (Zhu et al., 2015); studies in rats and animals indicate that less than 10% of orally administered ^{14}C-labeled triterpenyl esters of ferulic acid, which were labeled at the C3 position of the ferulic acid, are absorbed via the mesenteric vein (Fujiwara et al., 1980; Fujiwara et al., 1983); and clinical studies have reported that greater than 80% of the campesteryl ferulate, campestanyl ferulate, sitosteryl ferulate, cycloartanyl ferulate, cycloartenyl ferulate, and 24-methylenecycloartanyl ferulate ingested with γ-oryzanol can be recovered in the feces (Lubinus et al., 2013). Once absorbed the sterol ferulates are distributed to a variety of tissues, such as the adrenal glands, lungs, spleen, and liver, and metabolized to ferulic acid, *m*-coumaric acid, *m*-hydroxyhippuric acid, hippuric acid, *m*-hydroxyphenyl propionic acid, and dihydroferulic acid (Fujiwara et al., 1983; Noda et al., 1974; Noda et al., 1975; Kobayashi et al., 2016).

B. GENOTOXICITY STUDIES

The genotoxicity of ORYZA GAMMAX is corroborated by DNA repair (Rec assay), bacterial reverse mutation (Ames assay), and *in vivo* chromosomal aberration tests conducted by using a preparation of γ-oryzanol resuspended in acetone with an unknown sterol ferulate composition (Tsushimoto et al., 1991). To assess the potential to induce DNA damage, the Rec assay was performed with doses of 0.1, 1, 10, 100, and 1000 µg γ-oryzanol/plate and *Bacillus subtilis* H17 (Rec^+) and M45 (Rec^-). None of these doses elicited a DNA damage response in when compared to the positive control, 0.2 µg mitomycin C/plate. The mutagenic potential of γ-oryzanol at 156, 313, 625, 1250, 2500 and 5000 µg/plate was tested in *Salmonella typhimurium* TA1535, TA1537, TA98, and TA100, and in *Escherichia coli* B/r WP2 uvrA. None of the γ-oryzanol treated bacterial strains produced more colonies than vehicle control. Therefore, γ-oryzanol was not mutagenic in these two assays. The clastogenic potential of γ-oryzanol was assessed through a chromosome aberration test in Sprague-Dawley rats. Either vehicle or γ-Oryzanol was administered to rats at 40, 400, and 4000 mg/kg in a single oral gavage treatment either once or once daily for 5 consecutive days. Three mg/kg of mitomycin C was used as a positive control. After dosing on day 1 and day 5, the bone marrow was isolated and assessed for

chromosomal aberrations. Although γ-oryzanol bioavailability was not confirmed, none of the doses of γ-oryzanol induced chromosomal aberrations greater than the vehicle control after a after one day of dosing. Similar results were observed after five daily doses of γ-oryzanol.

C. TOXICOLOGY STUDIES

1. Summary

The safety of ORYZA GAMMAX was evaluated in a 90-day subchronic toxicity study in Sprague Dawley rats. The no observed adverse effect level (NOAEL) for ORYZA GAMMAX was determined to be 3000 mg/kg/day, which was the highest dose tested. Other studies, such as carcinogenicity, chronic and developmental toxicity studies have also been performed with other preparations of γ-oryzanol and corroborate the safety of ORYZA GAMMAX. Specifically, γ-oryzanol did not increase the incidence of tumors over the control mice and rats and did not induce adverse effects in utero or during development. Together, these studies support the safety of ORYZA GAMMAX.

2. Subchronic Toxicity of ORYZA GAMMAX in Rats (Moon et al. 2017)

The subchronic toxicity of ORYZA GAMMAX manufactured by Oryza Oil & Fat Chemical Co., Ltd was determined in Sprague-Dawley rats (10 per sex per group) for 90 consecutive days by oral gavage at 0, 1,000, 2,000 and 3000 mg/kg/day to determine a NOAEL for ORYZA GAMMAX and confirm the results of other published studies conducted with other γ-oryzanol preparations (Moon et al., 2017). The study was performed in accordance with cGLP [OECD EVN/MC/CHEM (98)17 and Notification No. 2014-240 of the Ministry of Environment, Republic of Korea] and as per OECD guideline No. 408.

a. Methods

Male and female Sprague-Dawley [Crl: CD (SD)] rats aged 5 weeks were obtained from Charles River Laboratories Japan, Inc. (Atsugi, Japan). Body weight ranged from 111.0 to 140.3 g, males (n = 46), and from 103.8 to 127.7 g, females (n = 46). The rats were acclimatized for 12 days at 20.6–22.8°C and 44–45% humidity with a 12 h light (150–300 lx)/dark cycle. Pellet rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and DI water were provided *ad libitum*. After the quarantine-acclimation period, 40 males and 40 females were randomly assigned to 4 groups (10 animals of each sex per group) with equalization of the mean group body weight, which ranged from 224.2 to 259.8 g for males (n = 40) and from 167.1 to 205.1 g for females (n = 40). The rats were housed individually in stainless steel wire mesh cages during the study period.

The γ-oryzanol used in this study was manufactured by Oryza Oil & Fat Chemical Co. Ltd. (ORYZA GAMMAX; Lot. M-506). In a previous study, the maximum dose of γ-oryzanol was 1000 mg/kg body weight/day (Hasato et al., 1974). Doses of 1000, 2000, and 3000 mg/kg body weight/day were chosen for this study. Because ORYZA GAMMAX accounts for 0.5 to 1% of rice bran oil, 1000 and 3000 mg of ORYZA GAMMAX are equivalent to 100 – 300 g of rice bran oil. ORYZA GAMMAX was suspended in corn oil (1000, 2000, and 3000 mg in a volume of 6 mL). A fresh suspension was prepared daily and was used within 4 h after preparation. Rats were administered the ORYZA GAMMAX suspension (6 mL/kg body weight/day) once daily for 90 consecutive days by gavage with a gastric tube connected to a 3 or 5 mL disposable syringe. The suspension was thoroughly stirred just prior to administration to maintain homogeneity. Control animals received the vehicle (corn oil) at 6 mL/kg body weight/day. Body weight was measured before administration.

Throughout the study, all rats were observed once daily to assess clinical observations and twice daily for mortality and morbidity. Detailed clinical examination was done on Day 1 prior to treatment of test article and weekly thereafter. Functional observations (visual response, proprioceptive stimuli, auditory stimuli, pain response, aerial righting reflex, hind limb landing foot splay, and grip strength) were performed on all survivors in all the groups at Week 13 according to the method of Mattsson et al. (1996) and MacDaniel et al. (1993). Individual body weight was recorded on Day 1 prior to test article administration and at weekly intervals. Feed consumption was measured at weekly intervals. Observation involved assessment of the skin, fur, eyes, mucous membranes, secretions, and excretion, as well as checking autonomic activity (lacrimation, piloerection, pupil size, and unusual respiration), stereotypic behavior (excessive grooming and repetitive circling), bizarre behavior (self-mutilation and walking backward), changes in gait, posture, and the response to handling, and the presence of chronic or tonic convulsions. Daily feed consumption was calculated from total feed consumption over 7 days.

Ophthalmological examination was conducted on both eyes of all rats prior to dosing and on both eyes of all rats in the control and 2000 mg/kg/day groups at the end of the 13-week administration period. A mydriatic agent (1% isoptoatropine) was instilled into the eyes prior to examination and the anterior segment, transparent media, and ocular fundus were observed using an ophthalmoscope.

On day 90, fresh urine was collected for 3 h from 5 rats of each sex per group (0, 1000, and 2000 mg/kg) in the fasting state, and a 21 h urine sample was subsequently collected while allowing access to feed. Rats in metabolic cages (Biotoxtech, Cheongju, Republic of Korea) were allowed free access to water during both urine collection periods. The urine samples were stored at 4°C until analysis was performed, within 2 h of sample collection. The Combur10Test®M stick (Roche Diagnostics, Mannheim, Germany) was used to measure pH, protein, glucose, and occult blood, while urine chemistry was done with a Cobasu 411 urine

analyzer (F. Hoffmann-La Roche, Basel, Switzerland). Urine color and turbidity were checked by visual inspection. The 24 h urine was used for measurement of the urine volume and specific gravity. The specific gravity was measured with a Gravimeter (VET 360, Reichert Technologies, New York, USA).

Rats were fasted overnight for approximately 18 h before blood collection. Then the rats were anesthetized with isoflurane on day 91 and blood was collected from the abdominal aorta. For hematology tests, approximately 3 mL of blood sample volume was collected and placed in a vacutainer containing 5.4 mg of K_2 EDTA. For clinical chemistry tests, approximately 5 mL of blood was collected. The blood samples for hematology tests were stored at 4◦C and analysis was carried out within 2 or 3 h after collection. A sample of approximately 1 mL was placed in a vacutainer containing EDTA and the following parameters were measured using an auto analyzer (ADVIA 2120i, Siemens, Berlin, Germany): total erythrocyte count (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, total leukocyte count (WBC), and differential WBC counts (including neutrophils, lymphocytes, monocytes, eosinophils, basophils, and reticulocytes). In addition, approximately 2 mL of blood mixed with 3.2% sodium citrate was centrifuged at 3000 rpm for 10 min to obtain plasma for evaluation of the prothrombin time and activated partial thromboplastin time using an automated coagulation meter (Coaprest 2000, Sekisui Medical Co. Ltd., Tokyo, Japan). For coagulation tests, blood samples were centrifuged within 1h after collection. Furthermore, blood for clinical chemistry tests collected from the abdominal aorta into a vacutainer was centrifuged at 3000 rpm for 10 min to obtain serum within 1h after collection and stored at −20◦C until examination. The following parameters were analyzed using an auto analyzer (7180, Hitachi High-Technologies Co., Tokyo, Japan) and an electrolyte analyzer (EasyLyte, MedicaCo., Bedford, MA, USA): alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptitase (GGT), blood urea nitrogen (BUN), creatinine, total protein, albumin, albumin/globulin (A/G) ratio, total cholesterol, triglycerides, glucose, phosphorus (P), calcium (Ca), chloride (Cl), sodium (Na), and potassium (K).

All rats remaining on the study were terminated by exsanguination from the abdominal aorta under isoflurane anesthesia on day 91. A macroscopic postmortem examination was performed on all rats, including the external surfaces of the carcass and the internal organs. All grossly visible abnormalities were recorded. Organs were weighed (paired organs were weighed together) and the organ-to-body weight (final body weight) ratios were calculated based on the fasting weight. The following organs were weighed: brain, thymus, heart, liver, kidney, spleen, adrenal, testis, epididymis, ovary and uterus. At necropsy, the following organs and tissues were harvested and stored in 10% neutral buffered formalin (the testes and eyes including the optic nerve were fixed in Davidson's fixative before storage in 10% neutral buffered formalin): brain

(cerebrum, cerebellum, and pons), pituitary, thyroid, parathyroid, thymus, lung including bronchi, trachea, heart, liver, spleen, kidney, adrenal, aorta, salivary glands (submandibular, sublingual and parotid glands), esophagus, stomach, duodenum, ileum, jejunum, colon, cecum, rectum, pancreas, epididymis, testis, seminal vesicle with coagulating gland, prostate, uterus including cervix, ovary, fallopian tube, vagina, urinary bladder, submandibular lymph node, mesenteric lymph node, mammary gland (inguinal),skin (inguinal), skeletal muscle (thigh), sciatic nerve, eye, optic nerve, Harderian gland, nasal turbinates, sternum including bone marrow, femur including bone marrow, spinal cord (cervical, mid-thoracic, and lumbar), and any gross lesions. For histopathological evaluation, specimens of the preserved tissues were trimmed, dehydrated, and embedded in paraffin by standard methods, after which the paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin. All residual organs and tissues were preserved in 10% neutral buffered formalin. Histopathological examination was performed on all tissues from the control and 2000 mg/kg/day group.

Statistical analysis was performed using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA). Data on the body weight, feed consumption, urine volume, functional observational battery, hematology, clinical chemistry, and organ weight were analyzed by using Bartlett's test for homogeneity of variance (significance level: $P < 0.05$). One-way analysis of variance (ANOVA) was initially employed for homogeneous data. If a significant difference was found, Dunnett's test was used for multiple comparisons (significance levels: $P < 0.05$ and 0.01, two-tailed. The Kruskal-Wallis test was performed on heterogeneous data. If a significant difference was identified, Steel's test was applied for multiple comparisons (significance levels: $P < 0.05$ and 0.01, two-tailed).

b. Results

Two male rats died in the 3000 mg/kg body weight-treated group. One died on day 2 and one died on day 6 after dosing. During necropsy, it was noted that the test article had disseminated into the thoracic cavity due to the rupture of their esophagi. It was also noted that the test article was poorly soluble at 3000 mg/6 mL and difficult to administer due its adherence to the intubation tube and the animals were in pain during dosing. The deaths in the 3000 mg/kg body weight-treated group were therefore considered to be due to the physiochemical properties of the dosing formulation. The study was subsequently amended and dosing at 3000 mg/kg body weight/day was discontinued. All rats in the remaining groups survived until the end of the study. No abnormal clinical observations or body weight changes (Figure 4) were observed in the control group. No abnormalities were identified by detailed examination of males and females in the 1000 and 2000 mg/kg body weight/day groups. Compared with the control group, there were no statistically significant differences of the body weight (Figure 4) and feed consumption of males and females (Figure 5) in the 1000 and 2000 mg/kg body weight/day groups.

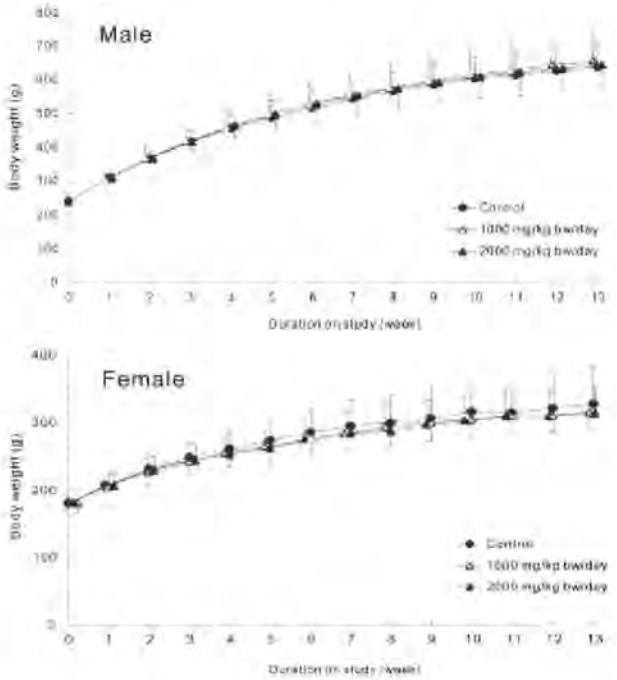

Figure 4. Body Weight of Male and Female Rats

Values represent the mean ± SD (n=10). No significant differences were observed.

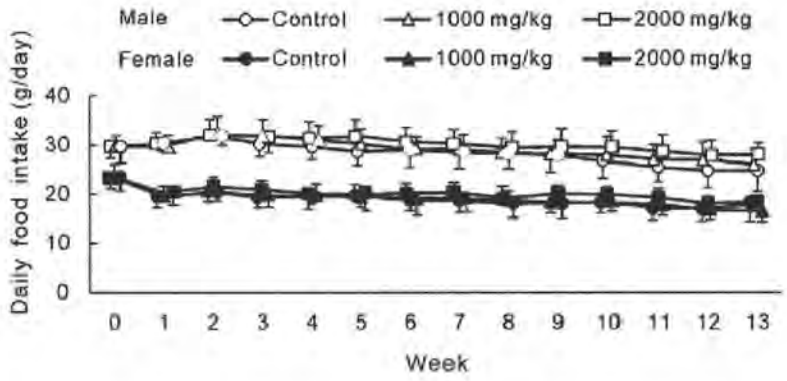

Figure 5. Daily Feed Intake

Each point represents the mean ± SD (n=10). No significant differences were observed.

A white substance was observed in the feces of all males and females from day 57–58 to day 90 in the 1000 mg/kg body weight/day group and all males and females from day 2–3 to day 90 in the 2000 mg/kg body weight/day group. Previous work has found that γ-oryzanol is poorly absorbed (Mandak and Nystrom, 2012). Although not confirmed, the white substance was assumed to be ORYZA GAMMAX due to the presence of the white substance in treated, but not control rats. Importantly, the white substance was not observed consistently throughout the study, and the observation did not appear to reflect a dose response. This clinical sign was not considered to be toxicologically significant because it suggested that the test article was excreted without being completely absorbed in the intestinal tract and the histopathology examination showed that no grossly visible findings or lesions were noted in the gastrointestinal tract in all animals. There were no abnormal ophthalmological findings in any rats. In addition, an open toe wound with nail damage, hemorrhage, and/or crust formation was observed on the left hind limb of one male on days 50–57 and on the right forelimb of one female on days 44–49 in the 2000 mg/kg body weight/day group. These findings were incidental changes unrelated to ORYZA GAMMAX, since they were not observed in any other rats throughout the study.

There were no significant differences of urine volume between control group and the ORYZA GAMMAX groups in either males or females. The urine color, transparency, pH, protein content, glucose content and specific gravity were not affected by ORYZA GAMMAX. Occult blood in urine (>25 Ery/μL) was detected in one male in the 1,000 mg/kg/day group and in one female in the 2,000 mg/kg/day group. They were not considered test article-related because the changes were slight and showed an incidental distribution.

There were not significant differences in hematology parameters in males and females in the treatment groups when compared to control (Table 15). Blood urea nitrogen (BUN) and total cholesterol (T-Chol) were significantly increased in males from the 2000 mg/kg/day group (Table 16). However, they were not considered test article-related effect because the levels were within the historical data range for control animals (male: 44-132 mg/dL, female 44-146 mg/dL). There were no significant differences of blood chemistry parameters in males and females from the 1000 mg/kg/day groups and males and females from the 2000 mg/kg/day group compared with the control values.

Compared with the control group, there were no significant differences in organ weights in males and females from the 1000 and 2000 mg/kg body weight/day groups (Table 17). At necropsy, macroscopic examination did not reveal any changes related to administration of γ-oryzanol and the macroscopic findings observed in this study were considered to be incidental. Microscopic examination also did not reveal any test substance-related changes (Tables 18 and 19).

Table 15. Hematological Parameters

Males	RBC ($\times 10^6$ cells/μL)	Hemoglobin (g/dL)	Hematocrit (%)	RBC Indices MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets ($\times 10^3$ cells/μL)	Reticulocytes (%)
Control	8.62 ± 0.28	14.9 ± 0.7	44.8 ± 1.8	52.0 ± 1.7	17.3 ± 0.6	33.3 ± 0.4	843 ± 50	1.75 ± 0.18
1000 mg/kg bw/day	8.74 ± 0.29	15.1 ± 0.5	45.2 ± 1.2	51.8 ± 1.4	17.3 ± 0.5	33.3 ± 0.3	818 ± 100	1.95 ± 0.22
2000 mg/kg bw/day	8.61 ± 0.24	15.2 ± 0.5	45.5 ± 1.4	52.9 ± 1.9	17.7 ± 0.7	33.4 ± 0.4	860 ± 95	1.95 ± 0.33
	WBC ($\times 10^3$ cells/μL)	Differential WBC counts (%) Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	PT (Sec)	APTT (Sec)
Control	8.39 ± 1.88	19.8 ± 4.7	75.7 ± 4.8	2.1 ± 0.5	1.6 ± 0.3	0.2 ± 0.1	17.5 ± 0.7	15.8 ± 1.2
1000 mg/kg bw/day	10.89 ± 2.02	18.1 ± 8.5	77.6 ± 8.5	1.9 ± 0.6	1.2 ± 0.8	0.3 ± 0.1	17.8 ± 0.5	16.3 ± 1.4
2000 mg/kg bw/day	10.13 ± 3.02	18.7 ± 3.9	76.8 ± 4.0	2.3 ± 0.4	1.3 ± 0.5	0.3 ± 0.1	17.8 ± 0.6	16.2 ± 1.0
Females	RBC ($\times 10^6$ cells/μL)	Hemoglobin (g/dL)	Hematocrit (%)	RBC Indices MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets ($\times 10^3$ cells/μL)	Reticulocytes (%)
Control	7.84 ± 0.23	14.6 ± 0.5	43.1 ± 1.1	55.1 ± 1.4	18.6 ± 0.5	33.8 ± 0.5	849 ± 93	1.87 ± 0.36
1000 mg/kg bw/day	7.89 ± 0.57	14.7 ± 0.5	42.7 ± 2.2	54.2 ± 2.0	18.7 ± 1.4	34.4 ± 1.9	863 ± 45	1.89 ± 0.39
2000 mg/kg bw/day	8.10 ± 0.34	15.0 ± 0.8	44.0 ± 2.2	54.3 ± 1.6	18.5 ± 0.5	34.0 ± 0.2	894 ± 96	1.84 ± 0.35
	WBC ($\times 10^3$ cells/μL)	Differential WBC counts (%) Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	PT (Sec)	APTT (Sec)
Control	4.91 ± 1.50	18.2 ± 8.5	77.5 ± 9.0	2.2 ± 0.9	1.4 ± 0.4	0.2 ± 0.1	17.5 ± 0.9	14.4 ± 1.9
1000 mg/kg bw/day	5.86 ± 1.82	14.2 ± 5.8	81.0 ± 6.5	2.2 ± 0.7	1.3 ± 0.3	0.2 ± 0.1	17.8 ± 0.6	14.5 ± 1.3
2000 mg/kg bw/day	5.01 ± 1.22	17.1 ± 5.7	77.4 ± 6.3	2.5 ± 0.9	1.7 ± 0.5	0.3 ± 0.1	17.8 ± 0.7	14.3 ± 1.4

Abbreviations: RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration (MCHC); WBC, white blood cells; PT, prothrombin time; APTT, activated partial thromboplastin time.
Values represent the mean ± SD (n = 10). No significant differences were observed.

Table 16. Blood Chemistry Parameters

Males	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)
Control	36.9 ± 17.2	91.7 ± 23.9	302.4 ± 75.7	0.55 ± 0.10	131 ± 13	10.8 ± 1.1	0.44 ± 0.03	64 ± 9
1000 mg/kg bw/day	30.7 ± 10.9	84.4 ± 20.7	325.3 ± 101.5	0.59 ± 0.24	134 ± 12	11.8 ± 1.1	0.45 ± 0.05	74 ± 10
2000 mg/kg bw/day	47.9 ± 35.9	121.4 ± 59.3	299.3 ± 66.4	0.56 ± 0.11	123 ± 6	12.2 ± 1.4*	0.46 ± 0.03	90 ± 19**
	Triglyceride (mg/dL)	Phospholipid (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
Control	66 ± 25	5.8 ± 0.1	0.68 ± 0.04	5.93 ± 0.49	10.1 ± 0.2	140 ± 1	4.50 ± 0.32	104.7 ± 1.0
1000 mg/kg bw/day	69 ± 36	5.9 ± 0.4	0.65 ± 0.04	5.98 ± 0.48	10.1 ± 0.3	141 ± 1	4.45 ± 0.26	105.1 ± 0.6
2000 mg/kg bw/day	67 ± 19	6.0 ± 0.2	0.66 ± 0.08	5.90 ± 0.34	10.2 ± 0.2	141 ± 1	4.38 ± 0.19	105.6 ± 0.7
Females	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glucose (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)
Control	41.4 ± 31.9	93.3 ± 76.5	137.2 ± 39.7	0.21 ± 0.15	115 ± 11	14.2 ± 1.9	0.54 ± 0.03	87 ± 13
1000 mg/kg bw/day	33.6 ± 8.7	78.1 ± 20.7	176.8 ± 94.0	0.33 ± 0.20	116 ± 6	13.4 ± 0.7	0.53 ± 0.04	84 ± 12
2000 mg/kg bw/day	31.8 ± 11.6	78.6 ± 37.3	160.7 ± 45.1	0.31 ± 0.18	113 ± 8	13.9 ± 1.9	0.52 ± 0.03	96 ± 27
	Triglyceride (mg/dL)	Phospholipid (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
Control	42 ± 37	6.8 ± 0.2	0.81 ± 0.05	4.22 ± 0.46	10.5 ± 0.3	141 ± 1	3.92 ± 0.18	106.9 ± 0.8
1000 mg/kg bw/day	34 ± 16	6.6 ± 0.2	0.81 ± 0.06	4.60 ± 0.60	10.5 ± 0.2	141 ± 1	3.93 ± 0.18	106.8 ± 1.5
2000 mg/kg bw/day	34 ± 24	6.7 ± 0.5	0.83 ± 0.05	4.54 ± 0.56	10.6 ± 0.5	141 ± 1	3.97 ± 0.22	106.6 ± 0.7

Values represent the mean ± SD (n = 10). Asterisks denote significant differences from the control group.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase, ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptitase; BUN, blood urea nitrogen; A/G, albumin/globulin; P, phosphorus; Ca, calcium; Cl, chloride; Na, sodium; K, potassium.
* $p < 0.05$.
** $p < 0.01$.

Table 17. Absolute Organ Weights (g)

Males	Final body weight	Brain	Thymus	Heart	Liver	Spleen	Kidneys	Adrenals	Testes	Epididymis
Control	635.5 ± 66.9	2.18 ± 0.13	0.37 ± 0.11	1.66 ± 0.18	16.8 ± 2.2	0.85 ± 0.17	3.47 ± 0.27	0.058 ± 0.006	3.74 ± 0.42	1.45 ± 0.17
1000 mg/kg bw/day	656.3 ± 87.8	2.21 ± 0.13	0.36 ± 0.07	1.62 ± 0.19	16.4 ± 2.2	0.93 ± 0.22	3.34 ± 0.45	0.059 ± 0.007	3.84 ± 0.34	1.58 ± 0.12
2000 mg/kg bw/day	643.1 ± 63.6	2.18 ± 0.09	0.33 ± 0.12	1.60 ± 0.18	16.5 ± 2.9	0.86 ± 0.15	3.47 ± 0.41	0.060 ± 0.009	3.74 ± 0.40	1.48 ± 0.16
Females		Brain	Thymus	Heart	Liver	Spleen	Kidneys	Adrenals	Ovaries	Uterus
Control	327.2 ± 55.8	1.99 ± 0.13	0.29 ± 0.06	0.96 ± 0.07	8.5 ± 0.9	0.51 ± 0.12	1.87 ± 0.12	0.066 ± 0.011	0.081 ± 0.016	0.80 ± 0.30
1000 mg/kg bw/day	315.0 ± 34.7	1.96 ± 0.08	0.30 ± 0.09	0.95 ± 0.10	7.9 ± 0.8	0.51 ± 0.21	1.90 ± 0.21	0.072 ± 0.010	0.078 ± 0.015	0.81 ± 0.29
2000 mg/kg bw/day	313.2 ± 23.1	1.97 ± 0.12	0.30 ± 0.09	0.95 ± 0.08	7.7 ± 0.8	0.55 ± 0.06	1.90 ± 0.119	0.066 ± 0.009	0.075 ± 0.015	0.94 ± 0.49

Values represent the mean ± SD (n = 10). No significant differences were observed.

Table 18. Summary of Histopathological Findings in Males

Organs	Observations	Control	1000 mg/kg bw/day	2000 mg/kg bw/day
Adrenal	No. of microscopic findings	10/10	N/A	10/10
	Vacuolation, zona fasciculata	Grade 1: 2	/	Grade 1: 2
		Grade 2: 0	/	Grade 2: 1
Eyes	No. of microscopic findings	10/10	N/A	10/10
	Focal retinal dysplasia	0/10	/	Grade 1: 1
Heart	No. of microscopic findings	10/10	N/A	10/10
	Focal infiltration of monocytes	Grade 1: 2	/	Grade 1: 1
Kidneys	No. of microscopic findings	10/10	N/A	10/10
	Focal hyaline cast	Grade 1: 1	/	0/10
	Pyelitis	0/10	/	Grade 2: 1
	Tubular basophilia	Grade 1: 5	/	Grade 1: 3
Liver	No. of microscopic findings	10/10	N/A	10/10
	Focal infiltration of monocytes	Grade 1: 2	/	Grade 1: 2
	Periportal vacuolation	Grade 1: 4	/	Grade 1: 2
		Grade 2: 3	/	Grade 2: 3
	Sporadic hepatocyte vacuolation	Grade 2: 1	/	Grade 2: 3
Lungs including bronchi	No. of microscopic findings	10/10	N/A	10/10
	Alveolar focal macrophage aggregation	0/10	/	Grade 1: 1
	Focal fibrosing alveolitis	0/10	/	Grade 1: 1
	Focal osseous metaplasia	Grade 1: 1	/	Grade 1: 1
Prostate	No. of microscopic findings	10/10	N/A	10/10
	Interstitial lymphocyte infiltration	Grade 1: 1	/	0/10
Salivary gland, parotid	No. of microscopic findings	10/10	N/A	10/10
	Focal infiltration of monocytes	Grade 1: 1	/	0/10
Spleen	No. of microscopic findings	10/10	N/A	10/10
	Deposition of brown pigment	Grade 1: 3	/	Grade 1: 1
		Grade 2: 0	/	Grade 2: 1
	Extramedullary hematopoiesis	Grade 1: 1	/	Grade 1: 2
Testes	No. of microscopic findings	10/10	N/A	10/10
	Unilateral dilation of rete testis	0/10	/	Grade 1: 1

Abbreviations: /, not examined; N/Anot applicable (only animals with gross lesions were examined). Severity was classified into 4 grades: grade 1: minimalgrade 2: slight, grade 3: moderate, grade 4: severe.

Table 19. Summary of Histopathological Findings in Females

Organs	Observations	Control	1000 mg/kg bw/day	2000 mg/kg bw/day
Harderian gland	No. of microscopic findings	10/10	N/A	10/10
	Focal infiltration of lymphocytes	Grade 1: 1	/	0/10
	Focal hyperplasia	0/10	/	Grade 1: 1
Kidneys	No. of microscopic findings			
	Focal hyaline cast	Grade 1: 1	/	0/10
	Focal infiltration of monocytes	0/10	/	Grade 1: 2
	Cystic tubules	3/10	/	0/10
	Pyelitis	Grade 1: 1	/	0/10
		Grade 2: 1	/	0/10
Liver	No. of microscopic findings	10/10	N/A	10/10
	Focal infiltration of monocytes	Grade 1: 1	/	Grade 1: 5
	Periportal vacuolation	Grade 1: 1	/	0/10
	Sporadic hepatocyte vacuolation	Grade 2: 1	/	Grade 2: 3
Lungs including bronchi	No. of microscopic findings	10/10	N/A	10/10
	Alveolar focal macrophage aggregation	0/10	/	Grade 1: 1
	Focal fibrosing alveolitis	0/10	/	Grade 1: 1
Mammary gland: inguinal	No. of microscopic findings	10/10	N/A	10/10
	Lobuloalveolar hypertrophy	0/10	/	Grade 1: 1
Ovaries	No. of microscopic findings	10/10	N/A	10/10
	Follicular cyst	0/10	/	1/10
Spleen	No. of microscopic findings	10/10	N/A	10/10
	Deposition of brown pigment	Grade 1: 6	/	Grade 1: 5
		Grade 2: 1	/	Grade 2: 3
	Extramedullary hepatopoiesis	0/10	/	Grade 1: 1
Thyroid	No. of microscopic findings	0/10	N/A	1/10
Uterus including cervix	No. of microscopic findings	10/10	1/10	10/10
	Cyst in cervix lined by stratum germinativum	0/10	1/10/	0/10
Vagina	No. of microscopic findings	10/10	N/A	10/10
	Mucification of epithelium	0/10	/	Grade 1: 1

Abbreviations: /, not examined; N/A, not applicable (only animals with gross lesions were examined). Severity was classified into 4 grades: grade 1: minimal, grade 2: slight, grade 3: moderate, grade 4: severe.

In conclusion, the NOAEL for ORYZA GAMMAX following gavage was determined to be 2000 mg/kg body weight/day under the test conditions employed. With the application of a 100-fold safety factor to account for the inter- and intra-species variability, the acceptable daily intake (ADI) for ORYZA GAMMAX is 20 mg/kg body weight/day. Assuming the ORYZA GAMMAX is consumed by a 60 kg human, the resulting daily intake per person would be 1.2 g/day.

3. Corroborative Animal Studies with γ-Oryzanol

Two carcinogenicity studies in rats and mice, a chronic toxicity study in rats, and a developmental toxicity study in mice and rats corroborate the safety of ORYZA GAMMAX (Tamagawa et al., 1992a; Tamagawa et al., 1992b; Maruoka and Kume, 1972; Hasato et al., 1974). Importantly, these studies were conducted with γ-oryzanol preparations with unreported levels of the different sterol ferulates. Thus, the studies collectively show that γ-oryzanol does not induce general and developmental toxicity up to 1000 and 600 mg/kg/day, respectively, and is not carcinogenic up to 2000 mg/kg/day.

a. Carcinogenicity

The carcinogenic potential of γ-oryzanol was tested in male and female B6C3F$_1$ mice (Tamagawa et al., 1992a). The mice were fed 0, 200, 600, and 2000 mg/kg/day γ-oryzanol for 78 weeks. γ-Oryzanol powder was mixed into the animal feed to the desired dose levels. There were no treatment-related changes in general condition, body weight, feed consumed, mortality, organ weight or hematology in either sex during the study. Pathological examination at the end of the study found tumors in all treatment groups of both sex and in the control mice. There was a high incidence of liver tumors in both the male 0 and 2000 mg/kg/day groups. There was an increase in the incidence of lymphoma in the 2000 mg/kg/day female group, but this difference was not significant compared to the 0 mg/kg/day females. Animals in the 2000 mg/kg/day group of both males and females did not show any statistically significant difference in the incidence of tumors compared with the control group. Additionally, there was a high incidence of thymus involution in both the control- and γ-Oryzanol-treated animals, which was considered to be typical of the natural decline of the immune system over time. The thymus involution was therefore considered to be within the normal range for age-matched mice and not treatment-related. γ-Oryzanol was not carcinogenic in this strain of mice at doses up to 2000 mg/kg/day.

The carcinogenic potential of γ-oryzanol was further tested using both male and female F344 rats (Tamagawa et al., 1992b). These rats were fed 0, 200, 600, or 2000 mg/kg/day γ-oryzanol for two years. γ-Oryzanol powder was mixed into the rat feed to the desired dose levels.

Females in the 2000 mg/kg/day group showed a slight decrease in body weight at the end of the study, but there were no treatment related changes in general condition, feed consumed, mortality, organ weight or hematology. This change was also within the normal body weight for female F344 rats, so the change was not considered treatment related or adverse. There were no changes in body weight in any of the male treatment groups through the course of the study. Overall the tumor incidence was high in both the control and 2000 mg/kg male and female rats. In both the control and 2000 mg/kg-treated male rats, the most frequently found tumors were in the testes followed by tumors in the pituitary, thyroid, adrenals, hemopoietic system, and skin/subcutis. In both the control and 2000 mg/kg-treated females, the most frequently found tumors were in the pituitary, uterus, mammary gland, thyroid, adrenal, and hemopoietic system. Importantly, there were no significant differences in the different tumors incidences between the control and 2000 mg/kg/day groups. γ-Oryzanol was therefore determined to be not carcinogenic at doses up to 2000 mg/kg/day.

b. Chronic Toxicity Study

In a chronic toxicity study, Hasato et al. (1974) administered either control (carboxymethylcellulose) or 30, 100, 300, and 1000 mg/kg/day γ-oryzanol to Wistar-Imamichi rats (15 males and 15 females/group) by gavage for 6 months. Body weights and feed intake were measured weekly throughout the administration period. On the 27th day of treatment, blood was collected from 6 males and 6 females randomly sampled from each group, except the group receiving 30 mg/kg/day, to evaluate the effect of the γ-oryzanol on hematology (red blood cell count, white blood cell count, and white blood cell type) and clinical chemistries (S-GPT, S-GOT, blood sugar, S-alkaline phosphatase, cholesterol, urea nitrogen, LDH, Na, K, Cl levels, A/G ratio, and total protein). In the group receiving 30 mg/kg/day only 5 males and 5 females were sampled. These animals were then euthanized for gross necropsy and organ weight. On the 181st day of treatment blood was collected from an additional 6 males and 6 females from control, 100, 300, and 1000 mg/kg/day treated groups and from and an additional 5 males and 5 females of 30 mg/kg/day-treated group to evaluate the effect of the γ-oryzanol on hematologies and clinical chemistries. These animals were then euthanized and necropsied, and their organ weight were determined.

In the 1000 mg/kg-treated group, one male rat died on the 17th day, one female rat died on the 73rd day, one female rat died on the 121st day, and on male rat died on the 128th day of the study. In the 300 mg/kg-treated group on male rat died on the 84th and one male rat died on 143rd day of the study. There were no deaths in the other groups. The causes of the deaths were not reported. Nasal inflammation was observed intermittently throughout the study in all groups

including control animals and was most severe at approximately the 100th day. Although there were no significant differences in the feed intakes or body weight gains between the 100, 300, and 1000 mg/kg/day and control-treated groups, there was a significant decrease in the body weights of the 30 mg/kg- and control-treated groups in the 14th week of the study, when it was reported that there was a sudden drop in ambient temperature. In the female rats, there was no significant difference in the weight change between 1000 mg/kg- and control-treated groups. In contrast, there were significant reductions in weight change in the 300, 100, and 30 mg/kg-treated groups. Importantly, because their weight range reductions were not dose-response, the changes were considered to be the result of having fewer numbers of rats after the interim investigation rather than a toxic effect of the test article.

For the hematology, there were no significant differences in male rats at the interim and final investigations. In the female rats, significant differences were observed in white blood cell count, red blood cell count, and percentage of lymphocytes and neutrophils in some of the γ-oryzanol-treated groups compared to the control group at the interim and final investigations. However, no positive correlation with dose was observed and they were considered to be not treatment-related. For the clinical chemistries, there were no significant differences S-GPT, S-GOT, blood sugar, S-alkaline phosphatase, cholesterol, urea nitrogen, LDH, Na, K, Cl levels, A/G ratio, and total protein between the γ-oryzanol- and control-treated groups.

c. Developmental Toxicity Study

In a developmental toxicity study, Maruoka et al. (1972) administered 0 (saline), 6 or 600 mg/kg of γ-oryzanol by gavage to ICR-JCL nulliparous mice from day 7 to 12 of pregnancy and to Wistar nulliparous rats from day 9 to 14 of pregnancy to assess fetal and perinatal development. Mice showing visual signs of estrus and rats determined to be in proestrus using the vaginal smear method were left with mature males overnight. The next morning was defined as day zero of pregnancy and only female mice with a copulatory plug and rats with sperm in their vagina were deemed pregnant. Pregnant dams were housed individually.

To assess fetal development, saline and γ-oryzanol-treated pregnant twenty mice and twenty rats were anesthetized by chloroform and euthanized by exsanguination on day 18 and 21, respectively. During the Caesarean section, the abdominothoracic area was observed. After the Caesarean section, the number of implants, live fetuses, and resorbed or dead fetuses were counted. The live fetuses were weighed, their sex was determined, and the external appearance was checked for abnormalities. Two-thirds of the total live fetuses were necropsied, fixed with alcohol, and stained with alizarin red S for skeletal analyses. In both the mice and rats, ossification of breast bone, tail caudal vertebra, and toe bone were observed and in the mice

ossification of the ankle and calcaneal bone was also observed. The remaining one-third of the fetuses, were placed in Bouin's solution and their internal parts were observed by the razor blade sectioning method. Partial inspection of the thoracoabdominal areas was also performed to determine if there were any abnormalities in the principal organs. Both the alizarin red S -stained skeleton samples and Bouin's-fixed fetuses were observed under a low-power stereoscopic microscope.

To assess perinatal development, five saline- and γ-oryzanol-treated dams were allowed to give natural birth. Then, the offspring were counted, their sex was determined, and their external appearance was checked for abnormalities within 24 h after birth. The dams were allowed to raise their offspring until the 21st day after birth, the date of delivery was counted as day zero. Differentiation conditions such as detachment of auricles, eruption of incisors, opening of eyelids, and fur appearance were observed. When differentiation conditions were observed, external appearance of the offspring and behavioral abnormalities behavior were monitored. The offspring were weighed every 4th day until the 21st day after the birth. External appearance of live offspring was examined, their behavior was observed, and auditory, pain, and righting reflex tests were performed at the time of weaning on the 21st day. Inspection was performed to check for visceral abnormalities. Skeleton samples from one male and one female offspring born from each dam were treated like fetuses' samples and checked for bone abnormalities.

In both the mouse and rat developmental toxicity studies, no dam died or miscarried during the test periods and there were no changes in their general condition. There was no significant difference in the weight of pregnant dams among the control and γ-oryzanol treated groups over the course of gestation, and no changes in the organs viewed during the Cesarean sections. Although abnormalities were seen in the fetuses of both the control and γ-oryzanol-treated groups, there were no significant differences in the occurrence rate of these abnormalities among groups. There were also no significant differences between the two groups in total implants, live fetus number, live fetus mean body weight, fetus physical appearance, stained skeleton samples or fetuses fixed in Bouin's solution.

When the offspring were observed, there were no γ-oryzanol-related changes in external features, body weight gain, behavior, organs or bones. The results therefore indicate that the γ-oryzanol used in this study had no effect on developmental toxicity.

D. CLINICAL STUDIES

1. Summary

Seven peer-reviewed studies that have assessed γ-oryzanol in human subjects were found in the publicly available literature (Table 20). All studies administered γ-oryzanol orally and none of them used ORYZA GAMMAX. Importantly, although many of these studies were performed to evaluate the efficacy of γ-oryzanol modulating serum lipid levels and exercise, none of these studies reported adverse effects, weight changes or abnormal laboratory values at doses up to 3.4 g/day for durations up to six months. These studies corroborate the results of the subchronic toxicity study, indicating that γ-oryzanol is well-tolerated in humans at the estimated daily intake level of 139 mg/person/day (2.0 mg/kg body weight/day).

2. Clinical Studies

The effect of γ-oryzanol alone or in combination with probucol was investigated in subjects with hyperlipidemia in a single arm, uncontrolled, non-randomized trial (Yoshino et al., 1989). Eighty subjects, 25 males and 55 females, with an average age of 61 years were given 300 mg/day of γ-oryzanol for 6 months and plasma cholesterol levels, serum chemistries, and blood cell counts were monitored. After three months, ten subjects who did not respond to the γ-oryzanol treatment were given 300 mg/day γ-oryzanol and 500 mg/day of probucol. Both the γ-oryzanol and the γ-oryzanol/probucol treatments were well tolerated in all subjects and the blood cell counts and serum chemistries reflected no adverse effects on hematopoietic, hepatic, or renal function.

The effect of γ-oryzanol on serum lipids was further investigated in schizophrenic, dyslipidemic subjects receiving major neuroleptics including phenothiazines (chlorpromazine, levomepromazine, perphenazine) for a mean of 10 years in a single arm, uncontrolled, non-randomized trial (Sasaki et al., 1990). This study was performed because schizophrenic patients often have elevated cholesterol as a comorbidity. Twenty subjects, all chronic schizophrenic patients with dyslipidemia, were given 100 mg γ-oryzanol orally three times daily for 16 weeks. This cohort was 51 years of age on average, and consisted of 11 males and 9 females. Although Sasaki et al. did not report the incidence or severity of adverse events, there were no body weight changes, adverse effects and abnormal laboratory values throughout the study.

γ-Oryzanol has also been used as a supplement for active adults during exercise training (Fry et al., 1997). Its effect was investigated in 22 college-aged male subjects during resistance exercise training in a double-blind, placebo-controlled trial. γ-Oryzanol was administered to 11

subjects at 500 mg/day for 9 weeks. Although adverse effects were not reported, there were no significant effects of resting heart rate, blood pressure, hematocrit, plasma cholesterol, serum triglycerides, serum albumin, serum calcium, serum magnesium, serum cortisol, plasma testosterone, serum estradiol, or serum insulin levels.

Rice bran oil supplemented with high amounts of γ-oryzanol was used to assess the effect of γ-oryzanol on cholesterol levels in men with mild hypercholesterolemia in a randomized, parallel group trial (Berger et al., 2005). Thirty males aged 38 to 64 years old were given 50 g of rice brain oil with either 0.05g or 0.8 g γ-oryzanol every day for 4 weeks via oral administration. The subjects kept written daily logs detailing consumption of cigarettes, alcohol, vitamins, medicines, food, physical activity and any sickness experienced. For the food recall, subjects recorded: all foods and beverages consumed; any reductions in habitual food intake; whether the amount of oil provided was too much in their judgement; and diarrhea incidence. Compliance and feedback were also monitored via random phone calls. There were no dropouts or expulsions during the study, and there were no adverse effects recorded in the daily logs.

γ-Oryzanol was supplemented with eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), vitamin E and niacin to determine their combined effect on lipid profile, inflammatory status and oxidative balance in a randomized, placebo-controlled trial (Accinni et al., 2006). Fifty seven male and female dyslipidemic subjects were randomly assigned to receive placebo (n=19), EPA + DHA and Vitamin E (n=18), or EPA + DHA, Vitamin E and γ-oryzanol (n=20). There were no drop-outs or reports of side effects during the study.

The fate of dietary ferulic acid esters (γ-oryzanol) was assessed after consumption by healthy human subjects in a crossover study (Lubinus et al., 2013). These subjects were 15 males average 28 years old. The subjects received skimmed milk drinking yogurt, Becel pro.active® (containing phytosteryl fatty acid esters), or a skimmed milk drinking yogurt enriched with γ-oryzanol as a source of phytosteryl/-stanyl ferulic acid esters. The subjects received 3.4 g of γ-oryzanol in the drinking yogurt per day for three days. Lubinus et al. did not report adverse events.

The effect of γ-oryzanol supplementation during resistance training was further assessed in a placebo-controlled, double-blinded study by administrating 600 mg γ-oryzanol/day for 9 weeks to 30 males aged 18 to 32 years old (placebo n=14, γ-oryzanol n=16) (Eslami et al. 2014). Eslami et al. did not report adverse events.

Table 20. Human Studies with γ-Oryzanol

Reference	Type and Purpose of Study	Subject Population (M/F)	Manufacturer	Route of Administration	Exposure	Duration	Safety Data
Yoshino *et al.*, 1989	Type: Single arm, uncontrolled, nonrandomized trial Purpose: Effect of γ-oryzanol or γ-oryzanol + probucol on hyperlipidemia	N=80 (25 males, 55 females), mean age 61 years. with varying degrees of hyperlipidemia. 70 subjects received γ-oryzanol alone	Not specified	Not reported	300 mg/day	6 months	γ-Oryzanol was well tolerated in all subjects. Blood cell count and serum chemistry showed no serious adverse effect of both γ-oryzanol or γ-oryzanol + probucol on hematopoietic, hepatic or renal function.
Sasaki *et al.*, 1990	Type: Single arm, uncontrolled, nonrandomized trial Purpose: Effect of γ-oryzanol on serum lipids and apolipoproteins in dyslipidemic schizophrenics receiving major tranquilizers	N=20 (11 males, 9 females), mean age 51 years. Chronic schizophrenic patients with dyslipidemia	Otsuka Pharmaceutical Co., Japan	Oral	100 mg three times daily,	16 weeks	No treatment side effects were recorded. No changes in body weight. No adverse effects or abnormal laboratory values were observed during study period.
Fry *et al.*, 1997	Type: Double-blind; placebo-controlled trial Purpose: Effect of γ-oryzanol supplementation during resistance exercise training	N=22, college aged males.	Not specified	Oral	Group 1 (placebo): lactose (n=11) Group 2: 500 mg γ-oryzanol (n=11)	9 weeks	Adverse events were not reported, but there were no significant effects on resting heart rate, blood pressure, hematocrit, plasma cholesterol, serum triglycerides, serum albumin, serum calcium, serum magnesium, serum cortisol, plasma testosterone, serum estradiol, or serum insulin.

Table 20. Human Studies with γ-Oryzanol

Reference	Type and Purpose of Study	Subject Population (M/F)	Manufacturer	Route of Administration	Exposure	Duration	Safety Data
Berger *et al.*, 2005	Type: Randomized, parallel group trial Purpose: Evaluate cholesterol lowering effects of rice bran oil with low and high amounts of γ-oryzanol in mildly hypercholesterolemic men	N=30 males, 38-64 years old	Rito Inc, USA	Oral	Treatment 1: 50 g rice bran oil (RBO) with 0.05g γ-oryzanol/day Treatment 2: 50 g rice bran oil (RBO) with 0.8g/day γ-oryzanol	4 weeks	There were no dropouts and expulsions during the duration of the experiment. In the daily logs. Authors stated that were no serious adverse effects were reported. Both treatments lowered total plasma cholesterol, low density lipoprotein cholesterol (LDL-C), and the LDL-C/high density lipoprotein cholesterol ratio.
Accinni *et al.*, 2006	Type: Randomized, placebo-controlled Purpose: Effect of combined dietary supplements, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), vitamin E, γ-oryzanol and niacin, on lipid profile, inflammatory status and oxidative balance.	N = 57 (29 males, 28 females), median age 48 years.	Not specified	Oral	Group 1: Placebo (n=19) Group 2: 660 mg EPA, 440 mg DHA, 4 mg vitamin E (n=18) Group 3: 660 mg EPA, 440 mg DHA, 4 mg vitamin E 40.2 mg γ-oryzanol and 18 mg niacin. (n=20)	4 months	There were no dropouts and the authors stated that during treatment, no patients reported any particular side effects Compared to the placebo-treated group, there were significant decreases in plasma triglycerides, total cholesterol, LDL-C, total antioxidant capacity, reactive oxygen species, plasma interleukin 1β, plasma tumor necrosis α, and thromboxane B_2 levels in the γ-oryzanol-treated group. There was also a significant increase in plasma levels of vitamin E in the γ-oryzanol-treated group compared to the placebo-treated group.

Table 20. Human Studies with γ-Oryzanol

Reference	Type and Purpose of Study	Subject Population (M/F)	Manufacturer	Route of Administration	Exposure	Duration	Safety Data
Lubinus *et al.*, 2013	Type: Randomized, single-blind three group crossover trial Purpose: Fate of dietary ferulic acid esters after consumption by healthy humans	n=15 males, mean age 28±3 years old, three groups, crossover trial	Henry Lamotte Oils GmbH, Germany	Oral	Treatment 1: Emmi Benecol enriched with phytostanyl fatty acid esters Treatment 2: Becel pro.active enriched with phytosteryl fatty acids esters Treatment 3: Skimmed milk drinking yogurt enriched with phytosteryl/-stanyl ferulic acid esters (γ-oryzanol; resulting in 3.4g γ-oryzanol/day)	3 days	Adverse events were not reported.
Eslami *et al.*, 2014	Type: Randomized, double-blind, placebo-controlled Purpose: Determine the effects of γ-oryzanol supplementation during resistance training	N=30, all males, aged 18 to 32 years old.	Qingdao Reach International Inc., China	Oral	Group 1 (placebo): lactose (n=14) Group 2: 600 mg γ-oryzanol/day (n=16)	9 weeks	Adverse events were not reported.

Together these studies indicate that γ-oryzanol is safe and well-tolerated at multiple oral doses in adults. Taken together, the data supports that ORYZA GAMMAX is safe for consumption by both male and female adults.

E. ALLERGENICITY

Although studies have reported that γ-oryzanol and its components inhibit mast cell degranulation and passive cutaneous anaphylaxis reactions (Oka et al., 2010), and promote cell-mediated and humoral responses (Ghatak and Panchal, 2012), the allergenicity of γ-oryzanol itself has not been directly assessed. Importantly, rice is generally considered to be non-allergenic and only a few isolated cases of allergy to raw rice have been reported (for review see Cosmetic Ingredient Review Expert Panel 2006). Moreover, no published reports of allergic reactions to γ-oryzanol were retrieved in a search of PubMed and Google Scholar using the terms oryzanol and allergy, which was conducted on January 29, 2018.

F. REGULATORY APPROVALS ACROSS THE WORLD

γ-Oryzanol is an approved food additive and specified as a substance composed mainly of both esters consisting of each combination of sterols and ferulic acid, and triterpene alcohols and ferulic acid obtained from rice bran or germ oil in Japan. A preparation of γ-Oryzanol from another manufacturer is used as a pharmaceutical in South Korea. Although the regulatory approvals in other countries are not known, ORYZA GAMMAX is exported to Thailand, Vietnam, Indonesia, Philippines, Korea, Taiwan, and South Africa.

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 the use of ORYZA GAMMAX (γ-oryzanol) in selected foods and beverages in the U.S. food supply, including meat, poultry, and fish products, dried bean, pea, nut and seed products, grain products, fruit and vegetable products, oils and salad dressings, sugars, sweets, and beverages. This GRAS determination for the use of γ-oryzanol for the intended uses specified above 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 intake of ORYZA GAMMAX 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 substances directly added to food, and is based on generally available and accepted information.

The proposed use of ORYZA GAMMAX as an ingredient for the intended uses in foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. γ-Oryzanol is a dried, refined, and bleached extract of *Oryza sativa Japonica* that contains a mixture of sterol ferulates, including cycloartenol ferulate, 24-methylene cycloartanol ferulate, campesterol ferulate, β-sitosteryl ferulate, cycloartanol ferulate, and cyclobranol ferulate, which account for not less than 85% of the finished product.

2. All *O. sativa* rice bran and germ used to manufacture ORYZA GAMMAX are grown by farmers that comply with Good Agricultural Practices (GAPs) and all applicable regulations for import of raw agricultural commodities into the United States, including compliance with pesticide tolerances.

3. ORYZA GAMMAX is manufactured from *Oryza sativa Japonica* rice bran in a series of stages according to Good Manufacturing Practices (GMPs).

4. All processing aids used in the production of ORYZA GAMMAX are Food Chemicals Codex (FCC) grade.

5. Product specifications other quality testing (solvents, pesticides, mycotoxins, PCBs/dioxins/furans, allergens) are in place to control the levels of the

predominating sterol ferulates, heavy metals, and microbes, and to ensure a consistent and food grade finished product.

6. Sterol ferulates are poorly absorbed from the intestinal tract, similar to other plant sterols, are distributed to a variety of tissues, including the adrenal glands, lungs, spleen, and liver, and are metabolized to ferulic acid, *m*-coumaric acid, *m*-hydroxyhippuric acid, hippuric acid, *m*-hydroxyphenyl propionic acid, and dihydroferulic acid.

7. The safety of ORYZA GAMMAX was determined in a pivotal published 90-day toxicology study that identified a no observed adverse effect level (NOAEL) of 2000 mg γ-Oryzanol/kg/day (Moon et al., 2017).

8. Application of a 100-fold safety factor to the NOAEL determined in the pivotal 90-day toxicology study results in an acceptable daily intake for ORYZA GAMMAX of 20 mg/kg/day or 1.2 g/day for a 60 kg human.

9. The safety of ORYZA GAMMAX is corroborated by carcinogenicity studies conducted in rats and mice, a one-year chronic toxicity in rats, developmental toxicity in rats and mice, and genotoxicity studies using other γ-oryzanol-containing products.

10. Clinical studies have reported that γ-oryzanol-containing products are well tolerated up 3.4 g/day for up to six months.

11. The addition of ORYZA GAMMAX to the intended foods will result in a mean estimated daily intake (EDI) of 139 mg/day (2.0 mg/kg body weight/day) and a heavy consumer (90^{th} percentile) intake of 313 mg/day (4.6 mg/kg body weight/day). There are no known significant dietary sources of γ-oryzanol in the United States.

12. The EDI is substantially below the ADI, establishing the safety of ORYZA GAMMAX intake from the intended uses and use levels.

Determination of the GRAS status of ORYZA GAMMAX under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, CFS, FASN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, FACN, and Thomas Sox PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of ORYZA GAMMAX and the human exposure to ORYZA GAMMAX resulting from its intended use as an ingredient in selected foods:

> *There is no evidence in the available information on* ORYZA GAMMAX *that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when* ORYZA GAMMAX *is used at levels that might reasonably be expected from the proposed applications of* ORYZA GAMMAX *for use in selected food as proposed by Oryza Oil & Fat Chemical Co., Ltd.*

Therefore, ORYZA GAMMAX is safe and GRAS at the proposed levels of addition to the intended foods. ORYZA GAMMAX 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.

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

Signature: (b) (6)

Date: June 5, 2018

A. Wallace Hayes, PhD, DABT, FATS, ERT
GRAS Expert Panel Member
Harvard School of Public Health

Signature: (b) (6)

Date: June 5, 2018

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

Signature: (b) (6)

Date: June 5, 2018

Claire Kruger, PhD, DABT
Scientific Advisor to the Panel
ChromaDex Spherix Consulting

Signature: (b) (6)

Date: June 5, 2018

Bonnette, Richard

From: Claire Kruger <ClaireK@chromadex.com>
Sent: Friday, July 20, 2018 2:25 PM
To: Bonnette, Richard
Cc: Kathy Brailer; Dietrich Conze; Fred Lozy; Claire Kruger
Subject: RE: GRAS submission for gamma-oryzanol - use levels and USDA supporting information

Follow Up Flag: Follow up
Flag Status: Flagged

Dear Richard:
Thank you for bringing this issue to our attention. After review, we realize that due to a clerical error, an incorrect subcategory list was inserted into the document. The correction, which reflects the food codes used for the EDI assessment, is highlighted below and should replace that section in the GRAS Notice. There are no proposed foods that are regulated by USDA. Additionally, as indicated in Part 3 of the GRAS Notice, the level of use in the food products is 0.1%.

Please let me know if you have any additional questions.

D. ESTIMATED DAILY INTAKE

A fixed concentration of ORYZA GAMMAX was used for each food and beverage, rather than a fixed mass to accommodate variations in serving size for the intended food products. The optimal fixed concentration was determined from the literature (Lerma-Garcia, 2009). In Lerma-Garcia et al., oxidation of whole milk powder during storage was reported to be reduced by adding 0.1% rice bran oil, and when compared with control milk powder, consumers could not detect any effect on the flavor of the reconstituted whole milk powder containing 0.1% rice bran oil. As a result, the EDI was calculated using this 0.1% concentration for the following subcategories of food:

- milk and milk products
- soups, broths, extracts from meat, poultry, fish base (fish, shellfish soups)
- dry beans, peas, other legumes, nuts and seeds
- grain products
- fruits
- vegetables
- fats, oils and salad dressings
- sugars, sweets and beverages

ChromaDex Spherix Consulting has completed an assessment of the consumption of ORYZA GAMMAX by the U.S. population resulting from the proposed uses of ORYZA GAMMAX. Estimates for the

intake were based on the proposed food uses and maximum use level in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2012; Bodner-Montville et al, 2006). Calculations for the mean and 90th percentile intakes were performed for all proposed food uses of ORYZA GAMMAX combined. The intakes were reported for the following population groups:

- children, ages 2 to 5 years,
- children, ages 6 to 12 years,
- teenagers, ages 13 to 19 years,
- adults, ages 20 years and up,
- total population (all age groups combined, excluding infants of 0-2 years),

Best regards,
Claire

Claire Kruger, PhD, DABT, CFS
President
Senior Director Regulatory Affairs

ChromaDex

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From: Bonnette, Richard [mailto:Richard.Bonnette@fda.hhs.gov]
Sent: Tuesday, July 17, 2018 3:33 PM
To: Claire Kruger <ClaireK@chromadex.com>
Subject: GRAS submission for gamma-oryzanol - use levels and USDA supporting information

Hello Claire,
We've completed a preliminary pre-filing review of your submission dated June 26, 2018, for Y-oryzanol submitted on behalf of Oryza Oil and Fat Chemical Co, Ltd. We noticed two issues that need to be resolved before the submission can be filed as a GRAS notice. The first is that the notices describes uses in USDA-regulated meat and poultry products but does not contain USDA-specific information to support the use of the ingredient in those products. Typically, in the case of USDA-regulated uses described in a GRAS notice, suitability studies and other types of USDA-specific information would be included in a GRAS notice submitted to FDA, often as an appendix, and FDA would then pass it along with the entire GRAS notice to USDA for their concurrent evaluation. USDA has requested that we not forward USDA-relevant GRAS notices for their review if it lacks the specific data supporting this use of an ingredient. I'd recommend getting in touch with USDA regarding the kinds of information they will need (Valeria Green at valeria.green@fsis.usda.gov or (301) 504-0846), and then provide that material to us as part of a future submission. You may also ask us to remove the meat and poultry uses from this GRAS submission, which would of course remove the requirement for these data.

The second issue relates to use levels. While the food categories are described in form 3667 and in Part 3, neither section clearly describes the intended use levels of γ-oryzanol in those foods. I didn't see it obviously in any other sections of the notice. Can you provide more information?

If you have any questions, please let me know.

Thanks,
Richard

Richard E. Bonnette, M.S.
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1235
richard.bonnette@fda.hhs.gov

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71 pages have been removed in accordance with copyright laws. The removed references are:

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ANALYTIC AND REPORTING GUIDELINES; The National Health and Nutrition Examination Survey (NHANES); Last Update: December, 2005; Last Correction, September, 2006; National Center for Health Statistics; Centers for Disease Control and Prevention ;Hyattsville, MD (http://www.cdc.gov/nchs/about/major/nhanes/NHANES99 00.htm)