# Annex 15
# 28-Day Study Report- Draft

# *Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

## DATA REQUIREMENTS

OECD Guidelines for Testing of Chemicals, Section 4 (Part 407): Health Effects, *Repeated Dose 28-Day Oral Toxicity Study in Rodents* (2008).

US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 3. a. *Short-Term Toxicity Studies with Rodents* (November 2003).

## STUDY NUMBER

55164

## PERFORMING LABORATORY

Product Safety Labs
2394 US Highway 130
Dayton, New Jersey 08810

## STUDY COMPLETION DATE

## STUDY DIRECTOR

Mark R. Bauter, BA

## SPONSOR

Elanco Animal Health Incorporated
2500 Innovation Way
Greenfield, Indiana 46140

## SPONSOR STUDY NUMBER

ELA210070

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

*L. reuteri* 3630 & *L. reuteri* 3632

This study meets the requirements of 21 CFR 58: U.S. FDA GLP Standards, 1987, which are compatible with OECD Principles of GLP (as revised in 1997) published in ENV/MC/CHEM (98)17, OECD, Paris, 1998, with the following exception:

Analyses performed and study data collected at BioPrimate (translocation analysis) were not in compliance with GLPs.

Specific information related to the characterization of the test article as received and tested is the responsibility of the study Sponsor (Section 3.A).

Study Director:_____ Date:_____

Name of Signer: Mark R. Bauter, BA

Name of Company: Product Safety Labs

Sponsor:_____ Date:_____

Name of Signer:_____

Name of Company: Elanco Animal Health Incorporated

Submitter:_____ Date:_____

Name of Signer:_____

Name of Company: Elanco Animal Health Incorporated

## QUALITY ASSURANCE STATEMENT

The Product Safety Labs' Quality Assurance (QA) Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA Activities for This Study:

| QA Activity | Performed By | Date Conducted | Date Findings Reported To Study Director And Management |
|---|---|---|---|
| Protocol review | R. Krick;<br>B. Simms;<br>B. Simms;<br>B. Simms | Mar 29, 2021;<br>Jul 8, 2021;<br>Jul 23, 2021;<br>Aug 2, 2021 | Mar 29, 2021;<br>Jul 8, 2021;<br>Jul 23, 2021;<br>Aug 3, 2021 |
| Critical phase inspection:<br>*CFU Enumeration for Day 9* | K. Rea | Apr 21, 2021 | Apr 21, 2021 |
| Critical phase inspection:<br>*Dosing on Day 15* | B. Simms | Apr 27, 2021 | Apr 27, 2021 |
| Critical phase inspection:<br>*Tissue list in Provantis* | B. Simms | May 12, 2021 | May 13, 2021 |
| Critical phase inspection:<br>*Terminal necropsy and tissue collection Day 31* | B. Simms | May 13, 2021 | May 13, 2021 |
| Critical phase inspection:<br>*Coagulation sample analysis* | K. Rea | May 20, 2021 | May 20, 2021 |
| Raw data audit | B. Simms | Jul 6, 2021;<br>Jul 23, 2021;<br>Aug 2-3, 2021 | Jul 8, 2021;<br>Jul 23, 2021;<br>Aug, 3, 2021 |
| Draft report review | B. Simms | Aug 2-3, 2021 | Aug 3, 2021 |

QA Statements for the clinical pathology and histopathology phases of the study may be found in Appendices K and R, respectively.

Final report reviewed by:

Quality Assurance
Product Safety Labs

Date

## CERTIFICATIONS

We, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Mark R. Bauter, BA
Study Director/Associate Director of Toxicology
Product Safety Labs

Date

Daniel J. Merkel, BS, MBA
President
Product Safety Labs

Date

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF APPENDICES

## STUDY INFORMATION

| | |
|---|---|
| Protocol Number: | P713.01 DDU |
| Test article IDs: | 1) *L. reuteri ATCC PTA-126787 (L. reuteri* 3630*)*<br>2) *L. reuteri ATCC PTA-126788 (L. reuteri* 3632*)* |
| Test article Lot Numbers: | 1) Lot #: 201123LRE3630<br>2) Lot #: 201123LRE3632 |
| Physical Description: | White to Tan powder |
| Date Test article Received: | February 26, 2021, April 8, 2021, and May 12, 2021 |
| PSL IDs:<br>(Amendments 1 and 4) | 1) 210226-1D, 210408-3D, 210512-1D<br>2) 210226-2D, 210408-2D |
| PSL Study Number: | 55164 |
| Sponsor Study Number: | ELA210070 |
| Sponsor: | Elanco Animal Health Incorporated<br>2500 Innovation Way<br>Greenfield, Indiana 46140 |
| Study Initiated-Completed: | April 13, 2021 – (see report cover page) |
| In-Life Study Initiated-Completed: | April 13 – May 13, 2021 |
| Notebook Number: | 55164: pages 1-To be entered prior to final |

## KEY PERSONNEL

| | |
|---|---|
| Product Safety Labs: | |
| President: | Daniel J. Merkel, BS, MBA |
| Study Director/Associate Director of Toxicology: (Amendment 7) | Mark R. Bauter, BA |
| Primary Scientist: | Morgan Woods, BS |
| Contributing Personnel: | Brittany Bamonte, BS<br>Nicole Baudendistel, BS<br>Aubrey Blue<br>Jamie Boulet, RLATR<br>Janet Dell John, BA, RLATG<br>Anupama Dubey, MS<br>Denise DiCarlo-Emery, BS<br>Victoria Golanski, BS<br>Heylin Gonzales, BS<br>Brooke Levens<br>Harry Maselli, ALAT<br>Mary Matthews, BS<br>Stephanie Meade, BS<br>Jeanette Paez, MS<br>Andrew Rockal, BS<br>Mark Schooley<br>Matthew Sorber, BS<br>Jonathan Stanley, BS |
| Laboratory Director: | Daniel Merrill, BS, MBA |
| Director of Quality Assurance: | Edward Hymen, BA, MBA, RQAP-GLP, CQA |
| Technical Writing Supervisor: | Celeste Dunn, AS |

## KEY PERSONNEL (Cont).

The following were responsible for the in-life data collection and the clinical pathology, tissue enumeration, and test article dose sample collection and evaluation:

| | |
|---|---|
| In-life, clinical chemistry, coagulation, urinalysis, tissue enumeration, and test article dose analysis | Product Safety Labs<br>2394 US Highway 130<br>Dayton, NJ 08810<br>P.I. (Dose Analysis): Denise DiCarlo-Emery, BS |
| Hematology analysis | Corteva Agriscience<br>Haskell R&D Center<br>1090 Elkton Road<br>Newark, DE 19711<br>P.I. Eva Maria Silvestro, AAS, MLT (ASCP) |
| Clinical pathology evaluation | Eurofins Advinus Ltd<br>21 & 22 Phase II, Peenya Industrial Area<br>Bengaluru, 560 058, India<br>P.I. Dr. Jayachandra, K.C., M.V.Sc., DABT |
| Translocation Analysis: | BioPrimate<br>1 Oak Avenue<br>Newark, DE 19711<br>P.I. Frank Burns |

The following were responsible for the histological slide preparation and pathology evaluations:

| | |
|---|---|
| Histological slide preparation | StageBio<br>5930 Main Street<br>Mount Jackson, VA 22842, USA<br>P.I. (histology): Erin Galati, HT (ASCP) |
| Histological slide evaluation | StageBio<br>5930 Main Street<br>Mount Jackson, VA 22842, USA<br>P.I. (pathology) Allen W. Singer, DVM, DACVP, DABT |

## 1. OBJECTIVE

The objective of this study was to evaluate the potential toxicity of *Limosilactobacillus reuteri* (*L. reuteri*) ATCC PTA-126787 (*L. reuteri* 3630) and ATCC PTA-126788 (*L. reuteri* 3632) and the potential for translocation of the organisms from the GI tract in male and female rats likely to arise from repeated exposure via oral gavage over a test period of at least 28 days. A no-observed-adverse-effect-level (NOAEL) was also sought for each sex.

## 2. SUMMARY

Eighty (80) adult Crl: Sprague-Dawley CD® IGS rats (40 males and 40 females) were distributed into four groups (10/sex/group). Two bacterial strains, *L. reuteri* 3630 and *L. reuteri* 3632, were combined to form the combined test article, as it is referred to throughout the report.

Combined test article formulations used in this study:

| Group | Test Article (mg/kg bw/day) | | Test Article Concentration (mg/mL) | |
|---|---|---|---|---|
| 1 | 0 (vehicle only) | | 0 (vehicle only) | |
| 2 | *L. reuteri* 3630 | 126.5 | *L. reuteri* 3630 | 25.3 |
| | *L. reuteri* 3632 | 75.8 | *L. reuteri* 3632 | 15.2 |
| 3 | *L. reuteri* 3630 | 253.3 | *L. reuteri* 3630 | 50.7 |
| | *L. reuteri* 3632 | 151.6 | *L. reuteri* 3632 | 30.3 |
| 4 | *L. reuteri* 3630 | 760 | *L. reuteri* 3630 | 152 |
| | *L. reuteri* 3632 | 455 | *L. reuteri* 3632 | 91 |

Dose volume: 5 mL/kg/day; vehicle: normal saline

A vehicle (normal saline) control group was also dosed as part of this study. The doses were administered for 29 or 30 days, based on the animal's individual necropsy date.

The animals were observed at least once daily for viability, signs of gross toxicity, and behavioral changes, and weekly for a battery of detailed observations. Body weights were recorded two times during the acclimation period (considered prior to initial dose administration on Day 1), twice weekly thereafter, shortly before dose administration in order to adjust the dose volume on body weight, and immediately prior to sacrifice. Individual food consumption was collected to coincide with body weight measurements. Urine was collected from animals fasted the day prior to respective sacrifice, Day 31 for five animals per sex per group for animals assigned to translocation analysis and on Day 30 for the remaining animals. Blood was sampled from study animals just prior to scheduled necropsy for hematology, clinical chemistry, and coagulation assessments. Gross necropsies were performed on all surviving study animals, and histopathological analysis was performed on selected tissues. Blood, liver, and mesenteric lymph nodes were collected for translocation analysis from five animals per sex on Day 31.

Samples of the neat test article were collected each week to evaluate stability and for colony enumeration (concentration verification). Samples were also collected from the dose formulation mixtures to verify homogeneity (Test Day 1) and dose concentration verification weekly.

Dose verification by bacterial enumeration in test article formulations – target and nominal dose levels (geometric means; n=7):

| Dose Group | Target dose for each strain [CFU/kg] | Nominal dose level verified by bacterial enumeration for strain 3630 [CFU/kg]; geometric mean* | Nominal dose level verified by bacterial enumeration for strain 3632 [CFU/kg]; geometric mean* |
|---|---|---|---|
| 2 | 1.7E+10 | 3.1E+09 | 7.5E+09 |
| 3 | 3.3E+10 | 4.5E+09 | 1.4E+10 |
| 4 | 1.0E+11 | 1.6E+10 | 5.7E+10 |

* the nth root of the product of n bacterial counts over the entire study duration
$\sqrt[n]{x1 * x2 * xn}$;
n=7.

The results of the neat test article concentration verification were determined to be generally below the targeted concentrations, as indicated in the Certificate of Analysis, which is attributed to slight differences in sampling and enumeration. Due to the test article containing combined concentrations of *L. reuteri* 3630 and *L. reuteri* 3632 strains, the homogeneity of the distribution of these strains within the dose mixtures could not be determined to within an adequate margin of variation. Results show a variable dose concentration of each strain over the course of the study which is not unexpected with microbiological organisms. Overall, the animals are considered to have received sufficient CFU from each test article to evaluate the potential toxicity of the *L. reuteri* 3630 and 3632 strains in male and female rats from repeated exposure via oral gavage, and the potential for translocation of the organisms from the GI tract after administration.

There were no mortalities or clinical signs attributable to the test article. One Group 2, low dose male (Animal 3829), was humanely sacrificed. The death was determined to be the result of an esophageal perforation caused by a gavage error and unrelated to test article treatment. There were no test article-related changes in mean body weights, body weight gain, food consumption, or food efficiency in the study.

The test strains *L. reuteri* (genotypes 1A and 1B) did not translocate compared to the endogenous *L. reuteri* (genotype 2). A low incidence of bacteria found on plates from blood samples was not associated with treatment and likely resulted from environmental contamination during the study. Absent any pathological findings or other treatment related toxicity, the translocation findings would not indicate the test strains to be harmful.

There were no test article-related changes in hematology, coagulation, serum chemistry or urinalysis parameters. All statistically significant findings were either small in magnitude or did not correlate to any other clinical or histopathological finding and thus were within expected biological variation and considered not to be toxicologically relevant.

No macroscopic or microscopic findings were attributed to administration of the test article. Non-test article-related microscopic observations noted were either, present in all groups including vehicle control, and/or considered spontaneous, incidental background changes in laboratory rats.

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect-level (NOAEL) for the oral administration of the combined test article,*L. reuteri* 3630 & *L. reuteri* 3632, was determined to be the high dose group, 1.6E+10 CFU *L. reuteri* 3630/kg bw/day (760 mg/kg bw/day) plus 5.7E+10 CFU *L. reuteri* 3632/kg bw/day (455 mg/kg bw/day) for male and female Sprague Dawley rats.

## 3. TEST ARTICLE

### A. Source

The test article was provided by the Sponsor.

### B. Identification

The test article strains were identified using the following information provided by the Sponsor and Product Safety Labs (PSL) identification number. Test article strains 1 and 2 were mixed together to prepare a single dose solution which will be referred to heretofore as the "test article". Documentation of the methods of synthesis, fabrication, or derivation of the test article is retained by the BioSource Cultures & Flavors, S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777.

Test Article 1: *L. reuteri* ATCC PTA-126787 (*L. reuteri* 3630)
Lot #: 201123LRE3630
PSL ID: 210226-1D, 210408-3D, 210512-1D
Physical Description: White to Tan powder
Composition: 100% *L. reuteri* 3630 (see also Appendix C)
Storage Conditions: Frozen (store ~ -25 to -15° C until ready to use)
Expiration Date: 11/23/21

Test Article 2: *L. reuteri* ATCC PTA-126788 (*L. reuteri* 3632)
Lot #: 201123LRE3632
PSL ID: 210226-2D, 210408-2D
Physical Description: White to Tan powder
Composition: 100% *L. reuteri* 3632 (see also Appendix C)
Storage Conditions: Frozen (store ~ -25 to -15° C until ready to use)
Expiration Date: 11/23/21

Vehicle Control: Normal Saline
Batch #: 19F002
Storage Conditions: Ambient
Expiration Date: 06/2021
Supplier: Vedco

### C. Analysis

The Sponsor was responsible for all analytical work required to characterize the neat test article, and validate its stability.

### D. Hazards

Appropriate routine safety precautions were exercised in the handling of the test article.

## 4. GENERAL TEST SYSTEM PARAMETERS

### A. Animal Requirements

4.A.1 Number of Animals: 80

4.A.2 Number of Groups: 4 (3 dose levels and 1 vehicle control group/sex).

4.A.3 Number of Animals per Group: 20 (10 male, 10 female)

4.A.4 Sex: Male and female; females will be nulliparous and non-pregnant.

4.A.5 Species/Strain: CRL Sprague-Dawley CD® IGS rats.

4.A.6 Age/Weight: Approximately nine weeks at initiation; the weight variation did not exceed ± 20% of the mean weight for each sex.

4.A.7 Supplier: Charles River Laboratories, Inc. Rats were shipped from the Raleigh, North Carolina facility in filtered cartons by airfreight and/or truck.

On March 25, 2021, eighty-eight (88) CRL Sprague-Dawley CD® IGS rats (44 per sex) arrived from Charles River Laboratories, Raleigh, NC, with an assigned birth date of February 7, 2021. The rats were designated by the supplier to be seven to eight weeks of age upon arrival.

### B. Test System Justification

The Sprague-Dawley® rat was the system of choice because, historically, it has been a preferred and commonly used species for oral toxicity tests. The current state of scientific knowledge does not provide acceptable alternatives to the use of live animals to accomplish the objective of this study.

### C. Animal Husbandry

4.C.1 Housing

The animals were group housed (2 per cage) unless fighting ensued, in which case the specific animals were housed individually, in cages which conform to the size recommendations in the latest *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). The animal room had a 12-hour light/dark cycle and was kept clean and vermin free.

4.C.2 Animal Room Temperature and Relative Humidity Ranges

The temperature and humidity ranged from 20-22ºC and 43-58%, respectively.

4.C.3 Acclimation

The animals were conditioned to the housing facilities for 19 days prior to testing. Body weights and clinical observations were recorded prior to study start.

4.C.4 Animal Room Air Changes/Hour

Animals were housed in Room #21, with 11 air changes per hour. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

#### 4.C.5 Feed

2016 Certified Envigo Teklad Global Rodent Diet® (Envigo Teklad, Inc.) was stored in a dedicated temperature and humidity monitored feed storage site and available *ad libitum* during acclimation and throughout the study, except when animals were fasted for clinical sample collections and terminal sacrifice.

#### 4.C.6 Water

Filtered tap water was available *ad libitum* from an automatic watering access system. Water analysis was conducted by Precision Analytical Services, Inc., Toms River, NJ and South Brunswick Municipal Water Supply, South Brunswick, NJ.

#### 4.C.7 Contaminants

There are no known contaminants reasonably expected to be found in the food or water that would interfere with the results of this study. Routine analysis consisting of the lot of feed used in this study was received from Envigo Teklad, Madison, WI. Water analysis is conducted periodically and the records are kept on file at Product Safety Labs. The date of the most recent analysis is reported in Appendix B.

#### 4.C.8 Viral Screen

The animals used in this study were considered to be pathogen-free as received from the vendor (Section 4.A.). Rodent-health surveillance for study animals was monitored by designating three rats as "sentinels" for the study room (Animals 356F 5/13/21, 374F 5/13/21 and 362F 5/13/21). Sentinels were housed under the conditions of the study, on racks alongside study animals, for the duration of the study Room #21 (from April 13, 2021, to May 13, 2021). These animals were not a part of the study, and were clearly marked as such. A serum sample was collected from each sentinel rat for screening of common rat pathogens (Rat Parvovirus, Toolan's H-1 Virus, Kilham Rat Virus, Rat Minute Virus, Parvovirus NS-1, Rat Coronavirus, Rat Theilovirus, and Pneumocystis carinii). The serum samples were sent on ice to IDEXX BioAnalytics (Columbia, MO) for evaluation. Serological pathogen screening results for the sentinels Animals 356F 5/13/21, 374F 5/13/21 and 362F 5/13/21, corresponding with this study, are reported in Appendix B. The sentinel samples were negative for all pathogens evaluated and therefore, the study animals were considered to be healthy and reasonably free of common rat pathogens.

### D. Identification

#### 4.D.1 Cage

Each cage was identified by a cage card indicating the study number, dose level, group assignment, individual identification, and sex of the animals.

#### 4.D.2 Animal

Each animal was given a sequential number in addition to being uniquely identified with a Monel® self-piercing stainless steel ear tag. Only the sequential animal number is presented in this report.

## 5. EXPERIMENTAL DESIGN

### A. Route of Administration

The test article was administered by oral gavage (PO).

### B. Justification of Route of Administration

The oral route of administration was selected by the Sponsor as the clinical route of exposure will be via oral capsule or oral liquid. This route of administration is recommended in the referenced guidelines.

### C. Control of Bias

Animals were randomly assigned, stratified by body weight, to test groups.

### D. Dose Levels

Ten animals per sex were assigned to each of the following test groups for toxicity evaluation:

| Group | No. Animals/ Group M/F | Test Article (mg/kg bw/day) | Targeted Dose Level (CFU/kg bw/day) | Calculated[b] Dose (CFU/kg bw/day) | Dose Volume (mL/kg bw/day) | Expected Test Article Concentration (mg/mL[a]) |
|---|---|---|---|---|---|---|
| 1 | 10/10 | 0 Vehicle only | 0 | 0 | 5 | 0 |
| 2 | 10/10 | *L. reuteri* 3630 (126.5) + *L. reuteri* 3632 (75.8) | 1.668E+10 + 1.668E+10 | 3.1E+09 + 7.5E+09 | | *L. reuteri* 3630 (25.3) + *L. reuteri* 3632 (15.2) |
| 3 | 10/10 | *L. reuteri* 3630 (253.3) + *L. reuteri* 3632 (151.6) | 3.337E+10 + 3.337E+10 | 4.5E+09 + 1.4E+10 | | *L. reuteri* 3630 (50.7) + *L. reuteri* 3632 (30.3) |
| 4 | 10/10 | *L. reuteri* 3630 (760) + *L. reuteri* 3632 (455) | 1.001E+11 + 1.001E+11 | 1.6E+10 + 5.7E+10 | | *L. reuteri* 3630 (152) + *L. reuteri* 3632 (91) |

[a] LR3630 = 132 000 000 000 CFU/g (1.32E+11) and LR3632 = 220 000 000 000 CFU/g (2.2E+11), as determined by Product Safety Labs prior to study initiation (Section 6.B).

[b] Calculated using the geometric mean (see Table 1C).

### E. Justification of Dose Level Selection

Dosing was selected based on the proposed clinical dose and was not expected to cause marked toxicity. The high dose (Group 4) corresponds to a margin of safety of 700x, based on a daily oral dose of 2.0E+10 CFU (for the two LR strains combined in equal proportions) per person (2.86E+08 CFU/kg; assuming a mean human body weight of 70 kg). The low dose (Group 2) corresponds to a Margin of Safety of 117x.

## 6. GENERAL PROCEDURES

### A. Selection of Animals

After acclimating to the laboratory environment for nineteen days, the rats were examined for general health and weighed. Only those rats free of clinical signs of disease or injury and having a body weight range within ±20% of the mean for each sex were selected for test. Eighty (80) healthy rats (40 males; 40 females) were selected for test. The animals weighed 312-350 grams (males) and 174-219 grams (females) and were approximately nine weeks of age at initiation of dosing. The rats that were used on test were randomly distributed, stratified by body weight, among the dose and control groups on the day of study start.

### B. Dose Preparations and Procedures

#### 6.B.1 Test Article Preparation

Product Safety Labs analyzed individual samples of *L. reuteri* 3630 and *L. reuteri* 3632 provided by the Sponsor to determine the actual recovery of viable CFU. These values were used to calculate the appropriate dose indicated in Section 5.D. Actual doses administered were determined and reported at least weekly during the study (see Table 1C).

Each test article component strain (*L. reuteri* 3630 or *L. reuteri* 3632) was received by the testing facility (PSL). Multiple shipments of the same lot of each strain (see Section 3.A.) were received from the Sponsor on an as-needed basis in order to complete the study. After receipt and prior to use, each component strain was appropriately portioned into foil pouches (Ted Pella Inc, 139-311) by weighing approximately 15.2 g of *L. reuteri* 3630 or 9.1 g of *L. reuteri* 3632 into separate pouches. The pouches were closed/sealed after expelling excess air to minimize exposure to oxygen and the weight of each pouch was recorded. The pouches were stored in a freezer at approximately -20°C until use.

Fresh formulations of the test article were prepared daily. On the day of use, one sealed pouch containing each strain was brought to room temperature. The contents of each pouch were combined into an appropriately sized sterile bottle and 100 mL of vehicle (sterile saline) was added. This concentration was used as the high dose solution. The solution was allowed to sit for ~30 minutes and then placed on a stir plate, stirring continuously while preparing the low (1:6 dilution in sterile saline) and mid dose (1:3 dilution in sterile saline) solutions. The formulations were stirred at ambient temperature until visually homogeneous suspensions were achieved. The dose solutions were continuously stirred until administration. Final formulations were not stored longer than four 4 hours at room temperature prior to dosing.

Weekly samples of the final dose formulations were collected for concentration verification of each strain. Fresh formulations of the test article were prepared daily. The preparation of the dose solutions was documented in the raw data. As a control for growth conditions and media, a pure culture of the respective strains were streaked and observed for growth.

#### 6.B.2 Dosing

Each animal was dosed by oral intubation using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Dose administration was daily (7 days/week). All doses were administered volumetrically at 5 mL/kg body weight. Due to a technical oversight, one Group 2 male (Animal 3829) was not dosed on Day 13 (Deviation 1). The animal was subsequently euthanized due to dosing injury on Day 19; therefore, this missed dose had no impact on the outcome of study. The control group received vehicle (sterile saline)

only, at the same dose volume as the test animals. The dose suspension was maintained and stirred continuously on a magnetic stir plate during dose administration. The first day of administration was considered Day 1 of the study. Dosing was at approximately the same time each day +/- 2 hours.

## C. Analysis of Test Article and Dose Preparations

### 6.C.1 Sample Collection

The neat test article and prepared dosing mixtures were sampled. Additional neat test samples were collected and analyzed weekly.

### 6.C.2 Test Article and Dose Preparation Stability

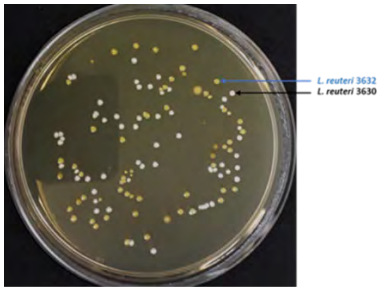
The neat test article was expected to be stable over the course of the study under the conditions of storage. All dose formulations were freshly prepared daily prior to dosing. A sample of the neat test article was collected weekly during the in-life phase.

### 6.C.3 Dose Preparation Homogeneity

Prior to initial dosing on Day 1 of the study, samples from the dose preparations were collected from the top, middle, and bottom for each concentration. The vehicle control mixture was sampled from the middle of the dose preparation only.

### 6.C.4 Dose Preparation Concentration Verification

The dose preparations (treatment and control groups) were sampled at the beginning of the study (as part of the homogeneity assessment) for verification of dose concentration. Dose preparations were also sampled weekly for verification of dose concentration of each strain. The strains were distinguished based on the colony morphology and color (see example below).




*L. reuteri* 3632 is morphologically distinct from *L. reuteri* 3630 - the two strains can be easily differentiated based on the color of the colonies. *L. reuteri 3632* produces orange/yellow colored colonies, while *L. reuteri* 3630 produces creamish white colonies.

### 6.C.5 Sample Analysis

The dosing preparations sampled prior to administration on Day 1 for homogeneity, and weekly thereafter, for concentration verification (as described above) were processed for enumeration as soon as possible (within 2-4 hours of preparation, stored at 4+/-2°C if not analyzed immediately) for viable CFU content according to methods provided by the Sponsor and recorded in the study records. The strains were distinguished based on the colony morphology and color as described in 6.C.4. For comparison and to confirm growing conditions were adequate for the test organisms, a sample of each neat test article was cultured concurrently with the dose solution as described in 6.B.1.

### D. Clinical Observations

All animals were observed at least twice daily for viability. Cage-side observations of all animals were performed daily during the study. All findings were recorded.

On Day 1, prior to test article administration, and weekly thereafter, a detailed observation was conducted while handling the animal. Potential signs noted included, but were not limited to: changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Likewise, changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling), or bizarre behavior (e.g., self-mutilation, walking backwards) were also recorded. The date and clock time of all observations and/or mortality checks were recorded.

When an animal was noticed with severe/remarkable clinical observations (Animal 3829), the animal was euthanized and a gross necropsy was performed. Organs and tissues were excised and submitted for histopathological examination of moribund condition. No further examination or data was collected for this animal (Deviation 2).

### E. Body Weight and Body Weight Gain

Individual body weights were recorded at least two times during acclimation. All animals were weighed on Day 1 (prior to study start) and twice weekly thereafter shortly before dose administration in order to adjust the dose volume on body weight. The animals were weighed prior to scheduled sacrifice in order to calculate organ-to-body weight ratios. Body weight gain was calculated for selected intervals and for the study overall.

### F. Food Consumption

Individual food consumption was measured and recorded twice weekly to coincide with body weight measurements.

### G. Clinical Pathology

Clinical pathology was performed on all surviving animals prior to death for coagulation, hematology, clinical chemistry and urinalysis prior to their respective necropsies. Animals were fasted overnight prior to blood collection. Blood samples were collected from the vena cava, under isoflurane anesthesia.

Additional blood samples were collected (maximum feasible quantity) and processed for serum. Samples were stored at -80ºC for future possible serum cytokine analysis (5 animals/sex/group). Serum samples were sent to BioPrimate for translocation analysis.

All blood samples were evaluated for quality by visual examination.

### 6.G.1 Hematology

Approximately 500 µL of blood was collected in a pre-calibrated tube containing $K_2$EDTA for hematology assessments. The whole blood samples were transferred to the clinical pathology department at Product Safety Labs. Whole blood samples were maintained on ice prior to analysis. All blood samples were evaluated for quality by visual examination.

| | |
|---|---|
| red blood cells count | hemoglobin concentration |
| Hematocrit | mean corpuscular volume |
| mean corpuscular hemoglobin | red cell distribution width |
| reticulocytes count | platelet count |

white blood cells and differential leukocyte counts of neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells.

Mean corpuscular hemoglobin concentration was calculated.

In addition, separate blood smears were prepared from each animal undergoing hematological evaluation and stained with Wright-Giesma stain for future possible examination.

### 6.G.2 Coagulation

Approximately 1.8 mL of blood was collected in a pre-calibrated tube containing 3.2% sodium citrate. These samples were centrifuged in a refrigerated centrifuge and the plasma was transferred to labeled tubes. Plasma samples were stored in a -80ºC freezer until analysis. The following parameters were evaluated:

prothrombin time
activated partial thromboplastin time

### 6.G.3 Clinical chemistry

Approximately 1000 µL of blood was collected into a tube containing no preservative for clinical chemistry assessments. These samples were centrifuged in a refrigerated centrifuge and the serum was transferred to a labeled tube. Serum samples were stored in a -80ºC freezer until analysis. The following parameters were evaluated:

| | |
|---|---|
| Serum aspartate aminotransferase | Serum alanine aminotransferase |
| Sorbitol dehydrogenase | Alkaline phosphatase |
| Gamma-glutamyl transferase | Total bilirubin |
| Urea nitrogen | Total cholesterol |
| Blood creatinine | Fasting glucose |
| Triglycerides | Albumin |
| Total serum protein | Calcium |
| Globulin | Sodium |
| Inorganic phosphorus | Chloride |
| Potassium | |
| L- and D-Lactic acid* | |

*(additional serum samples were sent to the Sponsor and were not evaluated further; Amendment 8)

6.G.4 Urinalysis

The day before their respective collection of samples for the clinical pathology evaluation, animals were placed in metabolism cages. Animals were fasted after 2 pm (at least 16 hours prior to blood collection) and urine was collected from each animal. Urine samples were stored on ice or under refrigeration until analysis.

| | | |
|---|---|---|
| Bilirubin | Ketone | Specific gravity |
| Blood | Microscopic urine sediments | Volume |
| Color | pH | Urobilinogen |
| Clarity | Protein (total) | |
| Glucose | Quality | |

6.G.5 Clinical Pathology Report and Records to be Maintained:

Data generated at Corteva Agriscience was provided to PSL for incorporation into the clinical pathology phase report provided by Eurofins Advinus. Clinical pathology data generated at PSL was provided to Eurofins Advinus. A signed, clinical pathology phase report was provided by Eurofins Advinus to the Study Director. This report includes the methodology, pertinent measurements, evaluation results, GLP compliance statement signed by the Principal Investigator (PI), Quality Assurance statement, and tabulated results. The phase report was incorporated into the main study report (Appendix K).

## H. Terminal Sacrifice and Histopathology

Terminal sacrifice and tissue collection took place over two consecutive days (Study Days 30 and 31; Amendment 3). On Day 30, animals were randomly selected from each group and designated for translocation analysis. All animals selected for translocation analysis were designated for termination on Day 31. All remaining surviving animals were sacrificed as scheduled on Study Day 30. Priority was given to providing the translocation samples; therefore, for Group 2 males, which had one animal sacrificed for humane reasons, five were assigned to Day 31 for translocation and the remaining animals were terminated as originally scheduled. Animals were fasted overnight prior to their scheduled terminal sacrifice.

Animals designated for sacrifice on Study Day 31 were treated with test article per study design on Day 30.

6.H.1 Scheduled Sacrifice

At terminal sacrifice, all survivors were euthanized by exsanguination from the inferior vena cava under isoflurane anesthesia. All surviving animals in the study (up to 10 animals/sex/group) were subjected to a full necropsy on their scheduled day, which included examination of the external surface of the body, all orifices, musculoskeletal system and the thoracic, abdominal, pelvic and cranial cavities and their contents.

6.H.2 Samples for Translocation and Future Evaluations

Samples were excised from the same area of the tissue in all animals that underwent necropsy on Day 31, placed in normal saline, and stored at approximately -80ºC for future possible microbiome analysis. The following tissues were collected:

| | |
|---|---|
| cecum (~0.5 cm, with contents) | jejunum (~0.5 cm segment with contents) |
| lungs (~2 cm diameter) | trachea (~0.5 cm segment, middle section) |

The following tissues were collected from the designated animals into ~10 volumes of RNALater, stored at approximately 4ºC overnight prior to storage at approximately -80ºC, for future possible cytokine analysis:

| | |
|---|---|
| trachea (~0.5 cm segment, distal) | cecum (~0.5 cm with contents) |
| lungs (~ 2 cm diameter) | jejunum (~0.5 cm segment diameter) |

The following tissues were collected from the designated animals into a sterile tube, snap frozen and stored at approximately -80ºC for future possible metabolic analysis. The remaining portion of each tissue/organ was preserved in neutral buffered formalin for histopathological evaluation.

| | |
|---|---|
| cecum (~0.5 cm, with contents) | jejunum (~0.5 cm segment with contents) |
| lungs (~ 2 cm diameter) | trachea (~0.5 cm segment, middle section) |

### 6.H.3 Samples for Histopathological Evaluation

The following tissues (from all animals sacrificed by design) were weighed wet as soon as possible after dissection to avoid drying (prior to sampling for additional testing):

| | | |
|---|---|---|
| adrenals (combined) | kidneys (combined) | spleen |
| brain | liver | thymus |
| epididymides (combined) | ovaries with oviducts (combined) | uterus |
| heart | testes (combined) | |

The following organs and tissues from all animals were preserved in 10% neutral buffered formalin for possible future histopathological examination:

| | | |
|---|---|---|
| accessory genital organs (prostate and seminal vesicles) | ileum with Peyer's patches | rectum |
| adrenals | **jejunum | salivary glands (sublingual submandibular, and parotid) |
| all gross lesions | kidneys | skeletal muscle |
| aorta | larynx | skin |
| bone (femur) | *liver | spinal cord - 3 levels: cervical, mid- thoracic, and lumbar |
| bone marrow (from femur & sternum) | **lungs | spleen |
| brain – sections including medulla/pons, cerebellar, and cerebral cortex | lymph node mandibular | sternum |
| **cecum | *lymph node mesenteric | stomach |
| cervix | mammary gland | thymus |
| colon | nasal turbinates | thyroid |
| duodenum | nose | **trachea |
| esophagus | ovaries | urinary bladder |
| Harderian gland | oviducts | uterus |
| heart | pancreas | vagina |
| | parathyroid | |
| | peripheral nerve (sciatic) | |
| | pharynx | |
| | pituitary gland | |

*Portions of these tissues were harvested from animals necropsied on Day 31 for translocation analysis.

** Portions of these tissues were harvested for future evaluations described in Section 6.H.2.

The following organs and tissues from all animals were preserved in modified Davidson's fixative and then stored in ethanol, for possible future histopathological examination:

| | |
|---|---|
| eyes | optic nerve |
| epididymides (combined) | testes |

6.H.4 Translocation Analysis

A sample of whole blood was collected from animals designated for translocation analysis. Sections of liver (median lobe) and mesenteric lymph nodes were excised and maintained on ice. Approximate sample sizes were 0.5 mL of blood and 0.5 - 2 grams of tissue, without compromising any other possible study endpoints. Aseptic and other appropriate methods were utilized in order to avoid contamination of samples (e.g., the use of alcohol to clean instruments and surfaces). Aliquots of selected homogenized and/or diluted samples were plated on MRS agar plates and incubated at approximately 37°C under anaerobic conditions (for *L. reuteri* 3630 and *L. reuteri* 3632) for 24-48 hours or until colony growth was adequate for counting. The average CFU/gram of tissue was calculated based on the amount of sample collected and dilution factors for each specific sample.

All plates were visually inspected for CFU growth after incubation and individual colonies were counted. An automated plate counter was utilized to assist in enumerating highly populated plates. The mean CFU/gram of tissue was calculated using the amount of respective sample evaluated, plate colony growth and factoring for applicable dilutions.

The sensitivity of the method was determined for each sample type using the average amount of tissue in each preparation, assuming a minimum detection limit of 1 CFU/plate and factoring for dilution of samples including plated volumes. Dilutions of the sample preparations either prior to inoculation or prior to plating were accounted for in the calculations by additional multiplication of the DF (Dilution Factor) by the appropriate factor.

$$\text{Sensitivity (CFU/g or mL)} = \frac{\text{Minimum Detectable CFU/plate (1 CFU)}}{\text{Avg amount of sample (g or mL)}} \text{ X DF}$$

Details of processing and enumeration procedures, as provided by the Sponsor (Appendix 1 of the Protocol, located in Appendix A) and PSL standard operating procedures, are documented in the raw data.

All plates were provided to BioPrimate, where colonies were further characterized as needed using 16S rRNA sequencing or equivalent methods. Detailed methods of processing and analysis are described in study records and in the report provided by BioPrimate (see Appendix L).

6.H.5 Unscheduled Sacrifice

Group 2 male (Animal 3829) was humanely sacrificed on Day 19. The animal was examined for the cause of moribund condition and evaluated for gross lesions. Organs and tissues were excised preserved as described for those animals sacrificed by design (Deviation 2).

6.H.6 Histopathology

Histological examination was performed on the preserved organs and tissues of all animals from the control and high dose groups (Groups 1 and 4) the unscheduled death animal, and gross lesions from all animals were processed. The fixed tissues were trimmed, processed, embedded in paraffin, sectioned with a microtome, placed on glass microscope slides, stained with hematoxylin and eosin and examined by light microscopy. Slide preparation and histological assessment, by a board-certified veterinary pathologist, was performed at StageBio.

## 7. STATISTICAL ANALYSIS

Product Safety Labs performed statistical analysis of all data collected during the in-life phase of the study. The use of the word "significant" or "significantly" indicates a statistically significant difference between the control and the experimental groups. Significance was judged at a probability value of $p<0.05$. Male and female rats were evaluated separately.

Statistical analysis was conducted using Provantis™ version 10, Tables and Statistics, Instem LSS, Staffordshire UK (Amendment 6).

### A. Statistical Methods

In-Life Data

For all in-life endpoints that are identified as multiple measurements of continuous data over time (e.g. body weight parameters, food consumption, or food efficiency), treatment and control groups were compared using a two-way analysis of variance (ANOVA), testing the effects of both time and treatment, with methods accounting for repeated measures in one independent variable (time; Mutulsky, 2014). Significant interactions observed between treatment and time, as well as main effects, are further analyzed by a *post hoc* multiple comparisons test (e.g. Dunnett's test; Dunnett, 1964, 1980) of the individual treated groups to control.

Organ Weight Data

All endpoints with single measurements of continuous data within groups (e.g., organ weight and relative organ weight) were evaluated for homogeneity of variances (Bartlett, 1937) and normality (Shapiro, 1965). Where homogeneous variances and normal distribution was observed, treated and control groups were compared using a one-way ANOVA. When one-way ANOVA was significant, a comparison of the treated groups to control was performed with a multiple comparisons test (e.g., Dunnett's test; Dunnett, 1964 and 1980). Where variance was considered significantly different, groups were compared using a non-parametric method (e.g., Kruskal-Wallis non-parametric analysis of variance; Kruskal-Wallis, 1952). When non-parametric analysis of variance was significant, a comparison of treated groups to control was performed (e.g., Dunn's test; Dunn, 1964).

Clinical Pathology

Significance was judged at a probability value of $p<0.05$. Males and females were analyzed separately.

| | | Method of Statistical Analysis | |
|---|---|---|---|
| Parameter | Preliminary Test | If preliminary test is not significant | If preliminary test is significant |
| Clinical Pathology[a] | Barlett's test for homogeneity and Shapiro-Wilk test for normality | One-way analysis of variance followed with Dunnett's test | Log transforms of the data to achieve normality and variance homogeneity were used. If the log transform failed, a non-parametric method (e.g., Kruskal-Wallis non-parametric analysis of variance) was used. When non-parametric analysis of variance was significant, a comparison of treated groups to control was performed (e.g., Dunn's test). |

[a] When an individual observation was recorded as being less than a certain value (e.g., below the lower limit of quantitation), calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1 (or ≤0.1), 0.05 was used for any calculations performed with that bilirubin data or when GGT is reported as <3.0 or ≤3.0, 3.0 was used for the GGT result. When an individual observation was recorded as being greater than a certain value (e.g., above the upper limit of quantitation), calculations were performed on the recorded value. For example, if specific gravity was reported as >1.100 (or ≥1.100), 1.100 was used for any calculation performed with that specific gravity data.

## 8. STUDY CONDUCT

### A. Laboratory

Testing Facility

| | |
|---|---|
| In-life, clinical chemistry, coagulation, Urinalysis, tissue enumeration, and test article dose analysis | Product Safety Labs<br>2394 US Highway 130<br>Dayton, NJ 08810 |
| P.I. (Dose Analysis): | Denise DiCarlo-Emery, BS |

Test Sites

| | |
|---|---|
| Clinical pathology evaluation | Eurofins Advinus Ltd<br>21 & 22 Phase II, Peenya Industrial Area<br>Bengaluru, 560 058, India |
| Principle Investigator: | Dr. Jayachandra, K.C., M.V.Sc., DABT |
| Hematology analysis (Amendment 5) | Corteva Agriscience<br>Haskell R&D Center<br>1090 Elkton Road<br>Newark, DE 19711 |
| P.I. (Clinical Pathology): | Eva Maria Silvestro, AAS, MLT (ASCP) |
| Translocation Analysis | BioPrimate<br>1 Oak Avenue<br>Newark, DE 19711 |
| Principal Investigator: | Frank Burns |

| | |
|---|---|
| Histological slide preparation | StageBio<br>5930 Main Street<br>Mount Jackson, VA 22842, USA |
| Principle Investigator: | Erin Galati, HT (ASCP) |
| Histological slide evaluation | StageBio<br>5930 Main Street<br>Mount Jackson, VA 22842, USA |
| Principle Investigator: | Allen W. Singer, DVM, DACVP, DABT |

### B. GLP Compliance

This study was conducted in compliance with the following regulations, with the exception of the analysis of translocation data:

- U.S. FDA GLP: 21 CFR Part 58, 1987.

Which are compatible with:

- OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM (98)17, OECD, Paris, 1998.

### C. Test Procedure Guidelines

This study design conformed to the following guidelines:

- OECD Guidelines for Testing of Chemicals and Food Ingredients, Section 4 (Part 407): Health Effects, Repeated Dose 28-Day Oral Toxicity Study in Rodents (2008).
- US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 3. a. Short-Term Toxicity Studies with Rodents (November 2003).

## 9. QUALITY ASSURANCE

The Quality Assurance Unit (QAU) of PSL has reviewed this report for GLP compliance and has conducted in-process inspections of selected procedures during the study. The clinical pathology phase report and final report has been audited for agreement with the raw data records and for compliance with the protocol and Product Safety Labs SOPs.

In addition, PSL QAU has functioned as lead QA for this study and monitored QA activities at StageBio and Eurofins Advinus. For portions of the study conducted by a subcontractor or partner laboratory, the QAU for that facility had conducted necessary critical phase inspections and audited respective results and reports for the study phase according to the SOPs of that facility.

Corteva Agriscience conducted a records inspection for the hematology under GLP compliance. Data generated at the test site was provided to PSL for incorporation into the clinical pathology phase report provided by Eurofins Advinus.

The QA Units from StageBio and Eurofins Advinus has sent all GLP audit reports to the Study Director, Study Director's management, and PSL QAU as soon as they were issued.

## 10. FINAL REPORT AND RECORDS TO BE MAINTAINED

The electronic copies of the signed report, and the protocol, associated amendments and/or deviations if applicable will be send to the sponsor. The original signed final report and all the raw data generated at PSL will be maintained in the Product Safety Labs archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor of the study will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL.

The following records will be maintained:

**A.** Information on test article will include but not be limited to the following:

| | |
|---|---|
| Storage | Test article analysis |
| Usage | Dose preparation analysis |
| Disposition | |

**B.** Information on animals will include but not be limited to the following:

| | |
|---|---|
| Receipt, date of birth | Clinical observations |
| Initial health assessment | Histopathology data |
| Dosing | Individual necropsy records |
| Body weights | Organ weights |
| Food consumption | |

Hematology, clinical chemistry, coagulation, and urinalysis data (as appropriate)

**C.** All other records that would demonstrate adherence to the protocol.

Any electronic raw data generated by the Test Site are maintained in accordance to the Test Site's procedures.

## 11. PROTOCOL, PROTOCOL AMENDMENTS, AND PROTOCOL DEVIATIONS

See Appendix A for the Protocol, Protocol Amendments, and Protocol Deviations.

## 12. RESULTS

### A. Test Article and Dose Preparation Analysis – Bacterial Enumeration (Tables 1A-1C; Appendix D)

The results of the neat test article concentration verification were determined to be generally below the targeted concentrations, as indicated in the Certificate of Analysis, which is attributed to slight differences in sampling and enumeration. Due to the test article containing combined concentrations of *L. reuteri* 3630 and *L. reuteri* 3632 strains, the homogeneity of the distribution of these strains within the dose mixtures could not be determined to within an adequate margin of variation. Results show a variable dose concentration of each strain over the course of the study which is not unexpected with microbiological organisms. Overall, the animals are considered to have received sufficient CFU from each article to evaluate the potential for translocation of the organisms from the GI tract after administration as well as the potential for toxicity.

12.A.1 Neat Test Article Concentration Verification

The concentration of viable cells detected in the test articles as received ranged from 1.7 x $10^{10}$ to 1.1 x $10^{11}$ CFU/g for *L. reuteri* 3630 and 1.0 x $10^{10}$ to 2.0 x $10^{11}$ CFU/g for *L. reuteri* 3632 across the sampling time points throughout the study. These results are generally below the anticipated concentration as reported in the C of A provided (see Appendix C), which is attributed to slight differences in the sampling and enumeration (Table 1A).

12.A.2 Homogeneity and Concentration Verification of Dose Solutions

Homogeneity analysis of the dose preparations resulted in a relative standard deviation (RSD) of 81.55%, 7.27%, and 21.78% for *L. reuteri* 3630 and 603.63%, 4.54%, and 4.78% for *L. reuteri* 3632 for Groups 2-4, respectively. Due to the test article containing combined concentrations of *L. reuteri* 3630 and *L. reuteri* 3632, homogeneity of the distribution of the strains within the dose mixtures could not be determined to be within an adequate margin of variation (Table 1B).

Weekly oral dose solution enumeration results confirm that animals in Group 2 (low dose) received 8.5 x $10^{8}$ to 2.3 x $10^{11}$ CFU/kg bw/day *L. reuteri* 3630 and 2.4 x $10^{9}$ to 4.6 x $10^{11}$ CFU/kg bw/day *L. reuteri* 3632. Animals in Group 3 (mid dose) received between 1.6 x $10^{9}$ and 2.7 x $10^{11}$ CFU/kg bw/day *L. reuteri* 3630 and 5.0 x $10^{9}$ to 5.5 x $10^{11}$ CFU/kg bw/day *L. reuteri* 3632. Group 4 animals (high dose) received 5.5 x $10^{9}$ to 2.9 x $10^{11}$ CFU/kg bw/day viable cells of *L. reuteri* 3630 and 1.9 x $10^{10}$ to 4.4 x $10^{12}$ CFU/kg bw/day *L. reuteri* 3632. The statistics of the dose preparations are given in Table 1C, including geometric and arithmetic means, median, standard deviation, min/max values and the standard error of the mean (SEM). Results show a variable dose concentration of each strain over the course of the study which is not unexpected with microbiological organisms. Overall, the animals are considered to have received sufficient CFU from each article to evaluate the potential for translocation of the organisms from the GI tract after administration as well as the potential for toxicity. Therefore, the nominal exposure of the animals within each dose group over the entire treatment duration is expressed as the geometric mean over 7 bacterial dose solution enumerations.

Dose solution enumeration results - target and nominal dose level (geometric means; n=7):

| Dose Group | Target dose for each strain [CFU/kg bw/day] | Dose level verified by bacterial enumeration for strain 3630 [CFU/kg bw/day]; geometric mean* | Dose level verified by bacterial enumeration for strain 3632 [CFU/kg bw/day]; geometric mean* |
|---|---|---|---|
| 2 | 1.7E+10 | 3.1E+09 | 7.5E+09 |
| 3 | 3.3E+10 | 4.5E+09 | 1.4E+10 |
| 4 | 1.0E+11 | 1.6E+10 | 5.7E+10 |

* the n$^{th}$ root of the product of n bacterial counts over the entire study duration $\sqrt[n]{x1 * x2 * xn}$; n=7.

### B. Mortality and Clinical Observations (Tables 2-3; Appendices E-G and M)

There were no mortalities or clinical signs attributable to the test article strain treatment. Animal 3829 had macro and microscopically evident ruptures of the esophagus, indicative of gavage error.

Mortality

One Group 2 male (Animal Number 3829) was humanely sacrificed on Day 19. Prior to death, the animal exhibited irregular respiration, slight piloerection, and superficial eschar on the head. Corresponding detailed clinical observations for this animal included eschar.

The fate of all animals is presented in Appendix M.

Males

In-life clinical observations for male rats included: slight to moderate hypersalivation in 2/10 Group 1 animals and 6/9 Group 2 animals; slight aggression in 2/10 Group 1 animals; slight hyperactivity in 2/9 Group 2 animals; and superficial eschar on the neck of 1/9 Group 2 animals. All males in Groups 3 and 4 (10/10) were active and healthy upon observation throughout the study.

One male rat in Group 2 exhibited eschar during detailed clinical observations. All other animals were normal during detailed clinical observation assessment.

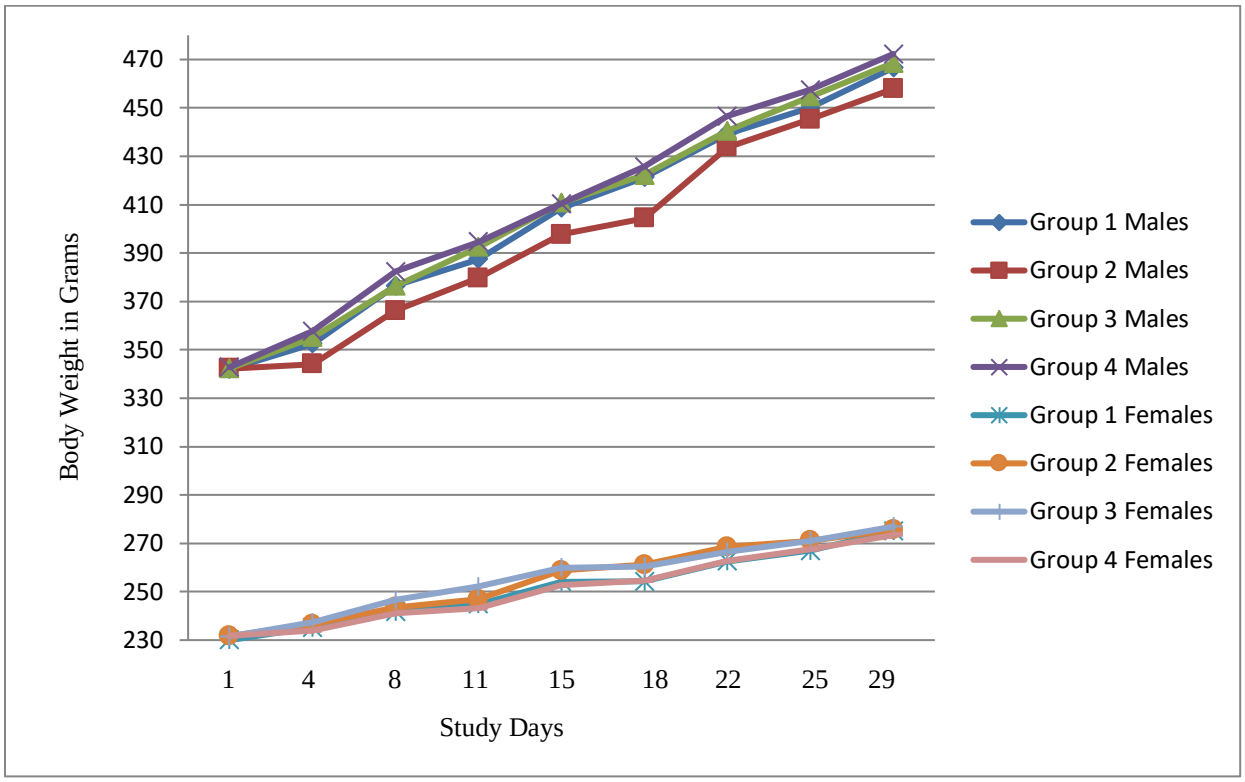
Females

In-life clinical observations for male rats included: slight hypersalivation in 1/10 Group 1 animals; slight aggression in 1/10 Group 1 animals; slight to moderate hyperactivity in 4/10 Group 1 animals; and slight hypoactivity in 1/10 Group 1 females. All females in Groups 2-4 (10/10) were active and healthy upon observation throughout the study. All animals were normal during detailed clinical observation assessment.

### C. Body Weight and Body Weight Gain (Tables 4-5; Appendices H-I)

There were no significant test article related changes in body weight parameters for male or female rats. Mean body weights and mean daily body weight gain for treated male and female rats in Groups 2-4 were comparable to control Group 1 animals throughout the study.

**Figure 1: Average Group Body Weights (Males and Females)**




See Table 4 for data points and intervals.

## D. Food Consumption (Table 6; Appendix J)

There were no significant test article related changes in food consumption parameters for male or female rats. Aside from a statistically significant increase ($p<0.05$) in Group 4 male food consumption on Days 15-22, all other treated male and female rats in Groups 2-4 were comparable to control Group 1 animals throughout the study.

## E. Clinical Pathology (Appendix K)

Administration of test articles *Limosilactobacillus reuteri* (*L. reuteri*) ATCC PTA-126787 (*L. reuteri* 3630) and ATCC PTA-126788 (*L. reuteri* 3632) in male and female rats by the oral gavage route over a test period of at least 28 days did not induce any changes in hematology, coagulation, clinical chemistry and urinalysis parameters.

### 12.E.1 Hematology

There were no test article-related changes in hematology parameters at all dose levels tested.

Decreased hemoglobin in Group 3 males, and reduced hematocrit in Group 3 males and females were considered toxicologically insignificant as there was no dose progression, the magnitude of the change was minimal, and the values were within ranges of historical controls. Further, there were no corresponding changes in MCH, MCHC, or red blood cell count. The increased neutrophil count in Group 3 males also was considered toxicologically insignificant as there was no dose progression and/or magnitude of the change was minimal

All other changes in hematology parameters were considered unrelated to test article, because they occurred sporadically, or were considered due to biological variance among rats as magnitude of variation was minimal.

12.E.2 Coagulation

There were no test article-related changes in coagulation parameters at all dose levels tested.

12.E.3 Clinical Chemistry

There were no test article-related changes in clinical chemistry parameters at all dose levels tested.

Increased creatinine in Group 4 females was considered toxicologically insignificant as it was not associated with any renal lesions and there were no effects of the test material on urinalysis.

Increased creatinine in Group 4 females was considered toxicologically insignificant as it was not associated with any renal lesions, values were within ranges of historical controls, and there were no effects of the test material on urinalysis.

12.E.4 Urinalysis

There were no test article-related changes in urinalysis parameters at all dose levels tested.

## F. Translocation Analysis (Appendix L)

Animals that are subject to either physical or chronic psychological stress experience extra intestinal bacterial translocation. The finding of translocated facultative anaerobes was not unexpected in animals subject to daily gavage over multiple rounds. The frequent finding of translocated bacteria across the vehicle control as well as treatment groups was consistent with expectations. The high levels of *L. reuteri* test strains administered in this study, coupled with *Limosilactobacillus*, being a commonly translocated genera, makes the finding of the apparent test strains contributing to the translocated bacteria not unexpected. Similar results have been seen in previous studies with high level dosing of frequently translocated genera (Mukerji et al, 2016).

In no instance were test article treated group translocated bacterial colony counts significantly different than the counts seen in the same sex vehicle control group. The male high dose group results were nominally higher than the vehicle control group. However, no adverse clinical signs or findings regarding clinical pathology and urinalysis and no morphological changes indicate any correlation to treatment. Therefore, these higher numbers are considered to be without toxicological significance.

The test strains *L. reuteri* (genotypes 1A and 1B) did not translocate in a greater abundance than the endogenous *L. reuteri* (genotype 2).

A low incidence of bacteria found on plates from blood samples (3 of 40 animals) was not associated with treatment and likely resulted from environmental contamination during the study.

Absent any pathological findings or other treatment related toxicity the translocation findings would not indicate the test strains to be harmful.

12.F.1 Number of Animals Showing Translocation by Tissue and Group

Five animals of each sex were analyzed from each dose group, vehicle control (Group 1), low dose (Group 2), mid dose (Group 3) and high dose (Group 4).

Males

Animals with translocated bacteria in blood included 1/5 Group 2 animals and 1/5 Group 4 animals. Animals with translocated bacteria in the liver included 2/5 Group 1 animals, 1/5 Group 2 animals, 3/5 Group 3 animals and 3/5 Group 4 animals. Animals with translocated bacteria in the mesentery included 4/5 Group 1 animals, 3/5 Group 2 animals, 3/5 Group 3 animals and 5/5 Group 4 animals. The total number of animals per group showing translocation in the collected samples included 5/5 Group 1 animals, 4/5 Group 2 animals, 4/5 Group 3 animals and 5/5 Group 4 animals.

Females

Animals with translocated bacteria in blood included 1/5 Group 1 animals. Animals with translocated bacteria in the liver included 3/5 Group 1 animals, 2/5 Group 2 animals, 2/5 Group 3 animals and 4/5 Group 4 animals. Animals with translocated bacteria in the mesentery included 4/5 Group 1 animals, 3/5 Group 2 animals, 3/5 Group 3 animals and 3/4 Group 4 animals. The total number of animals per group showing translocation in the collected samples was included 5/5 Group 1 animals, 4/5 Group 2 animals, 3/5 Group 3 animals and 5/5 Group 4 animals.

| Incidence of translocation of any bacteria: Group (treatment and sex) | Number of animals with translocated bacteria in blood | Number of animals with translocated bacteria in liver | Number of animals with translocated bacteria in mesentery | Number of animals showing any translocation |
|---|---|---|---|---|
| 1 Male | 0 | 2 | 4 | 5 |
| 1 Female | 1 | 3 | 4 | 5 |
| 2 Male | 1 | 1 | 3 | 4 |
| 2 Female | 0 | 2 | 3 | 4 |
| 3 Male | 0 | 3 | 3 | 4 |
| 3 Female | 0 | 2 | 3 | 3 |
| 4 Male | 1 | 3 | 5 | 5 |
| 4 Female | 0 | 4 | 3 (of 4*) | 5 |
| Total All Groups | 3 | 20 | 28 | 35 |

n = 5/sex/group
*No evaluable plates received for this tissue from one animal in this group.

Number of Animals Showing Translocation by Tissue and Group: Overall Result

Translocated bacteria were found among both males and females in each group, including vehicle control animals. The number of animals with translocated bacteria in each group of five animals (grouped by treatment and sex) ranged from 3 to 5. When both sexes within a treatment group are considered together translocations were observed in; 10/10 animals from the control Group 1, 8/10 animals from Group 2, 7/10 animals from Group 3, and 10/10 animals from Group 4. The differences among groups, either as a whole or when each sex is considered, are not statistically significant.

12.F.2 Evidence of Translocation in the Blood and Tissues

Blood

Translocated bacteria were found in one animal each from three groups. This included one control Group 1 animal. It is likely that these represent environmental contamination of the samples during necropsy or plating (absent clinical findings consistent with bacteremia).

Three animals, one from a control Group 1, one from Group 2 and one Group 4 had colonies grow on the blood plates.

One animal from the control Group 1 (Animal 3819) had 16 colonies identified as Staphylococcus.

One animal in Group 2 (Animal 3825) had four colonies, one Lactobacillus, two *L. reuteri* genotype 2 (of rodent origin, not test article related) and one of *L. reuteri* genotype 1B, consistent with the white colony variety present in test article.

Liver

Translocated bacteria in the liver were found in one to four animals from each group including three animals from control Group 1. Uptake of bacteria by Kupfer cells lining the sinusoids of the liver is a normal physiological response during bacterial translocation. Kupfer cells of the liver represent 80-90% of all tissue macrophages and are well known to take up bacteria delivered via the portal circulation where they can help orchestrate tolerance or inflammation. Differences between vehicle control and other dose groups is not statistically significant.

In each group of five animals per sex, three to five animals (at least one, and up to four), had detectable bacterial in the liver. The largest difference was between the low dose (3/10) and high dose (7/10) animals. The differences among dose groups, either as a whole or when each sex is considered independently, are not statistically significant.

Mesenteric Lymph Nodes

Translocated bacteria were identified in the mesenteric lymph nodes of both female and male rats from each treatment group with from three to five animals having translocated bacteria in the mesentery. Differences among the groups are not statistically significant with or without separating out animals by sex.

12.F.3 Enumeration of Translocated Bacteria, CFU/ gram in Tissues

When bacteria were detected the nominal CFU/gram is listed. In instances where no bacteria are detected the limit of detection based on the amount of tissue interrogated is also listed as < the 95% confidence limit of detection.

The average calculated CFU/gram in the liver, was calculated to be 12 CFU/gram for males and 40 CFU/gram in females in Group 1, 13 CFU/gram for males and 27 CFU/gram in females in Group 2, 53 CFU/gram for males and females in Group 3, and 1067 CFU/gram for males and 67 CFU/gram for females in Group 4.

The average calculated CFU/gram in the mesentery was calculated to be 240 CFU/gram for males and 886 CFU/gram in females in Group 1, 160 CFU/gram for males and 100 CFU/gram in females in Group 2, 5233 CFU/gram for males and 460 CFU/gram for females in Group 3, and 33947 CFU/gram for males and 3467 CFU/gram for females in Group 4. For Group 4 females, only four plates were available for enumeration and one plate generated colonies that were too numerous to count.

Mean and median detected CFU/gram of tissue of any bacteria in rats:

| Group (treatment and sex) | Mean CFU/gram in liver | Median CFU/gram in liver | Mean CFU/gram in mesentery | Median CFU/gram in mesentery |
|---|---|---|---|---|
| 1 Male | 12 | None detected | 240 | 200 |
| 1 Female | 40 | 33 | 886 | 300 |
| 2 Male | 13 | None detected | 160 | 33 |
| 2 Female | 27 | None detected | 100 | 33 |
| 3 Male | 53 | 33 | 5233 | 67 |
| 3 Female | 53 | None detected | 460 | 267 |
| 4 Male | 1067 | 133 | 33947* | 3667 |
| 4 Female | 67 | 33 | 3467** | 2150** |

None detected = value below 33

*Should be considered a minimum. One animal generated plates with colonies too numerous to accurately count. The count generated was based on identifiable single colonies and represents an estimated minimum.

**Interpretable plates from only 4 animals were received.

Group 2 Compared to Group 1 Control

Low dose male and female groups showed equivalent to nominally lower levels of translocation to both mesentery and liver in comparison to vehicle control groups.

Group 3 Compared to Group 1

Mid dose groups showed values not significantly different from vehicle control with the exception of mean CFU in mesentery from mid dose male animals. Occasional findings of a large number of translocated bacterial in a tissue are not abnormal in these studies and they have been observed in vehicle control as well as test article administered groups. The high mean value in Group 3 is driven by such an event, as indicated by the elevated mean, and lack of elevated median value. This is due to the mesentery sample from a single animal (Animal 3846) that yielded 775 colonies on the three plates (representing 0.03 grams of tissue), resulting in a CFU/gram of 25,383 for mesentery from that animal.

Taxonomic assignment of the colonies from this sample indicates that only 3 (0.38%) were consistent with a test strain (*L. reuteri* genotypes 1A or 1B) derived bacterium (see Appendix K for break-down by taxa).

Given the single elevating the mean, the low level of contribution of test article derived bacteria to the translocated population and the finding of such events among vehicle control in the past, this finding does not raise concerns.

Male Group 4 Compared to Group 1

High dose males exhibited nominally higher CFU means and medians in both liver and mesentery when compared to vehicle control animals. Actual colony counts for male animals in the mesentery plates were 11, 6, 4, 0, and 15 for control Group 1 animals and 110, 10, 4800, 47 and 125 for Group 4 high dose animals. Actual colony counts for male animals in the liver plates were 0, 1, 0, 1, and 0 for control Group 1 animals and 4, 0, 147, 0, and 5 for Group 4 high dose animals.

These findings fail to find a significant difference between vehicle control and high dose groups. In light of the small count of animals in this study and nominal increase in translocation seen in Group 4 it is suggested that signs of clinical impact in high dose males, particularly Animal 3868 (4800 mesentery and 142 liver counts) should be carefully checked for. It should be noted that most translocated bacteria in the mesentery were not of test article origin and none of the translocated bacteria in the liver of this animal were of test article origin (see Appendix L for break-down by taxa).

Colonies counted by animal in liver and mesentery of control and high dose male rats:

| Vehicle Control Male | High Dose Male | Vehicle Control Male | High Dose Male |
|---|---|---|---|
| Mesentery | Mesentery | Liver | Liver |
| 11 | 110 | 0 | 4 |
| 6 | 10 | 1 | 0 |
| 4 | 4800 | 0 | 147 |
| 0 | 47 | 1 | 0 |
| 15 | 125 | 0 | 5 |

Female Group 4 Compared to Group 1

No statistically significant differences were observed between the female high dose and the female vehicle control group. Additionally, there were no nominal findings indicative of need for enhanced scrutiny.

Actual colony counts for female animals in the mesentery plates were 0, 4, 90, 30, and 9 for control Group 1 animals and 92, 37, -(no plate), and 387 (results from only 4 animals) for Group 4 high dose animals. Actual colony counts for female animals in the liver plates were 0, 3, 0, 3, and 1 for control Group 1 animals and 5, 3, 0, 1, and 1 for Group 4 high dose animals.

## G. Terminal Sacrifice, Necropsy Observations, and Histopathology (Tables 7-10; Appendices M-R)

There were no gross or microscopic findings attributed to administration with the test articles, *L. reuteri* 3630 and *L. reuteri* 3632.

12.G.1 Mortality

There was one unscheduled death, Group 2 male (Animal 3829), on Day 19, among the animals submitted for histopathological evaluation. At necropsy, this animal had a visible tear of the esophagus, with fluid/edema noted in the thoracic cavity and around the lungs; these changes were attributed to inadvertent esophageal dosing trauma and served as the cause of death. No evidence of *L. reuteri* 3630 and *L. reuteri* 3632 toxicity was observed in this animal.

The animal had several gross findings, including small esophageal tear with food particles near the opening; lungs red and swollen with clear, colorless fluid; and clear colorless fluid in the thoracic cavity. Although the physical esophageal perforation was not present on the microscopic slide presented, there was significant chronic-active inflammation surrounding the esophagus, trachea, pharynx and into mediastinal tissues, consistent with a trauma-induced tear. The lungs also had significant inflammation of the pleural surfaces, related to spread of the mediastinal infection caused by the tear. Similar inflammation was found microscopically around the epicardial surfaces of the heart and surrounding the thymic tissue.

12.G.2 Macroscopic

All remaining animals survived to the scheduled in-life termination necropsy. Among these, only a few necropsy findings were noted as follows (with microscopic correlates in parentheses): Group 1 female (Animal 3819) had a fluid filled uterus (dilatation); Group 2 male (Animal 3828) had small, bilateral testes (right – 11 mm x 6 mm, and left – 16 mm x 4 mm; atrophy), and small, bilateral epididymides (right – 32 mm x 3 mm, and left –

42 mm x 3 mm; hypospermia); Group 3 male (Animal 3850) had a small, right testis (1.8 cm x 1.2 cm; atrophy); and Group 3 female (Animal 3854) had a fluid filled uterus (dilatation).

All these were common incidental macroscopic findings in control rats of this age and strain, and were unrelated to the test article administration.

12.G.3 Microscopic

There were no microscopic changes noted that were related to administration of the test articles. Findings, morphologies, severities observed, and incidences of changes seen, including that of early-stage "chronic progressive renal nephropathy", a very common spontaneous change in rats, were consistent with those expected in untreated rats of this age, sex, and strain. Most of these were simple cellular infiltrates of various organs.

CPN is a spontaneous renal disease of rats. The disease is most severe in Sprague Dawley and F344 strains, and there is a distinct male predisposition to CPN in respect of onset, incidence, and severity progression. The weight of evidence supports an absence of a renal counterpart in humans (Hard *et al.*, 2009). The finding of early-stage CPN was observed with higher incidence in high dose males (6/10) compared to control male rats (3/10). Due to the lack of elevated blood urea nitrogen (BUN) or creatinine (CREAT) values in the male high dose group, no further correlated findings, the minimal severity level and the well-known etiology of this spontaneously occurring disease (especially in the Sprague-Dawley rat strain), the higher incidence of CPN in Group 4 males compared to Group 1 males is not considered to be of toxicological significance.

12.G.4 Organ Weights and Ratios

Absolute organ weights for male and female rats in Groups 2-4 were comparable to the control Group 1 animals.

A statistically significant increase occurred in Group 2 male spleen to body weight ratios ($p<0.05$). This increase was not considered toxicologically relevant due to lack of a dose-response relationship. All other organ weights relative to brain and body weights for male and female rats in Groups 2-4 were comparable to control Group 1 animals.

## 13. CONCLUSION

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect-level (NOAEL) for the oral administration of the combined test article, *L. reuteri* 3630 plus *L. reuteri* 3632, was determined to be high dose group, 1.6E+10 CFU *L. reuteri* 3630/kg bw/day (760 mg/kg bw/day) plus 5.7E+10 CFU *L. reuteri* 3632/kg bw/day (455 mg/kg bw/day) for male and female Sprague Dawley rats.

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## TABLE 1A: BACTERIAL ENUMERATION OF NEAT TEST ARTICLE

| Sampling Day | Strain 3630 | Strain 3632 |
|---|---|---|
| | CFU/g | CFU/g |
| 1 | 9.5E+10 | 5.9E+10 |
| 9 | 7.1E+10 | 1.0E+10 |
| 15 | 6.6E+10 | 7.9E+10 |
| 18 | 2.0E+10 | 3.8E+10 |
| 22 | 2.6E+10 | 4.8E+10 |
| 25 | 1.1E+11 | 1.3E+10 |
| 30 | 1.7E+10 | 2.0E+11 |

## TABLE 1B: BACTERIAL ENUMERATION OF HOMOGENIETY SAMPLES

| Day Collected | Dose Group | Strata | CFU in Doing Solutions for Strain 3630 | | CFU in Doing Solutions for Strain 3632 | |
|---|---|---|---|---|---|---|
| | | | CFU/mL | CFU/kg bw/day[1] | CFU/mL | CFU/kg bw/day[1] |
| 1 | 2 | Top | 2.00E+08 | 1.00E+09 | 1.80E+09 | 9.00E+09 |
| | | Middle | 5.33E+08 | 2.67E+09 | 9.00E+08 | 4.50E+09 |
| | | Bottom | 1.00E+08 | 5.00E+08 | 5.33E+08 | 2.67E+09 |
| | | **Average** | **2.78E+08** | **1.39E+09** | **1.08E+08** | **5.39E+09** |
| | | **%RSD** | **81.55** | **-** | **603.63** | **-** |
| | 3 | Top | 7.30E+08 | 3.65E+09 | 1.57E+09 | 7.85E+09 |
| | | Middle | 6.33E+08 | 3.17E+09 | 1.53E+09 | 7.65E+09 |
| | | Bottom | 6.67E+08 | 3.34E+09 | 1.67E+09 | 8.35E+09 |
| | | **Average** | **6.77E+08** | **3.38E+09** | **1.59E+09** | **7.95E+09** |
| | | **%RSD** | **7.27** | **-** | **4.54** | **-** |
| | 4 | Top | 1.43E+09 | 7.15E+09 | 4.70E+09 | 2.35E+10 |
| | | Middle | 1.00E+09 | 5.00E+09 | 4.70E+09 | 2.35E+10 |
| | | Bottom | 1.00E+09 | 5.00E+09 | 5.10E+09 | 2.55E+10 |
| | | **Average** | **1.14E+09** | **5.72E+09** | **4.83E+09** | **2.42E+10** |
| | | **%RSD** | **21.78** | **-** | **4.78** | **-** |

[1] CFU/mL x 5 mL.

## TABLE 1C: BACTERIAL ENUMERATION OF DOSE SOLUTIONS CONCENTRATION VERIFICATION

| Day Collected | Dose Group | CFU in Doing Solutions for Strain 3630 | | CFU in Doing Solutions for Strain 3632 | |
|---|---|---|---|---|---|
| | | CFU/mL | CFU/kg bw/day[1] | CFU/mL | CFU/kg bw/day[1] |
| 1[2] | 2 | 2.8E+08 | 1.4E+09 | 1.1E+08 | 5.4E+09 |
| | 3 | 6.8E+08 | 3.4E+09 | 1.6E+09 | 8.0E+09 |
| | 4 | 1.1E+09 | 5.7E+09 | 4.8E+09 | 2.4E+10 |
| 9 | 2 | 3.1E+08 | 1.6E+09 | 9.5E+08 | 4.8E+09 |
| | 3 | 4.5E+08 | 2.3E+09 | 1.0E+09 | 5.0E+09 |
| | 4 | 3.6E+09 | 1.8E+10 | 9.1E+09 | 4.6E+10 |
| 15 | 2 | 4.5E+10 | 2.3E+11 | 9.1E+10 | 4.6E+11 |
| | 3 | 5.4E+10 | 2.7E+11 | 1.1E+11 | 5.5E+11 |
| | 4 | 5.7E+10 | 2.9E+11 | 8.8E+11 | 4.4E+12 |
| 18 | 2 | - | - | 6.6E+08 | 3.3E+09 |
| | 3 | 4.7E+08 | 2.4E+09 | 1.5E+09 | 7.5E+09 |
| | 4 | 1.3E+09 | 6.5E+09 | 3.7E+09 | 1.9E+10 |
| 22 | 2 | 3.3E+08 | 1.7E+09 | 1.0E+09 | 5.0E+09 |
| | 3 | 4.8E+08 | 2.4E+09 | 3.3E+09 | 1.7E+10 |
| | 4 | 2.8E+09 | 1.4E+10 | 7.2E+09 | 3.6E+10 |
| 25 | 2 | 1.7E+08 | 8.5E+08 | 4.7E+08 | 2.4E+09 |
| | 3 | 4.2E+08 | 2.1E+09 | 1.2E+09 | 6.0E+09 |
| | 4 | 1.1E+09 | 5.5E+09 | 4.5E+09 | 2.3E+10 |
| 30 | 2 | 2.4E+08 | 1.2E+09 | 5.7E+08 | 2.9E+09 |
| | 3 | 3.2E+08 | 1.6E+09 | 1.1E+09 | 5.5E+09 |
| | 4 | 3.2E+09 | 1.6E+10 | 5.5E+09 | 2.8E+10 |

[1] CFU/mL x 5 mL

[2] As part of the Homogeneity Assessment (Table 1B) average results presented.

## TABLE 1C (cont.): BACTERIAL ENUMERATION OF DOSE SOLUTIONS CONCENTRATION VERIFICATION

| Dose Group | Result | CFU in Doing Solutions for Strain 3630 | CFU in Doing Solutions for Strain 3632 |
|---|---|---|---|
| | | CFU/kg bw/day | CFU/kg bw/day |
| 2 | Mean | 3.9E+10 | 6.8E+10 |
| | Geometric Mean[1] | 3.1E+09 | 7.5E+09 |
| | Median | 1.5E+09 | 4.8E+09 |
| | STDev | 1.04E+11 | 1.65E+12 |
| | Min | 8.5E+08 | 2.4E+09 |
| | Max | 2.3E+11 | 4.6E+11 |
| | SEM | 3.45E+10 | 6.44E+11 |
| 3 | Mean | 4.1E+10 | 8.6E+10 |
| | Geometric Mean | 4.5E+09 | 1.4E+10 |
| | Median | 2.4E+09 | 7.5E+09 |
| | STDev | 1.01E+11 | 2.05E+11 |
| | Min | 1.6E+09 | 5.0E+09 |
| | Max | 2.7E+11 | 5.5E+11 |
| | SEM | 3.82E+10 | 7.74E+10 |
| 4 | Mean | 5.0E+10 | 6.5E+11 |
| | Geometric Mean | 1.6E+10 | 5.7E+10 |
| | Median | 1.4E+10 | 2.8E+10 |
| | STDev | 1.04E+11 | 1.65E+12 |
| | Min | 5.5E+09 | 2.4E+09 |
| | Max | 2.9E+11 | 4.4E+12 |
| | SEM | 3.92E+10 | 6.24E+11 |

[1] The geometric mean is a mean or average, which indicates the central tendency or typical value of a set of numbers by using the product of their values (as opposed to the arithmetic mean which uses their sum). The geometric mean is defined as the nth root of the product of n numbers.

## TABLE 2: SUMMARY OF IN-LIFE CLINICAL OBSERVATIONS

| Observation Type: All Types<br>Sex: Male<br>From Day 1 (Start Date) to 9999 (Start Date) | 0 CFU<br>Group 1 | 1.668E+10<br>CFU<br>Group 2 | 3.337E+10<br>CFU<br>Group 3 | 1.001E+11<br>CFU<br>Group 4 |
|---|---|---|---|---|
| Piloerection | 0 | 1 | 0 | 0 |
| Hypersalivation | 2 | 6 | 0 | 0 |
| Aggressive | 2 | 0 | 0 | 0 |
| Eschar | 0 | 2 | 0 | 0 |
| Hyperactivity | 0 | 2 | 0 | 0 |
| Irregular Respiration | 0 | 1 | 0 | 0 |

Values = Number of Animals Affected

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

| Observation Type: All Types<br>Sex: Female<br>From Day 1 (Start Date) to 9999 (Start Date) | 0 CFU<br>Group 1 | 1.668E+10<br>CFU<br>Group 2 | 3.337E+10<br>CFU<br>Group 3 | 1.001E+11<br>CFU<br>Group 4 |
|---|---|---|---|---|
| Hypersalivation | 1 | 0 | 0 | 0 |
| Aggressive | 1 | 0 | 0 | 0 |
| Hyperactivity | 4 | 0 | 0 | 0 |
| Hypoactivity | 1 | 0 | 0 | 0 |

Values = Number of Animals Affected

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## TABLE 3: SUMMARY OF DETAILED CLINICAL OBSERVATIONS

## TABLE 3: SUMMARY OF DETAILED CLINICAL OBSERVATIONS
### Males
### Days 1, 8, 15, 22, 29

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Dose Level (CFU/kg bw/day[1])** | **0** | **$1.668 \times 10^{10}$** | **$3.337 \times 10^{10}$** | **$1.001 \times 10^{11}$** |
| **Number of Animals in Group** | **10** | **10/9[2]** | **10** | **10** |
| **Observations During Removal From Cage and Handling** | **Score[3]** | | | |
| Handling Reactivity | 0 | 0 | 0 | 0 |
| Vocalization | 0 | 0 | 0 | 0 |
| Palpebral | 0 | 0 | 0 | 0 |
| Lacrimation | 0 | 0 | 0 | 0 |
| Eyes | 0 | 0 | 0 | 0 |
| Mucous Membranes | 0 | 0 | 0 | 0 |
| Salivation | 0 | 0 | 0 | 0 |
| Emaciation | 0 | 0 | 0 | 0 |
| Piloerection | 0 | 0 | 0 | 0 |
| Fur/Skin | 0 | 2(4[4]) | 0 | 0 |
| Muscle Tone | 0 | 0 | 0 | 0 |
| Respiratory Pattern | 0 | 0 | 0 | 0 |
| **Open Field Observations** | | | | |
| Activity/Arousal | 0 | 0 | 0 | 0 |
| Convulsions | 0 | 0 | 0 | 0 |
| Tremors | 0 | 0 | 0 | 0 |
| Posture | 0 | 0 | 0 | 0 |
| Gait | 0 | 0 | 0 | 0 |
| Locomotion | 0 | 0 | 0 | 0 |
| Vocalizations | 0 | 0 | 0 | 0 |
| Defecation | 0 | 0 | 0 | 0 |
| Urination | 0 | 0 | 0 | 0 |
| Unusual Behaviors | 0 | 0 | 0 | 0 |
| Twitches | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 |
| **Pupillary Response** | | | | |
| Pupillary Reflex | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

[1] CFU level is equal for both strains in the dose solution.

[2] Group 2 male (Animal 3829) was humanely sacrificed on Day 19.

[3] An entry of 0 indicates that all animals in the group appeared normal when evaluated for the specified observation, or that all animals did not exhibit the specific clinical sign. An entry greater than 0 indicates the number of animals in the group that exhibited the specific clinical sign. A number in the parenthesis (if present) represents the score given for the observed clinical sign.

[4] Eschar.

## TABLE 3 (cont.): SUMMARY OF DETAILED CLINICAL OBSERVATIONS
### Females
### Days 1, 8, 15, 22, 29

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Dose Level (CFU/kg bw/day[1])** | **0** | **$1.668 \times 10^{10}$** | **$3.337 \times 10^{10}$** | **$1.001 \times 10^{11}$** |
| **Number of Animals in Group** | **10** | **10** | **10** | **10** |
| **Observations During Removal From Cage and Handling** | **Score[2]** | | | |
| Handling Reactivity | 0 | 0 | 0 | 0 |
| Vocalization | 0 | 0 | 0 | 0 |
| Palpebral | 0 | 0 | 0 | 0 |
| Lacrimation | 0 | 0 | 0 | 0 |
| Eyes | 0 | 0 | 0 | 0 |
| Mucous Membranes | 0 | 0 | 0 | 0 |
| Salivation | 0 | 0 | 0 | 0 |
| Emaciation | 0 | 0 | 0 | 0 |
| Piloerection | 0 | 0 | 0 | 0 |
| Fur/Skin | 0 | 0 | 0 | 0 |
| Muscle Tone | 0 | 0 | 0 | 0 |
| Respiratory Pattern | 0 | 0 | 0 | 0 |
| **Open Field Observations** | | | | |
| Activity/Arousal | 0 | 0 | 0 | 0 |
| Convulsions | 0 | 0 | 0 | 0 |
| Tremors | 0 | 0 | 0 | 0 |
| Posture | 0 | 0 | 0 | 0 |
| Gait | 0 | 0 | 0 | 0 |
| Locomotion | 0 | 0 | 0 | 0 |
| Vocalizations | 0 | 0 | 0 | 0 |
| Defecation | 0 | 0 | 0 | 0 |
| Urination | 0 | 0 | 0 | 0 |
| Unusual Behaviors | 0 | 0 | 0 | 0 |
| Twitches | 0 | 0 | 0 | 0 |
| Other | 0 | 0 | 0 | 0 |
| **Pupillary Response** | | | | |
| Pupillary Reflex | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

[1] CFU level is equal for both strains in the dose solution.

[2] An entry of 0 indicates that all animals in the group appeared normal when evaluated for the specified observation, or that all animals did not exhibit the specific clinical sign. An entry greater than 0 indicates the number of animals in the group that exhibited the specific clinical sign. A number in the parenthesis (if present) represents the score given for the observed clinical sign.

## TABLE 4: SUMMARY OF MEAN BODY WEIGHTS

Bodyweight (g)

| Sex: Male<br>Day(s) Relative to Start Date | | 0 CFU<br>Group 1 | 1.668E+10 CFU<br>Group 2 | 3.337E+10 CFU<br>Group 3 | 1.001E+11 CFU<br>Group 4 |
|---|---|---|---|---|---|
| 1 [a] | Mean | 342.0 | 342.4 | 342.3 | 342.8 |
| | SD | 18.0 | 17.4 | 18.3 | 18.0 |
| | N | 10 | 10 | 10 | 10 |
| 4 [a1] | Mean | 352.5 | 344.1 | 355.2 | 357.7 |
| | SD | 25.0 | 24.9 | 20.0 | 19.3 |
| | N | 10 | 10 | 10 | 10 |
| 8 [a] | Mean | 376.5 | 366.2 | 376.4 | 382.3 |
| | SD | 27.0 | 27.2 | 23.1 | 25.3 |
| | N | 10 | 10 | 10 | 10 |
| 11 [a] | Mean | 387.5 | 379.7 | 392.5 | 394.6 |
| | SD | 29.2 | 31.0 | 24.8 | 24.9 |
| | N | 10 | 10 | 10 | 10 |
| 15 [a] | Mean | 408.6 | 397.6 | 410.7 | 410.3 |
| | SD | 31.7 | 35.2 | 30.4 | 28.8 |
| | N | 10 | 10 | 10 | 10 |
| 18 [a] | Mean | 421.3 | 404.4 | 422.2 | 425.7 |
| | SD | 34.9 | 46.8 | 32.2 | 31.7 |
| | N | 10 | 10 | 10 | 10 |
| 22 [a] | Mean | 439.3 | 433.7 | 440.5 | 446.6 |
| | SD | 36.5 | 37.1 | 35.4 | 34.4 |
| | N | 10 | 9 | 10 | 10 |
| 25 [a] | Mean | 450.0 | 445.3 | 454.6 | 457.4 |
| | SD | 35.9 | 37.7 | 36.7 | 37.6 |
| | N | 10 | 9 | 10 | 10 |
| 29 [a] | Mean | 466.6 | 457.9 | 468.6 | 472.3 |
| | SD | 38.7 | 35.2 | 40.0 | 40.0 |
| | N | 10 | 9 | 10 | 10 |

[a] - Anova & Dunnett(Rank)
[a1] - Anova & Dunnett

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

Bodyweight (g)

| Sex: Female<br>Day(s) Relative to Start Date | | 0 CFU<br>Group 1 | 1.668E+10 CFU<br>Group 2 | 3.337E+10 CFU<br>Group 3 | 1.001E+11 CFU<br>Group 4 |
|---|---|---|---|---|---|
| 1 [a] | Mean | 230.0 | 231.7 | 231.5 | 231.8 |
| | SD | 13.7 | 11.4 | 12.3 | 15.2 |
| | N | 10 | 10 | 10 | 10 |
| 4 [a] | Mean | 235.2 | 236.5 | 237.3 | 233.9 |
| | SD | 13.7 | 10.5 | 10.2 | 16.2 |
| | N | 10 | 10 | 10 | 10 |
| 8 [a] | Mean | 241.8 | 243.6 | 246.6 | 241.1 |
| | SD | 15.7 | 12.2 | 11.8 | 20.0 |
| | N | 10 | 10 | 10 | 10 |
| 11 [a] | Mean | 244.9 | 246.9 | 252.2 | 243.2 |
| | SD | 15.3 | 12.2 | 8.7 | 19.0 |
| | N | 10 | 10 | 10 | 10 |
| 15 [a1] | Mean | 254.1 | 258.7 | 259.9 | 252.7 |
| | SD | 16.8 | 12.7 | 7.8 | 22.5 |
| | N | 10 | 10 | 10 | 10 |
| 18 [a1] | Mean | 254.3 | 261.1 | 260.4 | 254.5 |
| | SD | 20.2 | 14.8 | 8.8 | 23.4 |
| | N | 10 | 10 | 10 | 10 |
| 22 [a] | Mean | 262.6 | 268.5 | 266.4 | 262.7 |
| | SD | 21.4 | 17.0 | 10.5 | 23.5 |
| | N | 10 | 10 | 10 | 10 |
| 25 [a] | Mean | 267.0 | 271.0 | 271.0 | 267.5 |
| | SD | 20.0 | 16.0 | 13.2 | 23.2 |
| | N | 10 | 10 | 10 | 10 |
| 29 [a] | Mean | 275.1 | 275.6 | 276.9 | 273.4 |
| | SD | 18.8 | 14.5 | 14.2 | 26.9 |
| | N | 10 | 10 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Rank)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## TABLE 5: SUMMARY OF MEAN DAILY BODY WEIGHT GAIN

Mean Daily Body Weight Gain (g/day)

| Sex: Male<br>Day(s) Relative to Start Date | | 0 CFU<br>Group 1 | 1.668E+10 CFU<br>Group 2 | 3.337E+10 CFU<br>Group 3 | 1.001E+11 CFU<br>Group 4 |
|---|---|---|---|---|---|
| 1 → 4 [a] | Mean | 3.50 | 0.57 | 4.30 | 4.97 |
| | SD | 3.59 | 6.26 | 7.40 | 2.39 |
| | N | 10 | 10 | 10 | 10 |
| 4 → 8 [a] | Mean | 6.00 | 5.53 | 5.30 | 6.15 |
| | SD | 1.05 | 1.95 | 6.57 | 1.77 |
| | N | 10 | 10 | 10 | 10 |
| 8 → 11 [a1] | Mean | 3.67 | 4.50 | 5.37 | 4.10 |
| | SD | 1.24 | 1.96 | 1.05 | 2.18 |
| | N | 10 | 10 | 10 | 10 |
| 11 → 15 [a] | Mean | 5.28 | 4.48 | 4.55 | 3.93 |
| | SD | 1.39 | 1.76 | 1.98 | 1.58 |
| | N | 10 | 10 | 10 | 10 |
| 15 → 18 [a] | Mean | 4.23 | 2.27 | 3.83 | 5.13 |
| | SD | 1.83 | 5.63 | 1.63 | 1.97 |
| | N | 10 | 10 | 10 | 10 |
| 18 → 22 [a1] | Mean | 4.50 | 4.78 | 4.58 | 5.23 |
| | SD | 1.13 | 1.73 | 1.72 | 0.91 |
| | N | 10 | 9 | 10 | 10 |
| 22 → 25 [a1] | Mean | 3.57 | 3.89 | 4.70 | 3.60 |
| | SD | 1.23 | 1.60 | 1.49 | 1.68 |
| | N | 10 | 9 | 10 | 10 |
| 25 → 29 [a1] | Mean | 4.15 | 3.14 | 3.50 | 3.73 |
| | SD | 1.14 | 1.13 | 1.20 | 1.06 |
| | N | 10 | 9 | 10 | 10 |

[a] - Anova & Dunnett(Rank)
[a1] - Anova & Dunnett

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

Mean Daily Body Weight Gain (g/day)

| Sex: Female<br>Day(s) Relative to Start Date | | 0 CFU<br>Group 1 | 1.668E+10 CFU<br>Group 2 | 3.337E+10 CFU<br>Group 3 | 1.001E+11 CFU<br>Group 4 |
|---|---|---|---|---|---|
| 1 → 4 [a] | Mean | 1.73 | 1.60 | 1.93 | 0.70 |
| | SD | 2.37 | 1.36 | 1.76 | 1.52 |
| | N | 10 | 10 | 10 | 10 |
| 4 → 8 [a] | Mean | 1.65 | 1.78 | 2.33 | 1.80 |
| | SD | 0.84 | 0.83 | 1.31 | 1.49 |
| | N | 10 | 10 | 10 | 10 |
| 8 → 11 [a] | Mean | 1.03 | 1.10 | 1.87 | 0.70 |
| | SD | 2.25 | 1.30 | 3.14 | 1.78 |
| | N | 10 | 10 | 10 | 10 |
| 11 → 15 [a1] | Mean | 2.30 | 2.95 | 1.93 | 2.38 |
| | SD | 1.06 | 0.94 | 1.54 | 1.56 |
| | N | 10 | 10 | 10 | 10 |
| 15 → 18 [a] | Mean | 0.07 | 0.80 | 0.17 | 0.60 |
| | SD | 2.39 | 1.69 | 1.55 | 2.18 |
| | N | 10 | 10 | 10 | 10 |
| 18 → 22 [a1] | Mean | 2.08 | 1.85 | 1.50 | 2.05 |
| | SD | 0.70 | 0.92 | 0.86 | 1.14 |
| | N | 10 | 10 | 10 | 10 |
| 22 → 25 [a1] | Mean | 1.47 | 0.83 | 1.53 | 1.60 |
| | SD | 2.15 | 1.87 | 2.45 | 2.30 |
| | N | 10 | 10 | 10 | 10 |
| 25 → 29 [a] | Mean | 2.03 | 1.15 | 1.48 | 1.48 |
| | SD | 1.03 | 0.74 | 1.17 | 1.20 |
| | N | 10 | 10 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Rank)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## TABLE 6: SUMMARY OF MEAN DAILY FOOD CONSUMPTION

Mean Daily Food Consumption (g/day)

| Sex: Male<br>Day(s) Relative to Start Date | | 0 CFU<br>Group 1 | 1.668E+10 CFU<br>Group 2 | 3.337E+10 CFU<br>Group 3 | 1.001E+11 CFU<br>Group 4 |
|---|---|---|---|---|---|
| 1 → 4 [a] | Mean | 23.97 | 20.70 | 24.37 | 24.80 |
| | SD | 2.89 | 5.41 | 1.84 | 2.00 |
| | N | 10 | 10 | 10 | 10 |
| 4 → 8 [a] | Mean | 25.73 | 22.98 | 25.05 | 27.25 |
| | SD | 1.27 | 5.15 | 1.05 | 2.30 |
| | N | 10 | 10 | 10 | 10 |
| 8 → 11 [a] | Mean | 25.63 | 25.60 | 25.97 | 28.70 |
| | SD | 1.63 | 1.89 | 1.32 | 3.84 |
| | N | 10 | 10 | 10 | 10 |
| 11 → 15 [a] | Mean | 26.03 | 25.90 | 25.08 | 27.40 |
| | SD | 1.75 | 1.68 | 0.93 | 2.83 |
| | N | 10 | 10 | 10 | 10 |
| 15 → 22 [a] | Mean | 25.71 | 26.41 | 26.10 | 31.33* |
| | SD | 1.34 | 2.24 | 1.68 | 6.99 |
| | N | 10 | 8 | 10 | 10 |
| 22 → 25 [a1] | Mean | 27.33 | 27.15 | 27.97 | 28.03 |
| | SD | 1.01 | 1.33 | 1.55 | 1.93 |
| | N | 10 | 9 | 10 | 10 |
| 25 → 29 [a] | Mean | 28.55 | 27.31 | 28.53 | 30.10 |
| | SD | 1.24 | 1.76 | 1.72 | 2.68 |
| | N | 10 | 9 | 10 | 10 |

[a] - Anova & Dunnett(Rank): * = p < 0.05
[a1] - Anova & Dunnett

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

Mean Daily Food Consumption (g/day)

| Sex: Female<br>Day(s) Relative to Start Date | | 0 CFU<br>Group 1 | 1.668E+10 CFU<br>Group 2 | 3.337E+10 CFU<br>Group 3 | 1.001E+11 CFU<br>Group 4 |
|---|---|---|---|---|---|
| 1 → 4 [a] | Mean | 18.00 | 17.60 | 17.73 | 17.57 |
| | SD | 0.56 | 1.05 | 0.29 | 1.15 |
| | N | 10 | 10 | 10 | 10 |
| 4 → 8 [a] | Mean | 18.15 | 17.73 | 18.38 | 17.70 |
| | SD | 0.94 | 1.10 | 0.89 | 1.58 |
| | N | 10 | 10 | 10 | 10 |
| 8 → 11 [a] | Mean | 17.60 | 17.83 | 17.93 | 16.80 |
| | SD | 0.76 | 1.56 | 0.77 | 2.34 |
| | N | 10 | 10 | 10 | 10 |
| 11 → 15 [a1] | Mean | 18.73 | 19.40 | 18.88 | 18.18 |
| | SD | 1.22 | 1.09 | 1.05 | 1.47 |
| | N | 10 | 10 | 10 | 10 |
| 15 → 22 [a1] | Mean | 17.59 | 17.90 | 17.66 | 17.64 |
| | SD | 0.75 | 1.50 | 1.12 | 1.39 |
| | N | 10 | 10 | 10 | 10 |
| 22 → 25 [a] | Mean | 19.13 | 18.53 | 19.33 | 18.43 |
| | SD | 1.78 | 1.92 | 1.71 | 1.69 |
| | N | 10 | 10 | 10 | 10 |
| 25 → 29 [a1] | Mean | 19.28 | 19.15 | 19.25 | 18.88 |
| | SD | 1.08 | 1.23 | 1.03 | 1.60 |
| | N | 10 | 10 | 10 | 10 |

[a] - Anova & Dunnett(Rank)
[a1] - Anova & Dunnett

* The doses listed are the nominal values for each dose group.

## TABLE 7: SUMMARY OF NECROPSY OBSERVATIONS

| Removal Reason(s): ALL | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| Summary: Count | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
| Number of Animals: | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **esophagus** | | | | | | | | |
| Submitted | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 |
| hole | . | 1 | . | . | . | . | . | . |
| **lungs** | | | | | | | | |
| Submitted | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 |
| enlarged; red | . | 1 | . | . | . | . | . | . |
| **testes-combined** | | | | | | | | |
| Submitted | 10 | 10 | 10 | 10 | . | . | . | . |
| bilateral; small | . | 1 | 0 | . | . | . | . | . |
| right; small | . | 0 | 1 | . | . | . | . | . |
| **uterus** | | | | | | | | |
| Submitted | . | . | . | . | 10 | 10 | 9 | 9 |
| fluid filled | . | . | . | . | 1 | . | 1 | . |
| **all gross lesions** | | | | | | | | |
| Submitted | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 |
| fluid filled; clear | . | 1 | . | . | . | . | . | . |
| **epididymides-combined** | | | | | | | | |
| Submitted | 10 | 10 | 10 | 10 | . | . | . | . |
| bilateral; small | . | 1 | . | . | . | . | . | . |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## TABLE 8: SUMMARY OF MEAN TERMINAL BODY AND ORGAN WEIGHTS

| Sex: Male | Day(s) Relative to Start Date | | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
|---|---|---|---|---|---|---|
| Adrenal Glands Wt (g) | Day 30/31 [a] | Mean | 0.0766 | 0.0714 | 0.0665 | 0.0693 |
| | | SD | 0.0137 | 0.0130 | 0.0151 | 0.0112 |
| | | N | 9 | 8 | 10 | 10 |
| Brain Wt (g) | Day 30/31 [a1] | Mean | 2.190 | 2.210 | 2.218 | 2.185 |
| | | SD | 0.079 | 0.107 | 0.089 | 0.157 |
| | | N | 10 | 9 | 10 | 10 |
| Epididymides Wt (g) | Day 30/31 [a1] | Mean | 1.3202 | 1.2923 | 1.3119 | 1.4279 |
| | | SD | 0.1914 | 0.2937 | 0.0885 | 0.1041 |
| | | N | 10 | 9 | 10 | 9 |
| Heart Wt (g) | Day 30/31 [a] | Mean | 1.373 | 1.417 | 1.353 | 1.422 |
| | | SD | 0.148 | 0.129 | 0.110 | 0.113 |
| | | N | 10 | 9 | 10 | 10 |
| Kidneys Wt (g) | Day 30/31 [a] | Mean | 3.189 | 3.250 | 3.212 | 3.287 |
| | | SD | 0.391 | 0.286 | 0.403 | 0.283 |
| | | N | 10 | 9 | 10 | 10 |
| Liver Wt (g) | Day 30/31 [a2] | Mean | 12.381 | 13.009 | 12.807 | 13.406 |
| | | SD | 1.847 | 2.010 | 1.327 | 3.129 |
| | | N | 10 | 9 | 10 | 10 |
| Spleen Wt (g) | Day 30/31 [a] | Mean | 0.768 | 0.896 | 0.890 | 0.853 |
| | | SD | 0.113 | 0.135 | 0.148 | 0.144 |
| | | N | 10 | 9 | 10 | 10 |
| Terminal BW (g) | Day 30/31 | Mean | 440.8 | 430.9 | 442.8 | 446.2 |
| | | SD | 36.5 | 34.4 | 34.1 | 40.0 |
| | | N | 10 | 9 | 10 | 10 |
| Testes Wt (g) | Day 30/31 [a1] | Mean | 3.474 | 3.177 | 3.384 | 3.467 |
| | | SD | 0.356 | 0.911 | 0.390 | 0.276 |
| | | N | 10 | 9 | 10 | 10 |
| Thymus Wt (g) | Day 30/31 [a] | Mean | 0.3644 | 0.4412 | 0.3605 | 0.3735 |
| | | SD | 0.0795 | 0.1226 | 0.0972 | 0.0973 |
| | | N | 10 | 9 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Rank)
[a2] - Anova & Dunnett(Log)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

| Sex: Female | Day(s) Relative to Start Date | | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
|---|---|---|---|---|---|---|
| Adrenal Glands Wt (g) | Day 30/31 [a] | Mean | 0.0727 | 0.0733 | 0.0758 | 0.0676 |
| | | SD | 0.0125 | 0.0181 | 0.0107 | 0.0146 |
| | | N | 10 | 10 | 10 | 10 |
| Brain Wt (g) | Day 30/31 [a] | Mean | 1.942 | 1.948 | 1.963 | 2.000 |
| | | SD | 0.076 | 0.083 | 0.088 | 0.077 |
| | | N | 10 | 10 | 10 | 10 |
| Heart Wt (g) | Day 30/31 [a] | Mean | 0.879 | 0.933 | 0.876 | 0.841 |
| | | SD | 0.060 | 0.078 | 0.063 | 0.063 |
| | | N | 10 | 10 | 10 | 10 |
| Kidneys Wt (g) | Day 30/31 [a] | Mean | 1.845 | 1.836 | 1.790 | 1.823 |
| | | SD | 0.153 | 0.155 | 0.109 | 0.153 |
| | | N | 10 | 10 | 10 | 10 |
| Liver Wt (g) | Day 30/31 [a1] | Mean | 7.424 | 7.479 | 7.450 | 7.617 |
| | | SD | 0.943 | 0.931 | 0.495 | 1.154 |
| | | N | 10 | 10 | 10 | 10 |
| Ovaries with Oviducts Wt (g) | Day 30/31 [a] | Mean | 0.1250 | 0.1202 | 0.1277 | 0.1366 |
| | | SD | 0.0135 | 0.0097 | 0.0227 | 0.0211 |
| | | N | 10 | 10 | 10 | 10 |
| Spleen Wt (g) | Day 30/31 [a2] | Mean | 0.563 | 0.486 | 0.573 | 0.512 |
| | | SD | 0.141 | 0.039 | 0.066 | 0.047 |
| | | N | 10 | 10 | 10 | 10 |
| Terminal BW (g) | Day 30/31 | Mean | 254.9 | 257.4 | 258.8 | 254.2 |
| | | SD | 19.4 | 14.2 | 9.7 | 23.9 |
| | | N | 10 | 10 | 10 | 10 |
| Thymus Wt (g) | Day 30/31 [a] | Mean | 0.3588 | 0.3383 | 0.3612 | 0.2993 |
| | | SD | 0.0787 | 0.0827 | 0.0777 | 0.0915 |
| | | N | 10 | 10 | 10 | 10 |
| Uterus Wt (g) | Day 30/31 [a2] | Mean | 0.647 | 0.495 | 0.535 | 0.567 |
| | | SD | 0.252 | 0.055 | 0.165 | 0.345 |
| | | N | 10 | 10 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Log)
[a2] - Anova & Dunnett(Rank)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## TABLE 9: SUMMARY OF MEAN ORGAN-TO-BODY WEIGHT RATIOS

| Sex: Male | Day(s) Relative to Start Date | | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
|---|---|---|---|---|---|---|
| Adrenal /TBW (Ratio) | Day 30/31 [a] | Mean | 0.1716 | 0.1683 | 0.1506 | 0.1564 |
| | | SD | 0.0250 | 0.0292 | 0.0334 | 0.0291 |
| | | N | 9 | 8 | 10 | 10 |
| Brain /TBW (Ratio) | Day 30/31 [a] | Mean | 4.992 | 5.164 | 5.028 | 4.908 |
| | | SD | 0.359 | 0.544 | 0.312 | 0.237 |
| | | N | 10 | 9 | 10 | 10 |
| Epididymides /TBW (Ratio) | Day 30/31 [a1] | Mean | 3.0054 | 3.0193 | 2.9772 | 3.2775 |
| | | SD | 0.4428 | 0.7138 | 0.2917 | 0.4525 |
| | | N | 10 | 9 | 10 | 9 |
| Heart /TBW (Ratio) | Day 30/31 [a] | Mean | 3.115 | 3.291 | 3.060 | 3.194 |
| | | SD | 0.237 | 0.216 | 0.193 | 0.177 |
| | | N | 10 | 9 | 10 | 10 |
| Kidneys /TBW (Ratio) | Day 30/31 [a] | Mean | 7.222 | 7.549 | 7.251 | 7.393 |
| | | SD | 0.452 | 0.418 | 0.710 | 0.614 |
| | | N | 10 | 9 | 10 | 10 |
| Liver /TBW (Ratio) | Day 30/31 [a1] | Mean | 27.998 | 30.152 | 28.897 | 29.908 |
| | | SD | 2.398 | 3.582 | 1.581 | 5.559 |
| | | N | 10 | 9 | 10 | 10 |
| Spleen /TBW (Ratio) | Day 30/31 [a] | Mean | 1.748 | 2.084 * | 2.004 | 1.902 |
| | | SD | 0.271 | 0.333 | 0.252 | 0.178 |
| | | N | 10 | 9 | 10 | 10 |
| Testes /TBW (Ratio) | Day 30/31 [a1] | Mean | 7.921 | 7.439 | 7.681 | 7.792 |
| | | SD | 0.983 | 2.227 | 1.007 | 0.522 |
| | | N | 10 | 9 | 10 | 10 |
| Thymus /TBW (Ratio) | Day 30/31 [a] | Mean | 0.8303 | 1.0185 | 0.8123 | 0.8394 |
| | | SD | 0.1867 | 0.2519 | 0.1948 | 0.2136 |
| | | N | 10 | 9 | 10 | 10 |

[a] - Anova & Dunnett: * = p < 0.05
[a1] - Anova & Dunnett(Rank)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

| Sex: Female | Day(s) Relative to Start Date | | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
|---|---|---|---|---|---|---|
| Adrenal /TBW (Ratio) | Day 30/31 [a] | Mean | 0.2877 | 0.2869 | 0.2926 | 0.2691 |
| | | SD | 0.0593 | 0.0790 | 0.0376 | 0.0662 |
| | | N | 10 | 10 | 10 | 10 |
| Brain /TBW (Ratio) | Day 30/31 [a] | Mean | 7.650 | 7.581 | 7.592 | 7.912 |
| | | SD | 0.526 | 0.391 | 0.387 | 0.575 |
| | | N | 10 | 10 | 10 | 10 |
| Heart /TBW (Ratio) | Day 30/31 [a] | Mean | 3.458 | 3.627 | 3.385 | 3.317 |
| | | SD | 0.242 | 0.279 | 0.202 | 0.170 |
| | | N | 10 | 10 | 10 | 10 |
| Kidneys /TBW (Ratio) | Day 30/31 [a] | Mean | 7.252 | 7.134 | 6.919 | 7.208 |
| | | SD | 0.515 | 0.488 | 0.377 | 0.691 |
| | | N | 10 | 10 | 10 | 10 |
| Liver /TBW (Ratio) | Day 30/31 [a1] | Mean | 29.148 | 29.014 | 28.782 | 30.065 |
| | | SD | 3.227 | 2.740 | 1.494 | 4.360 |
| | | N | 10 | 10 | 10 | 10 |
| Ovaries with oviducts/TBW (Ratio) | Day 30/31 [a] | Mean | 0.4927 | 0.4686 | 0.4918 | 0.5399 |
| | | SD | 0.0636 | 0.0494 | 0.0744 | 0.0868 |
| | | N | 10 | 10 | 10 | 10 |
| Spleen /TBW (Ratio) | Day 30/31 [a2] | Mean | 2.212 | 1.891 | 2.212 | 2.022 |
| | | SD | 0.545 | 0.153 | 0.225 | 0.189 |
| | | N | 10 | 10 | 10 | 10 |
| Thymus /TBW (Ratio) | Day 30/31 [a] | Mean | 1.4114 | 1.3100 | 1.4004 | 1.1678 |
| | | SD | 0.3012 | 0.2936 | 0.3139 | 0.2912 |
| | | N | 10 | 10 | 10 | 10 |
| Uterus /TBW (Ratio) | Day 30/31 [a2] | Mean | 2.539 | 1.934 | 2.064 | 2.241 |
| | | SD | 0.991 | 0.285 | 0.635 | 1.338 |
| | | N | 10 | 10 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Log)
[a2] - Anova & Dunnett(Rank)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## TABLE 10: SUMMARY OF MEAN ORGAN-TO-BRAIN WEIGHT RATIOS

| Sex: Male | Day(s) Relative to Start Date | | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
|---|---|---|---|---|---|---|
| Adrenal /BrW (Ratio) | Day 30/31 [a] | Mean | 0.0350 | 0.0324 | 0.0300 | 0.0319 |
| | | SD | 0.0063 | 0.0064 | 0.0070 | 0.0063 |
| | | N | 9 | 8 | 10 | 10 |
| Epididymides /BrW (Ratio) | Day 30/31 [a1] | Mean | 0.6023 | 0.5838 | 0.5930 | 0.6637 |
| | | SD | 0.0808 | 0.1314 | 0.0561 | 0.0867 |
| | | N | 10 | 9 | 10 | 9 |
| Heart /BrW (Ratio) | Day 30/31 [a] | Mean | 0.626 | 0.643 | 0.610 | 0.652 |
| | | SD | 0.052 | 0.070 | 0.047 | 0.047 |
| | | N | 10 | 9 | 10 | 10 |
| Kidneys /BrW (Ratio) | Day 30/31 [a1] | Mean | 1.455 | 1.474 | 1.448 | 1.509 |
| | | SD | 0.162 | 0.154 | 0.173 | 0.135 |
| | | N | 10 | 9 | 10 | 10 |
| Liver /BrW (Ratio) | Day 30/31 [a2] | Mean | 5.650 | 5.889 | 5.771 | 6.118 |
| | | SD | 0.783 | 0.855 | 0.514 | 1.226 |
| | | N | 10 | 9 | 10 | 10 |
| Spleen /BrW (Ratio) | Day 30/31 [a] | Mean | 0.350 | 0.406 | 0.401 | 0.389 |
| | | SD | 0.048 | 0.063 | 0.061 | 0.046 |
| | | N | 10 | 9 | 10 | 10 |
| Testes /BrW (Ratio) | Day 30/31 [a1] | Mean | 1.585 | 1.434 | 1.527 | 1.588 |
| | | SD | 0.139 | 0.416 | 0.183 | 0.088 |
| | | N | 10 | 9 | 10 | 10 |
| Thymus /BrW (Ratio) | Day 30/31 [a] | Mean | 0.1670 | 0.2009 | 0.1629 | 0.1711 |
| | | SD | 0.0386 | 0.0601 | 0.0443 | 0.0443 |
| | | N | 10 | 9 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Rank)
[a2] - Anova & Dunnett(Log)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

| Sex: Female | Day(s) Relative to Start Date | | 0 CFU Group 1 | 1.668E+10 CFU Group 2 | 3.337E+10 CFU Group 3 | 1.001E+11 CFU Group 4 |
|---|---|---|---|---|---|---|
| Adrenal /BrW (Ratio) | Day 30/31 [a] | Mean | 0.0376 | 0.0377 | 0.0387 | 0.0338 |
| | | SD | 0.0072 | 0.0098 | 0.0059 | 0.0072 |
| | | N | 10 | 10 | 10 | 10 |
| Heart /BrW (Ratio) | Day 30/31 [a] | Mean | 0.453 | 0.479 | 0.447 | 0.420 |
| | | SD | 0.034 | 0.039 | 0.035 | 0.025 |
| | | N | 10 | 10 | 10 | 10 |
| Kidneys /BrW (Ratio) | Day 30/31 [a] | Mean | 0.951 | 0.943 | 0.913 | 0.912 |
| | | SD | 0.080 | 0.079 | 0.061 | 0.079 |
| | | N | 10 | 10 | 10 | 10 |
| Liver /BrW (Ratio) | Day 30/31 [a1] | Mean | 3.825 | 3.841 | 3.805 | 3.810 |
| | | SD | 0.502 | 0.446 | 0.340 | 0.575 |
| | | N | 10 | 10 | 10 | 10 |
| Ovaries with oviducts/BrW (Ratio) | Day 30/31 [a] | Mean | 0.0644 | 0.0618 | 0.0652 | 0.0683 |
| | | SD | 0.0069 | 0.0051 | 0.0121 | 0.0099 |
| | | N | 10 | 10 | 10 | 10 |
| Spleen /BrW (Ratio) | Day 30/31 [a2] | Mean | 0.290 | 0.250 | 0.292 | 0.256 |
| | | SD | 0.074 | 0.020 | 0.033 | 0.022 |
| | | N | 10 | 10 | 10 | 10 |
| Thymus /BrW (Ratio) | Day 30/31 [a] | Mean | 0.1839 | 0.1740 | 0.1836 | 0.1484 |
| | | SD | 0.0342 | 0.0445 | 0.0358 | 0.0406 |
| | | N | 10 | 10 | 10 | 10 |
| Uterus /BrW (Ratio) | Day 30/31 [a2] | Mean | 0.334 | 0.254 | 0.273 | 0.286 |
| | | SD | 0.133 | 0.029 | 0.086 | 0.183 |
| | | N | 10 | 10 | 10 | 10 |

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Log)
[a2] - Anova & Dunnett(Rank)

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.in terms of CFU/kg bw/day.

## APPENDIX A: PROTOCOL, PROTOCOL AMENDMENTS AND PROTOCOL DEVIATIONS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

**Product Safety Labs**



# *Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

## PSL PROTOCOL NO.

P713.01 DDU

## PERFORMING LABORATORY

Product Safety Labs
2394 US Highway 130
Dayton, New Jersey 08810, USA

## PSL STUDY NUMBER

55164

## STUDY DIRECTOR

Raghavendra Gowda Ph.D.

## SPONSOR

Elanco Animal Health Incorporated
2500 Innovation Way
Greenfield, Indiana, 46140

## SPONSOR STUDY NUMBER

ELA210070

**Product Safety Labs**

28-Day Oral Toxicity/Translocation
Protocol # P713.01 DDU
PSL ID:210226-1D- 2D; 210408-2D-3D
Study No: 55164

**TABLE OF CONTENTS**

1. **TITLE OF STUDY: *Limosilactobacillus reuteri*: 28-Day repeat-dose oral gavage toxicity study in rats.**

2. **OBJECTIVE**

The objective of this study is to evaluate the potential toxicity of *Limosilactobacillus reuteri* (*L. reuteri*) ATCC PTA-126787 (*L. reuteri* 3630) and ATCC PTA-126788 (*L. reuteri* 3632) in male and female rats likely to arise from repeated exposure via oral gavage over a test period of 28 days. A no-observed-adverse-effect-level (NOAEL) is also sought for each sex.

3. **STUDY DIRECTOR**

Raghavendra Gowda P.D
Study Director
Tel: 732-438-5100 x1542
Email: Raghavendragowda@ProductSafetyLabs.com

4. **NAME AND ADDRESS OF THE TESTING FACILITY**

Product Safety Labs (PSL)
2394 US Highway 130, USA
Dayton, NJ 08810
Tel: 732 438 5100

5. **SPONSOR**

Elanco Animal Health Incorporated
2500 Innovation Way
Greenfield, Indiana, 46140

6. **SPONSOR REPRESENTATIVE**

Dr. Olaf Will
Senior Scientific Expert Toxicology
Bayer Animal Health GmbH
An Elanco Affiliate
Alfred-Nobel-Str. 50
40789 Monheim, Germany
Email: olaf.will@elancoah.com

7. **STUDY MONITOR**

Laurie C. Dolan, PhD, DABT, FACN
Senior Staff Toxicologist at GRAS Associates
a Nutrasource Pharmaceutical and Nutraceutical Services Company
1180 Grand Park Avenue, Suite 500
North Bethesda, MD 20852

8. **DATES**

Proposed In-Life Start Date: 04/13/2021

Proposed Experimental Termination Date: 06/12/2021

## 9. TEST SUBSTANCE

### 9.A Source

The test article will be provided by the Sponsor.

### 9.B Identification

The test article will be identified using the following information provided by the Sponsor and Product Safety Labs (PSL) identification number. Test article 1 and 2 will be mixed together to prepare a single dose solution of the "test article". Documentation of the methods of synthesis, fabrication, or derivation of the test article is retained by the BioSource Cultures & Flavors, S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777.

Test Article 1: *L. reuteri* ATCC PTA-126787 (*L. reuteri* 3630)
Lot #: 201123LRE3630
PSL ID: 210226-1D
Physical Description: White to Tan powder
Composition: 100% *L. reuteri* 3630
Storage Conditions: Frozen (store ~ -25 to -15° C until ready to use).
Expiration Date: 11/23/21

Test Article 2: *L. reuteri* ATCC PTA-126788 (*L. reuteri* 3632)
Lot #: 201123LRE3632
PSL ID: 210226-1D
Physical Description: White to Tan powder
Composition: 100% *L. reuteri* 3632
Storage Conditions: Frozen (store ~ -25 to -15° C until ready to use).
Expiration Date: 11/23/21

Vehicle Control: Dulbecco's Phosphate Buffer Saline (DPBS) (Mediatech Inc.)
Batch #: 29620002
PH: 7.4
Storage Conditions: Ambient
Expiration Date: 10/2023

Vehicle Control: Normal Saline (Vedco Inc.)
Batch #: 19F002
Storage Conditions: Ambient
Expiration Date: 06/2021

### 9.C Analysis

The Sponsor will be responsible for all analytical work required to characterize the neat test substance, and validate its stability.

### 9.D Hazards

Appropriate routine safety precautions will be exercised in the handling of the test article unless otherwise indicated by the Sponsor.

## 10. GENERAL TEST SYSTEM PARAMETERS

### 10.A Animal Requirements

10.A.1 Number of Animals: 80

10.A.2 Number of Groups: 4 (3 dose level per sex/test article + 1 control group per sex)

10.A.3 Number of Animals per Group: 20 (10 male, 10 female)

10.A.4 Sex: Male and female; females will be nulliparous and non-pregnant.

10.A.5 Species/Strain: CRL Sprague-Dawley CD® IGS rats

10.A.6 Age/Weight: Approximately eight to nine weeks at initiation; the weight variation will not exceed ± 20% of the mean weight for each sex.

10.A.7 Supplier: Charles River Laboratories, Inc. Rats will be shipped from the Raleigh, North Carolina facility in filtered cartons by airfreight and/or truck.

### 10.B Test System Justification

The Sprague-Dawley® rat is the system of choice because, historically, it has been the preferred and most commonly used species for oral toxicity tests. The current state of scientific knowledge does not provide acceptable alternatives to the use of live animals to accomplish the objective of this study.

### 10.C Husbandry

10.C.1 Housing

Animals will be group housed in cages which conform to the size recommendations in the most recent Guide for the Care and Use of Laboratory Animals (Natl. Res. Council, 2011) [1] and according to PSL SOP #503 and 504. The animal room will have a 12-hour light/dark cycle and will be kept clean and vermin free. Environmental controls are set to maintain temperature and relative humidity ranges of 21 ± 2°C and 30-70%, respectively. The observed values/ranges will be documented in the raw data. In addition, airflow in the animal room will be maintained at or above 10 air changes per hour.

10.C.2 Acclimation

The animals will be conditioned to the housing facilities for at least five days prior to testing. Body weights and clinical observations will be recorded at least two times prior to study start.

10.C.3 Feed

2016 Certified Envigo Teklad Global Rodent Diet® (Envigo Teklad, Inc.) will be stored in a dedicated temperature and humidity monitored feed storage site and available *ad libitum* during acclimation and throughout the study, except when animals are fasted for clinical sample collections and terminal sacrifice

10.C.4 Water

Filtered tap water will be available *ad libitum* from individual bottles attached to the cages or from an automatic watering access system. Water analysis is conducted by Precision Analytical

[1] National Research Council. (2011). Guide for the Care and Use of Laboratory Animals (8th ed.). Washington, DC: The National Academies Press.

Services, Inc., Toms River, NJ and South Brunswick Municipal Water Supply, South Brunswick, NJ.

10.C.5 Contaminants

There are no known contaminants reasonably expected to be found in the food or water that would interfere with the results of this study. Routine analysis for each lot of feed used in this study will be received from Envigo Teklad, Inc. (Madison, WI). Water analysis is conducted periodically and the records are kept on file at Product Safety Labs. The date(s) of the most recent analyses will be reported in the final report.

10.C.6 Viral Screen

Serum samples from naive rats housed in the same room as test animals, as part of PSL's sentinel health monitoring program, will be evaluated for the absence of viruses near the end of the in-life portion of the study (PSL SOP # 755).

### 10.D Identification

10.D.1 Cage

Each cage will be identified by a cage card indicating at least the study number, dose level, group assignment, individual animal identification (ear tag and animal number), and sex of the animal.

10.D.2 Animal

Each animal will be given a sequential number in addition to being uniquely identified with a Monel® self-piercing stainless steel ear tag or color marking.

## 11. EXPERIMENTAL DESIGN

### 11.A Route of Administration

The test article will be administered by oral gavage (PO).

### 11.B Justification of Route of Administration

The oral route of administration was selected by the Sponsor as the clinical route of exposure will be via oral capsule or oral liquid. This route of administration is recommended in the referenced guidelines (15.C).

### 11.C Control of Bias

Animals will be randomly assigned to test groups according to PSL SOP #714).

### 11.D Dose Levels

Ten male and ten female test animals will be randomly assigned to each of the following groups:

| Group | No. Animals/ Group M/F | Test article (mg/kg bw/day) | Dose Level Equivalent (CFU/kg/day) | Dose Volume mL/kg/day | Test Article Concentration (mg/mL[a]) |
|---|---|---|---|---|---|
| 1 | 10/10 | 0 Vehicle only | 0 | 5 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | 10/10 | *L. reuteri* 3630 **(126.5)** + *L. reuteri* 3632 **(75.8)** | 1.668E+10 + 1.668E+10 | | *L. reuteri* 3630 **(25.3)** + *L. reuteri* 3632 **(15.2)** |
| 3 | 10/10 | *L. reuteri* 3630 **(253.3)** + *L. reuteri* 3632 **(151.6)** | 3.337E+10 + 3.337E+10 | | *L. reuteri* 3630 **(50.7)** + *L. reuteri* 3632 **(30.3)** |
| 4 | 10/10 | *L. reuteri* 3630 **(760)** + *L. reuteri* 3632 **(455)** | 1.001E+11 + 1.001E+11 | | *L. reuteri* 3630 **(152)** + *L. reuteri* 3632 **(91)** |

[a] LR3630 = 132 000 000 000 CFU/g (1.32E+11) and LR3632 = 220 000 000 000 CFU/g (2.2E+11)

### 11.E Justification of Dose Level Selection

The dose is selected based on a proposed clinical dose and is not expected to cause marked toxicity. The high dose (group 4) corresponds to a margin of exposure of 700, based on a daily oral dose of 2.0E+10 CFU (for the two LR strains combined in equal proportions) per person (2.86E+08 **CFU/kg**; assuming a mean human body weight of **70 kg**). The low dose (group 2) corresponds to a Margin of Safety of 117.

## 12. GENERAL PROCEDURES

### 12.A Selection of Animals

Eighty (80) healthy rats (forty males; forty females) will be used on test. Animals will be selected for this study on the basis of adequate body weight gain, absence of clinical signs of disease or injury, and a body weight within ±20% of the mean within a sex. Selected rats will be distributed by randomization according to stratification by body weight so that there will be no statistically significant difference among group body weight means within a sex.

### 12.B Dose Preparations and Procedures

#### 12.B.1 Test Article Calculation and Preparation

In order to ensure the target doses are attained, dose concentrations are based on actual recovery of viable CFU demonstrated by Product Safety Labs using the individual samples of L. reuteri 3630 and *L. reuteri* 3632 provided, which differ from the reported CFU concentrations provided in the certificates of analyses. Actual doses administered will be determined and reported at least weekly during the study.

The test article will be aliquoted into at least 28 sealed foil pouches (Ted Pella Inc, 139-311) of each strain prior to study initiation. Each pouch will be weighed based on the amounts required for each strain in the above table (sec 11.D). The pouches will be closed/sealed after expelling any air inside to minimize exposure to oxygen and stored in a freezer at approximately -20°C until each individual pouch is used.

At least 28 pouches with **15.2** g LR 3630 each
At least 28 pouches with **9.1** g LR 3632 each

Fresh formulations of the test article will be prepared daily. On the day of use, thaw two pouches (one of each strain) still closed and bring to room temperature. Then open and combine both powders quantitatively and suspend in 100 mL of vehicle (PBS, phosphate buffered saline or normal saline). This results in a suspension with a concentration for Group 4 (high dose) of **152 mg/mL** LR3630 and **91 mg/mL** LR 3632. The solution will be mixed thoroughly by vortex or stomacher until a uniform suspension is achieved.

Dilute accordingly to achieve concentrations for Group 3 (mid-take 20 mL from the high dose, and add 40 mL PBS (or sterile saline) to achieve mid dose a 1:3 dilution) dose levels and Group 2 (take 10 mL from the high dose, and add 50 mL PBS (or normal saline) to achieve a low dose a 1:6 dilution) dose levels on each single day. The formulations will be stirred at ambient temperature until a visually homogeneous suspension is achieved. The dose solutions will be continuously stirred until administration. Final formulation cannot be stored longer than four 4 hours at room temperature prior to dosing.

Weekly samples of the final dose formulation will be collected for concentration verification of each strain. Fresh formulations of the test article will be prepared daily. The preparation of the dose solutions will be documented in the raw data. ***As a control for growth conditions and media, a pure culture of the respective strains or another reference L. reuteri strain will be streaked and observed for growth.***

12.B.2 Dosing

Each animal will be dosed by oral intubation using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Dose administration will be daily (7 days/week). All doses will be administered volumetrically at 5 mL/kg body weight. The control group will receive vehicle (PBS or normal saline) only, at the same dose volume as the test animals. The dose suspension will be maintained and stirred continuously on a magnetic stir plate during dose administration. The first day of administration will be considered Day 1 of the study. Dosing will be at approximately the same time each day $\pm$ 2 hours.

## 12.C Analysis of Test Article and Dose Preparations

12.C.1 Sample Collection

The neat test article and prepared dosing mixtures will be sampled. Additional samples may be collected and analyzed, at the discretion of the Study Director, to ensure stability, accuracy, and homogeneity of the dosing concentrations over the course of the study.

12.C.2 Test Article and Dose Preparation Stability

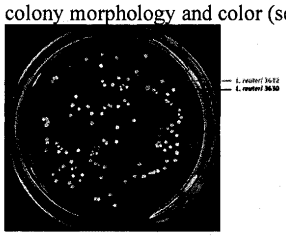
The neat test article is expected to be stable over the course of the study under the conditions of storage according to its CoA at Product Safety Labs. All dose formulations will be freshly prepared daily prior to dosing. A sample of the neat test article will be collected at the beginning, middle, and end of the in-life phase.

12.C.3 Dose Preparation Homogeneity

Prior to initial dosing on Day 1 of the study, samples from the dose preparations will be collected from the top, middle, and bottom for each concentration. The vehicle control mixture will be sampled from the middle of the dose preparation only.

12.C.4 Dose Preparation Concentration Verification

The dose preparations (treatment and control groups) will be sampled at the beginning of the study (as part of the homogeneity assessment, Section 12.C.3) for verification of dose concentration. Dose preparations will also be sampled weekly for verification of dose concentration of each strain. It is important to distinguish between the 2 strains based on the colony morphology and color (see example below).



*L. reuteri* 3632 is morphologically distinct from *L. reuteri* 3630 - the two strains can be easily differentiated based on the color of the colonies. *L. reuteri 3632* produces orange/yellow colored colonies, while *L. reuteri* 3630 produces creamish white colonies.

12.C.5 Sample Analysis

The dosing preparations sampled prior to administration on Day 1 for homogeneity, and weekly thereafter, for concentration verification (as described above) will be processed for enumeration as soon as possible (within 2-4 hours of preparation, stored at 4+/-2°C if applicable) for viable CFU content according to methods provided by the Sponsor and recorded in the study records. The analysis should consider each individual strain, based on the visual differences described above. For comparison and to confirm growing conditions are adequate for the test organisms, a sample of each neat test substance will be cultured concurrently with the dose solution.

## 12.D Clinical Observations

All animals will be observed at least twice daily for viability. Cage-side observations of all animals will be performed daily during the study. All findings will be recorded.

On Day 1, prior to the first treatment with the test article, and approximately weekly thereafter, a detailed observation will be conducted (PSL SOP #726) while handling the animal, generally on days that the animals are weighed and food consumption measurements are taken. Potential signs noted should include, but not be limited to: changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Likewise, changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling), or bizarre behavior (e.g., self-mutilation, walking backwards) should also be recorded. The date and clock time of all observations and/or mortality checks will be recorded.

The Study Director, the Study Monitor and Sponsor Representative will be promptly notified of severe/remarkable clinical observations, will be advised when an animal is found in a moribund

condition and may authorize euthanasia and necropsy as necessary to avoid the loss of quality data. All such authorizations will be recorded in the raw data.

### 12.E Body Weight and Body Weight Gain

Individual body weights will be recorded at least two times during acclimation. All animals will be weighed on Day 1 (prior to study start) and twice weekly thereafter shortly before dose administration in order to adjust the dose volume on body weight. Additional body weights may be taken for any animal with marked clinical observations, at the discretion of the Study Director. The animals will also be weighed prior to sacrifice in order to calculate organ-to-body weight ratios. Decedents need not be weighed. Body weight gain will be calculated for selected intervals and for the study overall.

### 12.F Food Consumption and Food Efficiency

Individual food consumption will be measured and recorded twice weekly to coincide with body weight measurements. Food efficiency will be calculated and reported, if warranted.

### 12.G Clinical Pathology

Clinical pathology will be performed on all surviving animals and on all animals killed in moribund condition for ethical reasons prior to death for coagulation, hematology clinical chemistry and urinalysis (if possible) prior to their respective necropsies. Animals will be fasted overnight prior to blood collection. Blood samples will be collected via sublingual bleeding or from the vena cava, under isoflurane anesthesia

Additional blood samples will be collected (maximum feasible quantity) and processed for serum. Samples will be stored at -80°C for future possible serum cytokine analysis (5 animals/sex/group).

All blood samples will be evaluated for quality by visual examination.

12.G.1 Hematology:

Approximately 500 µL of blood will be collected in a pre-calibrated tube containing $K_2$EDTA for hematology assessments. The whole blood samples will be transferred to the clinical pathology department at Product Safety Labs. Whole blood samples will be maintained on ice or equivalent conditions prior to analysis. All blood samples will be evaluated for quality by visual examination.

| | |
|---|---|
| red blood cells count | hemoglobin concentration |
| hematocrit | mean corpuscular volume |
| mean corpuscular hemoglobin | red cell distribution width |
| reticulocytes count | platelet count |

white blood cells and differential leukocyte counts of neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells.

Mean corpuscular hemoglobin concentration will be calculated.

In addition, separate blood smears will be prepared from each animal undergoing hematological evaluation and, if necessary, will be stained with New Methylene Blue or Wright-Giemsa stain and examined to substantiate or clarify the results of hematology findings.

12.G.2 Coagulation:

Approximately 1.8 mL of blood will be collected in a pre-calibrated tube containing 3.2% sodium citrate. These samples will be centrifuged in a refrigerated centrifuge and the plasma

will be transferred to labeled tubes. Plasma samples will be stored in a -80°C freezer until analysis. The following parameters will be evaluated:

prothrombin time
activated partial thromboplastin time

12.G.3 Clinical chemistry:

Approximately 1000 µL of blood will be collected into a tube containing no preservative for clinical chemistry assessments. These samples will be centrifuged in a refrigerated centrifuge and the serum will be transferred to a labeled tube. Serum samples will be stored in a -80°C freezer until analysis. The following parameters will be evaluated:

| | |
|---|---|
| Serum aspartate aminotransferase | Serum alanine aminotransferase |
| Sorbitol dehydrogenase | Alkaline phosphatase |
| Gamma-glutamyl transferase | Total bilirubin |
| Urea nitrogen | Total cholesterol |
| Blood creatinine | Fasting glucose |
| Triglycerides | Albumin |
| Total serum protein | Calcium |
| Globulin | Sodium |
| Inorganic phosphorus | Chloride |
| Potassium | |
| L- and D-Lactic acid* | |

*(additional serum sample will be sent to the Sponsor for evaluation)

12.G.4 Urinalysis

The day before their respective collection of samples for the clinical pathology evaluation, animals will be placed in metabolism cages. Animals will be fasted after 2 pm (at least 16 hours prior to blood collection) and urine will be collected from each animal. Urine samples will be stored on ice or under refrigeration until analysis. The urinalysis will be conducted only if any expected or observed toxicity indicated.

| | | |
|---|---|---|
| Bilirubin | Ketone | Specific gravity |
| Blood | Microscopic urine sediments | Volume |
| Color | pH | Urobilinogen |
| Clarity | Protein (total) | |
| Glucose | Quality | |

12.G.5 Clinical Pathology Report and Records to be Maintained:

Clinical pathology data generated at PSL will be provided to Eurofins Advinus. A signed, clinical pathology phase report will be provided by Eurofins Advinus to the Study Director. This report will include the methodology, pertinent measurements, evaluation results, GLP compliance statement signed by the Principal Investigator (PI), Quality Assurance statement, and tabulated results. The phase report will be incorporated into the main study report.

## 12.H Terminal Sacrifice and Histopathology

### 12.H.1 Scheduled Sacrifice

At terminal sacrifice, all survivors will be euthanized by exsanguination from the inferior vena cava under isoflurane anesthesia. All animals in the study (10 animals/sex/group) will be subjected to a full necropsy, which will include examination of the external surface of the body, all orifices, musculoskeletal system and the thoracic, abdominal, pelvic and cranial cavities and their contents.

### 12.H.2 Samples for Translocation and Future Evaluations

Samples will be excised from the same area of the tissue in all animals. The following tissues will be collected from five randomly selected animals/sex/group:

Collected in PBS or normal saline and stored at approximately -80°C for future possible microbiome analysis (5 animals/sex/group). Samples will be excised from the same area of the tissue in all animals.

| | |
|---|---|
| cecum (~0.5cm, with contents) | jejunum (~0.5cm segment with contents) |
| lungs (~ 2cm diameter) | trachea (~0.5 cm segment, middle section) |

Collected into a sterile tube, snap frozen and stored at approximately -80°C for future possible metabolic analysis. This will limit the amount of each tissue/organ submitted for histopathological evaluation in these animals.

| | |
|---|---|
| cecum (~0.5cm, with contents) | jejunum (~0.5cm segment with contents) |
| lungs (~ 2cm diameter) | trachea (~0.5 cm segment, middle section) |

Collected and preserved in neutral buffered formalin and stored at approximately 4°C for possible future histopathological analysis

| | |
|---|---|
| trachea (~0.5 cm segment, proximal) | cecum (~0.5cm, with contents) |
| lungs (~ 2cm diameter) | jejunum (~0.5cm segment) |

Collected into ~10 volumes of RNALater, stored at approximately 4°C overnight prior to storage at approximately -80°C, for future possible cytokine analysis

| | |
|---|---|
| trachea (~0.5 cm segment, distal) | cecum (~0.5cm with contents) |
| lungs (~ 2cm diameter) | jejunum (~0.5cm segment diameter) |

### 12.H.3 Samples for Histopathological Evaluation

The following tissues (from all animals sacrificed by design) will be weighed wet as soon as possible after dissection to avoid drying (prior to sampling for additional testing):

| | | |
|---|---|---|
| adrenals (combined) | kidneys (combined) | spleen |
| brain | liver | thymus |
| epididymides (combined) | ovaries with oviducts (combined) | uterus |
| heart | testes (combined) | |

The following organs and tissues from all animals will be preserved in 10% neutral buffered formalin for possible future histopathological examination:

| | | |
|---|---|---|
| accessory genital organs (prostate and seminal vesicles) | ileum with Peyer's patches *jejunum | rectum salivary glands (sublingual |

| | | |
|---|---|---|
| adrenals | kidneys | submandibular, and parotid) |
| all gross lesions | larynx | skeletal muscle |
| aorta | *liver | skin |
| bone (femur) | *lungs | spinal cord - 3 levels: cervical, mid- thoracic, and lumbar |
| bone marrow (from femur & sternum) | lymph node mandibular | spleen |
| brain – sections including medulla/pons, cerebellar, and cerebral cortex | *lymph node mesenteric | sternum |
| *cecum | mammary gland | stomach |
| cervix | nasal turbinates | thymus |
| colon | nose | thyroid |
| duodenum | ovaries | *trachea |
| esophagus | oviducts | urinary bladder |
| Harderian gland | pancreas | uterus |
| heart | parathyroid | vagina |
| | peripheral nerve (sciatic) | |
| | pharynx | |
| | pituitary gland | |

*Portions of these tissue will be harvested from 5 randomly selected animals per sex per group for translocation and other future evaluations.

The following organs and tissues from all animals will be preserved in modified Davidson's fixative and then stored in ethanol, for possible future histopathological examination:

| | |
|---|---|
| eyes | optic nerve |
| epididymides | testes |

12.H.4 Translocation Analysis

Additional samples from the 5 animals/sex/group selected above will be collected for translocation analysis. A sample of whole blood will be collected, and sections of liver (median lobe) and mesenteric lymph nodes will be excised and maintained on ice. Approximate sample sizes will be 0.5 mL of blood and 0.5 - 2 grams of tissue, without compromising any other possible study endpoints. Aseptic and other appropriate methods will be utilized in order to avoid contamination of samples (e.g., the use of alcohol to clean instruments and surfaces). Aliquots of selected homogenized and/or diluted samples will be plated on MRS agar plates and incubated at approximately 37-39°C under anaerobic conditions (for *L. reuteri* 3630 and *L. reuteri* 3632) for 24-48 hours or until colony growth is adequate for counting. The average CFU/gram of tissue will be calculated based on the amount of sample collected and dilution factors for each specific sample.

All plates will be visually inspected for CFU growth after incubation and individual colonies will be counted. An automated plate counter may be utilized to assist in enumerating highly populated plates. The mean CFU/gram of tissue will be calculated using the amount of respective sample evaluated, plate colony growth and factoring for applicable dilutions.

The sensitivity of the method will be determined for each sample type using the average amount of tissue in each preparation, assuming a minimum detection limit of 1 CFU/plate and factoring for dilution of samples including plated volumes. Dilutions of the sample preparations either prior to inoculation or prior to plating will be accounted for in the calculations by additional multiplication of the $DF^4$ by the appropriate factor.

$$\text{Sensitivity (CFU/g or mL)} = \frac{\text{Minimum Detectable CFU/plate (1 CFU)}}{\text{Avg amount of sample (g or mL)}} \text{ X DF}$$

Details of processing and enumeration procedures, as provided by the Sponsor (Appendix#1) and PSL standard operating procedures, will be documented in the raw data.

All plates will be provided to BioPrimate, where colonies will be further characterized as needed using 16S rRNA sequencing or equivalent methods. Detailed methods of processing and analysis will be described in study records and the final report.

12.H.5 Unscheduled Sacrifice

Any rat that dies or is euthanized because of a moribund condition will be examined for the cause of death or moribund condition on the day the observation is made. Rats will be evaluated for gross lesions. Organs and tissues will be excised, weighed (except for animals found dead), and preserved as described for those animals sacrificed by design.

12.H.6 Histopathology

Histological examination will be performed on the preserved organs and tissues of the all animals from the control and high dose group (Groups 1 and 4) as well as from any animal that dies during the course of the study, in the first instance. In addition, gross lesions of potential toxicological significance noted in any test groups at the time of terminal sacrifice will also be examined. The fixed tissues will be trimmed, processed, embedded in paraffin, sectioned with a microtome, placed on glass microscope slides, stained with hematoxylin and eosin and examined by light microscopy. Slide preparation and histological assessment, by a board-certified veterinary pathologist, will be performed at StageBio.

## 13. STATISTICAL ANALYSIS

Product Safety Labs will perform statistical analysis of all data collected during the in-life phase of the study, as well as clinical pathology and organ weight data. The use of the word "significant" or "significantly" indicates a statistically significant difference between the control and the experimental groups. Significance will be judged at a probability value of $p < 0.05$. Mean and standard deviations will be calculated for all quantitative data. Male and female rats will be evaluated separately.

Statistical analysis will be conducted by using one or more of the following software applications: Provantis® version 9, Tables and Statistics, Instem LSS, Stafforshire UK; Pristima® version 7, Statistical Analysis, Xybion Corporation, Lawrenceville, NJ; INSTAT or Prism Biostatistics, GraphPad Software, San Diego, CA; Statview, version 5, SAS Institute Inc., Cary, NC; and SigmaStat, version 2, Systat Software, San Jose, CA.

Other statistical methods will be used if appropriate, at the time of analysis and described in the final report.

### 13.A Statistical Methods

In-Life Data

For all in-life endpoints that are identified as multiple measurements of continuous data over time (e.g., body weight, food consumption, food efficiency), treatment and control groups will be compared using a two-way analysis of variance (ANOVA), testing the effects of both time and treatment, with methods

accounting for repeated measures in one independent variable (time)[1]. Further analysis of the p value for each individual factor may be conducted and ultimately by a *post hoc* multiple comparisons test (e.g. Dunnett's test) of the individual treated groups to control.

Organ Weight Data

If warranted by sufficient group sizes, all endpoints with single measurements of continuous data within groups (e.g., organ weight, relative organ weight, etc) will be evaluated for homogeneity of variances[2] and normality. Where homogeneous variances and normal distribution is observed, treatment and control groups will be compared using a one-way ANOVA. A comparison of the treated groups to control will be performed with a multiple comparisons test (e.g. Dunnett's test)[3,4]. Where variances are considered significantly different, groups will be compared using a non-parametric method (e.g. Kruskal-Wallis non-parametric analysis of variance[5]). When non-parametric analysis of variance is significant, a comparison of treated groups to control will be performed (e.g. Dunn's test[6]).

If warranted by sufficient group sizes, the incidence of clinical observations may be evaluated through sequential application of a trend test[7]. Other procedures will be used if appropriate, following consultation with the Sponsor, and will be described in the final report.

Clinical Pathology

Significance will be judged at a probability value of $p < 0.05$. Males and females will be analyzed separately.

| | | Method of Statistical Analysis | |
|---|---|---|---|
| Parameter | Preliminary Test | If preliminary test is not significant | If preliminary test is significant |
| Clinical Pathology[a] | Bartlett's test for homogeneity and Shapiro-Wilk[8] test for normality | One-way analysis of variance followed with Dunnett's test | Log transforms of the data to achieve normality and variance homogeneity may be used. If the log transform fails, a non-parametric method (Kruskal-Wallis non-parametric analysis of variance) will be used. When non-parametric analysis of variance is significant, a comparison of treated groups to control will be performed (e.g. Dunn's test). |

[1] Motulsky,H (2014). Intuitive biostatistics, a nonmathematical guide to statistical thinking (3rd Edition). Oxford University Press, New York, NJ.

[2] Bartlett, MS. (1937). Properties of sufficiency and statistical tests. *Proceeding of the Royal Statistical Society Series A*, 160, 268-82.
[3] Dunnett, C.W. (1980). Pairwise multiple comparisons in the unequal variance case. *J. Amer. Statist. Assoc.* 75, 796-800.
[4] Dunnett, C.W. (1964). New tables for multiple comparisons with control. *Biometrics*, 482-491.
[5] Kruskal, W.H. and Wallis W.A. (1952). Use of ranks in one-criterion analysis of variance. *J. Amer. Statist. Assoc.* 47, 583-621.
[6] Dunn, O.J. (1964). Multiple contrasts using rank sums. *Technometrics*, 6, 241-252.
[7] Agresti, Alan (2013). Categorical Data Analysis (3rd Edition). John Wiley & Sons, Inc. Hoboken, NJ.
[8] Shapiro, S.S. & Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4), 591–611.

| | | Method of Statistical Analysis | |
|---|---|---|---|
| Parameter | Preliminary Test | If preliminary test is not significant | If preliminary test is significant |

[a] When an individual observation is recorded as being less than a certain value (e.g. below the lower limit of quantitation), calculations are performed on half the recorded value. For example, if bilirubin is reported as <0.1, 0.05 is used for any calculations performed with that bilirubin data. When an individual observation is recorded as being greater than a certain value, calculations are performed on the recorded value. For example, if specific gravity was reported as ≥1.100, 1.100 is used for any calculation performed with that specific gravity data.

## 14. FINAL REPORT

A signed study report will be provided to the Sponsor. This report will include, but not be limited to, the following information:

- individual animal data (and averages where appropriate) for actual concentration of test article received; time of observation of each abnormal sign and its subsequent course;
- body weights, food and water consumption, and food efficiency values (if warranted);
- hematology, clinical chemistry, coagulation, and urinalysis results;
- organ weights, organ to body weight, and organ to brain weight ratios;
- necropsy and pathology findings (phase report);
- test article and dose preparation analysis;
- enumeration and translocation data (phase report);
- Serum L- and D-Lactic acid Analysis (phase report);
- Certificates of analysis from test articles, animal feed, most recent tap water analysis, and the results of the sentinel health monitoring animals,
- a compliance statement signed by the Study Director that states that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study;
- a Quality Assurance statement summarizing QA activities performed for the study;
- a certification statement signed by the Study Director and test facility management that the report accurately reflects the raw data obtained during the performance of the study.

## 15. STUDY CONDUCT

### 15.A Laboratory

Test Facility

| | |
|---|---|
| In-life, clinical pathology, tissue enumeration and test article dose analysis | Product Safety Labs<br>2394 US Highway 130<br>Dayton, NJ 08810, USA |

Test Site

| | |
|---|---|
| Clinical pathology evaluation | Eurofins Advinus<br>21 & 22, Phase II, Peenya Industrial Area<br>Bengaluru, 560 058, India<br>Principal Investigator (P.I.):<br>Dr. Jayachandra, K.C., M.V.Sc., DABT |

| | |
|---|---|
| Translocation Analysis | BioPrimate<br>1 Oak Avenue<br>Newark, DE 19711<br>Principal Investigator (P.I.): Frank Burns |
| Serum L- and D-Lactic acid | Elanco Animal Health Incorporated<br>2500 Innovation Way<br>Greenfield, Indiana, 46140<br>Principal Investigator (P.I.): Dharanesh Mahimapura Gangaiah |
| Histological slide preparation | StageBio<br>5930 Main Street<br>Mount Jackson, VA 22842, USA<br>P.I. (histology): Erin Galati, HT (ASCP) |
| Histological slide evaluation | StageBio<br>5930 Main Street<br>Mount Jackson, VA 22842, USA<br>Prospective PI(s) (pathology):<br>Laura E. Elcock, DVM, PhD, DACVP<br>Katherine A.B. Knostman, DVM, PhD, DACVP<br>Allen Singer, DVM, DACVP, DABT<br>Jessica S. Hoane, DVM, MTOX, DACVP, DABT<br>Stephanie M. Shrader, DVM, PhD, DACVP<br>Jamie K. Young, DVM, PhD, DACVP<br>Cindy Farman, DVM, PhD, DACVP<br>Brett Saladino, DVM, DACVP |

### 15.B GLP Compliance

This study will be conducted in compliance with the following regulations:

U.S. FDA GLP: 21 CFR Part 58, 1987; which is compatible with OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM (98)17, OECD, Paris, 1998. Exceptions for translocation analysis data at BioPrimate and the Serum L- and D- Lactic acid analysis at sponsors lab.

### 15.C Test Procedure Guidelines

This study design is based on the following guidelines:

OECD Guidelines for Testing of Chemicals and Food Ingredients, Section 4 (Part 407): Health Effects, *Repeated Dose 28-Day Oral Toxicity Study in Rodents* (2008).

US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 3. a. *Short-Term Toxicity Studies with Rodents* (November 2003).

## 16. QUALITY ASSURANCE

The Quality Assurance Unit (QAU) of PSL has reviewed this protocol for GLP compliance and will conduct in-process inspections of selected procedures during the study. The analytical and final study reports will be audited for agreement with the raw data records and for compliance with the protocol and PSL SOPs.

In addition, PSL QAU will function as lead QA for this study and will monitor QA activities at StageBio. For portions of the study conducted by a subcontractor, the QAU for that facility will conduct necessary critical phase inspections and audit respective results and reports for the study phase according to the SOPs of that facility.

The QA Units from StageBio will send all GLP audit reports to the Study Director, Study Director's management, and PSL QAU as soon as they are issued.

## 17. RECORDS TO BE MAINTAINED

The electronic copies of the signed report, and the protocol, associated amendments and/or deviations if applicable will be send to the sponsor. The original signed final report and all the raw data generated at PSL will be maintained in the Product Safety Labs archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor of the study will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL.

The following records will be maintained:

**A.** Information on test article will include but not be limited to the following:

| | |
|---|---|
| Storage | Test article analysis |
| Usage | Dose preparation analysis |
| Disposition | |

**B.** Information on animals will include but not be limited to the following:

| | |
|---|---|
| Receipt, date of birth | Clinical observations |
| Initial health assessment | Histopathology data |
| Dosing | Individual necropsy records |
| Body weights | Organ weights |
| Food consumption | |

Hematology, clinical chemistry, coagulation, urinalysis data

**C.** All other records that would demonstrate adherence to the protocol.

Raw data related to clinical pathology evaluations will be maintained by Product Safety Labs. Tissues, blocks, and slides, and associated records will be retained and archived by StageBio, Mount Jackson, VA, 22842. The raw histopathology data (StageBio), translocation analysis data (BioPrimate) and the Serum L- and D- Lactic acid analysis data (Elanco Animal Health Incorporated) will be transferred to Product Safety Labs.

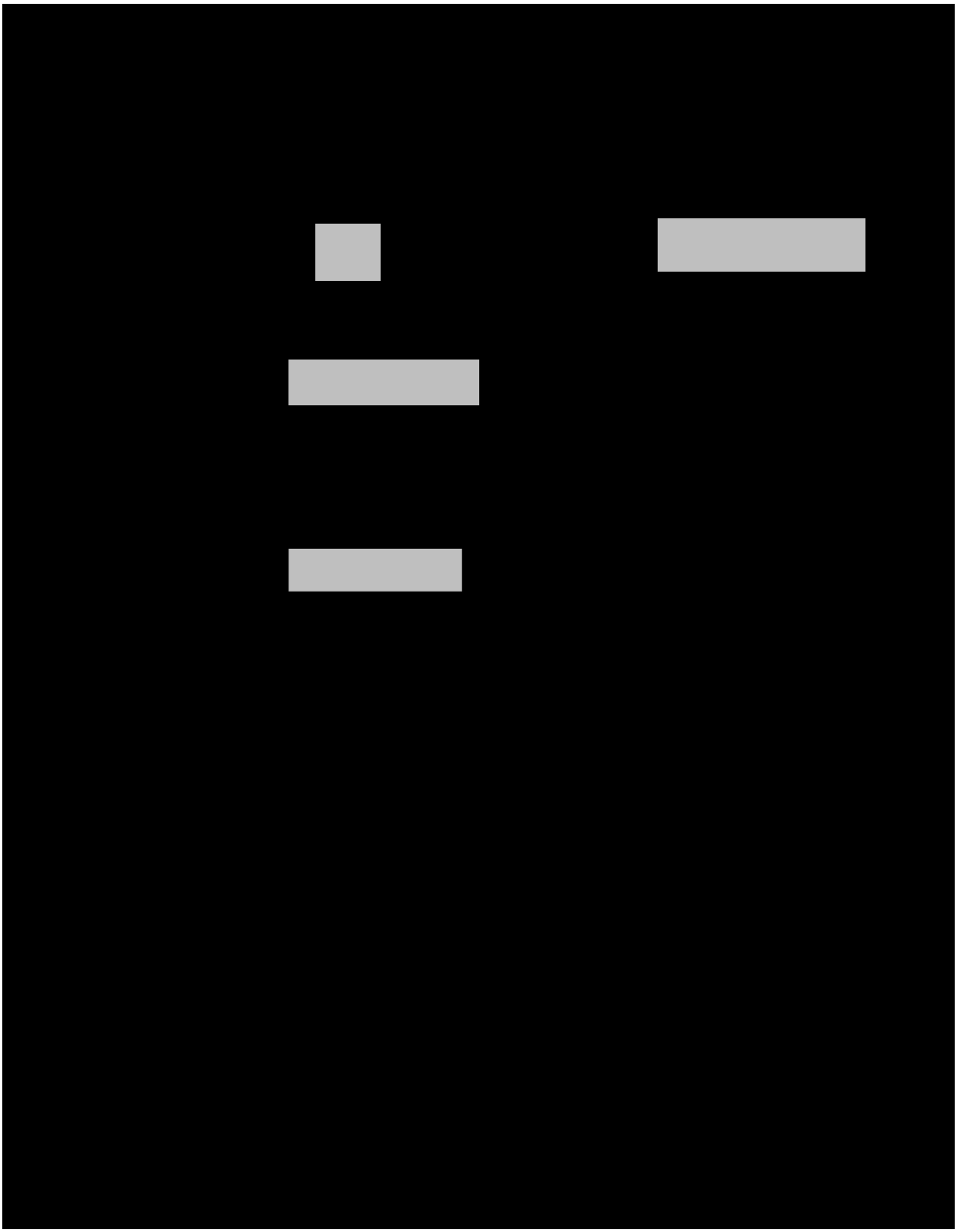
Any electronic raw data generated by the Test Site will be maintained by the Test Site in accordance with their GLP archiving procedures.

## 18. PROTOCOL AMENDMENTS AND DEVIATIONS

All amendments and/or deviations to this protocol and the reasons therefore, shall be appropriately documented, signed by the Study Director, Study Monitor and Sponsor Representative, and described in the final report.

## 19. DISPOSITION OF TEST ARTICLE

A retention sample will be kept at PSL. All remaining test article will be retained for at least one year from receipt, unless otherwise specified by the Sponsor. All remaining test article will be properly disposed, unless otherwise specified by the Sponsor.



**APPENDIX# 1**

**PROCEDURE FOR SAMPLING AND PROCESSING TO DETERMINE BACTERIAL TRANSLOCATION**

**MATERIALS (or equivalent)**

1. MRS agar: BD Difco™ Dehydrated Culture Media: Lactobacilli MRS Agar, catalog# DF0882-17-0, Fisher Scientific, originally from BD
2. Anaerobic box: AnaeroPak 2.5L Rectangular Jar, catalog# R685025, Thermo Scientific
3. Anaerobic packs: AnaeroPak – Anaero Anaerobic Gas Generator, catalog# R681001R681001
4. Buffered peptone: Buffered Peptone Water, catalog# 1072280500, Millipore Sigma
5. Stomacher: Stomacher® 80 Biomaster, Thomas Scientific, product# 3436A15

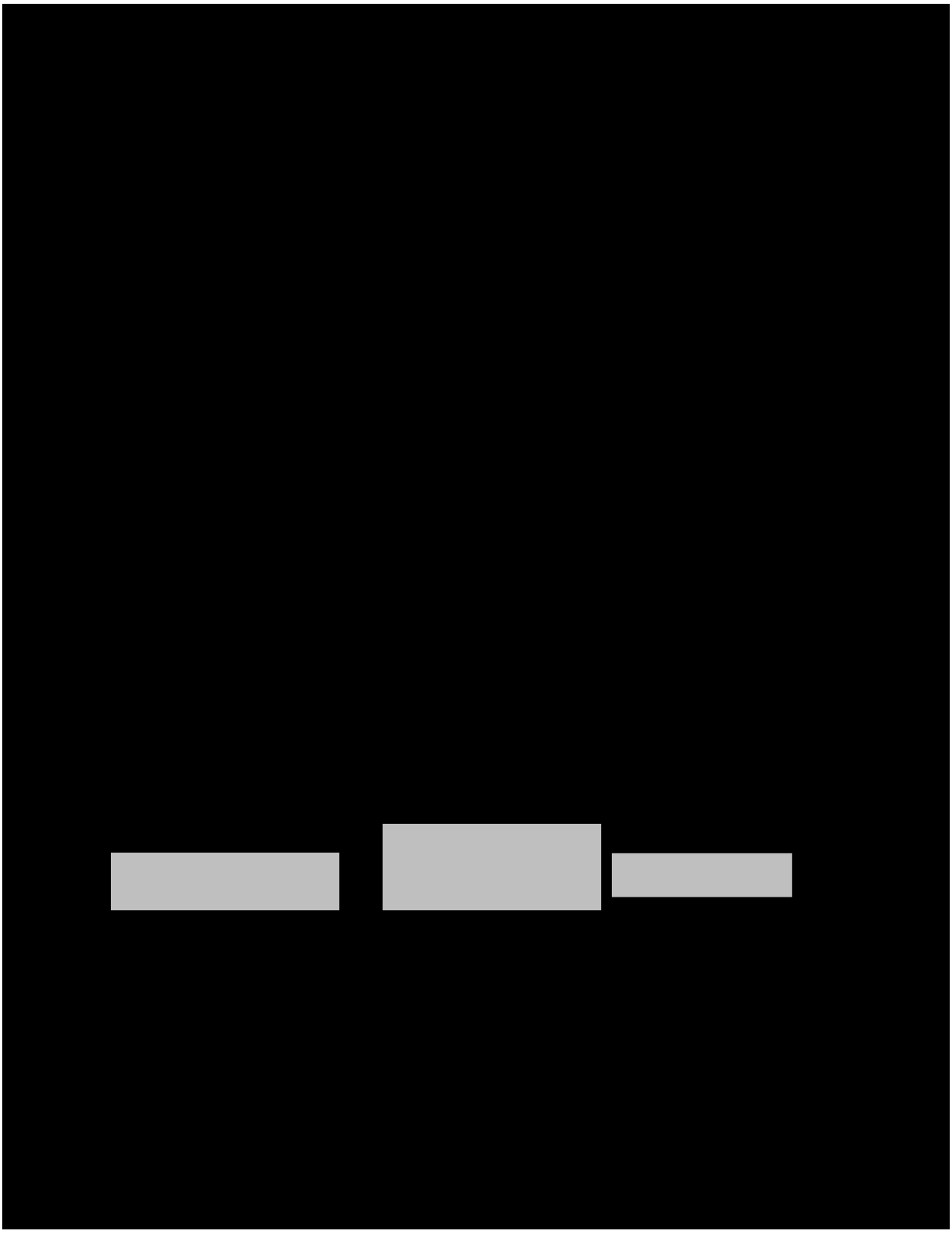
**METHODS**

1. 0.5 mL of blood and 0.5-2.0 grams of liver and mesenteric lymph nodes (collected from the same site for uniformity) from five randomly selected animals per sex per group will be harvested and maintained on ice (do not freeze) until analyzed for bacterial translocation as described below. The liver will be weighed prior to removal of the sample.
2. A 100 µL quantity of whole blood will be directly spread plated in duplicate on de Man, Rogosa, Sharpe (MRS) agar medium and incubated at 37°C for 48 hours or until colony growth is adequate for counting anaerobically (in an anaerobic box with AnaeroPaks).
3. Liver and mesenteric lymph nodes will be briefly rinsed after collection and homogenized using a mechanical homogenizer (Omni probe homogenizer Model TH) in 2 mL of buffered peptone water (1 g/mL) and 100 µL of the resulting homogenate will be plated in duplicate on MRS agar and incubated at 37°C for 24-48 hours or until colony growth is adequate for counting anaerobically. As a control for media and growth conditions, a known *Lactobacillus reuteri* strain (or even any *Lactobacillus* strain) will be streaked on a plate and incubated along with the above plates as described above.
4. Colony forming units (CFU) from liver, mesenteric lymph nodes and blood will be counted, averaged and tabulated for each animal and expressed as incidence of translocation (number of rats in which colony-forming units were detected/total number of rats. Positive growth on agar plates is defined by the presence of any microorganism (≥1 colony). All plates will be visually inspected for CFU growth after incubation and individual colonies will be counted. An automated plate counter may be utilized to assist in

enumerating highly populated plates. The mean CFU/gram of tissue will be calculated using the amount of respective sample evaluated, plate colony growth and factoring for applicable dilutions. For each tissue from each animal replicate plate will be examined under a stereo microscope to determine the number of distinct morphologies that have grown from the tissue using the characteristics of size, form, elevation, margin, surface, opacity and pigmentation (color). The characteristics and number of each morphology present will be recorded for the tissue.

5. The sensitivity of the method will be determined for each sample type using the average amount of tissue in each preparation, assuming a minimum detection limit of 1 CFU/plate and factoring for dilution of samples including plated volumes. Dilutions of the sample preparations either prior to inoculation or prior to plating will be accounted for in the calculations by additional multiplication of the dilution factor by the appropriate factor where applicable.

   Sensitivity (CFU/g or mL) = (Minimum Detectable CFU/plate (1 CFU))/(Avg amount of sample (g or mL)) X Dilution Factor
6. For each morphology identified on plates from each tissue, five colonies from each morphology positive plate will be randomly picked. In the case where less than five colonies of a particular morphology are present on the plates, all colonies will be picked.
7. Picked colonies will be regrown following initial plating conditions and time. DNA from each picked colony will be extracted and identified using 16S rRNA hypervariable region 4 (V4) sequencing and checked for identity to the test strains.
8. Any colonies do not differentiated from either of the test strains by sequence of 16S RNA gene V4 region will be further characterized by rapid amplification of polymorphic DNA (RAPD) DNA fingerprinting to determine if, by this criteria, the colony is or is not differentiable from the test strains.
9. Total colony counts, characteristics and numbers for each morphology detected, taxonomic designation based on 16S V4 sequencing of each isolate characterized and if it is not differentiable from a test strain, which test strain it could not be differentiated from will be reported.



# Product Safety Labs

## PROTOCOL AMENDMENT

*Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 DDU

AMENDMENT NO.: 2

STUDY NO.: 55164

PSL NO.: 210226-1D, 210226-2D, 210408-3D, 210408-2D

**PROTOCOL CHANGE:** APPENDIX# 1: PROCEDURE FOR SAMPLING AND PROCESSING TO DETERMINE BACTERIAL TRANSLOCATION

**CHANGE FROM:**

MATERIALS (or equivalent)

4. Buffered peptone: Buffered Peptone Water, catalog# 1072280500, Millipore Sigma
5. Stomacher: Stomacher® 80 Biomaster, Thomas Scientific, product# 3436A15

METHODS

3. Liver and mesenteric lymph nodes will be briefly rinsed after collection and homogenized using a mechanical homogenizer (Omni probe homogenizer Model TH) in 2 mL of buffered peptone water (1 g/mL) and 100 µL of the resulting homogenate will be plated in duplicate on MRS agar and incubated at 37°C for 24-48 hours or until colony growth is adequate for counting anaerobically. As a control for media and growth conditions, a known *Lactobacillus reuteri* strain (or even any *Lactobacillus* strain) will be streaked on a plate and incubated along with the above plates as described above.

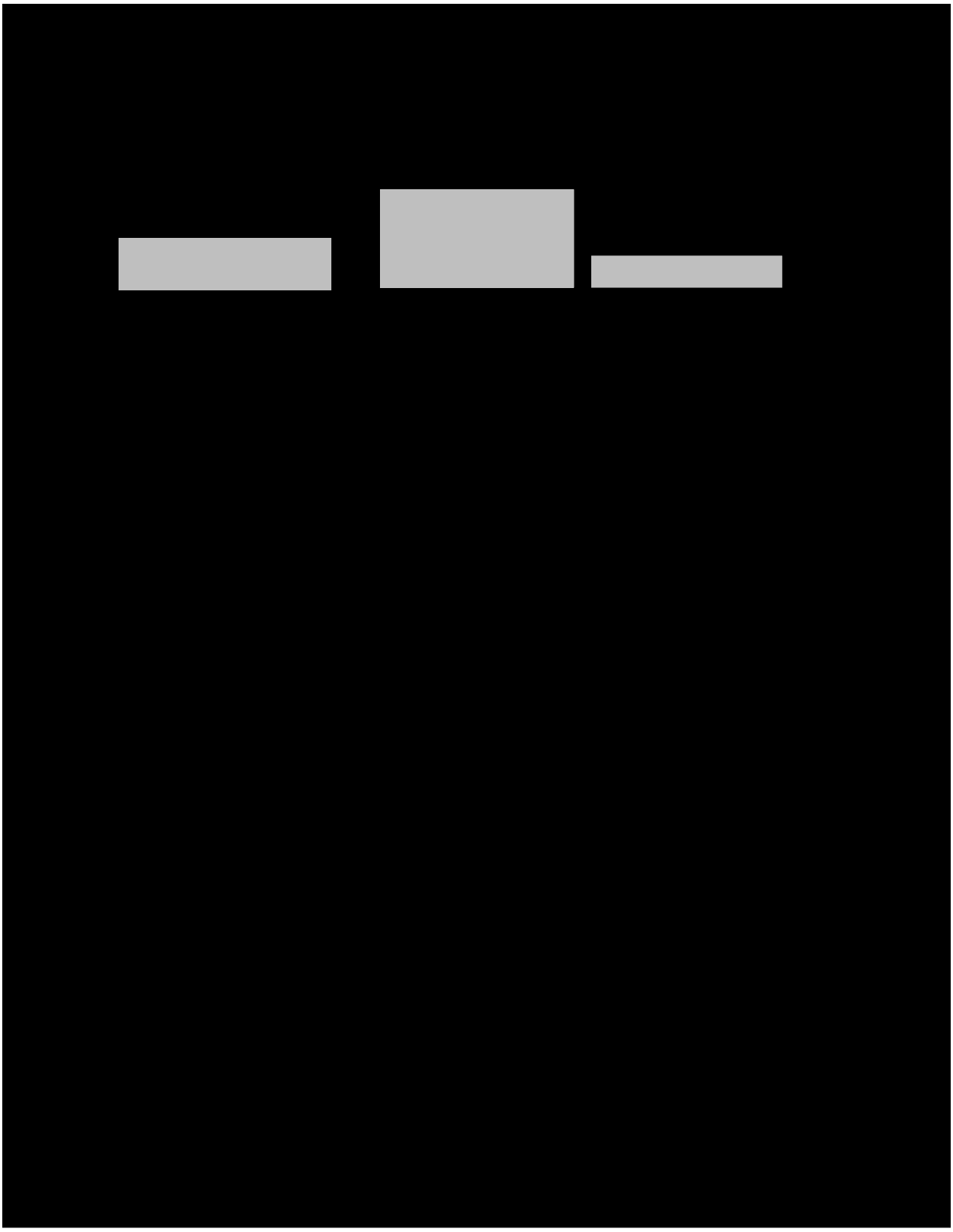
**CHANGE TO:**

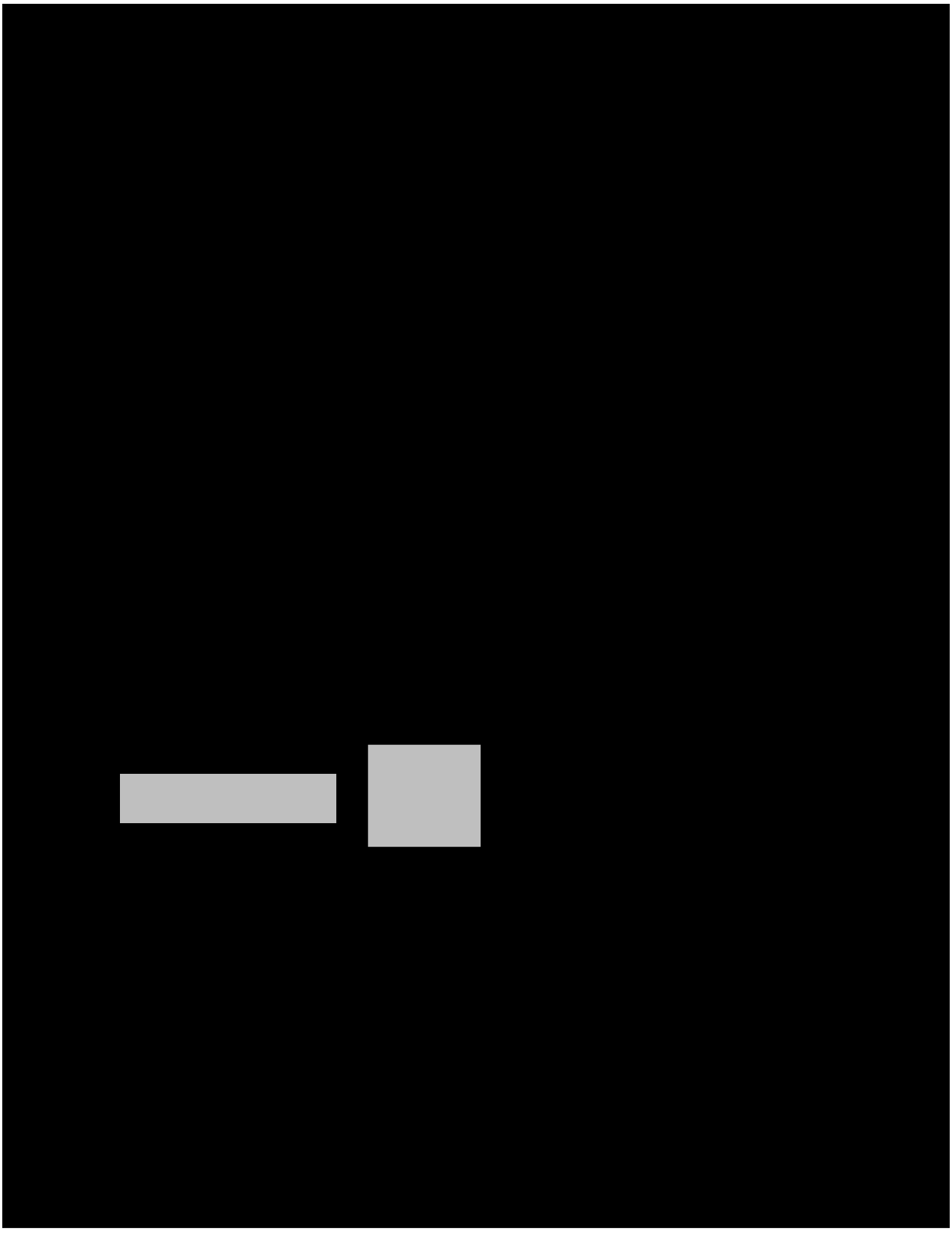
MATERIALS (or equivalent)

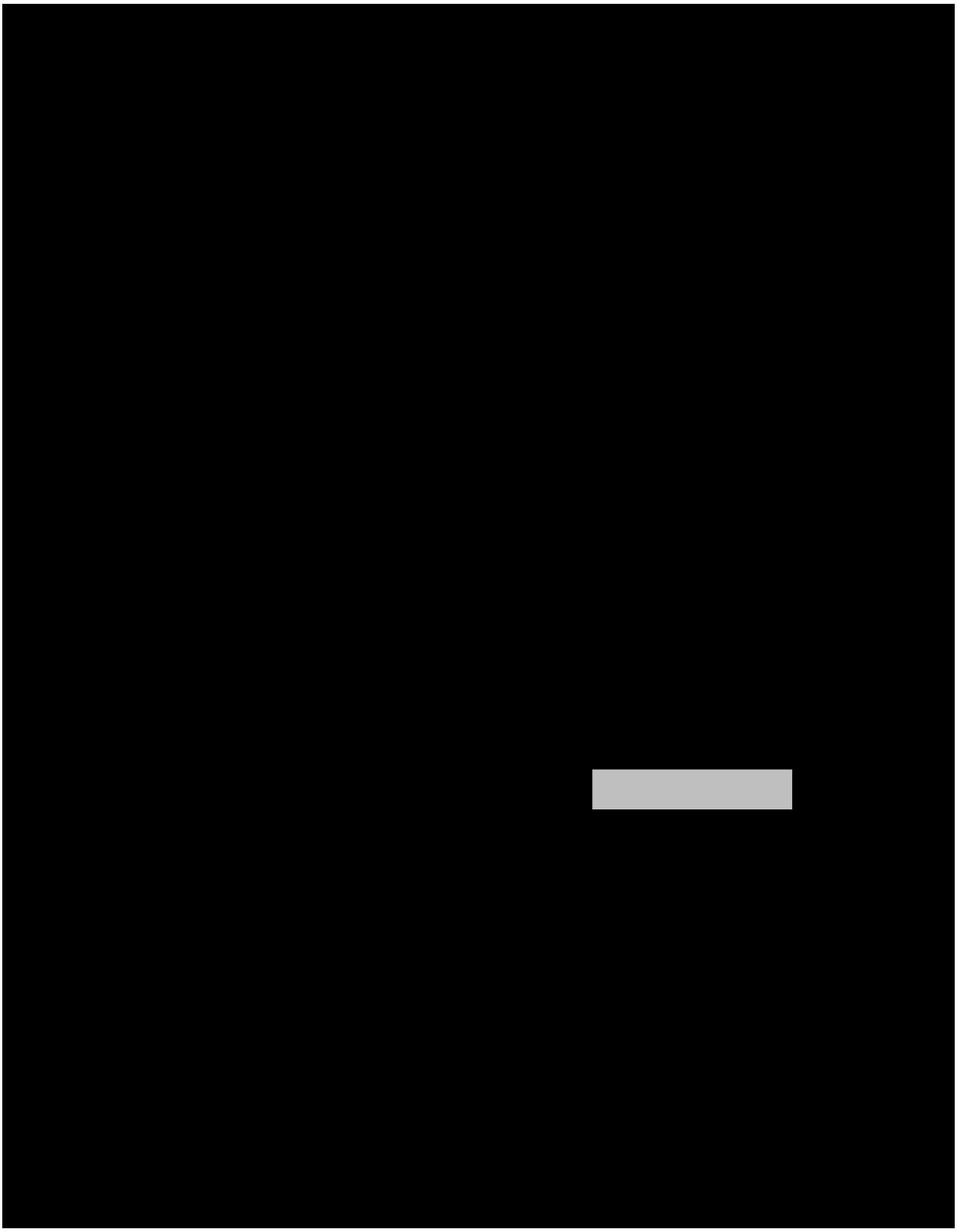
4. Dulbecco's Phosphate Buffered Saline from Mediatech Inc, Batch #: 29620002.
5. Mechanical homogenizer (Omni probe homogenizer Model TH).

METHODS

3. Liver and mesenteric lymph nodes will be briefly rinsed after collection and homogenized using a mechanical homogenizer (Omni probe homogenizer Model TH) in 10X volume of Dulbecco's Phosphate Buffered Saline (1 g/10 mL) and 100 µL of the resulting homogenate will be plated in triplicate on MRS agar and incubated with $CO_2$ gas packs at 37°C for 24-48 hours or until colony growth is adequate for counting. As a control for media and growth conditions, a known *Lactobacillus reuteri* strain (or even any *Lactobacillus* strain) will be streaked on a plate and incubated along with the above plates as described above.







**Product Safety Labs**

## PROTOCOL AMENDMENT

*Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 DDU

AMENDMENT NO.: 4

STUDY NO.: 55164

PSL NO.: 210226-1D, 210226-2D
210408-3D, 210408-2D

**PROTOCAL SECTION:** 9.B Identification

Test Article 1: *L. reuteri* ATCC PTA-126787 (*L. reuteri* 3630)
Lot #: 201123LRE3630
PSL ID: 210512-1D
Physical Description: White to Tan powder
Composition: 100% *L. reuteri* 3630
Storage Conditions: Frozen (store ~ -25 to -15° C until ready to use).
Expiration Date: 11/23/21

**REASON:** Additional test article was supplied by the Sponsor in order to complete the requested study. PSL ID 210512-1D will be used for testing. The additional material is from the same lot as the previous. This amendment has no adverse impact on the study.

Effective date: 05/12/2021

[redacted]

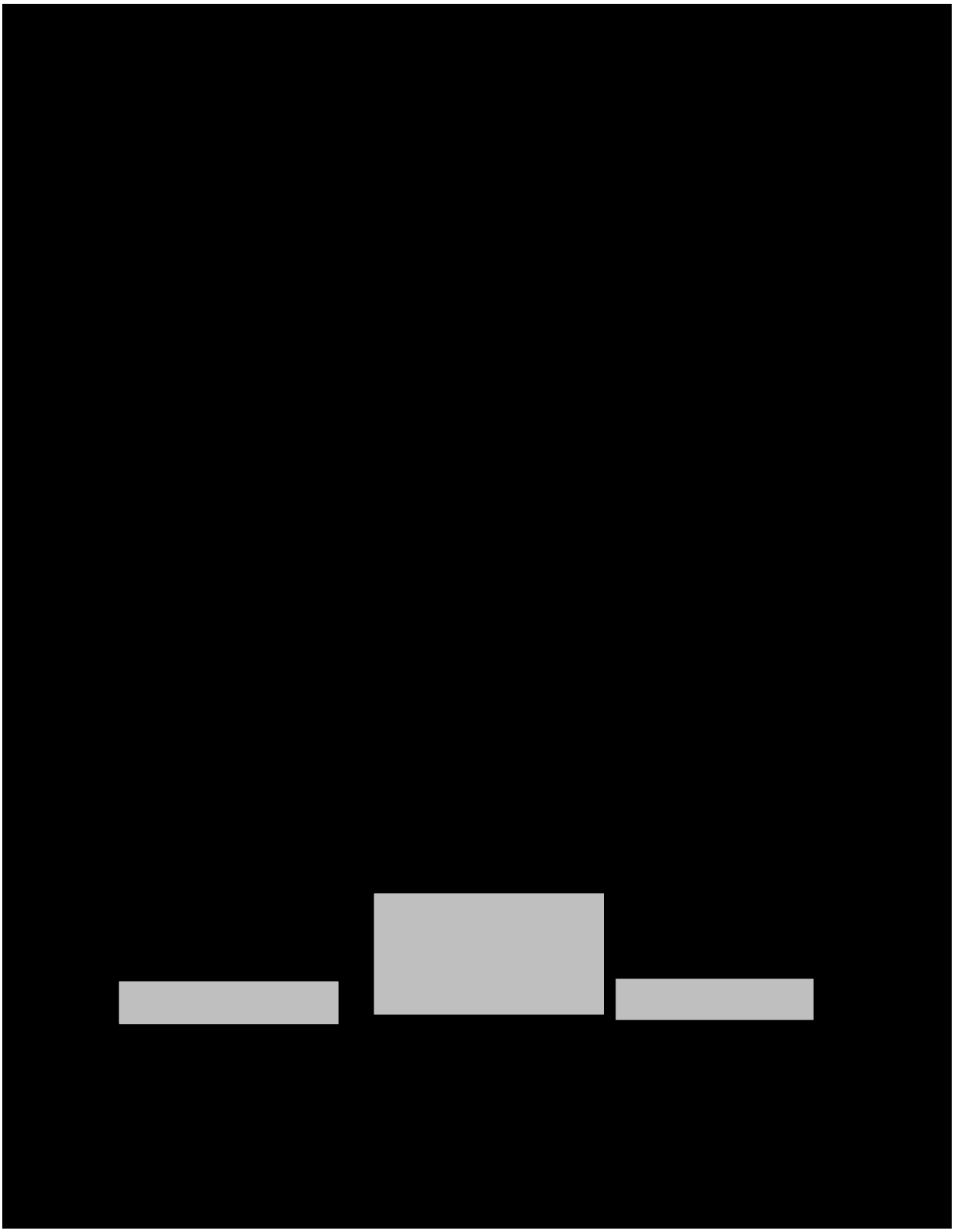
[redacted]
Digital unterschrieben von GLLUQ
Datum: 2021.05.12 20:04:08 +02'00'

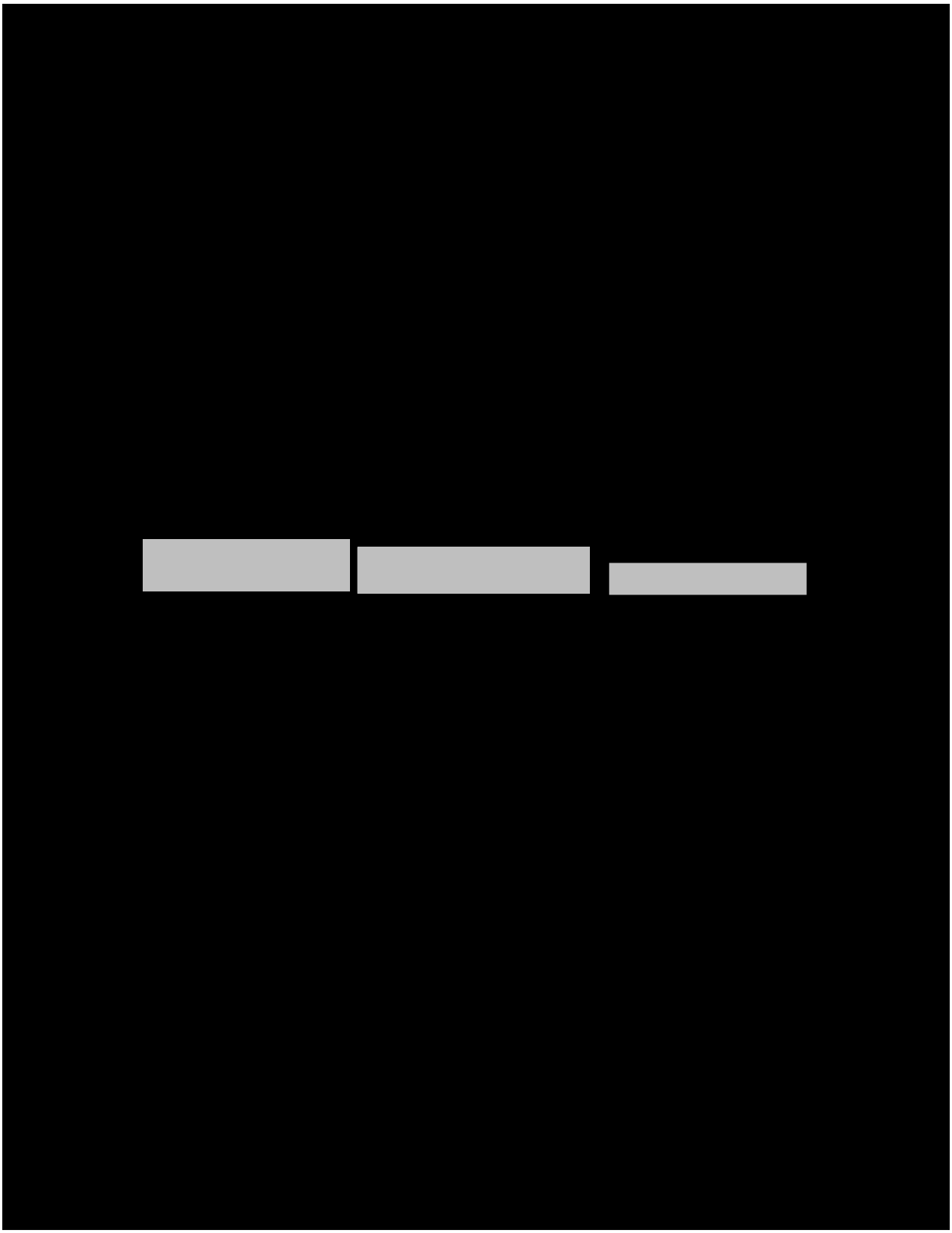
[redacted] 12May2021

Raghavendra Gowda Ph.D.
Study Director
Product Safety Laboratories

Dr. Olaf Will
Sponsor Representative
Bayer Animal Health GmbH
(An Elanco Affiliate)

Laurie C. Dolan
Study Monitor
GRAS Associates





**Product Safety Labs**

**PROTOCOL AMENDMENT**

*Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 DDU

AMENDMENT NO.: ① 7

STUDY NO.: 55164

PSL NO.: 210226-1D, 210226-2D 210408-3D, 210408-2D, 210512-1D

**PROTOCOL CHANGE:**

The Study Director, Raghavendra Gowda, is replaced by Mark Bauter, effective on 08/10/2021.

**REASON FOR CHANGE:**

Responsibility for this study has been re-assigned by test facility management.

New Study Director:

Signature: [redacted]
Mark Bauter, BS

8-17-21
Date

Management:

Signature: [redacted]
Daniel J. Merkel, BS, MBA

August 17, 2021
Date

[redacted]
Dr. Olaf Will
Sponsor Representative
Bayer Animal Health GmbH
(An Elanco Affiliate)

[redacted] Aug 16,2021
Laurie C. Dolan
Study Monitor
GRAS Associates

① CL MRB 8-18-21

**Product Safety Labs**

**PROTOCOL AMENDMENT**

*Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 DDU

AMENDMENT NO.: 8

STUDY NO.: 55164

PSL NO.: 210226-1D, 210226-2D
210408-3D, 210408-2D,
210512-1D

**PROTOCOL SECTION: 12.G.3: General Procedures; Clinical Pathology; Clinical Chemistry**

**PROTOCOL CHANGE:**

The additional serum sample collected for Sponsor analysis of L- and D – Lactic acid will not be evaluated for this study.

**REASON FOR CHANGE:**

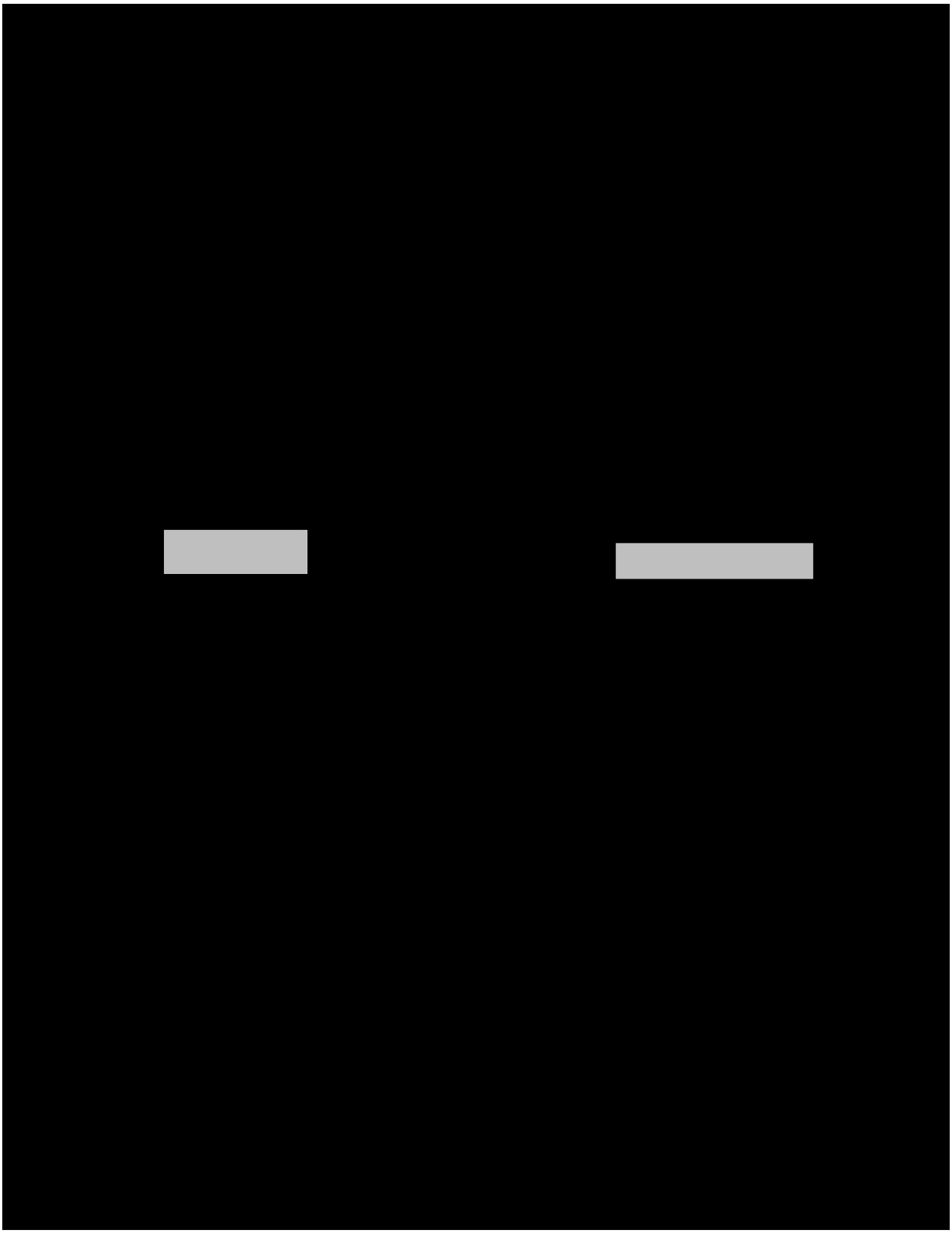
Sponsor Request

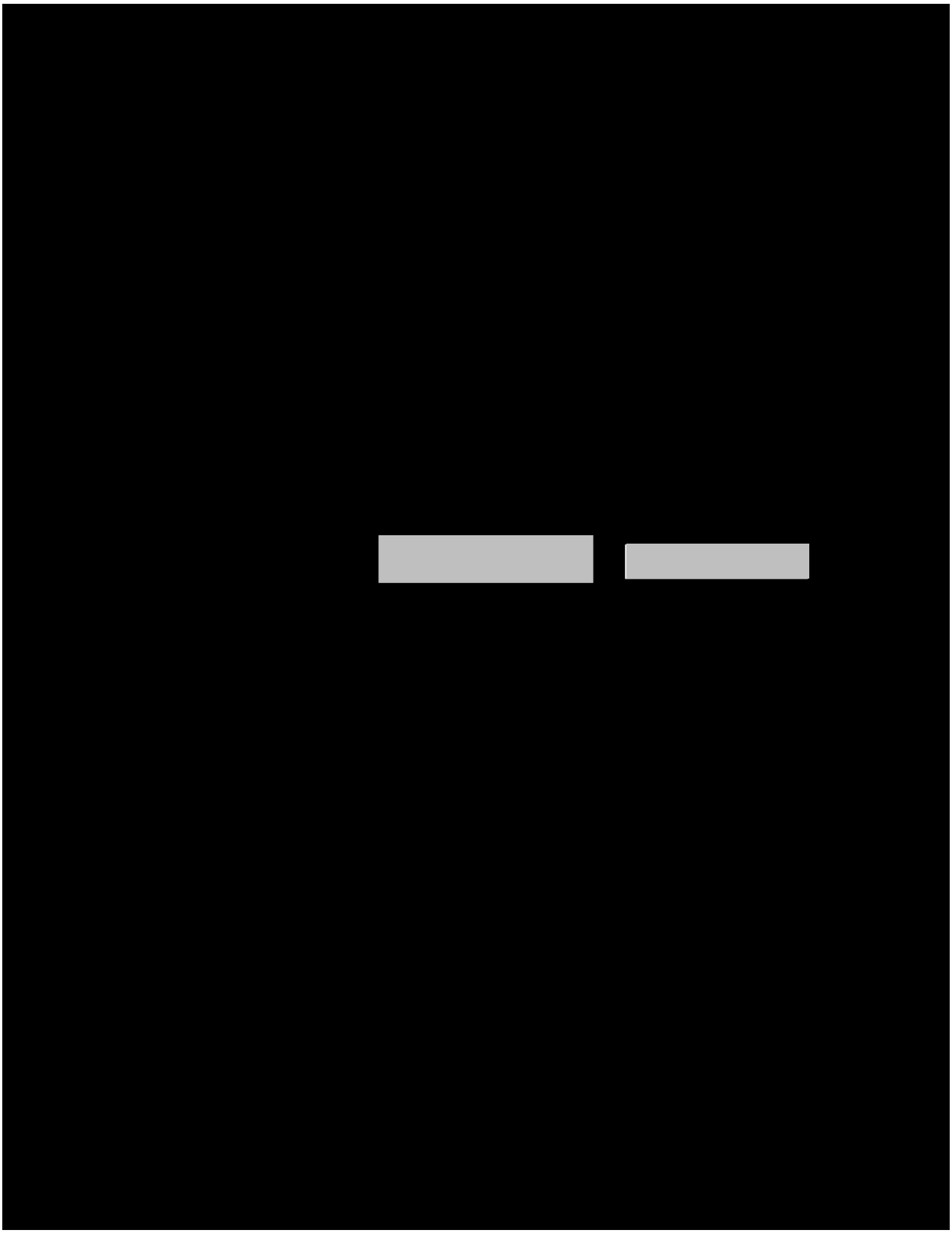
Signature: [redacted]
Mark Bauter, BA

8-18-21
Date

[redacted] Aug 18, 2021
Dr. Olaf Will
Sponsor Representative
Bayer Animal Health GmbH
(An Elanco Affiliate)

[redacted] August 18, 2021
Laurie C. Dolan
Study Monitor
GRAS Associates





# Product Safety Labs

**PROTOCOL DEVIATION**

*Limosilactobacillus reuteri*: 28-DAY REPEAT-DOSE ORAL GAVAGE TOXICITY STUDY IN RATS

**PROTOCOL NO.:** P713.01 DDU

**DEVIATION NO.:** 2

**STUDY NO.:** 55164

**PSL NO.:** 210226-1D, 210226-2D
210408-3D, 210408-2D

**PROTOCOL SECTION: 12.G& 12.H.5 Clinical Pathology and Unscheduled Sacrifice**

**Protocol Deviation**: The protocol indicates that clinical pathology and organ weight data will be collected for all animals killed in moribund condition for ethical reasons prior to death. These data were not collected for one animal that did not survive to terminal sacrifice

**Impact:** The absence of the data collected for this animal is not used in the evaluation of the data for the animals surviving to the end of the study.

[redacted] 8-20-21
Mark R. Bauter, BA
Associate Director, Toxicology
Product Safety Laboratories

[redacted] 20-Aug-2021
Dr. Olaf Will
Sponsor Representative
Bayer Animal Health GmbH
(An Elanco Affiliate)

[redacted] 8/19/2021
Laurie C. Dolan
Study Monitor
GRAS Associates

# APPENDIX B: FEED, WATER AND SEROLOGY ANALYSES

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

# APPENDIX B: FEED

2016C

+++ ENVIGO

Teklad Certified Global 16% Protein Rodent Diet

| | |
|---|---|
| Lot Number | 2016C-020321MA |
| Date of Manufacture | 03Feb2021 |
| Report Date | 12Feb2021 |

***Laboratory Diet Certification Report***

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Barbara Mickelson

**REVIEWED**
*By Barbara Mickelson at 4:10 pm, Feb 16, 2021*

| Analysis | Result (%) |
|---|---|
| **Proximate Analysis** | |
| Protein | **16.40** |
| Fat | **3.68** |
| Fiber | **3.69** |
| Moisture | **11.49** |
| Ash | **5.44** |
| Calcium | **0.99** |
| Phosphorus | **0.74** |

| Analysis | Result | Units | Established Maximum Concentration |
|---|---|---|---|
| **Heavy Metals** | | | |
| Arsenic | **< 0.10** | ppm | 1.00 |
| Cadmium | **< 0.10** | ppm | 0.50 |
| Lead | **< 0.20** | ppm | 1.50 |
| Mercury | **< 0.05** | ppm | 0.20 |
| Selenium | **0.23** | ppm | 0.50 |
| **Mycotoxin** | | | |
| Aflatoxin B1, B2, G1, G2 | **< 5.00** | ppb | 5.00 |
| **Chlorinated Hydrocarbons** | | | |
| Aldrin | **< 0.01** | ppm | 0.03 |
| Lindane | **< 0.01** | ppm | 0.05 |
| Chlordane | **< 0.01** | ppm | 0.05 |
| DDT & related substances | **< 0.03** | ppm | 0.15 |
| Dieldrin | **< 0.02** | ppm | 0.03 |
| Endrin | **< 0.02** | ppm | 0.03 |
| Heptachlor | **< 0.01** | ppm | 0.03 |
| Heptachlor Epoxide | **< 0.01** | ppm | 0.03 |
| Toxaphene | **< 0.10** | ppm | 0.15 |
| PCB's | **< 0.10** | ppm | 0.15 |
| a-BHC | **< 0.01** | ppm | 0.05 |
| b-BHC | **< 0.01** | ppm | 0.05 |
| d-BHC | **< 0.01** | ppm | 0.05 |
| Hexachlorobenzene | **< 0.01** | ppm | 0.03 |
| Mirex | **< 0.01** | ppm | 0.02 |
| Methoxychlor | **< 0.05** | ppm | 0.50 |
| **Organophosphates** | | | |
| Thimet | **< 0.15** | ppm | 0.50 |
| Diazinon | **< 0.14** | ppm | 0.50 |
| Disulfaton | **< 0.15** | ppm | 0.50 |
| Methyl Parathion | **< 0.14** | ppm | 0.50 |
| Malathion | **< 0.14** | ppm | 0.50 |
| Parathion | **< 0.12** | ppm | 0.50 |
| Thiodan | **< 0.02** | ppm | 0.50 |
| Ethion | **< 0.14** | ppm | 0.50 |
| Trithion | **< 0.15** | ppm | 0.50 |



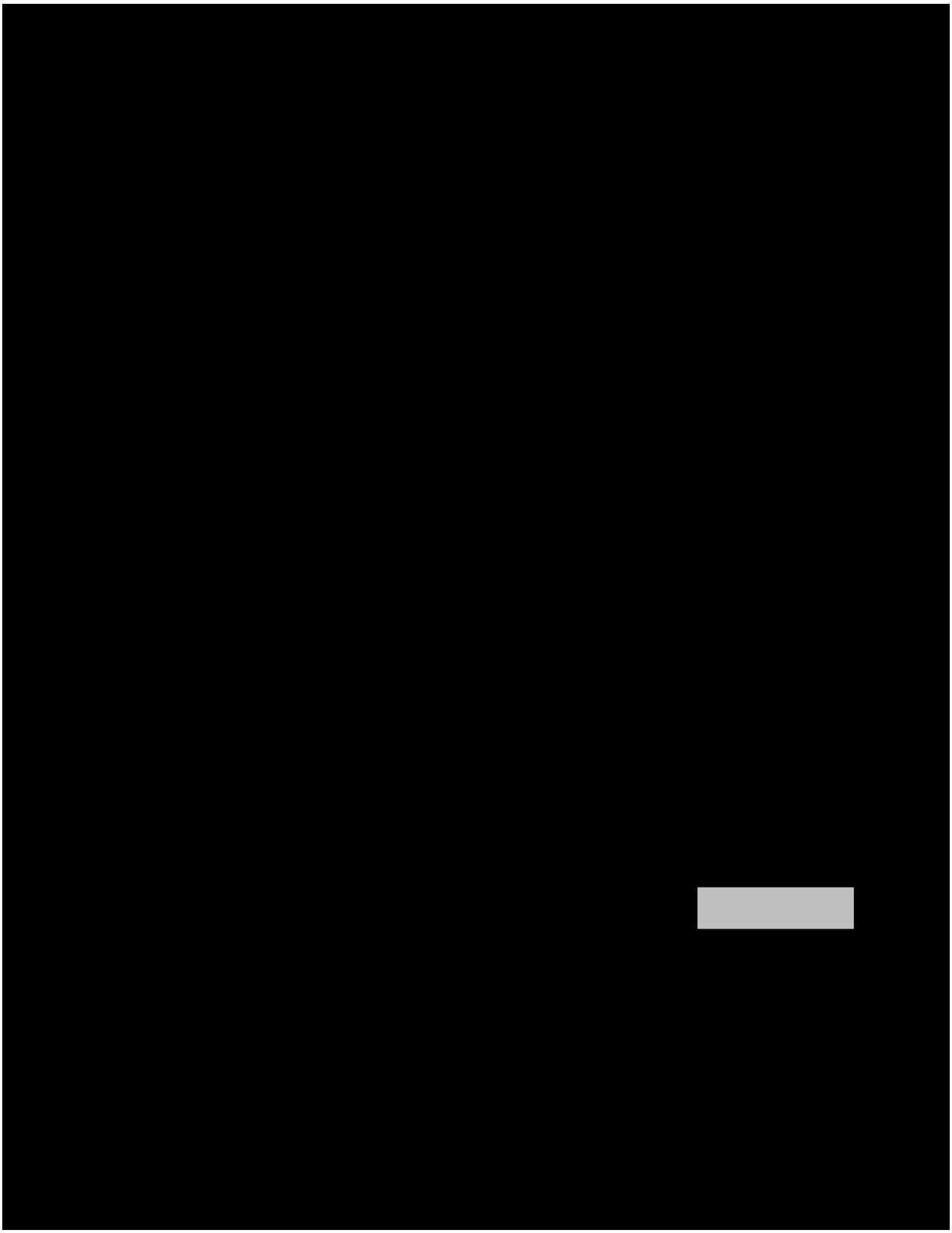
Envigo Teklad Diets + Madison WI + envigo.com + tekladinfo@envigo.com (800) 483-5523

## APPENDIX B (cont.): WATER

In March 2021, water was analyzed for contaminants.

LABORATORY: PRECISION ANALYTICAL SERVICES, INC.
2161 Whitesville Road
Toms River, NJ 08755

Results of water analysis for possible contaminants were acceptable within regulatory standards.



## APPENDIX B (cont.): SEROLOGY

In May 2021, serology from sentinel animals residing in Room #21, which also housed the study animals, was obtained from collected blood serum for a battery of common viral and microbiologic pathogens.

The sentinel animals along with the test animals were in Room #21 from April 13 to May 13, 2021, for the duration of the study. Blood samples were collected on May 13, 2021.

LABORATORY: IDEXX BioAnalytics
4011 Discovery Drive
Columbia, MO 65201

Results of the serology analyses for sentinel animals corresponding with this study are reported as samples 356F 5/13/21, 374F 5/13/21 and 362F 5/13/21. All samples were negative for microbial antibodies

IDEXX BioAnalytics

IDEXX

**FINAL REPORT OF LABORATORY EXAMINATION**

**4011 Discovery Drive, Columbia, MO 65201**
**1-800-669-0825 1-573-499-5700**
idexxbioanalytics@idexx.com www.idexxbioanalytics.com

**IDEXX BioAnalytics Case # 17682-2021**

**Received Date: 6/4/2021**
**Completed: 6/7/2021**

**Submitted By**

Andre Blue
Product Safety Labs
2394 US Highway 130
Dayton, NJ 08810
Phone: 732-230-5110 x 1158
Email: andreblue@productsafetylabs.com

**Specimen Description**

Species: rat
Breed/Strain: CD/CRL
Description: Opti-Spot; Opti-Spot strip(s)
Number of Specimens/Animals: 3

| Client ID | Investigator | Room # | Species | Strain /Breed | Sex | Study # |
|---|---|---|---|---|---|---|
| 356F 5/13/21 | Raghu Gowda | 21 | rat | CD/CRL | F | 55164 |
| 374F 5/13/21 | Raghu Gowda | 21 | rat | CD/CRL | F | 55164 |
| 362F 5/13/21 | Raghu Gowda | 21 | rat | CD/CRL | F | 55164 |

**Services/Tests Performed:** PSL Rat Serology Panel (1-3)

**Serologic evaluation for antibodies to:** H1, KRV, RCV/SDAV, RMV, RPV, RTV

**Summary:** All test results were negative.

## SEROLOGY SUMMARY

| | 356F 5/13/21 | 374F 5/13/21 | 362F 5/13/21 |
|---|---|---|---|
| RPV | - | - | - |
| RMV | - | - | - |
| KRV | - | - | - |
| H1 | - | - | - |
| RCV/SDAV | - | - | - |
| RTV | - | - | - |
| Rat IgG | N | N | N |

**Legend:** + = positive - = negative blank = test not performed EQ = equivocal HE = hemolysis precluded testing I = insufficient W = weak positive WB = Western Blot confirmatory analysis pending NS = non-specific reactivity N = normal IgG L = less than normal IgG

## SEROLOGY DETAILS

| | Baseline | 356F 5/13/21 | 374F 5/13/21 | 362F 5/13/21 |
|---|---|---|---|---|
| **RPV** | | | | |
| RPV purified virus | MFI > 2,500 | - | - | - |
| NS1[1] | MFI > 3,750 | - | - | - |
| **RMV** | | | | |
| RMV VP2 recombinant | MFI > 2,000 | - | - | - |
| NS1[1] | MFI > 3,750 | - | - | - |
| **KRV** | | | | |
| KRV purified virus | MFI > 4,250 | - | - | - |
| NS1[1] | MFI > 3,750 | - | - | - |
| **H1** | | | | |
| H1 purified virus | MFI > 1,750 | - | - | - |
| NS1[1] | MFI > 3,750 | - | - | - |
| **RCV/SDAV** | | | | |
| RCV/SDAV purified virus | MFI > 3,750 | - | - | - |
| RCV/SDAV Spike | MFI > 3,750 | - | - | - |
| **RTV** | | | | |
| RTV purified virus | MFI > 2,000 | - | - | - |
| TMEV purified virus | MFI > 2,000 | - | - | - |

**NS1[1]:** NS1 protein is highly conserved among rodent parvoviruses and thus serves as a generic assay for parvovirus seroconversion.

**Legend:** + = positive - = negative blank = test not performed EQ = equivocal HE = hemolysis precluded testing I = insufficient W = weak positive WB = Western Blot confirmatory analysis pending NS = non-specific reactivity N = normal IgG L = less than normal IgG

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.

# APPENDIX C: CERTIFICATES OF ANALYSIS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

BioSource
CULTURES & FLAVORS

S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777 • P: 414 422 9065; F: 414 422 9086 • info@biosource.us.com

Rev. 6 9/09/21 – added units to results

# Certificate of Analysis

Date: 9/9/2021
Customer: ELANCO
Product: L. reuteri 3630
Lot #: 201123LRE3630
Lab Report #: BQL201208, 3120523-0 (Eurofins)
Purchase Order #: Email

**Microbiological Analyses**

| Test | Method | Spec Limits | Results |
|---|---|---|---|
| Lactic Acid Bacteria Counts | BQL-LAB-001.3 | Not specified | 270.8 x $10^9$ cfu/g |
| Enterococcus | CMMEF, 4th Ed | < 100 CFU/g | <10 cfu/g |
| Non-Lactics | ISO 13559 | < 5000 CFU/g | <100 cfu/g |
| Enterobacteriaceae | USPN 2021 | < 10 cfu/g | < 10 cfu/g |
| E. coli | USPE 2022 | Absent/10 g | Absent/10g |
| Yeast & Mold | USPM 2021 | < 100 cfu/g | < 10 cfu/g |
| CP Staphylococcus | USPA 2022 | Absent/10 g | Absent /10g |
| Salmonella | USPS 2022 | Absent /25g | Absent /25g |
| Listeria | FDA BAM Ch. 10 | Not Detected /25 g | Not detected /25g |
| Appearance/Color | Visual | White to Tan powder | Pass |
| Identification | Riboprinter | >0.85 Similarity Index | 0.9 |
| Arsenic | AOAC 2011.19 and 993.14 | NMT 1 ppm | 0.194 ppm |
| Cadmium | AOAC 2011.19 and 993.14 | NMT 0.3 ppm | 0.115 ppm |
| Lead | AOAC 2011.19 and 993.14 | NMT 1 ppm | 0.046 ppm |
| Mercury | AOAC 2011.19 and 993.14 | NMT 0.05 ppm | <.005 ppm |

**Manufacturing Date**
11/23/2020

**Best Before Date**
11/23/2021

**Country of Origin**
US

**Ingredients**
*Lactobacillus reuteri* and no excipient

**Recommended Storage Conditions**
Store frozen at -20°C or colder

Approved by: Jerry Stoecklein, Quality Director

BioSource
CULTURES & FLAVORS

S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777 • P: 414 422 9065; F: 414 422 9086 • info@biosource.us.com

Rev. 6 – 9/09/21 – added units to results

# Certificate of Analysis

Date: 9/9/2021
Customer: ELANCO
Product: L. reuteri 3632
Lot #: 201123LRE3632
Lab Report #: BQL201208, 3121727-0 (Eurofins)
Purchase Order #: Email

**Microbiological and Analytical Analyses**

| Test | Method | Spec Limits | Results |
|---|---|---|---|
| Lactic Acid Bacteria Counts | BQL-LAB-001.3 | Not specified | 430.0 x $10^9$ cfu/g |
| Non-Lactics | ISO 13559 | < 5000 CFU/g | 300 cfu/g |
| Enterococcus | CMMEF, 4th Ed | < 100 CFU/g | <10 cfu/g |
| Enterobacteriaceae | USPN 2021 | < 10 cfu/g | < 10 cgu/g |
| E. coli | USPE 2022 | Absent/10 g | Absent /10g |
| Yeast & Mold | USPM 2021 | < 100 cfu/g | < 10 cfu/g |
| CP Staphylococcus | USPA 2022 | Absent/10 g | Absent /10g |
| Salmonella | USPS 2022 | Absent /25g | Absent /10g |
| Listeria | FDA BAM Ch. 10 | Not Detected /25 g | Not detected /25g |
| Appearance/Color | Visual | White to Tan powder | Pass |
| Identification | Riboprinter | >0.85 Similarity Index | 0.89 |
| Arsenic | AOAC 2011.19 and 993.14 | NMT 1 ppm | 0.282 ppm |
| Cadmium | AOAC 2011.19 and 993.14 | NMT 0.3 ppm | 0.195 ppm |
| Lead | AOAC 2011.19 and 993.14 | NMT 1 ppm | 0.103 ppm |
| Mercury | AOAC 2011.19 and 993.14 | NMT 0.05 ppm | 0.006 ppm |

**Manufacturing Date**
11/23/2020

**Best Before Date**
11/23/2021

**Country of Origin**
US

**Ingredients**
*Lactobacillus reuteri* and no excipient

**Recommended Storage Conditions**
Store frozen at -20°C or colder

Approved by: Jerry Stoecklein, Quality Director

# APPENDIX D: DOSE ENUMERATION ANALYSIS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

## NEAT TEST ARTICLE CONCENTRATION VERIFICATION

| Sampling Day | Plated Dilution Used[1] | CFU in Neat Test Article Strain 3630 | | | | | Plated Dilution Used[1] | CFU in Neat Test Article Strain 3632 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plates | | | Average | CFU\g | | Plates | | | Average | CFU\g |
| | | 1 | 2 | 3 | | | | 1 | 2 | 3 | | |
| 1 | $10^{-8}$ | 50 | 44 | 48 | 47 | 9.50E+10 | $10^{-8}$ | 31 | 25 | 32 | 29 | 5.90E+10 |
| 9 | $10^{-8}$ | 31 | 38 | 38 | 36 | 7.10E+10 | $10^{-8}$ | 50 | 54 | 52 | 52 | 1.00E+10 |
| 15 | $10^{-8}$ | 41 | 30 | 28 | 33 | 6.60E+10 | $10^{-8}$ | 39 | 41 | 39 | 40 | 7.90E+10 |
| 18 | $10^{-7}$ | 100 | 99 | 93 | 97 | 2.00E+10 | $10^{-8}$ | 19 | 17 | 21 | 19 | 3.80E+10 |
| 22 | $10^{-7}$ | 126 | 124 | 134 | 128 | 2.60E+10 | $10^{-8}$ | 21 | 23 | 28 | 24 | 4.80E+10 |
| 25 | $10^{-8}$ | 55 | 54 | 61 | 57 | 1.10E+11 | $10^{-8}$ | 59 | 68 | 62 | 63 | 1.30E+10 |
| 30 | $10^{-7}$ | 90 | 84 | 79 | 84 | 1.70E+10 | $10^{-8}$ | 88 | 93 | 120 | 100 | 2.00E+11 |

[1] Additional dilutions were performed ($10^{-6}$ to $10^{-9}$) and results are recorded in the raw data.

## DOSE SOLUTION HOMOGENEITY

| Day Collected | Dose Group | Strata | CFU in doing solutions for Strain 3630 | | | | | CFU in doing solutions for Strain 3632 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Plates ($10^{-7}$ Dilution)[1] | | | Average | CFU\mL | Plates ($10^{-7}$ Dilution)[1] | | | Average | CFU\mL |
| | | | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| 1 | 2 | Top | 1 | 1 | 4 | 2.00 | 2.00E+08 | 8 | 9 | 4 | 7.00 | 1.80E+09 |
| | | Middle | 6 | 5 | 5 | 5.33 | 5.33E+08 | 8 | 10 | 9 | 9.00 | 9.00E+08 |
| | | Bottom | 0 | 2 | 1 | 1.00 | 1.00E+08 | 7 | 4 | 5 | 5.33 | 5.33E+08 |
| | | | | | | Average CFU/mL | 2.78E+08 | | | | Average CFU/mL | 1.08E+08 |
| | 3 | Top | 8 | 7 | 7 | 7.33 | 7.30E+08 | 20 | 15 | 12 | 15.67 | 1.57E+09 |
| | | Middle | 4 | 7 | 8 | 6.33 | 6.33E+08 | 18 | 15 | 13 | 15.33 | 1.53E+09 |
| | | Bottom | 9 | 4 | 7 | 6.67 | 6.67E+08 | 17 | 20 | 13 | 16.67 | 1.67E+09 |
| | | | | | | Average CFU/mL | 6.77E+08 | | | | Average CFU/mL | 1.59E+09 |
| | 4 | Top | 10 | 14 | 19 | 14.33 | 1.43E+09 | 40 | 46 | 55 | 47.00 | 4.70E+09 |
| | | Middle | 12 | 10 | 8 | 10.00 | 1.00E+09 | 50 | 52 | 39 | 47.00 | 4.70E+09 |
| | | Bottom | 12 | 10 | 8 | 10.00 | 1.00E+09 | 56 | 40 | 57 | 51.00 | 5.10E+09 |
| | | | | | | Average CFU/mL | 1.14E+09 | | | | Average CFU/mL | 4.83E+09 |

[1] Additional dilutions were performed ($10^{-6}$ to $10^{-9}$) and results are recorded in the raw data.

## DOSE SOLUTION CONCENTRATION VERIFICATION

| Day Collected | Dose Group | Plated Dilution Used[1] | CFU in doing solutions for Strain 3630 | | | | | Plated Dilution Used | CFU in doing solutions for Strain 3632 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Plates | | | Average | CFU\mL | | Plates | | | Average | CFU\mL |
| | | | 1 | 2 | 3 | | | | 1 | 2 | 3 | | |
| 9 | 2 | $10^{-6}$ | 31 | 25 | 38 | 31 | 3.1E+08 | $10^{-6}$ | 99 | 93 | 92 | 95 | 9.5E+08 |
| | 3 | $10^{-6}$ | 49 | 40 | 46 | 45 | 4.5E+08 | $10^{-6}$ | 101 | 97 | 106 | 101 | 1.0E+09 |
| | 4 | $10^{-7}$ | 38 | 31 | 39 | 36.0 | 3.6E+09 | $10^{-7}$ | 88 | 90 | 94 | 90.7 | 9.1E+09 |
| 15 | 2 | $10^{-6}$ | 43 | 45 | 48 | 45.3 | 4.5E10 | $10^{-6}$ | 87 | 96 | 90 | 91.0 | 9.1E+10 |
| | 3 | $10^{-6}$ | 48 | 60 | 55 | 54.3 | 5.4E+10 | $10^{-6}$ | 110 | 107 | 105 | 107.3 | 1.1E+11 |
| | 4 | $10^{-6}$ | 54 | 65 | 53 | 57.3 | 5.7E+10 | $10^{-7}$ | 87 | 91 | 85 | 87.7 | 8.8E+11 |
| 18 | 2[2] | | | | | | | $10^{-7}$ | 6 | 8 | 6 | 6.7 | 6.6E+08 |
| | 3 | $10^{-7}$ | 3 | 4 | 7 | 4.7 | 4.7E+08 | $10^{-7}$ | 15 | 14 | 15 | 14.7 | 1.5E+09 |
| | 4 | $10^{-7}$ | 12 | 14 | 12 | 12.7 | 1.3E+09 | $10^{-7}$ | 30 | 44 | 37 | 37.0 | 3.7E+09 |
| 22 | 2 | $10^{-6}$ | 32 | 32 | 35 | 33 | 3.3E+08 | $10^{-6}$ | 100 | 97 | 103 | 100 | 1.0E+09 |
| | 3 | $10^{-6}$ | 45 | 53 | 46 | 48 | 4.8E+08 | $10^{-7}$ | 30 | 36 | 34 | 33.3 | 3.3E+09 |
| | 4 | $10^{-7}$ | 25 | 32 | 26 | 27.7 | 2.8E+09 | $10^{-7}$ | 68 | 71 | 76 | 71.7 | 7.2E+09 |
| 25 | 2 | $10^{-6}$ | 17 | 16 | 18 | 17 | 1.7E+08 | $10^{-6}$ | 42 | 49 | 50 | 47 | 4.7E+08 |
| | 3 | $10^{-6}$ | 42 | 41 | 44 | 42 | 4.2E+08 | $10^{-6}$ | 113 | 114 | 119 | 115 | 1.2E+09 |
| | 4 | $10^{-7}$ | 8 | 13 | 13 | 11.3 | 1.1E+09 | $10^{-7}$ | 47 | 48 | 39 | 44.7 | 4.5E+09 |
| 30 | 2 | $10^{-6}$ | 28 | 19 | 25 | 24 | 2.4E+08 | $10^{-6}$ | 52 | 64 | 56 | 57 | 5.7E+08 |
| | 3 | $10^{-6}$ | 34 | 32 | 31 | 32 | 3.2E+08 | $10^{-6}$ | 91 | 115 | 120 | 109 | 1.1E+09 |
| | 4 | $10^{-7}$ | 29 | 35 | 33 | 32.3 | 3.2E+09 | $10^{-7}$ | 59 | 51 | 55 | 55.0 | 5.5E+09 |

[1] Additional dilutions were performed ($10^{-6}$ to $10^{-9}$) and results are recorded in the raw data.

[2] Strain 3630 colonies failed to thrive; therefore, results could not be tabulated.

# APPENDIX E: INDIVIDUAL ANIMAL IN-LIFE CLINICAL OBSERVATIONS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 0 CFU<br>Group 1<br>Sex: Male | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3801 | Normal | 1 to 30 |
| 3802 | Normal | 1 to 30 |
| 3803 | Normal | 1 to 28 |
| | Hypersalivation, Slight | 29 to 30 |
| 3804 | Normal | 1 to 30 |
| 3805 | Normal | 1 to 28 |
| | Hypersalivation, Slight | 29 to 30 |
| | Aggressive, Slight | 29 to 30 |
| 3806 | Normal | 1 to 31 |
| 3807 | Normal | 1 to 31 |
| 3808 | Normal | 1 to 31 |
| 3809 | Normal | 1 to 28,31 |
| | Aggressive, Slight | 29 to 30 |
| 3810 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 1.668E+10<br>CFU<br>Group 2<br>Sex: Male | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3821 | Normal | 1 to 30 |
| 3822 | Normal | 1 to 30 |
| 3823 | Normal | 1 to 28,30 |
| | Hypersalivation, Moderate | 29 |
| 3824 | Normal | 1 to 30 |
| 3825 | Normal | 1 to 28,30 to 31 |
| | Hypersalivation, Slight | 29 |
| 3826 | Normal | 1 to 28,31 |
| | Hypersalivation, Moderate | 29 to 30 |
| 3827 | Normal | 1 to 28,31 |
| | Hypersalivation, Slight | 29 to 30 |
| 3828 | Normal | 1 to 28,30 to 31 |
| | Hypersalivation, Moderate | 29 |
| | Hyperactivity, Slight | 29 |
| 3829 | Normal | 1 to 3,15 to 16 |
| | Eschar, Head, Superficial | 4 to 14 |
| | Piloerection, Slight | 17 to 18 |
| | Irregular Respiration | 17 to 18 |
| | Humane Sacrifice | 19 |
| 3830 | Normal | 1 to 3,15 to 28,30 to 31 |
| | Eschar, Neck, Superficial | 4 to 14 |
| | Hypersalivation, Slight | 29 |
| | Hyperactivity, Slight | 29 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 3.337E+10<br>CFU<br>Group 3<br>Sex: Male | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3841 | Normal | 1 to 30 |
| 3842 | Normal | 1 to 30 |
| 3843 | Normal | 1 to 30 |
| 3844 | Normal | 1 to 30 |
| 3845 | Normal | 1 to 30 |
| 3846 | Normal | 1 to 31 |
| 3847 | Normal | 1 to 31 |
| 3848 | Normal | 1 to 31 |
| 3849 | Normal | 1 to 31 |
| 3850 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 1.001E+11<br>CFU<br>Group 4<br>Sex: Male | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3861 | Normal | 1 to 30 |
| 3862 | Normal | 1 to 30 |
| 3863 | Normal | 1 to 30 |
| 3864 | Normal | 1 to 30 |
| 3865 | Normal | 1 to 30 |
| 3866 | Normal | 1 to 31 |
| 3867 | Normal | 1 to 31 |
| 3868 | Normal | 1 to 31 |
| 3869 | Normal | 1 to 31 |
| 3870 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 0 CFU<br>Group 1<br>Sex: Female | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3811 | Normal | 1 to 30 |
| 3812 | Normal | 1 to 28,30 |
| | Aggressive, Slight | 29 |
| | Hyperactivity, Slight | 29 |
| 3813 | Normal | 1 to 28,30 |
| | Hyperactivity, Moderate | 29 |
| 3814 | Normal | 1 to 28,30 |
| | Hyperactivity, Moderate | 29 |
| 3815 | Normal | 1 to 30 |
| 3816 | Normal | 1 to 28,31 |
| | Hyperactivity, Slight | 29 to 30 |
| 3817 | Normal | 1 to 31 |
| 3818 | Normal | 1 to 31 |
| 3819 | Normal | 1 to 28,30 to 31 |
| | Hypersalivation, Slight | 29 |
| | Hypoactivity, Slight | 29 |
| 3820 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 1.668E+10<br>CFU<br>Group 2<br>Sex: Female | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3831 | Normal | 1 to 30 |
| 3832 | Normal | 1 to 30 |
| 3833 | Normal | 1 to 30 |
| 3834 | Normal | 1 to 30 |
| 3835 | Normal | 1 to 30 |
| 3836 | Normal | 1 to 31 |
| 3837 | Normal | 1 to 31 |
| 3838 | Normal | 1 to 31 |
| 3839 | Normal | 1 to 31 |
| 3840 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 3.337E+10<br>CFU<br>Group 3<br>Sex: Female | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3851 | Normal | 1 to 30 |
| 3852 | Normal | 1 to 30 |
| 3853 | Normal | 1 to 30 |
| 3854 | Normal | 1 to 30 |
| 3855 | Normal | 1 to 30 |
| 3856 | Normal | 1 to 31 |
| 3857 | Normal | 1 to 31 |
| 3858 | Normal | 1 to 31 |
| 3859 | Normal | 1 to 31 |
| 3860 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal In-Life Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

| 1.001E+11<br>CFU<br>Group 4<br>Sex: Female | Observation Type: All Types | From Day 1 (Start Date) to 31 (Start Date) |
|---|---|---|
| 3871 | Normal | 1 to 30 |
| 3872 | Normal | 1 to 30 |
| 3873 | Normal | 1 to 30 |
| 3874 | Normal | 1 to 30 |
| 3875 | Normal | 1 to 30 |
| 3876 | Normal | 1 to 31 |
| 3877 | Normal | 1 to 31 |
| 3878 | Normal | 1 to 31 |
| 3879 | Normal | 1 to 31 |
| 3880 | Normal | 1 to 31 |

Values=Clin Obs Range

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

## APPENDIX F: DETAILED CLINICAL OBSERVATIONS METHODS SCORING KEY

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

| **Removal from Cage/Hand-held Observations** | |
|---|---|
| Handling Reactivity | 0. Slight/moderate resistance – animal is easy to handle, may squirm or vocalize occasionally<br>1. No resistance – animal is flaccid when being handled<br>2. High resistance – animal is difficult to handle, and/or squirms continuously<br>3. Aggressive – biting or lunging behavior specifically directed at handler |
| Emaciation | 0. Absent<br>1. Present (confirmed using body weights) |
| Eyes | 0. Normal<br>1. Exophthalmos – abnormal protrusion of eyeball present<br>2. Enophthalmus – posterior displacement of the eye (sunken eyeball)<br>3. Eye lesion – mechanical damage or other (e.g., orbital bleeding) |
| Fur/Skin Appearance | 0. Normal<br>1. Unkempt – coat rough or ungroomed, may be slightly stained<br>2. Stained/wetness (e.g., ano-genital staining)<br>3. Hair loss<br>4. Other – includes but is not limited; eschar, wound, laceration or other skin lesions |
| Lacrimation | 0. Absent<br>1. Present – lacrimation noticeable<br>2. Excessive – animal has excessive amount of tearing |
| Mucous Membranes (color) | 0. Normal<br>1. Blanch to pink tone<br>2. Dusky rose to deep flush<br>3. Cyanosis (blue)<br>4. Excessive or abnormal secretion |
| Muscle Tone | 0. Normal – muscles are resilient and firm and the hind legs go through their full range of motion<br>1. Increased – muscles are rigid; hind limbs will not go through their full range of motion<br>2. Decreased – muscles are flaccid; hind limbs have little or no resistance to movement |
| Palpebral Closure | 0. Eyes wide open<br>1. Eyes halfway shut<br>2. Eyes completely shut |
| Piloerection | 0. Absent<br>1. Present |
| Respiratory Pattern | 0. Normal<br>1. Slow<br>2. Rapid<br>3. Rales (Moist or Dry)<br>4. Gasping<br>5. Labored - Dyspnea |
| Salivation | 0. None<br>1. Present - salivation is noticeable around the edge of the mouth<br>2. Excessive - salivation extends to the fur around the jaw |
| Vocalization | 0. Absent<br>1. Present - animal vocalizes unprovoked or continuously vocalizes when being handled. |

| **Open Field Observations** | |
|---|---|
| Activity/Arousal | 0. Alternating behaviors – animal goes through normal repertoire of behaviors during observation period; these consist of exploring, sniffing, grooming, rearing, etc.<br>1. Inactive/Alert – animal sits in one place during the observation period but appears to be aware of its surroundings. It may go through its normal repertoire of activities but the majority of the observation period is spent not moving.<br>2. Hypoactive/Not alert – animal sits in one place during the observation period; animal appears to be unaware of its surroundings or in a stupor.<br>3. Hyperactive/Hyperalert – animal appears excited; animal may dart and freeze during the observation period or animal may sit in one place and jump at any sound or movement. |
| Convulsions | 0. None<br>1. Clonic – alternating periods of contraction and relaxation of muscles<br>2. Tonic – prolonged period of muscle contractions |
| Defecation | 0. None/Normal<br>1. Soft (partially formed)<br>2. Diarrhea (watery feces usually of increased volume) |
| Gait | 0. Normal<br>1. Ataxic Gait – inability of truncal, pelvic and limb muscles to move in unison so animal is not able to move in straight line (lurch).<br>2. Hypotonic gait – impaired gait (limp) due to limb weakness or paralysis in which the animal is unable to support is weight but can move forward in a straight line without lurching.<br>3. Impaired Gait – includes steppage (due to dorsiflexion of foot or toe the animal drags its forelimbs, walks on its knuckles or lifts its forelimbs unusually high to avoid dragging its toes over the ground); spastic (shuffling gait with legs rigidly extended and not lifted during movement; waddling (lateral wobbling of the pelvis); dysmetric (incoordinating movement with a coarse tremor due to overshooting goal).<br>4. Total gait incapacity – applies when these are severe gait abnormalities or combinations of gait abnormality. |
| Locomotion (speed and vigor of movement) | 0. Normal<br>1. Somewhat impaired<br>2. Totally impaired |
| Other | 0. Absent<br>1. Present<br>NOTE: When present, a comment will identify finding |
| Posture | 0. Normal (awake) – e.g., alert, sitting, standing, or rearing or Normal (sleeping) – e.g. curled up, usually with head down<br>1. Hunched – e.g., abnormal posture<br>2. Flattened (prone) –e.g., limbs spread out lying flat or on one side |
| Tremors | 0. None<br>1. Slight – e.g., localized involuntary oscillatory movement<br>2. Severe – e.g., more to more than one area or involving whole body |
| Twitches | 0. None<br>1. Slight – brief coarse involuntary muscle contraction<br>2. Moderate – increased frequency and severity<br>3. Fasciculation – wave-like ripples of a muscle or group of muscles |
| Unusual Behaviors | 0. Absent<br>1. Present – Stereotypies/Bizarre behavior/Aggression be specific in describing all unusual behaviors on data sheet |
| Urination | 0. None/Normal<br>1. Excessive |
| Vocalizations | 0. Absent<br>1. Present |

# APPENDIX G: INDIVIDUAL ANIMAL DETAILED CLINICAL OBSERVATIONS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3801 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3803 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3804 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3805 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3806 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3807 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3809 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3810 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | 0 | . | . | 0 | 4 [1] | 0 | . | . | 0 | 0 | 0 | . | . | 0 |
| 3830 | 0 | 0 | 0 | 0 | 4 [1] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[1] [RC:has eschar from fighting]

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3821 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3822 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3823 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3824 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3825 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3826 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3828 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3829 | 0 | . | . | 0 | 0 | 0 | . | . | 0 | 0 | 0 | . | . |
| 3830 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3841 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3842 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3843 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3844 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3847 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3848 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3849 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3850 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3861 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3862 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3863 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3864 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3865 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3866 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3867 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3868 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3869 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3870 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 0 CFU Group 1 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3811 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3814 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3815 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3816 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3817 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3818 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3819 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3820 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.668E+10 CFU Group 2 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3831 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3832 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3835 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3836 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3837 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3838 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3839 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 3.337E+10 CFU Group 3 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3851 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3854 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3855 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3856 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3857 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3858 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3859 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3860 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Handling Reactivity | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Vocalization (RC) | Palpebral Closure | Palpebral Closure | Palpebral Closure | Palpebral Closure |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palpebral Closure | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Lacrimation | Eyes | Eyes | Eyes | Eyes | Eyes | Mucous Membranes | Mucous Membranes | Mucous Membranes |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mucous Membranes | Mucous Membranes | Salivation | Salivation | Salivation | Salivation | Salivation | Emaciation | Emaciation | Emaciation | Emaciation | Emaciation | Piloerection | Piloerection |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Piloerection | Piloerection | Piloerection | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Fur/Skin | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Muscle Tone | Respiratory Pattern |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Removal from Cage) | | | | | | | | | DetClinObs (Open Field Obs) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Respiratory Pattern | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Pupillary Reflex | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal | Activity/ Arousal |
| | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Convulsions | Convulsions | Convulsions | Convulsions | Convulsions | Tremors | Tremors | Tremors | Tremors | Tremors | Posture | Posture | Posture | Posture |
| | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Posture | Gait | Gait | Gait | Gait | Gait | Locomotion | Locomotion | Locomotion | Locomotion | Locomotion | Defecation | Defecation | Defecation |
| | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Defecation | Defecation | Urination | Urination | Urination | Urination | Urination | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Unusual Behaviors | Vocalization (OF) | Vocalization (OF) |
| | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Detailed Clinical Observations
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Day(s) Relative to Start Date

| 1.001E+11 CFU Group 4 | DetClinObs (Open Field Obs) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vocalization (OF) | Vocalization (OF) | Vocalization (OF) | Other | Other | Other | Other | Other | Twitches | Twitches | Twitches | Twitches | Twitches |
| | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 | 1 | 8 | 15 | 22 | 29 |
| 3871 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3872 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3874 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3875 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3876 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3877 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3878 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3879 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

# APPENDIX H: INDIVIDUAL ANIMAL BODY WEIGHTS

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3801 | 340 | 352 | 379 | 388 | 402 | 414 | 433 |
| 3802 | 336 | 321 | 345 | 356 | 382 | 399 | 414 |
| 3803 | 344 | 364 | 392 | 406 | 433 | 456 | 469 |
| 3804 | 361 | 382 | 405 | 420 | 446 | 456 | 479 |
| 3805 | 349 | 364 | 390 | 404 | 423 | 440 | 458 |
| 3806 | 326 | 336 | 352 | 354 | 366 | 374 | 386 |
| 3807 | 365 | 375 | 398 | 411 | 432 | 445 | 459 |
| 3808 | 324 | 327 | 345 | 355 | 380 | 385 | 405 |
| 3809 | 363 | 384 | 413 | 424 | 449 | 464 | 490 |
| 3810 | 312 | 320 | 346 | 357 | 373 | 380 | 400 |
| Mean | 342.0 | 352.5 | 376.5 | 387.5 | 408.6 | 421.3 | 439.3 |
| SD | 18.0 | 25.0 | 27.0 | 29.2 | 31.7 | 34.9 | 36.5 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 0 CFU Group 1 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3801 | 441 | 456 |
| 3802 | 426 | 440 |
| 3803 | 473 | 493 |
| 3804 | 485 | 512 |
| 3805 | 470 | 490 |
| 3806 | 399 | 410 |
| 3807 | 472 | 486 |
| 3808 | 415 | 430 |
| 3809 | 506 | 521 |
| 3810 | 413 | 428 |
| Mean | 450.0 | 466.6 |
| SD | 35.9 | 38.7 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3821 | 343 | 346 | 373 | 393 | 411 | 433 | 467 |
| 3822 | 337 | 343 | 358 | 361 | 374 | 384 | 399 |
| 3823 | 316 | 320 | 344 | 357 | 381 | 391 | 410 |
| 3824 | 325 | 330 | 337 | 346 | 359 | 361 | 376 |
| 3825 | 347 | 356 | 383 | 394 | 418 | 427 | 446 |
| 3826 | 365 | 380 | 406 | 430 | 454 | 467 | 486 |
| 3827 | 364 | 371 | 390 | 406 | 419 | 435 | 445 |
| 3828 | 352 | 368 | 398 | 413 | 439 | 451 | 467 |
| 3829 | 321 | 323 | 338 | 348 | 352 | 313 | - |
| 3830 | 354 | 304 | 335 | 349 | 369 | 382 | 407 |
| Mean | 342.4 | 344.1 | 366.2 | 379.7 | 397.6 | 404.4 | 433.7 |
| SD | 17.4 | 24.9 | 27.2 | 31.0 | 35.2 | 46.8 | 37.1 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 9 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3821 | 472 | 481 |
| 3822 | 409 | 422 |
| 3823 | 422 | 436 |
| 3824 | 387 | 399 |
| 3825 | 451 | 467 |
| 3826 | 504 | 509 |
| 3827 | 463 | 476 |
| 3828 | 478 | 488 |
| 3829 | - | - |
| 3830 | 422 | 443 |
| Mean | 445.3 | 457.9 |
| SD | 37.7 | 35.2 |
| N | 9 | 9 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3841 | 320 | 377 | 341 | 352 | 369 | 380 | 396 |
| 3842 | 362 | 326 | 400 | 419 | 445 | 458 | 479 |
| 3843 | 349 | 358 | 376 | 394 | 410 | 424 | 443 |
| 3844 | 325 | 337 | 354 | 368 | 390 | 392 | 405 |
| 3845 | 343 | 354 | 372 | 392 | 411 | 429 | 434 |
| 3846 | 365 | 383 | 409 | 422 | 447 | 455 | 473 |
| 3847 | 316 | 335 | 359 | 372 | 379 | 386 | 400 |
| 3848 | 360 | 373 | 403 | 422 | 449 | 461 | 485 |
| 3849 | 330 | 340 | 361 | 377 | 380 | 392 | 415 |
| 3850 | 353 | 369 | 389 | 407 | 427 | 445 | 475 |
| Mean | 342.3 | 355.2 | 376.4 | 392.5 | 410.7 | 422.2 | 440.5 |
| SD | 18.3 | 20.0 | 23.1 | 24.8 | 30.4 | 32.2 | 35.4 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3841 | 405 | 422 |
| 3842 | 492 | 508 |
| 3843 | 458 | 468 |
| 3844 | 423 | 430 |
| 3845 | 457 | 471 |
| 3846 | 488 | 508 |
| 3847 | 409 | 418 |
| 3848 | 497 | 513 |
| 3849 | 425 | 435 |
| 3850 | 492 | 513 |
| Mean | 454.6 | 468.6 |
| SD | 36.7 | 40.0 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3861 | 362 | 378 | 404 | 410 | 431 | 448 | 468 |
| 3862 | 316 | 330 | 345 | 357 | 365 | 374 | 388 |
| 3863 | 328 | 340 | 354 | 367 | 380 | 390 | 405 |
| 3864 | 338 | 352 | 377 | 385 | 402 | 414 | 435 |
| 3865 | 352 | 366 | 401 | 417 | 442 | 460 | 482 |
| 3866 | 360 | 382 | 410 | 410 | 430 | 441 | 465 |
| 3867 | 370 | 374 | 401 | 424 | 431 | 455 | 478 |
| 3868 | 332 | 359 | 386 | 397 | 416 | 425 | 449 |
| 3869 | 324 | 329 | 346 | 363 | 371 | 392 | 414 |
| 3870 | 346 | 367 | 399 | 416 | 435 | 458 | 482 |
| Mean | 342.8 | 357.7 | 382.3 | 394.6 | 410.3 | 425.7 | 446.6 |
| SD | 18.0 | 19.3 | 25.3 | 24.9 | 28.8 | 31.7 | 34.4 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Bodyweight (g)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3861 | 480 | 499 |
| 3862 | 392 | 404 |
| 3863 | 415 | 430 |
| 3864 | 446 | 456 |
| 3865 | 498 | 518 |
| 3866 | 469 | 484 |
| 3867 | 488 | 502 |
| 3868 | 460 | 468 |
| 3869 | 423 | 438 |
| 3870 | 503 | 524 |
| Mean | 457.4 | 472.3 |
| SD | 37.6 | 40.0 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3811 | 253 | 248 | 258 | 252 | 261 | 269 | 280 |
| 3812 | 236 | 233 | 234 | 250 | 261 | 250 | 257 |
| 3813 | 216 | 226 | 233 | 232 | 242 | 245 | 257 |
| 3814 | 225 | 234 | 237 | 248 | 251 | 256 | 267 |
| 3815 | 235 | 251 | 263 | 268 | 275 | 279 | 284 |
| 3816 | 203 | 206 | 211 | 212 | 219 | 206 | 209 |
| 3817 | 230 | 239 | 244 | 242 | 247 | 252 | 260 |
| 3818 | 231 | 241 | 251 | 255 | 273 | 269 | 278 |
| 3819 | 229 | 225 | 231 | 237 | 246 | 250 | 259 |
| 3820 | 242 | 249 | 256 | 253 | 266 | 267 | 275 |
| Mean | 230.0 | 235.2 | 241.8 | 244.9 | 254.1 | 254.3 | 262.6 |
| SD | 13.7 | 13.7 | 15.7 | 15.3 | 16.8 | 20.2 | 21.4 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3811 | 292 | 295 |
| 3812 | 269 | 279 |
| 3813 | 256 | 269 |
| 3814 | 263 | 263 |
| 3815 | 285 | 292 |
| 3816 | 221 | 233 |
| 3817 | 268 | 276 |
| 3818 | 276 | 288 |
| 3819 | 258 | 265 |
| 3820 | 282 | 291 |
| Mean | 267.0 | 275.1 |
| SD | 20.0 | 18.8 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3831 | 228 | 237 | 239 | 247 | 251 | 250 | 258 |
| 3832 | 244 | 256 | 264 | 268 | 279 | 278 | 283 |
| 3833 | 233 | 237 | 247 | 253 | 270 | 273 | 282 |
| 3834 | 230 | 233 | 243 | 248 | 257 | 255 | 260 |
| 3835 | 217 | 223 | 228 | 233 | 243 | 239 | 245 |
| 3836 | 229 | 233 | 240 | 237 | 249 | 250 | 254 |
| 3837 | 238 | 239 | 251 | 257 | 269 | 275 | 282 |
| 3838 | 234 | 237 | 245 | 248 | 260 | 262 | 269 |
| 3839 | 251 | 249 | 256 | 252 | 268 | 281 | 298 |
| 3840 | 213 | 221 | 223 | 226 | 241 | 248 | 254 |
| Mean | 231.7 | 236.5 | 243.6 | 246.9 | 258.7 | 261.1 | 268.5 |
| SD | 11.4 | 10.5 | 12.2 | 12.2 | 12.7 | 14.8 | 17.0 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3831 | 266 | 272 |
| 3832 | 281 | 280 |
| 3833 | 288 | 293 |
| 3834 | 269 | 272 |
| 3835 | 248 | 256 |
| 3836 | 260 | 267 |
| 3837 | 274 | 278 |
| 3838 | 275 | 283 |
| 3839 | 299 | 300 |
| 3840 | 250 | 255 |
| Mean | 271.0 | 275.6 |
| SD | 16.0 | 14.5 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3851 | 238 | 239 | 256 | 260 | 270 | 278 | 286 |
| 3852 | 222 | 233 | 240 | 243 | 253 | 253 | 260 |
| 3853 | 218 | 221 | 233 | 241 | 251 | 254 | 264 |
| 3854 | 234 | 237 | 253 | 245 | 254 | 254 | 256 |
| 3855 | 251 | 254 | 264 | 265 | 271 | 267 | 271 |
| 3856 | 248 | 248 | 259 | 263 | 268 | 266 | 272 |
| 3857 | 212 | 225 | 233 | 252 | 257 | 261 | 265 |
| 3858 | 230 | 240 | 249 | 255 | 252 | 248 | 249 |
| 3859 | 232 | 245 | 248 | 244 | 265 | 259 | 265 |
| 3860 | 230 | 231 | 231 | 254 | 258 | 264 | 276 |
| Mean | 231.5 | 237.3 | 246.6 | 252.2 | 259.9 | 260.4 | 266.4 |
| SD | 12.3 | 10.2 | 11.8 | 8.7 | 7.8 | 8.8 | 10.5 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3851 | 294 | 302 |
| 3852 | 272 | 278 |
| 3853 | 257 | 265 |
| 3854 | 261 | 269 |
| 3855 | 282 | 286 |
| 3856 | 280 | 288 |
| 3857 | 255 | 261 |
| 3858 | 257 | 256 |
| 3859 | 272 | 286 |
| 3860 | 280 | 278 |
| Mean | 271.0 | 276.9 |
| SD | 13.2 | 14.2 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 4 | 8 | 11 | 15 | 18 | 22 |
| 3871 | 214 | 217 | 223 | 223 | 232 | 232 | 238 |
| 3872 | 227 | 232 | 238 | 238 | 247 | 255 | 264 |
| 3873 | 205 | 200 | 198 | 208 | 214 | 219 | 228 |
| 3874 | 231 | 237 | 248 | 238 | 251 | 260 | 270 |
| 3875 | 240 | 236 | 246 | 254 | 261 | 259 | 278 |
| 3876 | 249 | 245 | 265 | 267 | 290 | 299 | 301 |
| 3877 | 257 | 261 | 268 | 272 | 283 | 284 | 294 |
| 3878 | 230 | 236 | 238 | 240 | 238 | 242 | 248 |
| 3879 | 236 | 240 | 243 | 246 | 255 | 250 | 255 |
| 3880 | 229 | 235 | 244 | 246 | 256 | 245 | 251 |
| Mean | 231.8 | 233.9 | 241.1 | 243.2 | 252.7 | 254.5 | 262.7 |
| SD | 15.2 | 16.2 | 20.0 | 19.0 | 22.5 | 23.4 | 23.5 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Mean Daily Body Weights
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Bodyweight (g)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | |
|---|---|---|
| | 25 | 29 |
| 3871 | 245 | 252 |
| 3872 | 267 | 270 |
| 3873 | 230 | 229 |
| 3874 | 277 | 283 |
| 3875 | 269 | 281 |
| 3876 | 298 | 310 |
| 3877 | 309 | 320 |
| 3878 | 256 | 255 |
| 3879 | 262 | 267 |
| 3880 | 262 | 267 |
| Mean | 267.5 | 273.4 |
| SD | 23.2 | 26.9 |
| N | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

# APPENDIX I: INDIVIDUAL ANIMAL MEAN DAILY BODY WEIGHT GAIN

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3801 | 4.0 | 6.8 | 3.0 | 3.5 | 4.0 | 4.8 | 2.7 |
| 3802 | -5.0 | 6.0 | 3.7 | 6.5 | 5.7 | 3.8 | 4.0 |
| 3803 | 6.7 | 7.0 | 4.7 | 6.8 | 7.7 | 3.3 | 1.3 |
| 3804 | 7.0 | 5.8 | 5.0 | 6.5 | 3.3 | 5.8 | 2.0 |
| 3805 | 5.0 | 6.5 | 4.7 | 4.8 | 5.7 | 4.5 | 4.0 |
| 3806 | 3.3 | 4.0 | 0.7 | 3.0 | 2.7 | 3.0 | 4.3 |
| 3807 | 3.3 | 5.8 | 4.3 | 5.3 | 4.3 | 3.5 | 4.3 |
| 3808 | 1.0 | 4.5 | 3.3 | 6.3 | 1.7 | 5.0 | 3.3 |
| 3809 | 7.0 | 7.3 | 3.7 | 6.3 | 5.0 | 6.5 | 5.3 |
| 3810 | 2.7 | 6.5 | 3.7 | 4.0 | 2.3 | 5.0 | 4.3 |
| Mean | 3.50 | 6.00 | 3.67 | 5.28 | 4.23 | 4.50 | 3.57 |
| SD | 3.59 | 1.05 | 1.24 | 1.39 | 1.83 | 1.13 | 1.23 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date<br>25 → 29 |
|---|---|
| 3801 | 3.8 |
| 3802 | 3.5 |
| 3803 | 5.0 |
| 3804 | 6.8 |
| 3805 | 5.0 |
| 3806 | 2.8 |
| 3807 | 3.5 |
| 3808 | 3.8 |
| 3809 | 3.8 |
| 3810 | 3.8 |
| Mean | 4.15 |
| SD | 1.14 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3821 | 1.0 | 6.8 | 6.7 | 4.5 | 7.3 | 8.5 | 1.7 |
| 3822 | 2.0 | 3.8 | 1.0 | 3.3 | 3.3 | 3.8 | 3.3 |
| 3823 | 1.3 | 6.0 | 4.3 | 6.0 | 3.3 | 4.8 | 4.0 |
| 3824 | 1.7 | 1.8 | 3.0 | 3.3 | 0.7 | 3.8 | 3.7 |
| 3825 | 3.0 | 6.8 | 3.7 | 6.0 | 3.0 | 4.8 | 1.7 |
| 3826 | 5.0 | 6.5 | 8.0 | 6.0 | 4.3 | 4.8 | 6.0 |
| 3827 | 2.3 | 4.8 | 5.3 | 3.3 | 5.3 | 2.5 | 6.0 |
| 3828 | 5.3 | 7.5 | 5.0 | 6.5 | 4.0 | 4.0 | 3.7 |
| 3829 | 0.7 | 3.8 | 3.3 | 1.0 | -13.0 | . | . |
| 3830 | -16.7 | 7.8 | 4.7 | 5.0 | 4.3 | 6.3 | 5.0 |
| Mean | 0.57 | 5.53 | 4.50 | 4.48 | 2.27 | 4.78 | 3.89 |
| SD | 6.26 | 1.95 | 1.96 | 1.76 | 5.63 | 1.73 | 1.60 |
| N | 10 | 10 | 10 | 10 | 10 | 9 | 9 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3821 | 2.3 |
| 3822 | 3.3 |
| 3823 | 3.5 |
| 3824 | 3.0 |
| 3825 | 4.0 |
| 3826 | 1.3 |
| 3827 | 3.3 |
| 3828 | 2.5 |
| 3829 | . |
| 3830 | 5.3 |
| Mean | 3.14 |
| SD | 1.13 |
| N | 9 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3841 | 19.0 | -9.0 | 3.7 | 4.3 | 3.7 | 4.0 | 3.0 |
| 3842 | -12.0 | 18.5 | 6.3 | 6.5 | 4.3 | 5.3 | 4.3 |
| 3843 | 3.0 | 4.5 | 6.0 | 4.0 | 4.7 | 4.8 | 5.0 |
| 3844 | 4.0 | 4.3 | 4.7 | 5.5 | 0.7 | 3.3 | 6.0 |
| 3845 | 3.7 | 4.5 | 6.7 | 4.8 | 6.0 | 1.3 | 7.7 |
| 3846 | 6.0 | 6.5 | 4.3 | 6.3 | 2.7 | 4.5 | 5.0 |
| 3847 | 6.3 | 6.0 | 4.3 | 1.8 | 2.3 | 3.5 | 3.0 |
| 3848 | 4.3 | 7.5 | 6.3 | 6.8 | 4.0 | 6.0 | 4.0 |
| 3849 | 3.3 | 5.3 | 5.3 | 0.8 | 4.0 | 5.8 | 3.3 |
| 3850 | 5.3 | 5.0 | 6.0 | 5.0 | 6.0 | 7.5 | 5.7 |
| Mean | 4.30 | 5.30 | 5.37 | 4.55 | 3.83 | 4.58 | 4.70 |
| SD | 7.40 | 6.57 | 1.05 | 1.98 | 1.63 | 1.72 | 1.49 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3841 | 4.3 |
| 3842 | 4.0 |
| 3843 | 2.5 |
| 3844 | 1.8 |
| 3845 | 3.5 |
| 3846 | 5.0 |
| 3847 | 2.3 |
| 3848 | 4.0 |
| 3849 | 2.5 |
| 3850 | 5.3 |
| Mean | 3.50 |
| SD | 1.20 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3861 | 5.3 | 6.5 | 2.0 | 5.3 | 5.7 | 5.0 | 4.0 |
| 3862 | 4.7 | 3.8 | 4.0 | 2.0 | 3.0 | 3.5 | 1.3 |
| 3863 | 4.0 | 3.5 | 4.3 | 3.3 | 3.3 | 3.8 | 3.3 |
| 3864 | 4.7 | 6.3 | 2.7 | 4.3 | 4.0 | 5.3 | 3.7 |
| 3865 | 4.7 | 8.8 | 5.3 | 6.3 | 6.0 | 5.5 | 5.3 |
| 3866 | 7.3 | 7.0 | 0.0 | 5.0 | 3.7 | 6.0 | 1.3 |
| 3867 | 1.3 | 6.8 | 7.7 | 1.8 | 8.0 | 5.8 | 3.3 |
| 3868 | 9.0 | 6.8 | 3.7 | 4.8 | 3.0 | 6.0 | 3.7 |
| 3869 | 1.7 | 4.3 | 5.7 | 2.0 | 7.0 | 5.5 | 3.0 |
| 3870 | 7.0 | 8.0 | 5.7 | 4.8 | 7.7 | 6.0 | 7.0 |
| Mean | 4.97 | 6.15 | 4.10 | 3.93 | 5.13 | 5.23 | 3.60 |
| SD | 2.39 | 1.77 | 2.18 | 1.58 | 1.97 | 0.91 | 1.68 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3861 | 4.8 |
| 3862 | 3.0 |
| 3863 | 3.8 |
| 3864 | 2.5 |
| 3865 | 5.0 |
| 3866 | 3.8 |
| 3867 | 3.5 |
| 3868 | 2.0 |
| 3869 | 3.8 |
| 3870 | 5.3 |
| Mean | 3.73 |
| SD | 1.06 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3811 | -1.7 | 2.5 | -2.0 | 2.3 | 2.7 | 2.8 | 4.0 |
| 3812 | -1.0 | 0.3 | 5.3 | 2.8 | -3.7 | 1.8 | 4.0 |
| 3813 | 3.3 | 1.8 | -0.3 | 2.5 | 1.0 | 3.0 | -0.3 |
| 3814 | 3.0 | 0.8 | 3.7 | 0.8 | 1.7 | 2.8 | -1.3 |
| 3815 | 5.3 | 3.0 | 1.7 | 1.8 | 1.3 | 1.3 | 0.3 |
| 3816 | 1.0 | 1.3 | 0.3 | 1.8 | -4.3 | 0.8 | 4.0 |
| 3817 | 3.0 | 1.3 | -0.7 | 1.3 | 1.7 | 2.0 | 2.7 |
| 3818 | 3.3 | 2.5 | 1.3 | 4.5 | -1.3 | 2.3 | -0.7 |
| 3819 | -1.3 | 1.5 | 2.0 | 2.3 | 1.3 | 2.3 | -0.3 |
| 3820 | 2.3 | 1.8 | -1.0 | 3.3 | 0.3 | 2.0 | 2.3 |
| Mean | 1.73 | 1.65 | 1.03 | 2.30 | 0.07 | 2.08 | 1.47 |
| SD | 2.37 | 0.84 | 2.25 | 1.06 | 2.39 | 0.70 | 2.15 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date<br>25 → 29 |
|---|---|
| 3811 | 0.8 |
| 3812 | 2.5 |
| 3813 | 3.3 |
| 3814 | 0.0 |
| 3815 | 1.8 |
| 3816 | 3.0 |
| 3817 | 2.0 |
| 3818 | 3.0 |
| 3819 | 1.8 |
| 3820 | 2.3 |
| Mean | 2.03 |
| SD | 1.03 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3831 | 3.0 | 0.5 | 2.7 | 1.0 | -0.3 | 2.0 | 2.7 |
| 3832 | 4.0 | 2.0 | 1.3 | 2.8 | -0.3 | 1.3 | -0.7 |
| 3833 | 1.3 | 2.5 | 2.0 | 4.3 | 1.0 | 2.3 | 2.0 |
| 3834 | 1.0 | 2.5 | 1.7 | 2.3 | -0.7 | 1.3 | 3.0 |
| 3835 | 2.0 | 1.3 | 1.7 | 2.5 | -1.3 | 1.5 | 1.0 |
| 3836 | 1.3 | 1.8 | -1.0 | 3.0 | 0.3 | 1.0 | 2.0 |
| 3837 | 0.3 | 3.0 | 2.0 | 3.0 | 2.0 | 1.8 | -2.7 |
| 3838 | 1.0 | 2.0 | 1.0 | 3.0 | 0.7 | 1.8 | 2.0 |
| 3839 | -0.7 | 1.8 | -1.3 | 4.0 | 4.3 | 4.3 | 0.3 |
| 3840 | 2.7 | 0.5 | 1.0 | 3.8 | 2.3 | 1.5 | -1.3 |
| Mean | 1.60 | 1.78 | 1.10 | 2.95 | 0.80 | 1.85 | 0.83 |
| SD | 1.36 | 0.83 | 1.30 | 0.94 | 1.69 | 0.92 | 1.87 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3831 | 1.5 |
| 3832 | -0.3 |
| 3833 | 1.3 |
| 3834 | 0.8 |
| 3835 | 2.0 |
| 3836 | 1.8 |
| 3837 | 1.0 |
| 3838 | 2.0 |
| 3839 | 0.3 |
| 3840 | 1.3 |
| Mean | 1.15 |
| SD | 0.74 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3851 | 0.3 | 4.3 | 1.3 | 2.5 | 2.7 | 2.0 | 2.7 |
| 3852 | 3.7 | 1.8 | 1.0 | 2.5 | 0.0 | 1.8 | 4.0 |
| 3853 | 1.0 | 3.0 | 2.7 | 2.5 | 1.0 | 2.5 | -2.3 |
| 3854 | 1.0 | 4.0 | -2.7 | 2.3 | 0.0 | 0.5 | 1.7 |
| 3855 | 1.0 | 2.5 | 0.3 | 1.5 | -1.3 | 1.0 | 3.7 |
| 3856 | 0.0 | 2.8 | 1.3 | 1.3 | -0.7 | 1.5 | 2.7 |
| 3857 | 4.3 | 2.0 | 6.3 | 1.3 | 1.3 | 1.0 | -3.3 |
| 3858 | 3.3 | 2.3 | 2.0 | -0.8 | -1.3 | 0.3 | 2.7 |
| 3859 | 4.3 | 0.8 | -1.3 | 5.3 | -2.0 | 1.5 | 2.3 |
| 3860 | 0.3 | 0.0 | 7.7 | 1.0 | 2.0 | 3.0 | 1.3 |
| Mean | 1.93 | 2.33 | 1.87 | 1.93 | 0.17 | 1.50 | 1.53 |
| SD | 1.76 | 1.31 | 3.14 | 1.54 | 1.55 | 0.86 | 2.45 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female  Mean Daily Body Weight Gain (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3851 | 2.0 |
| 3852 | 1.5 |
| 3853 | 2.0 |
| 3854 | 2.0 |
| 3855 | 1.0 |
| 3856 | 2.0 |
| 3857 | 1.5 |
| 3858 | -0.3 |
| 3859 | 3.5 |
| 3860 | -0.5 |
| Mean | 1.48 |
| SD | 1.17 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3871 | 1.0 | 1.5 | 0.0 | 2.3 | 0.0 | 1.5 | 2.3 |
| 3872 | 1.7 | 1.5 | 0.0 | 2.3 | 2.7 | 2.3 | 1.0 |
| 3873 | -1.7 | -0.5 | 3.3 | 1.5 | 1.7 | 2.3 | 0.7 |
| 3874 | 2.0 | 2.8 | -3.3 | 3.3 | 3.0 | 2.5 | 2.3 |
| 3875 | -1.3 | 2.5 | 2.7 | 1.8 | -0.7 | 4.8 | -3.0 |
| 3876 | -1.3 | 5.0 | 0.7 | 5.8 | 3.0 | 0.5 | -1.0 |
| 3877 | 1.3 | 1.8 | 1.3 | 2.8 | 0.3 | 2.5 | 5.0 |
| 3878 | 2.0 | 0.5 | 0.7 | -0.5 | 1.3 | 1.5 | 2.7 |
| 3879 | 1.3 | 0.8 | 1.0 | 2.3 | -1.7 | 1.3 | 2.3 |
| 3880 | 2.0 | 2.3 | 0.7 | 2.5 | -3.7 | 1.5 | 3.7 |
| Mean | 0.70 | 1.80 | 0.70 | 2.38 | 0.60 | 2.05 | 1.60 |
| SD | 1.52 | 1.49 | 1.78 | 1.56 | 2.18 | 1.14 | 2.30 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Numbers, Dose Groups, and Fates
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female  Mean Daily Body Weight Gain (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3871 | 1.8 |
| 3872 | 0.8 |
| 3873 | -0.3 |
| 3874 | 1.5 |
| 3875 | 3.0 |
| 3876 | 3.0 |
| 3877 | 2.8 |
| 3878 | -0.3 |
| 3879 | 1.3 |
| 3880 | 1.3 |
| Mean | 1.48 |
| SD | 1.20 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

# APPENDIX J: INDIVIDUAL ANIMAL MEAN DAILY FOOD CONSUMPTION

## PRODUCT IDENTIFICATION

*L. reuteri* 3630 & *L. reuteri* 3632

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3801 | 19.7 | 26.0 | 26.8 | 26.5 | 24.0 | 26.1 | 27.2 |
| 3802 | 19.7 | 26.0 | 26.8 | 26.5 | 24.0 | 26.1 | 27.2 |
| 3803 | 27.8 | 27.6 | 27.2 | 28.9 | 26.5 | 28.4 | 27.3 |
| 3804 | 27.8 | 27.6 | 27.2 | 28.9 | 26.5 | 28.4 | 27.3 |
| 3805 | 22.8 | 24.0 | 23.2 | 23.9 | 22.7 | 25.3 | 25.8 |
| 3806 | 22.8 | 24.0 | 23.2 | 23.9 | 22.7 | 25.3 | 25.8 |
| 3807 | 23.8 | 25.0 | 24.5 | 25.8 | 24.3 | 25.3 | 27.5 |
| 3808 | 23.8 | 25.0 | 24.5 | 25.8 | 24.3 | 25.3 | 27.5 |
| 3809 | 25.7 | 26.0 | 26.5 | 25.1 | 26.3 | 27.1 | 28.8 |
| 3810 | 25.7 | 26.0 | 26.5 | 25.1 | 26.3 | 27.1 | 28.8 |
| Mean | 23.97 | 25.73 | 25.63 | 26.03 | 24.77 | 26.43 | 27.33 |
| SD | 2.89 | 1.27 | 1.63 | 1.75 | 1.54 | 1.26 | 1.01 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date<br>25 → 29 |
|---|---|
| 3801 | 28.1 |
| 3802 | 28.1 |
| 3803 | 30.1 |
| 3804 | 30.1 |
| 3805 | 26.8 |
| 3806 | 26.8 |
| 3807 | 28.3 |
| 3808 | 28.3 |
| 3809 | 29.5 |
| 3810 | 29.5 |
| Mean | 28.55 |
| SD | 1.24 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3821 | 21.2 | 23.9 | 24.5 | 24.4 | 24.7 | 27.8 | 28.0 |
| 3822 | 21.2 | 23.9 | 24.5 | 24.4 | 24.7 | 27.8 | 28.0 |
| 3823 | 20.0 | 22.4 | 22.7 | 23.9 | 22.0 | 24.1 | 24.8 |
| 3824 | 20.0 | 22.4 | 22.7 | 23.9 | 22.0 | 24.1 | 24.8 |
| 3825 | 26.3 | 27.1 | 26.5 | 28.0 | 26.2 | 27.5 | 27.7 |
| 3826 | 26.3 | 27.1 | 26.5 | 28.0 | 26.2 | 27.5 | 27.7 |
| 3827 | 24.5 | 27.5 | 27.7 | 27.3 | 26.0 | 31.4 | 28.0 |
| 3828 | 24.5 | 27.5 | 27.7 | 27.3 | 26.0 | 31.4 | 28.0 |
| 3829 | 11.5 | 14.0 | 26.7 | 26.0 | . | . | . |
| 3830 | 11.5 | 14.0 | 26.7 | 26.0 | . | . | 27.3 |
| Mean | 20.70 | 22.98 | 25.60 | 25.90 | 24.71 | 27.69 | 27.15 |
| SD | 5.41 | 5.15 | 1.89 | 1.68 | 1.78 | 2.74 | 1.33 |
| N | 10 | 10 | 10 | 10 | 8 | 8 | 9 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3821 | 26.9 |
| 3822 | 26.9 |
| 3823 | 25.1 |
| 3824 | 25.1 |
| 3825 | 26.8 |
| 3826 | 26.8 |
| 3827 | 29.0 |
| 3828 | 29.0 |
| 3829 | . |
| 3830 | 30.3 |
| Mean | 27.31 |
| SD | 1.76 |
| N | 9 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3841 | 24.8 | 25.3 | 26.0 | 26.1 | 25.0 | 27.5 | 28.3 |
| 3842 | 24.8 | 25.3 | 26.0 | 26.1 | 25.0 | 27.5 | 28.3 |
| 3843 | 21.3 | 23.3 | 24.0 | 24.0 | 23.2 | 24.4 | 26.2 |
| 3844 | 21.3 | 23.3 | 24.0 | 24.0 | 23.2 | 24.4 | 26.2 |
| 3845 | 26.7 | 26.3 | 27.8 | 26.1 | 27.2 | 28.6 | 29.7 |
| 3846 | 26.7 | 26.3 | 27.8 | 26.1 | 27.2 | 28.6 | 29.7 |
| 3847 | 25.0 | 25.0 | 26.5 | 24.5 | 22.8 | 26.1 | 26.3 |
| 3848 | 25.0 | 25.0 | 26.5 | 24.5 | 22.8 | 26.1 | 26.3 |
| 3849 | 24.0 | 25.5 | 25.5 | 24.6 | 25.7 | 28.9 | 29.3 |
| 3850 | 24.0 | 25.5 | 25.5 | 24.6 | 25.7 | 28.9 | 29.3 |
| Mean | 24.37 | 25.05 | 25.97 | 25.08 | 24.77 | 27.10 | 27.97 |
| SD | 1.84 | 1.05 | 1.32 | 0.93 | 1.69 | 1.76 | 1.55 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3841 | 30.1 |
| 3842 | 30.1 |
| 3843 | 26.5 |
| 3844 | 26.5 |
| 3845 | 28.6 |
| 3846 | 28.6 |
| 3847 | 26.9 |
| 3848 | 26.9 |
| 3849 | 30.5 |
| 3850 | 30.5 |
| Mean | 28.53 |
| SD | 1.72 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3861 | 24.2 | 27.0 | 26.2 | 25.5 | 23.5 | 28.1 | 27.5 |
| 3862 | 24.2 | 27.0 | 26.2 | 25.5 | 23.5 | 28.1 | 27.5 |
| 3863 | 22.0 | 23.8 | 24.0 | 23.6 | 24.2 | 28.9 | 26.2 |
| 3864 | 22.0 | 23.8 | 24.0 | 23.6 | 24.2 | 28.9 | 26.2 |
| 3865 | 27.8 | 30.1 | 30.2 | 30.9 | 29.7 | 32.1 | 31.3 |
| 3866 | 27.8 | 30.1 | 30.2 | 30.9 | 29.7 | 32.1 | 31.3 |
| 3867 | 25.5 | 28.9 | 28.5 | 27.1 | 27.5 | 29.1 | 28.5 |
| 3868 | 25.5 | 28.9 | 28.5 | 27.1 | 27.5 | 29.1 | 28.5 |
| 3869 | 24.5 | 26.5 | 34.7 | 29.9 | 35.5 | 50.6 | 26.7 |
| 3870 | 24.5 | 26.5 | 34.7 | 29.9 | 35.5 | 50.6 | 26.7 |
| Mean | 24.80 | 27.25 | 28.70 | 27.40 | 28.07 | 33.78 | 28.03 |
| SD | 2.00 | 2.30 | 3.84 | 2.83 | 4.58 | 9.00 | 1.93 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3861 | 27.6 |
| 3862 | 27.6 |
| 3863 | 27.4 |
| 3864 | 27.4 |
| 3865 | 33.6 |
| 3866 | 33.6 |
| 3867 | 32.5 |
| 3868 | 32.5 |
| 3869 | 29.4 |
| 3870 | 29.4 |
| Mean | 30.10 |
| SD | 2.68 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3811 | 17.8 | 18.0 | 18.0 | 20.4 | 17.2 | 19.8 | 21.5 |
| 3812 | 17.8 | 18.0 | 18.0 | 20.4 | 17.2 | 19.8 | 21.5 |
| 3813 | 17.7 | 16.5 | 16.2 | 16.8 | 15.2 | 17.4 | 17.0 |
| 3814 | 17.7 | 16.5 | 16.2 | 16.8 | 15.2 | 17.4 | 17.0 |
| 3815 | 17.3 | 18.8 | 17.8 | 18.9 | 16.5 | 18.6 | 20.7 |
| 3816 | 17.3 | 18.8 | 17.8 | 18.9 | 16.5 | 18.6 | 20.7 |
| 3817 | 18.8 | 18.5 | 18.0 | 19.0 | 16.8 | 17.9 | 18.0 |
| 3818 | 18.8 | 18.5 | 18.0 | 19.0 | 16.8 | 17.9 | 18.0 |
| 3819 | 18.3 | 19.0 | 18.0 | 18.6 | 16.7 | 18.5 | 18.5 |
| 3820 | 18.3 | 19.0 | 18.0 | 18.6 | 16.7 | 18.5 | 18.5 |
| Mean | 18.00 | 18.15 | 17.60 | 18.73 | 16.47 | 18.43 | 19.13 |
| SD | 0.56 | 0.94 | 0.76 | 1.22 | 0.72 | 0.84 | 1.78 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 0 CFU<br>Group 1 | Day(s) Relative to Start Date<br>25 → 29 |
|---|---|
| 3811 | 18.9 |
| 3812 | 18.9 |
| 3813 | 17.5 |
| 3814 | 17.5 |
| 3815 | 20.5 |
| 3816 | 20.5 |
| 3817 | 19.9 |
| 3818 | 19.9 |
| 3819 | 19.6 |
| 3820 | 19.6 |
| Mean | 19.28 |
| SD | 1.08 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3831 | 18.0 | 18.1 | 17.2 | 17.5 | 16.0 | 17.9 | 20.3 |
| 3832 | 18.0 | 18.1 | 17.2 | 17.5 | 16.0 | 17.9 | 20.3 |
| 3833 | 16.8 | 18.3 | 18.5 | 19.6 | 17.5 | 18.5 | 20.2 |
| 3834 | 16.8 | 18.3 | 18.5 | 19.6 | 17.5 | 18.5 | 20.2 |
| 3835 | 16.2 | 17.0 | 15.3 | 19.3 | 14.7 | 16.8 | 17.2 |
| 3836 | 16.2 | 17.0 | 15.3 | 19.3 | 14.7 | 16.8 | 17.2 |
| 3837 | 18.0 | 19.1 | 19.7 | 20.3 | 18.0 | 18.6 | 15.7 |
| 3838 | 18.0 | 19.1 | 19.7 | 20.3 | 18.0 | 18.6 | 15.7 |
| 3839 | 19.0 | 16.1 | 18.5 | 20.4 | 19.0 | 21.0 | 19.3 |
| 3840 | 19.0 | 16.1 | 18.5 | 20.4 | 19.0 | 21.0 | 19.3 |
| Mean | 17.60 | 17.73 | 17.83 | 19.40 | 17.03 | 18.55 | 18.53 |
| SD | 1.05 | 1.10 | 1.56 | 1.09 | 1.61 | 1.47 | 1.92 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 1.668E+10 CFU Group 2 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3831 | 19.1 |
| 3832 | 19.1 |
| 3833 | 20.0 |
| 3834 | 20.0 |
| 3835 | 17.4 |
| 3836 | 17.4 |
| 3837 | 18.5 |
| 3838 | 18.5 |
| 3839 | 20.8 |
| 3840 | 20.8 |
| Mean | 19.15 |
| SD | 1.23 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3851 | 17.3 | 19.3 | 18.2 | 19.5 | 17.7 | 20.3 | 22.0 |
| 3852 | 17.3 | 19.3 | 18.2 | 19.5 | 17.7 | 20.3 | 22.0 |
| 3853 | 17.8 | 17.1 | 17.2 | 17.9 | 16.5 | 17.5 | 17.8 |
| 3854 | 17.8 | 17.1 | 17.2 | 17.9 | 16.5 | 17.5 | 17.8 |
| 3855 | 17.7 | 19.1 | 17.0 | 18.0 | 16.2 | 18.1 | 19.3 |
| 3856 | 17.7 | 19.1 | 17.0 | 18.0 | 16.2 | 18.1 | 19.3 |
| 3857 | 17.7 | 17.6 | 18.5 | 18.5 | 15.7 | 16.6 | 17.5 |
| 3858 | 17.7 | 17.6 | 18.5 | 18.5 | 15.7 | 16.6 | 17.5 |
| 3859 | 18.2 | 18.8 | 18.8 | 20.5 | 17.0 | 19.8 | 20.0 |
| 3860 | 18.2 | 18.8 | 18.8 | 20.5 | 17.0 | 19.8 | 20.0 |
| Mean | 17.73 | 18.38 | 17.93 | 18.88 | 16.60 | 18.45 | 19.33 |
| SD | 0.29 | 0.89 | 0.77 | 1.05 | 0.73 | 1.44 | 1.71 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 3.337E+10 CFU Group 3 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3851 | 20.1 |
| 3852 | 20.1 |
| 3853 | 18.6 |
| 3854 | 18.6 |
| 3855 | 18.0 |
| 3856 | 18.0 |
| 3857 | 18.9 |
| 3858 | 18.9 |
| 3859 | 20.6 |
| 3860 | 20.6 |
| Mean | 19.25 |
| SD | 1.03 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 → 4 | 4 → 8 | 8 → 11 | 11 → 15 | 15 → 18 | 18 → 22 | 22 → 25 |
| 3871 | 16.5 | 15.9 | 16.2 | 17.3 | 16.2 | 16.9 | 17.5 |
| 3872 | 16.5 | 15.9 | 16.2 | 17.3 | 16.2 | 16.9 | 17.5 |
| 3873 | 16.3 | 16.0 | 12.8 | 16.5 | 15.5 | 16.9 | 16.2 |
| 3874 | 16.3 | 16.0 | 12.8 | 16.5 | 15.5 | 16.9 | 16.2 |
| 3875 | 17.8 | 19.6 | 19.3 | 20.6 | 20.0 | 19.6 | 18.0 |
| 3876 | 17.8 | 19.6 | 19.3 | 20.6 | 20.0 | 19.6 | 18.0 |
| 3877 | 19.3 | 18.6 | 18.0 | 18.1 | 17.3 | 19.4 | 20.3 |
| 3878 | 19.3 | 18.6 | 18.0 | 18.1 | 17.3 | 19.4 | 20.3 |
| 3879 | 17.8 | 18.4 | 17.7 | 18.4 | 15.7 | 18.1 | 20.2 |
| 3880 | 17.8 | 18.4 | 17.7 | 18.4 | 15.7 | 18.1 | 20.2 |
| Mean | 17.57 | 17.70 | 16.80 | 18.18 | 16.93 | 18.18 | 18.43 |
| SD | 1.15 | 1.58 | 2.34 | 1.47 | 1.75 | 1.24 | 1.69 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.

Individual Animal Daily Food Consumption
PSL Study Number 55164: Limosilactobacillus Reuteri:
28-Day Repeat-Dose Oral Gavage Toxicity Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

| 1.001E+11 CFU Group 4 | Day(s) Relative to Start Date |
|---|---|
| | 25 → 29 |
| 3871 | 18.1 |
| 3872 | 18.1 |
| 3873 | 17.1 |
| 3874 | 17.1 |
| 3875 | 21.6 |
| 3876 | 21.6 |
| 3877 | 19.1 |
| 3878 | 19.1 |
| 3879 | 18.4 |
| 3880 | 18.4 |
| Mean | 18.88 |
| SD | 1.60 |
| N | 10 |

* The doses listed are the nominal values for each dose group in terms of CFU/kg bw/day.