TABLE 1: SUMMARY OF IN-LIFE CLINICAL OBSERVATIONS

Day numbers relative to Start Date

Sex: Male

		0 mg/kg/day	125 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Lesion					
	Number of Observations	.	.	.	1
	Number of Animals	.	.	.	1
	Days from - to	.	.	.	15 15
Eschar					
	Number of Observations	.	7	.	.
	Number of Animals	.	1	.	.
	Days from - to	.	8 15	.	.

TABLE 2: SUMMARY OF DETAILED CLINICAL OBSERVATIONS

TABLE 3 (cont.) SUMMARY OF DETAILED CLINICAL OBSERVATIONS

Males

Days 1, 8, and 15

Group	1	2	3	4
Dose Level (mg/kg/day)	0	125	250	500
Number of Animals in Group	5	5	5	5
Observations During Removal From Cage and Handling	**Score[1]**			
Handling Reactivity	0	0	0	0
Vocalization	0	0	0	0
Palpebral Closure	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	1(4[2]); 1(4[2])	0	1(4[3])
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

[1] 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.

[2] Eschar (other)/eschar (face).

[3] Tail lesion.

TABLE 3 (cont.) SUMMARY OF DETAILED CLINICAL OBSERVATIONS

Females

Days 1, 8, and 15

Group	1	2	3	4
Dose Level (mg/kg/day)	**0**	**125**	**250**	**500**
Number of Animals in Group	**5**	**5**	**5**	**5**
Observations During Removal From Cage and Handling	**Score[1]**			
Handling Reactivity	0	0	0	0
Vocalization	0	0	0	0
Palpebral Closure	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

[1] 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 3: SUMMARY OF MEAN BODY WEIGHTS

Bodyweight (g)

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1	Mean	236.2	237.0	233.4	236.6
	SD	16.7	18.9	18.4	15.4
	N	5	5	5	5
8	Mean	289.2	281.0	311.4	295.4
	SD	18.3	25.3	40.6	16.1
	N	5	5	5	5
15	Mean	344.8	331.8	344.0	351.6
	SD	30.6	36.7	29.6	21.1
	N	5	5	5	5

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: Not significant Time Factor: 1% significance level Group Factor: Not significant

Bodyweight (g)

Sex: Female Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1	Mean	151.2	151.4	152.6	154.2
	SD	5.5	15.0	8.0	7.0
	N	5	5	5	5
8	Mean	176.8	169.4	177.2	181.0
	SD	8.6	12.7	11.3	7.4
	N	5	5	5	5
15	Mean	200.6	196.2	198.4	204.8
	SD	11.6	9.7	13.2	9.9
	N	5	5	5	5

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: Not significant Time Factor: 1% significance level Group Factor: Not significant

TABLE 4: SUMMARY OF MEAN DAILY BODY WEIGHT GAIN

Mean Daily Body Weight Gain (g/day)

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1 → 8	Mean	7.57	6.29	11.14	8.40
	SD	2.27	1.33	6.35	0.40
	N	5	5	5	5
8 → 15	Mean	7.94	7.26	4.66	8.03
	SD	2.20	2.48	7.42	0.88
	N	5	5	5	5
1 → 15	Mean	7.76	6.77	7.90	8.21
	SD	1.34	1.77	1.24	0.55
	N	5	5	5	5

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: Not significant Time Factor: Not significant Group Factor: Not significant

Mean Daily Body Weight Gain (g/day)

Sex: Female Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1 → 8	Mean	3.66 *[1]	2.57	3.51	3.83
	SD	0.59	1.10	0.58	0.23
	N	5	5	5	5
8 → 15	Mean	3.40	3.83	3.03	3.40
	SD	0.55	0.80	0.36	0.57
	N	5	5	5	5
1 → 15	Mean	3.53	3.20	3.27	3.61
	SD	0.49	0.61	0.40	0.35
	N	5	5	5	5

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: 5% significance level Time Factor: Not significant Group Factor: Not significant

1 [* - (All Groups) Test: 2 Way ANOVA 5% significance level]

TABLE 5: SUMMARY OF MEAN DAILY FOOD CONSUMPTION

Mean Daily Food Consumption (g/day)

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
8 → 15	Mean	35.77 R[1]	26.80	24.94	29.20
	SD	11.62	0.40	5.40	1.49
	N	5	5	5	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

1 [R - Automatic Transformation: Rank]

Mean Daily Food Consumption (g/day)

Sex: Female Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
8 → 15	Mean	19.17 R[1]	17.71	19.11	23.03
	SD	0.89	0.72	0.81	3.81
	N	5	5	5	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

1 [R - Automatic Transformation: Rank]

TABLE 6: SUMMARY OF MEAN FOOD EFFICIENCY[1]

[1] Food efficiency = $\frac{\text{Mean Daily Body Weight Gain}}{\text{Mean Daily Food Consumption}}$

Food Efficiency

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
8 → 15	Mean	0.229 R[1]	0.271	0.167	0.276
	SD	0.059	0.093	0.320	0.034
	N	5	5	5	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

1 [R - Automatic Transformation: Rank]

Food Efficiency

Sex: Female Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
8 → 15	Mean	0.178 I,A[1]	0.216	0.159	0.148
	SD	0.030	0.046	0.022	0.018
	N	5	5	5	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

1 [I,A - Automatic Transformation: Identity (No Transformation), (All Groups) Test: Analysis of Variance $p < 0.05$]

APPENDIX A: PROTOCOL, PROTOCOL AMENDMENTS, AND PROTOCOL DEVIATION

PRODUCT IDENTIFICATION

Silk Fibroin

SILK FIBROIN SOLUTION: A 14-DAY REPEAT DOSE ORAL GAVAGE RANGE-FINDING STUDY IN RATS

PRODUCT IDENTIFICATION

Silk Fibroin solution

PSL PROTOCOL NO.

P710.01 CMR

PERFORMING LABORATORY

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

PSL STUDY NUMBER

50725

STUDY DIRECTOR

[redacted]

SPONSOR

Cambridge Crops Inc
444 Somerville Ave
Somerville, MA 02143

TABLE OF CONTENTS

1. **TITLE OF STUDY: SILK FIBROIN SOLUTION: A 14-DAY REPEAT DOSE ORAL GAVAGE RANGE-FINDING STUDY IN RATS**

2. **OBJECTIVE**

The objective of this range-finding study is to evaluate the potential subchronic toxicity of Silk fibrin solution in male and female rats that is likely to arise from repeated exposure via oral gavage over a test period of at least 14 days. These data will be used, along with existing data provided by Sponsor, to select dose levels for a subsequent longer toxicity study in rats.

3. **STUDY DIRECTOR**

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

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

5. **SPONSOR**

Cambridge Crops Inc
444 Somerville Ave
Somerville, MA 02143

6. **SPONSOR REPRESENTATIVE**

Cambridge Crops Inc
444 Somerville Ave
Somerville, MA 02143

7. **DATES**

Proposed in-life start date: June 24, 2019

Proposed experimental termination date: July 08, 2019

8. **TEST SUBSTANCE**

8.A Source

The test substance will be provided by the Sponsor.

8.B Identification

The test substance will be identified using the following information provided by the Sponsor and PSL identification number:

Test Substance: Silk fibroin solution
Batch #: 128
PSL ID: 190614-1D
Physical Description: Slightly yellow liquid
Composition: 5.0 % Silk Fibroin (CAS# 9007-65-5) & 95 % Water
Storage Conditions: -20 °C (thawed on ice before use at ambient temp)
Expiration Date: 07/20/2019
Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

8.C Analysis

The test substance, as received, is expected to be stable for the duration of the study. Stability of the neat test substance and concentrations of the test substance in vehicle will be determined as part of this study.

8.D Hazards

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

9. GENERAL TEST SYSTEM PARAMETERS

9.A Animal Requirements

9.A.1 Number of Animals: 40

9.A.2 Number of Groups: 4 (3 dose levels per sex + 1 control group per sex)

9.A.3 Number of Animals per Group: 10 (5 males, 5 females)

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

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

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

9.A.7 Supplier: Charles River Laboratories, Inc. Rats will be shipped in filtered cartons by airfreight and/or truck.

9.B Test System Justification

The Sprague-Dawley® rat is 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.

9.C Husbandry

9.C.1 Housing

The animals will be housed in regularly cleaned cages which conform to the size recommendations in the most recent Guide for the Care and Use of Laboratory Animals[1]. 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.

9.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.

9.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 terminal sacrifice.

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

9.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 consisting of 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.

9.D Identification

9.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, and sex of the animal.

9.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.

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

10. EXPERIMENTAL DESIGN

10.A Route of Administration

The test substance will be administered by oral gavage.

10.B Justification of Route of Administration

The oral route of administration was selected by the Sponsor. This route of administration is recommended in the referenced guidelines (Section 14.C) and a potential route of human exposure.

10.C Control of Bias

Animals will be randomly assigned to test groups according to PSL SOP #714. Five male and five female rats will be randomly assigned to each of the following test groups:

10.D Dose Levels

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

Group	No. Animals/Group (M/F)	Target Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Dose Concentration[a] (mg/mL)
1	5/5	Vehicle Control[b] 0	10	0
2	5/5	Low Dose 125		12.5
3	5/5	Intermediate Dose 250		25
4	5/5	High Dose 500		50

[a] Appropriate concentrations of the test substance in vehicle to achieve the target dose level.
[b] Water.

10.E Justification of Dose Level Selection

The dose levels of 0 (vehicle control), 125, 250, and 500 mg/kg/day of silk fibroin solution were selected by the Sponsor in consultation with the Study Director. The high dose is a tolerable dose and is not expected to cause marked toxicity. The intermediate and low dose levels are selected to derive a dose-response for any effects observed. These data will be used to select dose levels for a subsequent longer toxicity study.

11. GENERAL PROCEDURES

11.A Selection of Animals

Forty (40) healthy rats (twenty males; twenty 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.

11.B Dose Preparations and Procedures

11.B.1 Test Substance Preparation

The test substance will be mixed weight to volume (w/v) in water. Group 1 will receive distilled water alone, as a vehicle control. Fresh formulations containing 12.5 (low dose), 25 (intermediate dose), and 50 (high dose) mg/mL concentrations of the test substance will be prepared once a week. The formulations will be vertex if necessary at ambient temperature until a visually homogeneous mixture is achieved. Preparations of the test substance will be documented in the raw data.

11.B.2 Dose Calculations

Individual doses will be calculated based on the most recent weekly body weights and will be adjusted each week to maintain the targeted dose level for all rats (i.e., mg/kg/day). All doses will be administered volumetrically at 10 mL/kg. The control group will receive the vehicle only, at the same dose volume as the test animals.

11.B.3 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) for a period of at least 14 days. The dose mixtures will be maintained 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 (±2 hours). Residual dose mixtures will be properly discarded following daily administration and sampling (as required).

11.C Sampling of Test Substance and Dose Preparations

11.C.1 Sample Collections

The neat test substance and dose preparations will be sampled in duplicate. Additional samples may be collected and analyzed, at the discretion of the Study Director, to ensure stability, homogeneity, and accuracy of the dose concentrations over the course of the study.

11.C.2 Test Substance and Dose Preparation Stability

The test substance is expected to be stable over the course of the study under the conditions of storage at Product Safety Labs. Given that the dose preparations will be prepared daily, maintained on a stir plate during dose administration, and used within approximately two hours, the test substance in the preparations is considered to be stable. A sample of the test substance (neat) will be collected at the beginning and end of the in-life phase.

11.C.3 Dose Preparation Homogeneity

At the beginning of the study, formulation of each concentration will be prepared according to the procedures as will be used on test (Section 11.B.1). Samples from these preparations will be collected from the top, middle, and bottom of each concentration of test substance that was prepared in the vehicle. Sample of the vehicle control will be collected from the middle of the container only.

11.C.4 Dose Preparation Concentration Verification

Samples for concentration verification will be collected from dose preparations at the beginning of the study (as part of the homogeneity assessment, Section 11.C.3).

11.C.5 Sample Preservation

Upon sampling, dose preparations and neat test substance will be stored frozen. Samples will be considered stable from the point at which they are frozen. All samples will be retained until finalization of the study and discarded following the issuance of the final report.

11.C.6 Sample Analysis

Selected frozen samples described above will be sent to Product Safety Labs Analytical Services for future analysis of dose preparations (i.e., high and low doses), neat test substance samples. If necessary, the concentration verifications will also identified via BCA assay with silk fibroin solution as the standard. Alternatively, gravimetrical methods are also applicable (i.e. TGA, moisture analyzer).

11.D Analytical Chemistry

11.D.1 Sample Storage

Upon receipt, all samples will be stored and maintained frozen prior to analysis.

11.D.2 Reference Substance

An aliquot of the test substance will serve as the reference standard.

11.D.3 Chemical Analysis (PSL SOP #1104)

Analytical test methodology, if supplied by the client, may be adapted by PSL personnel and appropriately employed as needed. Samples will be analyzed in replicate. A detailed description of the analytical test method will be documented. Any remaining sample material will be retained until finalization of the study and discarded following the issuance of the final report.

11.D.4 Data Reporting

Data will be captured on standard raw data sheets and as instrument output, as necessary, and summarized in tabular form.

11.D.5 Analytical Report and Records to be Maintained

Summary data tables will be provided to the Study Director. Upon completion of the study, all raw data will be transferred to the Study Director.

11.E 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.

Prior to the first treatment with the test substance on Day 1, 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 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.

11.F 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 approximately weekly thereafter (intervals of 7 days ±1). Additional bodyweights 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. Decedents need not be weighed. Body weight gain will be calculated for selected intervals and for the study overall.

11.G Food Consumption and Food Efficiency

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

11.H Terminal Sacrifice

At terminal sacrifice, all surviving animals will be euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals in the study will be subjected to a gross necropsy, which will include examination of the external surface of the body, all orifices, musculoskeletal system, and the cranial, thoracic, abdominal, and pelvic cavities with their associated organs and tissues. All gross lesions will be recorded.

11.I Unscheduled Sacrifice

Any rat that dies or is sacrificed 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 may be excised and preserved as described for those animals sacrificed by design (Section 11.H) at the discretion of the Study Director.

12. STATISTICAL ANALYSIS

In-Life Data

Product Safety Labs will perform 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 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, Staffordshire UK; 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.

12.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, and 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)[2]. Significant interactions observed between treatment and time as well as main effects and non-significant findings will be further analyzed by a *post hoc* multiple comparisons test (e.g. Dunnett's test[3,4]) of the individual treated groups to control.

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

13. FINAL REPORT

A signed study report will be provided to the Sponsors. This report will include individual animal data (and means where appropriate) for concentrations of test substance received (if applicable); time of observation of each abnormal sign and its subsequent course; body weights; food consumption and food efficiency values; and necropsy findings,. The final report will also include the procedures and conclusions drawn by the Study Director. A signed study report will be provided to the Sponsors.

14. STUDY CONDUCT

14.A Laboratories

Testing Facility

In-life	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810
Test substance and dose preparation analysis	Product Safety Labs 2394 Highway 130 Dayton, NJ 08810 Prospective P.I. (s):

14.B GLP Compliance

This study will not be performed in full compliance with Good Laboratory Practice (GLP) standards, but will be conducted in a GLP-compliant facility.

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

[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 a control. *Biometrics*, 20(3), 482-491.

14.C Test Procedure Guidelines

This study design is based on the following guidelines:

- OECD Guidelines for Testing of Chemicals, Section 4, Test No. 407: Health Effects, *Repeated Dose 28-Day Oral Toxicity Study in Rodents* (adopted 1995; updated October 2008). US EPA Health Effects Test Guidelines: OPPTS 870.3050 Repeated Dose 28-day Oral Toxicity Study in Rodents (2000).
- US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, Revised 2007, IV.C. 4. a. *Subchronic Toxicity Studies with Rodents* (2003)

15. RECORDS TO BE MAINTAINED

The original signed report and all raw paper data will be sent to the Sponsor. A copy of the signed report, together with a copy of the protocol and all raw data generated at Product Safety Labs, will be maintained in the Product Safety Lab's Archives.

The following records will be maintained:

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

Storage	Disposition
Usage	Test substance and dose preparation analysis

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

Receipt, date of birth	Food consumption
Initial health assessment	Clinical observations
Dosing	Individual necropsy records
Body weights	

All other records that would demonstrate adherence to the protocol. Any electronic raw data generated will be maintained in accordance to the Test Site's procedures.

16. 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, and described in the final report.

17. DISPOSITION OF TEST SUBSTANCE

All remaining test substance will be properly disposed, unless otherwise specified by the Sponsor. Records of sample disposition will be maintained by PSL.

Product Safety Labs

14-Day Oral Toxicity Study
Protocol # P710.01
PSL ID: 190614-1D
Study No: 50725

18. PROTOCOL APPROVAL Obtained by:

Signature: [redacted]

[redacted]

Cambridge Crops, Inc

Date: 6/20/2019

Signature: [redacted]

[redacted]

Product Safety Labs

Date: 6/20/2019

Signature: [redacted]

[redacted]

Product Safety Labs

Date: 6/20/19

Product Safety Labs

PROTOCOL AMENDMENT

14-Day Oral Toxicity Study

PROTOCOL NO.: P710.01 CMR **AMENDMENT NO.:** 1

STUDY NO.: 50725 **PSL NO.:** 190614-1D; 190628-1D

ADD:

PSL No.:	190628-1D
Batch #:	136
Expiration Date:	07/20/2019

REASON:

An additional shipment of test substance was supplied by the Sponsor in order to complete the requested study. PSL No. 190628-1D will be used for testing. This amendment has no adverse impact on the study.

[redacted] 7/12/2019

Study Director
Product Safety Labs

Date

Product Safety Labs

PROTOCOL AMENDMENT

14-Day Oral Toxicity Study

PROTOCOL NO.: P710.01 CMR **AMENDMENT NO.:** 2

STUDY NO.: 50725 **PSL NO.:** 190614-1D; 190628-1D

Section: Study Title

Change from: Product ID "Silk Fibroin Solution"

Change to: Product ID "Silk Fibroin"

REASON:

The corrections required by the sponsor. This amendment has no adverse impact on the study.

[redacted]
Study Director
Product Safety Labs

9/6/2019
Date

Product Safety Labs

PROTOCOL AMENDMENT

14-Day Oral Toxicity Study

PROTOCOL NO.: P710.01 CMR **AMENDMENT NO.:** 3

STUDY NO.: 50725 **PSL NO.:** 190614-1D; 190628-1D

Section: 8.B Identification

Change from: Composition: 5.0% Silk Fibroin (CAS#9007-65-5) & 95% Water

Change to: Composition: 5.0% Silk Fibroin (CAS#9007-76-5) & 95% Water

REASON:

The protocol contained a typo in the CAS number for the test article. This amendment has no adverse impact on the study.

[redacted] 9/6/2019

Study Director
Product Safety Labs

Date

roduct Safety Labs

PROTOCOL DEVIATION

SILK FIBROIN SOLUTION: A 14-DAY REPEAT DOSE ORAL GAVAGE RANGE-FINDING STUDY IN RATS

PROTOCOL NO.: P710.01 CMR
DEVIATION NO.: 1
STUDY NO.: 50725
PSL NO.: 190614-1D

PROTOCOL SECTION: 11.E General Procedures; Clinical Observations

The protocol states:

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.

PROTOCOL DEVIATION:

The protocol requires that cage-side clinical observations be performed and recorded daily. Clinical observations were not recorded for all animals on Study Day 13.

REASON FOR DEVIATION: Scientist oversight.

IMPACT ON STUDY: All Animals were observed to be active and healthy on Day 12 and on Day 14 of the study. For purposes of the final report, these animals will be considered active and healthy throughout this period. This deviation does not adversely affect the outcome of the study or interpretation of the results

[redacted]

07/24/2019
Date

Study Director
Product Safety Labs

APPENDIX B: FEED AND WATER ANALYSES

PRODUCT IDENTIFICATION

Silk Fibroin

APPENDIX B (cont.): FEED ANALYSIS

2016C

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ENVIGO

Teklad Certified Global 16% Protein Rodent Diet

Lot Number	**2016C-041619MA**
Date of Manufacture	16Apr2019
Report Date	26Apr2019

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.

[redacted]
Nutritionist
2019.04.29
11:24:17 -05'00'

Analysis	Result (%)
Proximate Analysis	
Protein	16.90
Fat	3.56
Fiber	3.09
Moisture	12.31
Ash	4.81
Calcium	0.85
Phosphorus	0.68

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	< 0.10	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	0.42	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.21	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	< 0.16	ppm	0.50

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

2016C

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ENVIGO

Teklad Certified Global 16% Protein Rodent Diet

Lot Number	**2016C-032819MA**
Date of Manufacture	28Mar2019
Report Date	08Apr2019

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.

2019.04.08 19:23:49 -05'00'

Analysis	Result (%)
Proximate Analysis	
Protein	**16.50**
Fat	**3.56**
Fiber	**3.89**
Moisture	**11.93**
Ash	**4.92**
Calcium	**0.84**
Phosphorus	**0.68**

Analysis	Result	Units	Detection Limit
Heavy Metals			
Arsenic	< 0.50	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

APPENDIX B (cont.): WATER ANALYSIS

In June 2019, 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 C: INDIVIDUAL ANIMAL IN-LIFE OBSERVATIONS

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal In-Life Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male	Animal	Observation Type: All Types	From Day 1 (Start Date) to 15 (Start Date)
1	7001	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7002	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7003	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7004	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7005	Normal	1 to 14
		Scheduled Removal (Terminal)	15
2	7011	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7012	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7013	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7014	Normal	1 to 7
		Scheduled Removal (Terminal)	15
		Eschar, Face, Superficial	8 to 15
	7015	Normal	1 to 14
		Scheduled Removal (Terminal)	15
3	7021	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7022	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7023	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7024	Normal	1 to 14
		Scheduled Removal (Terminal)	15

Values = Clin Obs Range

Individual Animal In-Life Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male	Animal	Observation Type: All Types	From Day 1 (Start Date) to 15 (Start Date)
3	7025	Normal	1 to 14
		Scheduled Removal (Terminal)	15
4	7031	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7032	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7033	Normal	1 to 14
		Lesion, Tail	15
		Scheduled Removal (Terminal)	15
	7034	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7035	Normal	1 to 14
		Scheduled Removal (Terminal)	15

Values = Clin Obs Range

Individual Animal In-Life Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female	Animal	Observation Type: All Types	From Day 1 (Start Date) to 15 (Start Date)
1	7006	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7007	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7008	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7009	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7010	Normal	1 to 14
		Scheduled Removal (Terminal)	15
2	7016	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7017	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7018	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7019	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7020	Normal	1 to 14
		Scheduled Removal (Terminal)	15
3	7026	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7027	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7028	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7029	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7030	Normal	1 to 14

Values = Clin Obs Range

Individual Animal In-Life Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female	Animal	Observation Type: All Types	From Day 1 (Start Date) to 15 (Start Date)
3	7030	Scheduled Removal (Terminal)	15
4	7036	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7037	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7038	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7039	Normal	1 to 14
		Scheduled Removal (Terminal)	15
	7040	Normal	1 to 14
		Scheduled Removal (Terminal)	15

APPENDIX D: DETAILED CLINICAL OBSERVATIONS SCORING KEY

PRODUCT IDENTIFICATION

Silk Fibroin

Removal from Cage/Hand-held Observations	
Ease of Removal/Handling	0. Slight/moderate resistance – animal is easy to handle, may squirm or vocalize occasionally 1. No resistance – animal is flaccid when being handled 2. High resistance – animal is difficult to handle, and/or squirms continuously 3. Aggressive – biting or lunging behavior specifically directed at handler
Emaciation	0. Absent 1. Present (confirmed using body weights)
Eyes	0. Normal 1. Exophthalmos – abnormal protrusion of eyeball present 2. Enophthalmos – posterior displacement of the eye (sunken eyeball) 3. Eye lesion – mechanical damage or other (e.g., orbital bleeding)
Fur/Skin Appearance	0. Normal 1. Unkempt – coat rough or ungroomed, may be slightly stained 2. Stained/wetness (e.g., ano-genital staining) 3. Hair loss 4. Other – includes but is not limited; eschar, wound, laceration or other skin lesions
Lacrimation	0. Absent 1. Present – lacrimation noticeable 2. Excessive – animal has excessive amount of tearing
Mucous Membranes (color)	0. Normal 1. Blanch to pink tone 2. Dusky rose to deep flush 3. Cyanosis (blue) 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 1. Increased – muscles are rigid; hind limbs will not go through their full range of motion 2. Decreased – muscles are flaccid; hind limbs have little or no resistance to movement
Palpebral Closure	0. Eyes wide open 1. Eyes halfway shut 2. Eyes completely shut
Piloerection	0. Absent 1. Present
Pupillary reflex	0. Normal 1. Slow or absent- pupil reaction is slow or absent.
Respiratory Pattern	0. Normal 1. Slow 2. Rapid 3. Rales (Moist or Dry) 4. Gasping 5. Labored - Dyspnea
Salivation	0. None 1. Present - salivation is noticeable around the edge of the mouth 2. Excessive - salivation extends to the fur around the jaw
Vocalization	0. Absent 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. 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. 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. 3. Hyperactive/Hyper alert – 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 1. Clonic – alternating periods of contraction and relaxation of muscles 2. Tonic – prolonged period of muscle contractions
Defecation	0. None/Normal 1. Soft (partially formed) 2. Diarrhea (watery feces usually of increased volume)
Gait	0. Normal 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). 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. 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). 4. Total gait incapacity – applies when these are severe gait abnormalities or combinations of gait abnormality.
Locomotion (speed and vigor of movement)	0. Normal 1. Somewhat impaired 2. Totally impaired
Other	0. Absent 1. Present 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 1. Hunched – e.g., abnormal posture 2. Flattened (prone) –e.g., limbs spread out lying flat or on one side
Tremors	0. None 1. Slight – e.g., localized involuntary oscillatory movement 2. Severe – e.g., more to more than one area or involving whole body
Twitches	0. None 1. Slight – brief coarse involuntary muscle contraction 2. Moderate – increased frequency and severity 3. Fasciculation – wave-like ripples of a muscle or group of muscles
Unusual Behaviors	0. Absent 1. Present – Stereotypies/Bizarre behavior/Aggression be specific in describing all unusual behaviors on data sheet
Urination	0. None/Normal 1. Excessive
Vocalizations	0. Absent 1. Present

APPENDIX E: INDIVIDUAL ANIMAL DETAILED CLINICAL OBSERVATIONS

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7003	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7004	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7005	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7003	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7004	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7005	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7003	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7004	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7005	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7003	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7004	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7005	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7003	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7004	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7005	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7001	0	0	0	0	0
7002	0	0	0	0	0
7003	0	0	0	0	0
7004	0	0	0	0	0
7005	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7012	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7014	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7015	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7012	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7014	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7015	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7012	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7014	4 !¹	4 !²	0	0	0	0	0	0	0	0	0	0	0	0
7015	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1 [RC:Other- eschar]

2 [RC:eschar- face]

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7012	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7014	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7015	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7012	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7014	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7015	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7011	0	0	0	0	0
7012	0	0	0	0	0
7013	0	0	0	0	0
7014	0	0	0	0	0
7015	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7021	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7022	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7023	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7025	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7021	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7022	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7023	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7025	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7021	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7022	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7023	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7025	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7021	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7022	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7023	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7025	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7021	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7022	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7023	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7025	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7021	0	0	0	0	0
7022	0	0	0	0	0
7023	0	0	0	0	0
7024	0	0	0	0	0
7025	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7031	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7032	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7033	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7034	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7035	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7031	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7032	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7033	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7034	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7035	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7031	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7032	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7033	0	4 !1	0	0	0	0	0	0	0	0	0	0	0	0
7034	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7035	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1 [RC:Tail lesion; Tail was caught in the caging between rack and solid bottom box]

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7031	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7032	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7033	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7034	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7035	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7031	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7032	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7033	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7034	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7035	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7031	0	0	0	0	0
7032	0	0	0	0	0
7033	0	0	0	0	0
7034	0	0	0	0	0
7035	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7006	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7010	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7006	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7010	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7006	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7010	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
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Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7006	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7010	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7006	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7010	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

0 mg/kg/day Group 1	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7006	0	0	0	0	0
7007	0	0	0	0	0
7008	0	0	0	0	0
7009	0	0	0	0	0
7010	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7016	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7017	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7018	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7020	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7016	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7017	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7018	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7020	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7016	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7017	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7018	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7020	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7016	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7017	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7018	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7020	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7016	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7017	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7018	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7020	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

125 mg/kg/day Group 2	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7016	0	0	0	0	0
7017	0	0	0	0	0
7018	0	0	0	0	0
7019	0	0	0	0	0
7020	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7026	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7027	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7029	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7030	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7026	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7027	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7029	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7030	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7026	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7027	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7029	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7030	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7026	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7027	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7029	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7030	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7026	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7027	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7029	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7030	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

250 mg/kg/day Group 3	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7026	0	0	0	0	0
7027	0	0	0	0	0
7028	0	0	0	0	0
7029	0	0	0	0	0
7030	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Removal from Cage)													
	Handling Reactivity	Handling Reactivity	Handling Reactivity	Vocalization (RC)	Vocalization (RC)	Vocalization (RC)	Palpebral Closure	Palpebral Closure	Palpebral Closure	Lacrimation	Lacrimation	Lacrimation	Eyes	Eyes
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7036	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7037	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7038	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7039	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7040	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Removal from Cage)													
	Eyes	Mucous Membranes	Mucous Membranes	Mucous Membranes	Salivation	Salivation	Salivation	Emaciation	Emaciation	Emaciation	Piloerection	Piloerection	Piloerection	Fur/Skin
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7036	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7037	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7038	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7039	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7040	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Removal from Cage)											DetClinObs (Open Field Obs)		
	Fur/Skin	Fur/Skin	Muscle Tone	Muscle Tone	Muscle Tone	Respiratory Pattern	Respiratory Pattern	Respiratory Pattern	Pupillary Reflex	Pupillary Reflex	Pupillary Reflex	Activity/ Arousal	Activity/ Arousal	Activity/ Arousal
	8	15	1	8	15	1	8	15	1	8	15	1	8	15
7036	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7037	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7038	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7039	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7040	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Open Field Obs)													
	Convulsions	Convulsions	Convulsions	Tremors	Tremors	Tremors	Posture	Posture	Posture	Gait	Gait	Gait	Locomotion	Locomotion
	1	8	15	1	8	15	1	8	15	1	8	15	1	8
7036	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7037	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7038	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7039	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7040	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Open Field Obs)													
	Locomotion	Defecation	Defecation	Defecation	Urination	Urination	Urination	Unusual Behaviors	Unusual Behaviors	Unusual Behaviors	Vocalization (OF)	Vocalization (OF)	Vocalization (OF)	Other
	15	1	8	15	1	8	15	1	8	15	1	8	15	1
7036	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7037	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7038	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7039	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7040	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual Animal Detailed Clinical Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Day(s) Relative to Start Date

500 mg/kg/day Group 4	DetClinObs (Open Field Obs)				
	Other	Other	Twitches	Twitches	Twitches
	8	15	1	8	15
7036	0	0	0	0	0
7037	0	0	0	0	0
7038	0	0	0	0	0
7039	0	0	0	0	0
7040	0	0	0	0	0

APPENDIX F: INDIVIDUAL ANIMAL MEAN BODY WEIGHTS

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Bodyweight (g)

0 mg/kg/day Group 1	Day(s) Relative to Start Date		
	1	8	15
7001	241	316	376
7002	231	284	334
7003	212	265	297
7004	239	293	357
7005	258	288	360
Mean	236.2	289.2	344.8
SD	16.7	18.3	30.6
N	5	5	5

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Bodyweight (g)

125 mg/kg/day Group 2	Day(s) Relative to Start Date		
	1	8	15
7011	223	258	301
7012	248	284	316
7013	232	278	351
7014	264	322	387
7015	218	263	304
Mean	237.0	281.0	331.8
SD	18.9	25.3	36.7
N	5	5	5

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Bodyweight (g)

250 mg/kg/day Group 3	Day(s) Relative to Start Date		
	1	8	15
7021	235	281	323
7022	210	274	328
7023	220	376	317 <!1
7024	251	306	372
7025	251	320	380
Mean	233.4	311.4	344.0
SD	18.4	40.6	29.6
N	5	5	5

1 [<, RC:Animal reweighed, food and water checked]

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Bodyweight (g)

500 mg/kg/day Group 4	Day(s) Relative to Start Date		
	1	8	15
7031	239	297	348
7032	247	309	374
7033	219	280	331
7034	223	278	332
7035	255	313	373
Mean	236.6	295.4	351.6
SD	15.4	16.1	21.1
N	5	5	5

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Bodyweight (g)

0 mg/kg/day Group 1	Day(s) Relative to Start Date		
	1	8	15
7006	158	183	211
7007	155	183	207
7008	148	174	193
7009	144	163	184
7010	151	181	208
Mean	151.2	176.8	200.6
SD	5.5	8.6	11.6
N	5	5	5

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Bodyweight (g)

125 mg/kg/day Group 2	Day(s) Relative to Start Date		
	1	8	15
7016	154	176	195
7017	172	177	204
7018	130	147	180
7019	149	172	203
7020	152	175	199
Mean	151.4	169.4	196.2
SD	15.0	12.7	9.7
N	5	5	5

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Bodyweight (g)

250 mg/kg/day Group 3	Day(s) Relative to Start Date		
	1	8	15
7026	143	163	184
7027	165	194	219
7028	151	172	191
7029	154	179	201
7030	150	178	197
Mean	152.6	177.2	198.4
SD	8.0	11.3	13.2
N	5	5	5

Individual Animal Body Weights
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Bodyweight (g)

500 mg/kg/day Group 4	Day(s) Relative to Start Date		
	1	8	15
7036	149	178	201
7037	165	193	221
7038	148	174	201
7039	152	177	195
7040	157	183	206
Mean	154.2	181.0	204.8
SD	7.0	7.4	9.9
N	5	5	5

APPENDIX G: INDIVIDUAL ANIMAL DAILY BODY WEIGHT GAIN

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

0 mg/kg/day Group 1	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7001	10.7	8.6	9.6
7002	7.6	7.1	7.4
7003	7.6	4.6	6.1
7004	7.7	9.1	8.4
7005	4.3	10.3	7.3
Mean	7.57	7.94	7.76
SD	2.27	2.20	1.34
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

125 mg/kg/day Group 2	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7011	5.0	6.1	5.6
7012	5.1	4.6	4.9
7013	6.6	10.4	8.5
7014	8.3	9.3	8.8
7015	6.4	5.9	6.1
Mean	6.29	7.26	6.77
SD	1.33	2.48	1.77
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

250 mg/kg/day Group 3	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7021	6.6	6.0	6.3
7022	9.1	7.7	8.4
7023	22.3	-8.4	6.9
7024	7.9	9.4	8.6
7025	9.9	8.6	9.2
Mean	11.14	4.66	7.90
SD	6.35	7.42	1.24
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Body Weight Gain (g/day)

500 mg/kg/day Group 4	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7031	8.3	7.3	7.8
7032	8.9	9.3	9.1
7033	8.7	7.3	8.0
7034	7.9	7.7	7.8
7035	8.3	8.6	8.4
Mean	8.40	8.03	8.21
SD	0.40	0.88	0.55
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

0 mg/kg/day Group 1	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7006	3.6	4.0	3.8
7007	4.0	3.4	3.7
7008	3.7	2.7	3.2
7009	2.7	3.0	2.9
7010	4.3	3.9	4.1
Mean	3.66	3.40	3.53
SD	0.59	0.55	0.49
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

125 mg/kg/day Group 2	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7016	3.1	2.7	2.9
7017	0.7	3.9	2.3
7018	2.4	4.7	3.6
7019	3.3	4.4	3.9
7020	3.3	3.4	3.4
Mean	2.57	3.83	3.20
SD	1.10	0.80	0.61
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

250 mg/kg/day Group 3	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7026	2.9	3.0	2.9
7027	4.1	3.6	3.9
7028	3.0	2.7	2.9
7029	3.6	3.1	3.4
7030	4.0	2.7	3.4
Mean	3.51	3.03	3.27
SD	0.58	0.36	0.40
N	5	5	5

Individual Animal Mean Daily Body Weight Gain
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Body Weight Gain (g/day)

500 mg/kg/day Group 4	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
7036	4.1	3.3	3.7
7037	4.0	4.0	4.0
7038	3.7	3.9	3.8
7039	3.6	2.6	3.1
7040	3.7	3.3	3.5
Mean	3.83	3.40	3.61
SD	0.23	0.57	0.35
N	5	5	5

APPENDIX H: INDIVIDUAL ANIMAL MEAN DAILY FOOD CONSUMPTION

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

0 mg/kg/day Group 1	Day(s) Relative to Start
	8 → 15
7001	27.3
7002	27.3
7003	27.3
7004	48.5
7005	48.5
Mean	35.77
SD	11.62
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

125 mg/kg/day Group 2	Day(s) Relative to Start
	8 → 15
7011	27.1
7012	27.1
7013	27.1
7014	26.4
7015	26.4
Mean	26.80
SD	0.40
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

250 mg/kg/day Group 3	Day(s) Relative to Start
	8 → 15
7021	21.0
7022	21.0
7023	21.0
7024	30.9
7025	30.9
Mean	24.94
SD	5.40
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Mean Daily Food Consumption (g/day)

500 mg/kg/day Group 4	Day(s) Relative to Start
	8 → 15
7031	30.3
7032	30.3
7033	30.3
7034	27.6
7035	27.6
Mean	29.20
SD	1.49
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

0 mg/kg/day Group 1	Day(s) Relative to Start
	8 → 15
7006	18.5
7007	18.5
7008	18.5
7009	20.1
7010	20.1
Mean	19.17
SD	0.89
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

125 mg/kg/day Group 2	Day(s) Relative to Start
	8 → 15
7016	17.2
7017	17.2
7018	17.2
7019	18.5
7020	18.5
Mean	17.71
SD	0.72
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

250 mg/kg/day Group 3	Day(s) Relative to Start
	8 → 15
7026	18.5
7027	18.5
7028	18.5
7029	20.0
7030	20.0
Mean	19.11
SD	0.81
N	5

Individual Animal Mean Daily Food Consumption
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Mean Daily Food Consumption (g/day)

500 mg/kg/day Group 4	Day(s) Relative to Start
	8 → 15
7036	25.8
7037	25.8
7038	25.8
7039	18.9
7040	18.9
Mean	23.03
SD	3.81
N	5

APPENDIX I: INDIVIDUAL ANIMAL FOOD EFFICIENCY[1]

PRODUCT IDENTIFICATION

Silk Fibroin

[1] Food efficiency = Mean Daily Body Weight Gain / Mean Daily Food Consumption

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Food Efficiency

0 mg/kg/day Group 1	Day(s) Relative to Start
	8 → 15
7001	0.31
7002	0.26
7003	0.17
7004	0.19
7005	0.21
Mean	0.229
SD	0.059
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Food Efficiency

125 mg/kg/day Group 2	Day(s) Relative to Start
	8 → 15
7011	0.23
7012	0.17
7013	0.38
7014	0.35
7015	0.22
Mean	0.271
SD	0.093
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Food Efficiency

250 mg/kg/day Group 3	Day(s) Relative to Start
	8 → 15
7021	0.29
7022	0.37
7023	-0.40
7024	0.31
7025	0.28
Mean	0.167
SD	0.320
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Male Food Efficiency

500 mg/kg/day Group 4	Day(s) Relative to Start
	8 → 15
7031	0.24
7032	0.31
7033	0.24
7034	0.28
7035	0.31
Mean	0.276
SD	0.034
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Food Efficiency

0 mg/kg/day Group 1	Day(s) Relative to Start
	8 → 15
7006	0.22
7007	0.19
7008	0.15
7009	0.15
7010	0.19
Mean	0.178
SD	0.030
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Food Efficiency

125 mg/kg/day Group 2	Day(s) Relative to Start
	8 → 15
7016	0.16
7017	0.22
7018	0.27
7019	0.24
7020	0.19
Mean	0.216
SD	0.046
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Food Efficiency

250 mg/kg/day Group 3	Day(s) Relative to Start
	8 → 15
7026	0.16
7027	0.19
7028	0.15
7029	0.16
7030	0.14
Mean	0.159
SD	0.022
N	5

Individual Animal Mean Food Efficiency
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Sex: Female Food Efficiency

500 mg/kg/day Group 4	Day(s) Relative to Start
	8 → 15
7036	0.13
7037	0.15
7038	0.15
7039	0.14
7040	0.17
Mean	0.148
SD	0.018
N	5

APPENDIX J: ANIMAL NUMBERS, DOSE GROUPS AND FATES

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal Numbers, Dose Groups and Fates
PSL Study Number 50725
Silk Fibroin Solution: A 14-Day Repeat Dose Oral Gavage Range-Finding Study in Rats

Group	Dose Level	Sex	Animal	Cage	Removal Day	Removal Week	Removal Date	Removal Time	Time Slot	Removal Symptom	Pathology Reason
1	0 mg/kg/day	Male	7001	1	15	3	08/07/19	11:25	.	Term	Term
			7002	1	15	3	08/07/19	11:25	.	Term	Term
			7003	1	15	3	08/07/19	11:26	.	Term	Term
			7004	2	15	3	08/07/19	11:26	.	Term	Term
			7005	2	15	3	08/07/19	11:26	.	Term	Term
1	0 mg/kg/day	Female	7006	3	15	3	08/07/19	11:26	.	Term	Term
			7007	3	15	3	08/07/19	11:26	.	Term	Term
			7008	3	15	3	08/07/19	11:26	.	Term	Term
			7009	4	15	3	08/07/19	11:26	.	Term	Term
			7010	4	15	3	08/07/19	11:26	.	Term	Term
2	125 mg/kg/day	Male	7011	5	15	3	08/07/19	11:26	.	Term	Term
			7012	5	15	3	08/07/19	11:26	.	Term	Term
			7013	5	15	3	08/07/19	11:27	.	Term	Term
			7014	6	15	3	08/07/19	11:27	.	Term	Term
			7015	6	15	3	08/07/19	11:27	.	Term	Term
2	125 mg/kg/day	Female	7016	7	15	3	08/07/19	11:27	.	Term	Term
			7017	7	15	3	08/07/19	11:27	.	Term	Term
			7018	7	15	3	08/07/19	11:27	.	Term	Term
			7019	8	15	3	08/07/19	11:27	.	Term	Term
			7020	8	15	3	08/07/19	11:27	.	Term	Term
3	250 mg/kg/day	Male	7021	9	15	3	08/07/19	11:27	.	Term	Term
			7022	9	15	3	08/07/19	11:27	.	Term	Term
			7023	9	15	3	08/07/19	11:27	.	Term	Term
			7024	10	15	3	08/07/19	11:27	.	Term	Term
			7025	10	15	3	08/07/19	11:28	.	Term	Term
3	250 mg/kg/day	Female	7026	11	15	3	08/07/19	11:28	.	Term	Term

Individual Animal Numbers, Dose Groups and Fates
PSL Study Number 50725
Silk Fibroin Solution: A 14-Day Repeat Dose Oral Gavage Range-Finding Study in Rats

Group	Dose Level	Sex	Animal	Cage	Removal Day	Removal Week	Removal Date	Removal Time	Time Slot	Removal Symptom	Pathology Reason
3	250 mg/kg/day	Female	7027	11	15	3	08/07/19	11:28	.	Term	Term
			7028	11	15	3	08/07/19	11:28	.	Term	Term
			7029	12	15	3	08/07/19	11:28	.	Term	Term
			7030	12	15	3	08/07/19	11:28	.	Term	Term
4	500 mg/kg/day	Male	7031	13	15	3	08/07/19	11:28	.	Term	Term
			7032	13	15	3	08/07/19	11:28	.	Term	Term
			7033	13	15	3	08/07/19	11:28	.	Term	Term
			7034	14	15	3	08/07/19	11:29	.	Term	Term
			7035	14	15	3	08/07/19	11:29	.	Term	Term
4	500 mg/kg/day	Female	7036	15	15	3	08/07/19	11:29	.	Term	Term
			7037	15	15	3	08/07/19	11:29	.	Term	Term
			7038	15	15	3	08/07/19	11:29	.	Term	Term
			7039	16	15	3	08/07/19	11:29	.	Term	Term
			7040	16	15	3	08/07/19	11:29	.	Term	Term

APPENDIX K: INDIVIDUAL ANIMAL NECROPSY OBSERVATIONS

PRODUCT IDENTIFICATION

Silk Fibroin

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Animal:	7001	Group:	1	Sex:	Male
		Dose:	0		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7002	Group:	1	Sex:	Male
		Dose:	0		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7003	Group:	1	Sex:	Male
		Dose:	0		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7004	Group:	1	Sex:	Male
		Dose:	0		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7005	Group:	1	Sex:	Male
		Dose:	0		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7006	Group:	1	Sex:	Female
		Dose:	0		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7007 Group: 1 Sex: Female
Dose: 0
Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7008 Group: 1 Sex: Female
Dose: 0
Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7009 Group: 1 Sex: Female
Dose: 0
Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7010 Group: 1 Sex: Female
Dose: 0
Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7011 Group: 2 Sex: Male
Dose: 125
Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7012 Group: 2 Sex: Male
Dose: 125
Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7013 Group: 2 Sex: Male
Dose: 125

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7014 Group: 2 Sex: Male
Dose: 125

Necropsy Date: 7/8/2019

Last Clinical Observations:

Eschar, Face, Superficial

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7015 Group: 2 Sex: Male
Dose: 125

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7016 Group: 2 Sex: Female
Dose: 125

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7017 Group: 2 Sex: Female
Dose: 125

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7018 Group: 2 Sex: Female
Dose: 125

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7019	Group:	2	Sex:	Female
		Dose:	125		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7020	Group:	2	Sex:	Female
		Dose:	125		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7021	Group:	3	Sex:	Male
		Dose:	250		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7022	Group:	3	Sex:	Male
		Dose:	250		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7023	Group:	3	Sex:	Male
		Dose:	250		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7024	Group:	3	Sex:	Male

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Dose: 250

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7025 Group: 3 Sex: Male
Dose: 250

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7026 Group: 3 Sex: Female
Dose: 250

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7027 Group: 3 Sex: Female
Dose: 250

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7028 Group: 3 Sex: Female
Dose: 250

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal: 7029 Group: 3 Sex: Female
Dose: 250

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Animal:	7030	Group:	3	Sex:	Female
		Dose:	250		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7031	Group:	4	Sex:	Male
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7032	Group:	4	Sex:	Male
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7033	Group:	4	Sex:	Male
		Dose:	500		

Necropsy Date: 7/8/2019

Last Clinical Observations:

Lesion, Tail

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7034	Group:	4	Sex:	Male
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7035	Group:	4	Sex:	Male
		Dose:	500		

Necropsy Date: 7/8/2019

Individual Animal Necropsy Observations
PSL Study Number 50725
Silk Fibroin: A 14-Day Repeat Dose Oral Gavage
Range-Finding Study in Rats

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7036	Group:	4	Sex:	Female
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7037	Group:	4	Sex:	Female
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7038	Group:	4	Sex:	Female
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7039	Group:	4	Sex:	Female
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

Animal:	7040	Group:	4	Sex:	Female
		Dose:	500		

Necropsy Date: 7/8/2019

Gross Pathology Observations [Correlation]:

No observations found

Any remaining protocol required tissues, which have been examined, have no visible lesions

APPENDIX G4
MORI SILK 28-DAY ORAL TOXICITY STUDY IN RATS

Product Safety Labs

SILK FIBROIN:
A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RATS

PRODUCT IDENTIFICATION

Silk Fibroin

DATA REQUIREMENTS

OECD Guidelines for Testing of Chemicals and Food Ingredients, Section 4 (Test No. 407):
Health Effects, *Repeated Dose 28-Day Oral Toxicity Study in Rodents* (adopted 1995; updated October 2008)

US FDA Toxicological Principles for the Safety Assessment of Food Ingredients,
Redbook 2000, IV.C. 4. a. *Subchronic Toxicity Studies with Rodents* (2007)

STUDY NUMBER

51651

PERFORMING LABORATORY

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

STUDY COMPLETION DATE

April 2, 2020

STUDY DIRECTOR

SPONSOR

Cambridge Crops, Inc.
444 Somerville Ave.
Somerville, MA 02143

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Silk Fibroin

This study meets the requirements of 21 CFR Part 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.

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

Study Director: [redacted] Date: 04|02|2020

Name of Signer: [redacted]

Name of Company: Product Safety Labs

DocuSigned by:
Sponsor: [redacted] Date: 4/2/2020
347425AC6C4B482...

Name of Signer: [redacted]

Name of Company: Cambridge Crops, Inc.

DocuSigned by:
Submitter: [redacted] Date: 4/2/2020
D030DB2D76D94AD...

Name of Signer [redacted]

Name of Company: Cambridge Crops, Inc.

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		Oct 31, 2019; Feb 11, 2020; Feb 27, 2020	Oct 31, 2019; Feb 17, 2020; Feb 28, 2020
Critical phase inspection: *Study schedule review*		Nov 11, 2019	Nov 11, 2019
Critical phase inspection: *Tissue list in Provantis*		Dec 12, 2019	Dec 12, 2019
Critical phase inspection: *Sample preparation and sampling (Day 1)*		Nov 6, 2019	Nov 6, 2019
Critical phase inspection: *Necropsy with tissue and blood collection (Day 30)*		Dec 5, 2019	Dec 5, 2019
Critical phase inspection: *Clinical Chemistry (Day 31)*		Dec 17, 2019	Dec 17, 2019
Raw data audit		Feb 11-14, 2020	Feb 17, 2020
Draft report review		Feb 27-28, 2020	Feb 28, 2020

QA Statements for the chemical analysis, clinical pathology and histopathology phases of the study may be found in Appendices C, J, and P, respectively.

Final report reviewed by:

Quality Assurance Auditor
Product Safety Labs

4/2/2020
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.

[redacted]

Study Director
Product Safety Labs

04/02/2020
Date

[redacted]

04/02/2020
Date

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STUDY INFORMATION

Protocol No.:	P713.01 CMR
Test Substance:	Silk Fibroin
Physical Description:	Slightly yellow liquid
Dates Test Substance Received:	October 15, 2019
PSL IDs:	191015-2D
PSL Study Number:	51651
Sponsor:	Cambridge Crops, Inc. 444 Somerville Ave. Somerville, MA 02143
Study Initiated-Completed:	November 1, 2019 – (see report cover page)
In-Life Study Initiated-Completed:	November 6 – December 6, 2019
Notebook No.:	51651: pages 1-536

KEY PERSONNEL

Product Safety Labs:

President:

Laboratory Director:

Study Director:

Primary Scientist:

Contributing Personnel:

Director of Quality Assurance:

Technical Writing Supervisor:

The following individuals were responsible for the clinical pathology analysis and evaluation:

Clinical chemistry, hematology, coagulation and urinalysis analyses:	Product Safety Labs 2394 US-Highway 130 Dayton, New Jersey 08810
Principal Investigator:	Victor Ansah-Johnson, BS

KEY PERSONNEL (cont.)

Clinical pathology data evaluation:	Eurofins Advinus 21 & 22, Phase II, Peenya Industrial Area Bengaluru, 560 058, India
Principal Investigator:	

The following facility was responsible for the conduct and reporting of analysis of the neat test substance and dose preparations:

Dose Analysis:	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810
Principal Investigator:	

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

Histological slides preparation:	HSRL Histo-Scientific Research Laboratories 5930 Main Street Mount Jackson, VA 22842
Histology Principal Investigator:	Craig Zook
Histological slide evaluation by:	HSRL Histo-Scientific Research Laboratories 5930 Main Street Mount Jackson, VA 22842
Pathology Principal Investigator:	

1. OBJECTIVE

The objective of this study was to evaluate the potential subchronic toxicity of Silk Fibroin in male and female rats that is 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 sought for each sex.

2. SUMMARY

Eighty adult Crl: Sprague-Dawley CD® IGS rats (40 males and 40 females) were equally distributed into four groups (10/sex/group). Dose levels of 125, 250, and 500 mg/kg/day of Silk Fibroin (Groups 2-4, respectively), as well as a vehicle control (distilled water; Group 1) were selected for the study and administered for 29 days (males) or 30 days (females).

An appropriate amount of the vehicle control (distilled water) or test substance was administered once daily (7 days/week) via oral intubation to each rat for at least 28 days. The test substance was prepared at concentrations of 12.5, 25, and 50 mg/mL, w/v in distilled water. The vehicle control and test substance preparations were administered at a dose volume of 10 mL/kg daily. Samples of the test prep samples were collected at the beginning (Day 1) middle (Day 16) and end of the in-life phase of the study (Day 30) and analyzed to evaluate stability. Samples were also collected from the dose formulation solutions to verify homogeneity and (Day 1) and dose concentration (Days 16 and 30).

All dose preparations were considered to be homogeneously distributed and met the target concentration in the dosing mixtures. Based on the overall neat test substance stability, dose preparation homogeneity, and concentration verification results, the animals are considered to have received target concentrations of Silk Fibroin.

The animals were observed for viability, signs of gross toxicity, and behavior changes at least once daily during the study and weekly for a battery of detailed clinical observations. Body weights were recorded twice during acclimation, including prior to dosing on (Day 1), weekly thereafter, and prior to sacrifice. Individual food consumption was also recorded in conjunction with scheduled body weights. Food efficiency was calculated and reported. Urine and blood samples were collected on Day 30 and 31 (males and females, respectively), for urinalysis hematology, coagulation, and clinical chemistry determinations. Gross necropsies and histological evaluation of selected organs and tissues were performed on all study animals.

There were no mortalities or clinical observations attributed to the test substance administration. There were no changes in mean weekly body weights, daily body weight gain, food consumption and food efficiency attributable to the administration of Silk Fibroin in male or female rats.

There were no test substance-related changes in, hematology, coagulation, serum chemistry and urinalysis parameters. All significant findings were either small in magnitude, did not correlate to any other clinical or histopathological finding, or were within or close to PSL historical controls, and thus were within expected biological variation and of no toxicological relevance. There were no macroscopic, microscopic, or organ weight changes attributable to the test substance administration.

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect-level (NOAEL) for Silk Fibroin, administered orally for 29 days (males) or 30 days (females), was determined to be 500 mg/kg/day for both male and female Sprague-Dawley rats.

3. TEST SUBSTANCE

A. Source

The test substance was provided by the Sponsor.

B. Identification

The test substance was received from the Sponsor and identified using the following information provided by the Sponsor and PSL identification number.

Product Identifier: Silk Fibroin
Composition: 5.0% Silk Fibroin (CAS# 9007-76-5) & 95% Water

Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The Sponsor provided vials of a solution of the test substance at the highest dose concentration:

Identity: Silk fibroin solution
PSL ID: 191015-2D
Batch #: 215
Concentration: 50 mg/mL, aqueous
Physical Description: Slightly yellow liquid
Storage Conditions: -20°C (thawed in refrigerator before use)
Expiration Date: Stable at 4 °C for one month. Upon thawing, please use in one month

C. Analysis

The test substance, as received, was expected to be stable for the duration of the study. Verification of the test substance concentration in the dose preparations were determined as part of this study (Section 6.C) (Amendment 1).

D. Hazards

Appropriate routine safety precautions were exercised in the handling of the test substance and control substances.

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 per sex + 1 control group per sex)

4.A.3 Number of Animals per Group: 20 (10 males, 10 females)

4.A.4 Sex: Male and female. Females were nulliparous and non-pregnant.

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

4.A.6 Age/Weight: Animals were approximately eight 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 in filtered cartons by truck.

On October 29, 2019, eighty-four (84) CRL Sprague-Dawley CD® IGS rats (42M/42F) arrived from Charles River Laboratories (Raleigh, NC) with an assigned birth date of September 13, 2019. The rats were designated by the supplier to be approximately six to seven weeks of age upon arrival.

B. Test System Justification

The Sprague-Dawley® rat is 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. PSL is AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited and certified in the appropriate care of all live experimental animals and maintains current staff training, ensuring animals were handled humanely during the experimental phase of this study and met all guideline standards.

C. Husbandry

4.C.1 Housing

The animals were housed in regularly cleaned 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. In addition, airflow in the animal room was maintained at or above 10 air changes per hour. (Amendment 2).

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

Temperature and humidity was 19-22°C and 12-62%, respectively. Humidity was below the targeted lower limit on 12 days during the study, but no impact on study animals or study data was observed.

4.C.3 Acclimation

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

4.C.4 Feed

2016 Certified Envigo Teklad Global Rodent Diet® was stored in a dedicated temperature and humidity monitored feed storage site and available *ad libitum* during acclimation and throughout the study.

4.C.5 Water

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

4.C.6 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. Results of routine analysis consisting of each lot of feed used in this study were received from Envigo Teklad, Madison, WI. Water analysis was conducted periodically and the records are kept on file at Product Safety Labs. The date of the most recent analysis is reported in the final report (Appendix B).

4.C.7 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 from a few representative control animals, as part of PSL's sentinel health monitoring program (7004M 9/13/19, 7005M 9/13/19, and 7009M 9/13/19). A serum sample was collected from each 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 animals 7004M 9/13/19, 7005M 9/13/19, and 7009M 9/13/19, 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 (Amendment 4).

D. Identification

4.D.1 Cage

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

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 substance was administered by oral gavage.

B. Justification of Route of Administration

The oral route of administration was selected by the Sponsor. This route of administration is recommended in the referenced guidelines (Section 8.C), and a potential route of human exposure.

C. Control of Bias

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

D. Dose Levels

Ten male and ten female rats were randomly assigned to each of the following test groups:

Group	No. Animals/ Group (M/F)	Oral Gavage Dose of Test Substance (mg/kg/day)	Dose Volume (mL/kg)	Concentration mg/mL[b]
1	10/10	0 (Vehicle Control)[a]	10	0
2	10/10	125		12.5
3	10/10	250		25
4	10/10	500		50

[a] Distilled water (Lot #: 20490025; Exp. Date: September 25, 2021).
[b] Appropriate concentrations of the test substance as received in vehicle to achieve the target dose level.

E. Justification of Dose Level Selection

The dose levels of 0 (vehicle control), 125, 250, and 500 mg/kg/day of Silk Fibroin were selected by the Sponsor in consultation with the Study Director, based on the results of a previous study 14-Day range-finding study (PSL 50725, 2020). The high dose was previously selected due to solubility limitations. The high dose is a tolerable dose and was not expected to cause marked toxicity. The low and intermediate dose levels were selected to derive a dose response for any effects observed.

6. GENERAL PROCEDURES

A. Selection of Animals

After acclimating to the laboratory environment for eight 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 within a sex were selected for test. Eighty (80) healthy rats (40 males; 40 females) were selected for test. The animals weighed 211-253 grams (males) and 181-234 grams (females) and were approximately eight weeks of age at initiation of dosing.

B. Dose Preparations and Procedures

6.B.1 Test Substance Preparation

The test substance was provided by the Sponsor at the highest concentration (50 mg/mL). Vials were thawed overnight in refrigerator before use at ambient temperature, and vortexed thoroughly to ensure adequate mixing. Further dilutions were made with distilled water to produce formulations containing 25 (intermediate dose) and 12.5 (low dose) concentrations of the test substance. These were prepared daily. Formulations were mixed until a visually homogeneous mixture was achieved. Preparations of the test substance were documented in the raw data.

6.B.2 Dose Calculations

Individual doses were calculated based on the most recent weekly body weights and were adjusted each week to maintain the targeted dose level for all rats (i.e., mg/kg). All doses

were administered volumetrically at 10 mL/kg. The control group recieved the vehicle only, at the same dose volume as the test animals.

6.B.3 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) for a period of 29 days (males) or 30 days (females). The dose mixtures were maintained 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) with an exception on the day(s) hematology, coagulation, clinical chemistry, and urinalysis samples were collected. Residual dose mixtures were properly discarded following daily administration and sampling (as required).

C. Analysis of Test Substance and Dose Preparations

6.C.1 Sampling

The prepared dosing mixtures were sampled in duplicate. Additional samples were collected and analyzed, at the discretion of the Study Director, to ensure accuracy and homogeneity of the dosing concentrations over the course of the study. Samples not requiring analysis were discarded at study termination.

6.C.2 Homogeneity

At the beginning of the study, formulations of each concentration were prepared according to the procedures as were used on test (Section 6.B.1). Samples from these preparations were collected from the top, middle, and bottom of each concentration of test substance that was prepared in the vehicle. Samples of the vehicle control were collected from the middle of the container only.

6.C.3 Concentration Verification

Dose preparations were sampled at the beginning (as part of the homogeneity assessment, Section 6.C.3), near the middle, and again at the end of the study for verification of dose concentration. Samples were collected from preparation of each concentration of test substance, and one sample from the vehicle control (middle).

6.C.4 Sample Preservation

Samples of dose preparations were stored frozen. Samples were considered stable from the point at which they are frozen. All samples were retained until finalization of the study and discarded following the issuance of the final report.

6.C.5 Sample Analysis

The frozen samples described above were sent to Product Safety Labs Clinical Pathology Laboratory for analysis of dose preparations.

D. Analytical Chemistry

6.D.1 Sample Storage

Upon receipt, all samples were stored and maintained frozen (-20°C) prior to analysis.

6.D.2 Method Validation

Prior to sample analysis, the suitability of the Pierce BCA Protein assay (Catalog # 23225; Thermo Scientific) was demonstrated. Method validation included, but was not limited to determination of linearity, precision, and accuracy. In addition, QC samples were prepared in the vehicle at the low, middle, and high dose concentrations. These samples were analyzed the day they were prepared and then stored frozen. The frozen QC samples were re-analyzed after a storage period of at least the maximum number of days that the dose solutions samples were stored prior to analysis (Amendment 5).

6.D.3 Reference Substance

A Sponsor-provided AB Silk Fibroin solution (Catalog #5154; Advanced Biomatrix, Carlsbad, CA) served as the reference standard.

6.D.4 Chemical Analysis

Samples were analyzed in replicate. A detailed description of the analytical test method(s) was documented. Any remaining sample material will be retained until the issuance of the final report.

6.D.5 Data Reporting

Data was captured on standard raw data sheets and as instrument output, as necessary, and summarized in tabular form.

6.D.6 Analytical Report and Records to be Maintained

A signed, analytical report was provided to the Study Director. This report included the methodology, pertinent measurements, study results, and tabulated results. The finalized analytical chemistry report was provided to the Study Director, to be incorporated into the main study report.

E. 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 the first treatment with the test substance, and weekly thereafter, a detailed clinical observation was conducted while handling the animal, generally occurring on days that the animals were weighed and food consumption measurements were taken. 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.

F. 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 approximately weekly thereafter (intervals of 7 days ± 1). The animals were also weighed prior to sacrifice in order to calculate organ-to-body weight ratios. Body weight gain was calculated for selected intervals and for the study overall. A final fasted body weight was also obtained prior to scheduled terminal sacrifice.

G. Food Consumption and Food Efficiency

Individual food consumption was measured and recorded, to coincide with body weight measurements. Food efficiency was calculated and reported.

H. Clinical Pathology

Clinical pathology was performed on all animals for clinical chemistry, hematology, and coagulation, at necropsy. Blood was collected via the inferior vena cava, under isoflurane anesthesia at terminal sacrifice. All clinical pathology samples were evaluated for quality by visual examination. The animals were fasted overnight prior to blood collection.

6.H.1 Hematology

Approximately 500 µL of blood will be collected in a pre-calibrated tube containing K_2EDTA for hematology assessments. Whole blood samples will be stored under refrigeration or on ice and transferred to the clinical pathology department at Product Safety Labs on cold packs. The following parameters were evaluated.

Erythrocyte count	hemoglobin concentration
hematocrit	mean corpuscular volume
mean corpuscular hemoglobin	red cell distribution width
absolute reticulocytes count	platelet count

total white blood cells and differential leukocyte count

Mean corpuscular hemoglobin concentration was calculated.

In addition, separate, blood smears, stained with Wright-Giemsa stain, were prepared from each animal undergoing hematological evaluation.

6.H.2 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
total bilirubin	urea nitrogen
blood creatinine	total cholesterol
triglycerides	fasting glucose
total serum protein	albumin
globulin	calcium
inorganic phosphorus	sodium
potassium	chloride

Any remaining serum samples will be maintained frozen at approximately -80°C and discarded upon approval of the Sponsor at finalization.

6.H.3 Urinalysis

The day before collection of samples for the clinical pathology evaluations, the animals were placed in metabolism cages. Animals were fasted overnight and urine was collected

from each animal. Urine samples were stored on ice until analysis. The following parameters were evaluated.

quality	pH	ketone
color	glucose	bilirubin
clarity	specific gravity	blood
volume	protein	urobilinogen

microscopic urine sediment examination

6.H.4 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. In addition, a second blood sample was retained during the exsanguination procedure for possible future evaluation, if treatment-related effects were identified. Details of this evaluation will be added by amendment, if applicable. The following parameters were evaluated.

prothrombin time

activated partial thromboplastin time

6.H.5 Clinical Pathology Report

A signed, clinical pathology report was provided to the Study Director. This report included the methodology, pertinent measurements, study results, GLP compliance statement signed by the Principal Investigator, Quality Assurance statement, and tabulated results. Upon completion of the report, the finalized clinical pathology report was transferred to the Study Director to be incorporated into the main study report.

I. Terminal Sacrifice and Histopathology

6.I.1 Scheduled Sacrifice

At terminal sacrifice, all animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals in the study were subjected to a gross necropsy, which included examination of the external surface of the body, all orifices, musculoskeletal system, and the cranial, thoracic, abdominal, and pelvic cavities, with their associated organs and tissues. All gross lesions were recorded. The following tissues were weighed wet as soon as possible after dissection to avoid drying (Amendment 3):

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

The following tissues were weighed at least 24 hours after preservation in 10% neutral buffered formalin:

ventral prostate	thyroid/parathyroid
seminal vesicles with coagulating gland (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 to include 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	

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	testes

6.I.2 Histopathology

Histological examination was performed on the preserved organs and tissues of the animals from both the control and high dose groups (Groups 1 and 4, respectively) and gross lesions noted in any of the test groups at the time of terminal sacrifice were also examined. 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 preparations, and histological assessment by a board-certified veterinary pathologist, were performed at Histo-Scientific Research Laboratories (HSRL).

7. STATISTICAL ANALYSIS

Product Safety Labs performed statistical analysis of all data collected during the in-life phase of the study as well as organ weight data and clinical pathology results. 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 the following software applications: Provantis® version 9, Tables and Statistics, Instem LSS, Staffordshire UK; (Pristima® version 7, Statistical Analysis, Xybion Corporation, Lawrenceville, NJ); and INSTAT, GraphPad Software, San Diego, CA.

A. Statistical Methods

In-Life Data

Means and standard deviations were calculated for all quantitative data. For all in-life endpoints that were identified as multiple measurements of continuous data over time (e.g., body weight parameters, food consumption, and 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; Motulsky, 2014). Significant interactions observed between treatment and time as well as main effects were further analyzed by a *post hoc* multiple comparisons test (e.g., Dunnett's test; Dunnett, 1964 and 1980) of the individual treated groups to control.

Organ Weight Data

When warranted by sufficient group sizes, 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. 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).

B. Statistical Methods (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 was not significant	If preliminary test was significant
Clinical Pathology[a]	Bartlett's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed with Dunnett's test	Log transformations of the data to achieve normality and variance homogeneity were used. If the log transformation 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. 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

Test Facility

In-life portion	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810
Clinical pathology and Dose analysis (clinical chemistry, hematology, coagulation, and urinalysis) and dose formulation analysis	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810 [redacted]

Test Site

Clinical pathology data evaluation	Eurofins Advinus 21 & 22 Phase II, Peenya Industrial Area Bengaluru, 560 058, India Primary Investigator (P.I.): [redacted]
Test Site QA for Clinical Pathology Evaluation	[redacted]
Test Site Management for Clinical Pathology Evaluation	[redacted]
Histological slide preparation	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 P.I. (histology): [redacted]
Histological slide evaluation (Amendment 6)	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 P.I. (pathology): [redacted], [redacted]

B. GLP Compliance

This study was conducted in compliance with the following regulations:

- 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:

- US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, Revised 2007, IV.C. 4. a. Subchronic Toxicity Studies with Rodents (2003)
- OECD Guidelines for Testing of Chemicals, Section 4, Test No. 407: Health Effects, Repeated Dose 28-Day Oral Toxicity Study in Rodents (adopted 1995; updated October 2008)

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 analytical phase report, clinical pathology 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 HSRL and Eurofins Advinus Ltd. For portions of the study conducted by a subcontractor, 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.

The QA Units from HSRL 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

Information on care of the test system, equipment maintenance and calibration, storage, usage, and disposition of the test substance, and all other records that would demonstrate adherence to the protocol will be maintained. Facility records which are not specific to the subject study will be maintained by the testing facility and archived according to PSL SOP

An electronic signed copy of the report will be sent to the Sponsor. The original signed report, together with the protocol and all raw data generated at Product Safety Labs, are 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 may request continued archiving by PSL.

The following records are maintained:

A. Information on test substance includes but is not limited to the following:

Storage	Disposition
Usage	Dose preparation analysis

B. Information on animals includes but is not 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

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. Prepared slides and pathology data will be maintained by Product Safety Labs and/or by HSRL, 5930 Main Street, Mount Jackson, VA, 22842. Dose preparation analysis data will be maintained by Product Safety Labs, 2394 US Highway 130, Dayton, NJ 08810.

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

11. PROTOCOL AND PROTOCOL AMENDMENTS

See Appendix A for the Protocol and Protocol Amendments.

12. RESULTS

A. Test Substance and Dose Preparation Analysis (Tables 1A-B; Appendix C)

All dose preparations were considered to be homogeneously distributed and met the target concentration in the dosing mixtures. Based on the overall neat test substance stability, dose preparation homogeneity, and concentration verification results, the animals are considered to have received target concentrations of Silk Fibroin.

12.A.1 Homogeneity

Homogeneity analysis of the Day 1 dose preparations resulted in a relative standard deviation (RSD) of 10.4, 2.7, and 3.6 for Groups 2-4, respectively. (Table 1A, Appendix C). The test substance was considered to be homogeneously distributed in all dose solutions, to within an acceptable margin of variation.

12.A.2 Concentration Verification

Concentration verification samples were collected on the day of preparation for initial (Day 1, as part of the homogeneity assessment), middle (Day 16), and final preparations (Day 30). The analysis of the Day 1 samples were 104.4, 91.0, and 85.5% of target, the Day 16 samples resulted in 96.2, 99.8 and 90.2% of target, and the Day 30 samples resulted in 128.9, 90.7 and 89.6% of target concentrations of 12.5, 25, and 50 mg/mL of of Silk Fibroin for Groups 2-4, respectively (Table 1B, Appendix C).

B. Mortality and Clinical Observations (Tables 2-3; Appendices D-F and K)

There were no mortalities or clinical signs attributable to the test substance administration.

The fate of all animals is presented in Appendix K.

Males

Incidental in-life clinical signs included: superficial eschar on the head in 1/10 Group 3 animals with corresponding detailed clinical observations of eschar in 1/10 Group 3 animals.

Females

Incidental in-life clinical signs consisted of a unilateral slight swelling of the eye in 1/10 Group 2 animals. There were no corresponding detailed clinical observations findings noted in any of the animals.

C. Body Weight and Body Weight Gain (Table 4-5; Appendices G-H)

There were no test substance-related changes in mean weekly body weights or daily body weight gain attributable to the administration of test substance to male or female rats.

Males

Mean weekly body weights for male rats in Groups 2-4 were comparable to control Group 1 throughout the study.

A significant increase ($p<0.05$) in mean daily body weight gain occurred on Study Days 1-8 for Group 4 males, and a significant decrease ($p<0.05$) on Days 22-29 for Group 2 and Group 3 males as compared to control Group 1 throughout the study.

Females

Mean weekly body weights and daily body weight gain for female rats in Groups 2-4 were comparable to control Group 1 throughout the study.

D. Food Consumption (Table 6; Appendix I)

There were no test substance-related changes in mean daily food consumption or mean food efficiency attributable to the administration of test substance to male or female rats.

Mean daily food consumption for male and female rats in Groups 2-4 were comparable to control Group 1 throughout the study.

E. Clinical Pathology (Appendix J)

Administration of test substance Silk Fibroin by oral gavage route in Sprague Dawley rats for at least 28 consecutive days at dose levels of 0, 125, 250 and 500 mg/kg/day did not induce any test substance-related changes in hematology, coagulation, clinical chemistry and urinalysis parameters.

12.E.1 Hematology

There were no test substance-related changes in hematology parameters on Day 30 (males) and 31 (females).

All the changes in clinical chemistry were considered unrelated to test substance, because they occurred sporadically, were considered due to biological variance among rats as magnitude of variation was minimal.

12.E.2 Coagulation

There were no test substance-related changes in hematology parameters on Day 30 (males) and 31 (females).

12.E.3 Clinical Chemistry

There were no test substance-related changes in hematology parameters on Day 30 (males) and 31 (females).

All the changes in clinical chemistry were considered unrelated to test substance, because they occurred sporadically, were considered due to biological variance among rats as magnitude of variation was minimal.

12.E.4 Urinalysis

There were no test substance-related changes in hematology parameters on Day 30 (males) and 31 (females).

F. Sacrifice, Macroscopic Observations, and Histopathology (Tables 7-10; Appendices L-P)

The gross findings at terminal sacrifice on Day 30/31, were considered incidental, of the nature commonly observed in rats (background findings) and/or were of similar incidence in control and dosed rats and were not considered related to administration of test substance. No test substance-related microscopic findings were noted in terminal sacrifice animals on Day 30/31. The microscopic findings observed were considered incidental (background findings), of the nature commonly observed in rats, and/or were of similar incidence and severity in the control and dosed animals and were not considered related to the administration of the test substance.

12.F.1 Macroscopic

The gross findings at terminal sacrifice were considered incidental, of the nature commonly observed in rats (background findings) and/or were of similar incidence in control and dosed rats and were not considered related to administration of Silk Fibroin.

Animals 7009 and 7029 had macroscopic findings of unilateral (right) small and/or flaccid testes correlated microscopically with testicular atrophy. Animal 7009 had macroscopic findings of small right epididymis that microscopically correlated epididymal atrophy. Females in Groups 1 through 4 with a macroscopic finding of a fluid filled uterus was consistent with normal estrogen cycling of female rats.

12.F.2 Microscopic

No Silk Fibroin-related microscopic findings were noted in terminal sacrifice animals on Day 30/31. The microscopic findings observed were considered incidental (background findings), of the nature commonly observed in rats, and/or were of similar incidence and severity in the control and dosed animals and were not considered related to the administration of the test substance.

12.F.3 Organ Weights and Ratios

All absolute and relative organ weights in treated male and female rats were comparable to the respective controls.

13. CONCLUSION

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect-level (NOAEL) for Silk Fibroin, administered orally for over 28 days, was determined to be 500 mg/kg/day for both male and female Sprague-Dawley rats.

14. REFERENCES

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TABLE 1A: CHEMICAL ANALYSIS RESULTS

Results for Homogeneity

Group	Target Dose Level (mg/mL)	Sampling Location	Average Conc. (mg/mL)	Overall Average Conc. (mg/mL)	% of Target[1]	Average % of Target	%RSD
1	0	Middle	0	NA	NA	NA	NA
2	12.5	Top	12.7	13.1	101.5	104.4	10.4
		Middle	11.9		95.3		
		Bottom	14.6		116.4		
3	25	Top	22.1	22.8	88.2	91.0	2.7
		Middle	23.1		92.3		
		Bottom	23.1		92.6		
4	50	Top	42.5	42.7	85.0	85.5	3.6
		Middle	41.3		82.7		
		Bottom	44.4		88.7		

NA = Not Applicable

ND = None Detected

[1] % of Target = [Average Test Substance in dose (mg/mL)/Target Dose Concentration (mg/mL)] x 100.

TABLE 1B: CHEMICAL ANALYSIS RESULTS

Results for Concentration Verification

Study Day	Group	Dose Level (mg/mL)	Average Conc. (mg/mL)	% of Target
1	1	0	0	NA
	2	12.5	13.1	104.4
	3	25	22.8	91.0
	4	50	42.7	85.5
16	1	0	0	NA
	2	12.5	12.0	96.2
	3	25	24.9	99.8
	4	50	45.1	90.2
30	1	0	0	NA
	2	12.5	16.1	128.9
	3	25	22.7	90.7
	4	50	44.8	89.6

NA = Not Applicable

TABLE 2: SUMMARY OF IN-LIFE CLINICAL OBSERVATIONS

Day numbers relative to Start Date

Sex: Male

		0 mg/kg/day	125 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Eschar[1]					
	Number of Observations	.	.	4	.
	Number of Animals	.	.	1	.
	Days from - to	.	.	27 30	.

[1] Observation was not expected to be test substance related.

Day numbers relative to Start Date

Sex: Female

	0 mg/kg/day	125 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Swelling[1]				
Number of Observations	.	1	.	.
Number of Animals	.	1	.	.
Days from - to	.	3 3	.	.

[1] Observation was not expected to be test substance related.

TABLE 3: SUMMARY OF DETAILED CLINICAL OBSERVATIONS

Males

Days 1, 8, 15, 22, and 29

Group	1	2	3	4
Dose Level (mg/kg/day)	0	125	250	500
Number of Animals in Group	10	10	10	10
Observations During Removal From Cage and Handling	**Score[1]**			
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	1(4)[2]	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

[1] 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.

[2] Other: Superficial eschar, head (observation was not expected to be test substance related).

TABLE 3 (cont.): SUMMARY OF DETAILED CLINICAL OBSERVATIONS

Females

Days 1, 8, 15, 22, and 29

Group	1	2	3	4
Dose Level (mg/kg/day)	0	125	250	500
Number of Animals in Group	10	10	10	10
Observations During Removal From Cage and Handling	**Score[1]**			
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

[1] 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 WEEKLY BODY WEIGHTS

Bodyweight (g)

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1	Mean	231.9	232.2	233.0	231.7
	SD	12.5	12.3	13.6	12.6
	N	10	10	10	10
8	Mean	276.6	282.2	284.5	289.9
	SD	17.3	11.6	16.0	16.9
	N	10	10	10	10
15	Mean	332.1	327.5	335.2	340.8
	SD	17.4	14.2	14.4	20.1
	N	10	10	10	10
22	Mean	380.2	372.8	379.1	392.6
	SD	26.5	15.6	18.9	20.5
	N	10	10	10	10
29	Mean	428.6	410.1	416.6	438.2
	SD	26.9	18.7	18.4	22.3
	N	10	10	10	10

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: 5% significance level Time Factor: 1% significance level Group Factor: Not significant

Bodyweight (g)

Sex: Female Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1	Mean	204.2	204.9	205.3	205.9
	SD	13.9	12.8	13.3	11.8
	N	10	10	10	10
8	Mean	224.3	222.6	226.4	221.4
	SD	14.2	16.7	16.0	14.8
	N	10	10	10	10
15	Mean	243.2	240.9	241.5	239.7
	SD	16.0	23.7	18.0	16.2
	N	10	10	10	10
22	Mean	256.3	252.4	257.0	255.7
	SD	17.2	26.1	19.9	21.5
	N	10	10	10	10
29	Mean	268.6	267.0	269.3	273.2
	SD	18.4	30.1	23.9	25.3
	N	10	10	10	10

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: Not significant Time Factor: 1% significance level Group Factor: Not significant

TABLE 5: SUMMARY OF MEAN DAILY BODY WEIGHT GAIN

Mean Daily Body Weight Gain (g/day)

Sex: Male Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1 → 8	Mean	6.39 *1	7.14	7.36	8.31 *3
	SD	1.52	1.23	0.84	1.17
	N	10	10	10	10
8 → 15	Mean	7.93	6.47	7.24	7.27
	SD	2.14	0.62	0.92	1.03
	N	10	10	10	10
15 → 22	Mean	6.87	6.47	6.27	7.40
	SD	1.93	1.49	0.93	0.74
	N	10	10	10	10
22 → 29	Mean	6.91 *1	5.33 *3	5.36 *3	6.51
	SD	1.55	0.81	0.93	1.42
	N	10	10	10	10
1 → 29	Mean	7.03 **2	6.35	6.56	7.38
	SD	0.76	0.75	0.40	0.69
	N	10	10	10	10

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: 5% significance level Time Factor: 1% significance level Group Factor: 1% significance level

1 [* - (All Groups) Test: 2 Way ANOVA 5% significance level]
2 [** - (All Groups) Test: 2 Way ANOVA 1% significance level]
3 [* - Test: 2 Way ANOVA 5% significance level]

Mean Daily Body Weight Gain (g/day)

Sex: Female Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
1 → 8	Mean	2.87	2.53	3.01	2.21
	SD	0.82	0.96	0.68	1.07
	N	10	10	10	10
8 → 15	Mean	2.70	2.61	2.16	2.61
	SD	1.42	1.38	1.11	1.07
	N	10	10	10	10
15 → 22	Mean	1.87	1.64	2.21	2.29
	SD	1.37	1.25	0.91	1.25
	N	10	10	10	10
22 → 29	Mean	1.76	2.09	1.76	2.50
	SD	1.35	1.03	1.16	1.65
	N	10	10	10	10
1 → 29	Mean	2.30	2.22	2.29	2.40
	SD	0.42	0.72	0.54	0.64
	N	10	10	10	10

Statistical Test: 2 Way ANOVA Transformation: Automatic
Interaction Factor: Not significant Time Factor: 5% significance level Group Factor: Not significant

TABLE 6: SUMMARY OF FOOD CONSUMPTION BY CAGE

Day numbers relative to Start Date

Group	Sex		From: 1 / To: 6	6 / 8	8 / 13	13 / 15	15 / 20	20 / 22	22 / 27	27 / 29
1	m	Mean	25.12	23.60	27.40	29.70	28.88	27.50	29.80	28.20
		S.D.	1.63	3.85	1.93	2.29	2.31	1.79	2.51	2.61
		N	5	5	5	5	5	5	5	5
2	m	Mean	24.80	25.50	26.70	28.95	28.26	26.00	27.94	25.80
		S.D.	1.08	1.55	0.75	0.96	2.39	2.38	1.31	1.60
		N	5	5	5	5	5	5	5	5
3	m	Mean	25.72	26.30	28.36	30.35	28.54	27.65	28.86	27.55
		S.D.	1.90	2.42	1.48	2.01	1.56	0.38	1.28	1.51
		N	5	5	5	5	5	5	5	5
4	m	Mean	26.16	27.95*	28.56	29.30	29.52	29.60	30.90	29.35
		S.D.	1.43	1.47	1.30	2.29	0.73	2.07	1.28	2.32
		N	5	5	5	5	5	5	5	5

Food consumption units are g/animal/day

Group 1 - 0 mg/kg/day Group 1 Group 2 - 125 mg/kg/day Group 2
Group 3 - 250 mg/kg/day Group 3 Group 4 - 500 mg/kg/day Group 4

Day numbers relative to Start Date

Group	Sex		From: 1 / To: 6	6 / 8	8 / 13	13 / 15	15 / 20	20 / 22	22 / 27	27 / 29
1	f	Mean	20.42	19.75	20.88	22.20	20.28	18.85	20.60	18.65
		S.D.	1.34	1.66	1.69	1.62	1.46	1.42	1.70	1.97
		N	5	5	5	5	5	5	5	5
2	f	Mean	19.62	18.90	20.24	22.95	20.14	17.70	20.28	18.90
		S.D.	0.98	0.95	0.84	1.05	1.36	2.76	2.09	0.76
		N	5	5	5	5	5	5	5	5
3	f	Mean	19.88	19.50	20.02	21.45	19.96	19.10	20.60	18.70
		S.D.	1.51	1.79	1.79	1.42	1.49	1.23	1.57	1.93
		N	5	5	5	5	5	5	5	5
4	f	Mean	20.88	20.95	20.52	23.70	21.38	19.90	22.06	21.65*
		S.D.	1.88	1.55	0.70	1.69	1.49	2.93	1.69	1.81
		N	5	5	5	5	5	5	5	5

Food consumption units are g/animal/day

Group 1 - 0 mg/kg/day Group 1 Group 2 - 125 mg/kg/day Group 2
Group 3 - 250 mg/kg/day Group 3 Group 4 - 500 mg/kg/day Group 4

TABLE 7: SUMMARY OF NECROPSY OBSERVATIONS

Removal Reason: Killed Terminal	Male				Female			
	0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4	0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Number of Animals	10	10	10	10	10	10	10	10
testes-combined								
Submitted	10	10	10	10				
right: small	0	1						
flaccid	0	1						
right: flaccid	1	0						
uterus								
Submitted					10	10	10	10
fluid filled					4	3	5	1
epididymides-combined								
Submitted	10	10	10	10				
right: small	1							

TABLE 8: SUMMARY OF MEAN TERMINAL BODY AND ORGAN WEIGHTS

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Terminal BW (g)	Day 30	Mean	394.4	383.7	389.7	403.4
		SD	28.4	23.1	20.6	17.5
		N	10	10	10	10
Adrenal Glands Wt (g)	Day 30	Mean	0.0677	0.0684	0.0636	0.0708
		SD	0.0070	0.0065	0.0073	0.0110
		N	10	10	10	10
Brain Wt (g)	Day 30	Mean	2.190	2.217	2.225	2.203
		SD	0.082	0.100	0.071	0.086
		N	10	10	10	10
Epididymides Wt (g)	Day 30	Mean	1.0288	1.0398	1.0341	1.0403
		SD	0.1236	0.1334	0.1161	0.0977
		N	10	10	10	10
Heart Wt (g)	Day 30	Mean	1.330	1.290	1.378	1.409
		SD	0.243	0.153	0.149	0.101
		N	10	10	10	10
Kidneys Wt (g)	Day 30	Mean	2.961	2.868	2.891	3.005
		SD	0.266	0.300	0.257	0.367
		N	10	10	10	10
Liver Wt (g)	Day 30	Mean	11.992	11.860	12.789	13.046
		SD	1.702	1.254	1.750	2.104
		N	10	10	10	10
SV&CG Wt (g)	Day 30	Mean	1.324	1.420	1.312	1.540
		SD	0.199	0.234	0.213	0.291
		N	10	10	10	10
Spleen Wt (g)	Day 30	Mean	0.826	0.775	0.792	0.881
		SD	0.067	0.083	0.124	0.130
		N	10	10	10	10
Testes Wt (g)	Day 30	Mean	3.538	3.489	3.442	3.477
		SD	0.287	0.463	0.250	0.205
		N	10	10	10	10

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Thymus Wt (g)	Day 30	Mean	0.4691	0.4047	0.5215	0.5134
		SD	0.1203	0.1243	0.0869	0.1651
		N	10	10	10	10
Thyroid-Parathyroid Wt (g)	Day 30	Mean	0.0233	0.0293	0.0275	0.0278
		SD	0.0060	0.0062	0.0050	0.0042
		N	10	10	10	10
Ventral Prostate Wt (g)	Day 30	Mean	0.655	0.753	0.711	0.796
		SD	0.136	0.125	0.191	0.132
		N	10	10	10	10

Sex: Female	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Terminal BW (g)	Day 31	Mean	251.5	247.7	253.8	253.3
		SD	17.3	28.2	21.9	19.2
		N	10	10	10	10
Adrenal Glands Wt (g)	Day 31	Mean	0.0813	0.0829	0.0808	0.0811
		SD	0.0073	0.0099	0.0078	0.0100
		N	10	10	10	10
Brain Wt (g)	Day 31	Mean	2.005	2.020	2.035	2.075
		SD	0.128	0.115	0.046	0.116
		N	10	10	10	10
Heart Wt (g)	Day 31	Mean	0.935	0.941	0.924	0.917
		SD	0.051	0.153	0.082	0.075
		N	10	10	10	10
Kidneys Wt (g)	Day 31	Mean	1.936	1.847	1.920	1.927
		SD	0.131	0.281	0.073	0.215
		N	10	10	10	10
Liver Wt (g)	Day 31	Mean	8.390	8.382	8.669	8.546
		SD	0.894	1.430	0.750	1.171
		N	10	10	10	10
Ovaries with Oviducts Wt (g)	Day 31	Mean	0.1352	0.1232	0.1361	0.1341
		SD	0.0222	0.0114	0.0142	0.0150
		N	10	10	10	10
Spleen Wt (g)	Day 31	Mean	0.577	0.582	0.529	0.592
		SD	0.080	0.095	0.053	0.067
		N	10	10	10	10
Thymus Wt (g)	Day 31	Mean	0.4545	0.3845	0.4432	0.4426
		SD	0.0758	0.0777	0.0824	0.0754
		N	10	10	10	10
Thyroid-Parathyroid Wt (g)	Day 31	Mean	0.0226	0.0257	0.0243	0.0264
		SD	0.0056	0.0055	0.0026	0.0038
		N	10	10	10	10
Uterus Wt (g)	Day 31	Mean	0.743	0.682	0.849	0.648
		SD	0.286	0.249	0.368	0.280
		N	10	10	10	10

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

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Adrenal /TBW (Ratio)	Day 30	Mean	0.1719	0.1790	0.1636	0.1753
		SD	0.0159	0.0218	0.0203	0.0248
		N	10	10	10	10
Brain /TBW (Ratio)	Day 30	Mean	5.571	5.789	5.721	5.466
		SD	0.327	0.283	0.302	0.209
		N	10	10	10	10
Epididymides /TBW (Ratio)	Day 30	Mean	2.6177	2.7204	2.6523	2.5800
		SD	0.3377	0.3983	0.2448	0.2262
		N	10	10	10	10
Heart /TBW (Ratio)	Day 30	Mean	3.362	3.361	3.544	3.494
		SD	0.480	0.335	0.413	0.222
		N	10	10	10	10
Kidneys /TBW (Ratio)	Day 30	Mean	7.505	7.470	7.415	7.438
		SD	0.336	0.575	0.469	0.710
		N	10	10	10	10
Liver /TBW (Ratio)	Day 30	Mean	30.337	30.889	32.740	32.199
		SD	2.900	2.283	3.416	4.055
		N	10	10	10	10
SV&CG /TBW (Ratio)	Day 30	Mean	3.356	3.709	3.363	3.820
		SD	0.458	0.608	0.486	0.719
		N	10	10	10	10
Spleen /TBW (Ratio)	Day 30	Mean	2.101	2.020	2.032	2.178
		SD	0.196	0.185	0.295	0.265
		N	10	10	10	10
Testes /TBW (Ratio)	Day 30	Mean	9.020	9.107	8.841	8.622
		SD	1.053	1.183	0.577	0.409
		N	10	10	10	10
Thymus /TBW (Ratio)	Day 30	Mean	1.1843	1.0449	1.3381	1.2696
		SD	0.2733	0.2798	0.2136	0.4040
		N	10	10	10	10

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Thyroid-Parathyroid /TBW (Ratio)	Day 30	Mean	0.59412	0.75964	0.70711	0.68952
		SD	0.16255	0.13100	0.13645	0.10037
		N	10	10	10	10
Ventral Prostate /TBW (Ratio)	Day 30	Mean	1.663	1.960	1.816	1.971
		SD	0.323	0.281	0.436	0.294
		N	10	10	10	10

Sex: Female	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Adrenal /TBW (Ratio)	Day 31	Mean	0.3248	0.3363	0.3206	0.3211
		SD	0.0383	0.0368	0.0433	0.0406
		N	10	10	10	10
Brain /TBW (Ratio)	Day 31	Mean	7.994	8.249	8.066	8.222
		SD	0.592	0.999	0.646	0.612
		N	10	10	10	10
Heart /TBW (Ratio)	Day 31	Mean	3.726	3.789	3.649	3.623
		SD	0.217	0.287	0.266	0.185
		N	10	10	10	10
Kidneys /TBW (Ratio)	Day 31	Mean	7.721	7.439	7.601	7.611
		SD	0.647	0.532	0.508	0.687
		N	10	10	10	10
Liver /TBW (Ratio)	Day 31	Mean	33.317	33.728	34.258	33.689
		SD	1.957	3.333	2.860	3.494
		N	10	10	10	10
Ovaries with oviducts/TBW (Ratio)	Day 31	Mean	0.5363	0.5014	0.5375	0.5301
		SD	0.0675	0.0555	0.0504	0.0523
		N	10	10	10	10
Spleen /TBW (Ratio)	Day 31	Mean	2.293	2.348	2.086	2.341
		SD	0.261	0.257	0.141	0.247
		N	10	10	10	10
Thymus /TBW (Ratio)	Day 31	Mean	1.8188	1.5547	1.7538	1.7494
		SD	0.3537	0.2623	0.3460	0.2707
		N	10	10	10	10
Thyroid-Parathyroid /TBW (Ratio)	Day 31	Mean	0.89527	1.05138	0.96663	1.04373
		SD	0.19509	0.26845	0.16191	0.13698
		N	10	10	10	10
Uterus /TBW (Ratio)	Day 31	Mean	2.974	2.714	3.363	2.534
		SD	1.207	0.780	1.483	0.957
		N	10	10	10	10

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

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Adrenal /BrW (Ratio)	Day 30	Mean	0.0309	0.0309	0.0286	0.0321
		SD	0.0029	0.0028	0.0035	0.0045
		N	10	10	10	10
Epididymides /BrW (Ratio)	Day 30	Mean	0.4703	0.4691	0.4653	0.4724
		SD	0.0564	0.0580	0.0554	0.0419
		N	10	10	10	10
Heart /BrW (Ratio)	Day 30	Mean	0.608	0.583	0.621	0.641
		SD	0.113	0.079	0.080	0.053
		N	10	10	10	10
Kidneys /BrW (Ratio)	Day 30	Mean	1.352	1.293	1.300	1.363
		SD	0.105	0.118	0.120	0.152
		N	10	10	10	10
Liver /BrW (Ratio)	Day 30	Mean	5.470	5.344	5.747	5.905
		SD	0.676	0.429	0.766	0.829
		N	10	10	10	10
Seminal vesicles /BrW (Ratio)	Day 30	Mean	0.604	0.641	0.591	0.700
		SD	0.084	0.103	0.105	0.134
		N	10	10	10	10
Spleen /BrW (Ratio)	Day 30	Mean	0.377	0.350	0.356	0.399
		SD	0.031	0.041	0.055	0.051
		N	10	10	10	10
Testes /BrW (Ratio)	Day 30	Mean	1.617	1.572	1.549	1.579
		SD	0.143	0.186	0.128	0.084
		N	10	10	10	10
Thymus /BrW (Ratio)	Day 30	Mean	0.2150	0.1824	0.2342	0.2312
		SD	0.0579	0.0543	0.0372	0.0672
		N	10	10	10	10
Thyroid-Parathyroid /BrW (Ratio)	Day 30	Mean	0.00107	0.00132	0.00124	0.00126
		SD	0.00029	0.00028	0.00023	0.00020
		N	10	10	10	10

Sex: Male	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Ventral Prostate /BrW (Ratio)	Day 30	Mean	0.298	0.340	0.319	0.361
		SD	0.057	0.058	0.085	0.052
		N	10	10	10	10

Sex: Female	Day(s) Relative to Start Date		0 mg/kg/day Group 1	125 mg/kg/day Group 2	250 mg/kg/day Group 3	500 mg/kg/day Group 4
Adrenal /BrW (Ratio)	Day 31	Mean	0.0407	0.0410	0.0397	0.0391
		SD	0.0040	0.0044	0.0037	0.0048
		N	10	10	10	10
Heart /BrW (Ratio)	Day 31	Mean	0.467	0.468	0.454	0.443
		SD	0.020	0.088	0.036	0.039
		N	10	10	10	10
Kidneys /BrW (Ratio)	Day 31	Mean	0.967	0.917	0.944	0.929
		SD	0.062	0.153	0.040	0.096
		N	10	10	10	10
Liver /BrW (Ratio)	Day 31	Mean	4.192	4.154	4.258	4.120
		SD	0.434	0.711	0.339	0.544
		N	10	10	10	10
Ovaries with oviducts/BrW (Ratio)	Day 31	Mean	0.0675	0.0611	0.0669	0.0646
		SD	0.0104	0.0061	0.0065	0.0061
		N	10	10	10	10
Spleen /BrW (Ratio)	Day 31	Mean	0.287	0.289	0.260	0.286
		SD	0.032	0.053	0.026	0.033
		N	10	10	10	10
Thymus /BrW (Ratio)	Day 31	Mean	0.2268	0.1903	0.2175	0.2124
		SD	0.0345	0.0371	0.0386	0.0262
		N	10	10	10	10
Thyroid-Parathyroid /BrW (Ratio)	Day 31	Mean	0.00112	0.00128	0.00119	0.00127
		SD	0.00024	0.00029	0.00013	0.00016
		N	10	10	10	10
Uterus /BrW (Ratio)	Day 31	Mean	0.373	0.340	0.418	0.311
		SD	0.147	0.133	0.185	0.129
		N	10	10	10	10

APPENDIX A: PROTOCOL AND PROTOCOL AMENDMENTS

PRODUCT IDENTIFICATION

Silk Fibroin

SILK FIBROIN: A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RATS

PRODUCT IDENTIFICATION

Silk Fibroin

PSL PROTOCOL NO.

P713.01 CMR

PERFORMING LABORATORY

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

PSL STUDY NUMBER

51651

STUDY DIRECTOR

SPONSOR

Cambridge Crops, Inc.
444 Somerville Ave.
Somerville, MA 02143

TABLE OF CONTENTS

1. TITLE OF STUDY: SILK FIBROIN: A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RATS

2. OBJECTIVE

The objective of this study is to evaluate the potential subchronic toxicity of Silk Fibroin 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) will be determined.

3. STUDY DIRECTOR

Study Director
Tel: 732-438-5100 x1542
Email:

4. NAME AND ADDRESS OF THE TESTING FACILITY

5. SPONSOR

Cambridge Crops, Inc.
444 Somerville Ave.
Somerville, MA 02143

6. SPONSOR REPRESENTATIVE

Cambridge Crops, Inc.
444 Somerville Ave.
Somerville, MA 02143
Tel: 301-580-3965
Email:

7. DATES

Proposed In-Life Start Date: November 5, 2019

Proposed Experimental Termination Date: December 4, 2019

8. TEST SUBSTANCE

9. NAME AND ADDRESS OF THE TESTING FACILITY

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

9.A Source

The test substance will be provided by the Sponsor.

9.B Identification

The test substance is identified using the following information provided by the Sponsor:

Product Identifier: Silk Fibroin
Composition: 5.0% Silk Fibroin (CAS# 9007-76-5) & 95% Water

Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

Sponsor has provided vials of a solution of the test substance at the highest dose concentration:

Identity: Silk fibroin solution
PSL ID: 191015-2D
Batch #: 215
Concentration: 50 mg/mL, aqueous
Physical Description: Slightly yellow liquid
Storage Conditions: -20°C (thawed in refrigerator before use)
Expiration Date: Stable at 4 °C for one month. Upon thawing, please use in one month

9.C Analysis

The test substance, as received, is expected to be stable for the duration of the study. The stability of the test substance and verification of the test substance in the dose preparations will be determined as part of this study (Section 10.C).

9.D Hazards

Appropriate routine safety precautions will be exercised in the handling of the test and control substances 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 levels + 1 control group)

10.A.3 Number of Animals per Group: 20 (10 males, 10 females)

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: Seven to eight 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 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 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.

PSL is AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited and certified in the appropriate care of all live experimental animals and maintains current staff training, ensuring animals will be handled humanely during the experimental phase of this study, and will meet all guideline standards[1].

10.C Husbandry

10.C.1 Housing

The animals will be housed in cages which conform to the size recommendations in the latest *Guide for the Care and Use of Laboratory Animal*[1]. Litter paper placed beneath the cage will be changed at least three times/week. 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 23 ± 3°C and 30-70%, respectively. Observed ranges will be documented in the raw data.

10.C.2 Acclimation

The animals will be conditioned to the housing facilities for a minimum of 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® will be stored in a dedicated temperature and humidity monitored feed storage site and will be available *ad libitum* during acclimation and throughout the study except for times of fasting.

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 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 consisting of each lot of feed used in this study will be received from Envigo Teklad, 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 naïve 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).

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

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 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.

11. EXPERIMENTAL DESIGN

11.A Route of Administration

The test substance will be administered by oral gavage.

11.B Justification of Route of Administration

The oral route of administration will be used because it is recommended in the referenced guidelines (Section 14.C.), and because human exposure may occur via this route.

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 test groups:

Group	No. Animals/ Group (M/F)	Oral Gavage Dose of Test Substance (mg/kg/day)	Dose Volume (mL/kg)	Concentration (mg/mL)[b]
1	10/10	0 (Vehicle Control)[a]	10	0
2	10/10	125		12.5
3	10/10	250		25
4	10/10	500		50

[a] Distilled Water
[b] Appropriate concentrations of the test substance as received in vehicle to achieve the target dose level

11.E Justification of Dose Level Selection

The dose levels of 0, (vehicle control), 125, 250, and 500 mg/kg/day of Silk Fibroin were selected by the Sponsor in consultation with the Study Director and was based on a previous 14-Day range-

finding study (PSL Study#50725)[1]. The high dose was previously selected because of solubility limitations. The high dose is a tolerable dose and is not expected to cause marked toxicity. The intermediate and low dose levels were selected to derive a dose-response for any effects observed.

12. GENERAL PROCEDURES

12.A Selection of Animals

Eighty healthy male and female rats will be used on test. Animals will be selected for this study on the basis of adequate body weight gain, freedom from 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.

12.B Dose Preparations and Procedures

12.B.1 Test Substance Preparation

The test substance has been provided by the Sponsor at the highest concentration (50 mg/mL). Vials will be thawed overnight in refrigerator before use, and vortexed two (2) times in five (5) second intervals, with a ten (10) second waiting period between each vortex before use. Further dilutions will be made with distilled water to produce formulations containing 25 (intermediate dose) and 12.5 (low dose) concentrations of the test substance. These will be prepared daily. Formulations will be mixed until a visually homogeneous mixture is achieved. Preparations of the test substance will be documented in the raw data.

12.B.2 Dose Calculations

Individual doses will be calculated based on the most recent weekly body weights and will be adjusted to maintain the targeted dose level for all rats (i.e. mg/kg). All doses will be administered volumetrically at 10 mL/kg. The control group will receive vehicle only, at the same dose volume as the test animals.

12.B.3 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 once daily (7 days/week). The first day of administration will be considered Day 1 of the study. Dosing will be at approximately the same time each day (± 2 hours) with an exception on the day(s) hematology and/or clinical chemistry, and urinalysis samples are collected. Prepared dosing formulations remaining will be discarded following each administration and sampling (as required).

12.C Analysis of Test Substance and Dose Preparations

12.C.1 Sampling

The prepared dosing mixtures will be sampled in duplicate. 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. Samples not requiring analysis will be discarded at study termination.

[1] Silk Fibroin: A 14-day repeat dose oral gavage ranger finder study in rats; PSL#50725 (report in preparation).

12.C.2 Homogeneity

At the beginning of the study, formulation of each concentration will be prepared according to the procedures as will be used on test (Section 11.B). Samples from these preparations will be collected from the top, middle, and bottom of each concentration of test substance that was prepared in the vehicle. Sample of the vehicle control will be collected from the middle of the container only.

12.C.3 Concentration Verification

Dose preparations will be sampled at the beginning (as part of the homogeneity assessment, Section 12.C.2), near the middle, and again at the end of the study for verification of dose concentration. Samples will be collected from preparations of each concentration of test substance and one sample from the control (middle).

12.C.4 Sample Preservation

Samples of dose preparations will be stored frozen. Samples will be considered stable from the point at which they are frozen.

12.C.5 Sample Analysis

The frozen samples described above will be sent to Product Safety Labs Clinical Pathology Lab for analysis of dose preparations.

12.D Analytical Chemistry

12.D.1 Sample Storage

Upon receipt, all samples will be stored and maintained frozen (approximately -20°C) prior to analysis.

12.D.2 Method Validation

Prior to sample analysis, the suitability of the Pierce BCA Protein assay (Catalog # 23225; Thermo Scientific) will be demonstrated. Method validation will include, but is not limited to determination of linearity, precision, and accuracy. In addition, QC samples will be prepared in the vehicle at the low, middle, and high dose concentrations. These samples will be analyzed the day they are prepared and then stored frozen. The frozen QC samples will be re-analyzed after a storage period of at least the maximum number of days that the dose solutions samples were stored prior to analysis.

12.D.3 Reference Substance

A Sponsor-provided AB Silk Fibroin solution (Catalog #5154; Advanced Biomatrix, Carlsbad, CA) will serve as the reference standard.

12.D.4 Chemical Analysis

Samples will be analyzed in replicate. A detailed description of the analytical test method(s) will be documented. Any remaining sample material will be retained until the issuance of the final report.

12.D.5 Data Reporting

Data will be captured on standard raw data sheets and as instrument output, as necessary, and summarized in tabular form.

12.D.6 Analytical Report and Records to be Maintained

A signed, analytical report will be provided to the Study Director. This report will include the methodology, pertinent measurements, study results, and tabulated results. The finalized analytical chemistry report will be provided to the Study Director, to be incorporated into the main study report.

12.E 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 test substance administration, 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, stereotypes (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 will be promptly notified of severe/remarkable clinical observations and 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.F Body Weight and Body Weight Gain

Individual body weights will be recorded at least two times during acclimation. Test animals will be weighed on Day 1 (prior to study start) and approximately weekly thereafter (intervals of 7 days ± 1) for all animals. Decedents need not be weighed. Body weight gain will be calculated for selected intervals and for the study overall. A final fasted body weight will also be obtained prior to scheduled terminal sacrifice.

12.G Food Consumption and Food Efficiency

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

12.H Clinical Pathology

Clinical pathology will be performed on all surviving animals for clinical chemistry, hematology, and coagulation once, prior to or at necropsy. Blood will be collected via the inferior vena cava, under isoflurane anestbesia at terminal sacrifice. All clinical pathology samples will be evaluated for quality by visual examination. The animals will be fasted overnight prior to blood collection.

12.H.1 Hematology

Approximately 500 µL of blood will be collected in a pre-calibrated tube containing K_2EDTA for hematology assessments. Whole blood samples will be stored under refrigeration or on ice and transferred to the clinical pathology department at Product Safety Labs on cold packs. The following parameters will be evaluated.

erythrocyte count (RBC)	hemoglobin concentration (HGB)
hematocrit (HCT)	mean corpuscular volume (MCV)
mean corpuscular hemoglobin (MCH)	red cell distribution width (RDW)
absolute reticulocyte count (ARET)	platelet count (PLT)

total white blood cell (WBC) and differential leukocyte count

Mean corpuscular hemoglobin concentration (MCHC) will be calculated.

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

12.H.1 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 amino transferase (AST)	serum alanine aminotransferase (ALT)
sorbitol dehydrogenase (SDH)	alkaline phosphatase (ALKP)
total bilirubin (BILI)	urea nitrogen (BUN)
blood creatinine (CREA)	total cholesterol (CHOL)
triglycerides (TRIG)	fasting glucose (GLUC)
total serum protein (TP)	albumin (ALB)
globulin (GLOB)	calcium (CALC)
inorganic phosphorus (IPHS)	sodium (NA)
potassium (K)	chloride (CL)

12.H.1 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 overnight and urine will be collected from each animal. Urine samples will be stored on ice or under refrigeration until analysis.

quality (QUAL)	pH	ketone (KET)
color (COL)	glucose (UGLC)	bilirubin (UBIL)
clarity (CLAR)	specific gravity (SG)	blood (BLD)
volume (UVOL)	protein (UMTP)	urobilinogen (URO)

microscopic urine sediment examination

12.H.1 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. In possible, a second blood sample will be retained during the exsanguination procedure for future possible evaluation if treatment-related effects are identified. Details of this evaluation will be added by amendment. The following parameters will be evaluated.

prothrombin time (PT)

activated partial thromboplastin time (APTT)

12.H.2 Clinical Pathology Report

A signed, clinical pathology report will be provided to the Study Director. This report will include, but not be limited to the methodology, pertinent measurements, study results, a GLP compliance statement signed by the Principal Investigator (Section 13.B), a Quality Assurance statement, and tabulated results. The finalized clinical pathology report will be provided to the Study Director, to be incorporated into the main study report.

Any remaining serum samples will be maintained frozen at approximately -80°C and discarded upon approval of the Sponsor at finalization.

12.I Terminal Sacrifice and Histopathology

12.I.1 Scheduled Sacrifice

At respective terminal sacrifice, all survivors will be euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals in the study (including decedents) will be subjected to a gross necropsy, which will include examination of the external surface of the body, all orifices, musculoskeletal system, and the cranial, thoracic, abdominal, and pelvic cavities, with their associated organs and tissues. All gross lesions will be recorded.

The following tissues (of all animals sacrificed by design) will be weighed wet as soon as possible after dissection to avoid drying:

adrenals (combined)	kidneys (combined)	spleen
brain	liver	thymus
epididymides (combined)	heart	testes (combined)
Uterus	Ovaries w/o oviducts	

The following tissues will be weighed at least 24 hours after preservation in 10% neutral buffered formalin

ventral prostate	thyroid/parathyroid
seminal vesicles with coagulating gland (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	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
	lymph node mesenteric	
	mammary gland	
	nasal turbinates	
	nose	

cecum	ovaries	stomach
cervix	oviducts	thymus
colon	pancreas	thyroid
duodenum	parathyroid	trachea
esophagus	peripheral nerve (sciatic)	urinary bladder
Harderian gland	pharynx	uterus
heart	pituitary gland	vagina

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

eyes	optic nerve
epididymides	testes

Additional tissues will be preserved if indicated by signs of toxicity or target organ involvement. All tissues collected and preserved at terminal sacrifice will be scheduled for pick-up by courier and delivered to Histo-Scientific Research Laboratories (HSRL).

12.I.2 Unscheduled Sacrifice

Any rat that dies or is sacrificed 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 at the scheduled terminal sacrifices. Blood need not be collected from animals sacrificed prior to study termination unless requested by the Study Director.

12.I.3 Histopathology

Histological examination will be performed on the preserved organs and tissues of the animals from both the control and high dose groups (Groups 1 and 4, respectively) as well as from any animal that dies during the course of the study. In addition, gross lesions noted in any test groups at the time of terminal sacrifice will also be examined. These examinations may be extended to other tissues and organs from the low and intermediate groups at the request of Pathologist in consultation with the Study Director and Sponsor to further investigate changes observed in the high dose group. 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 HSRL (Section 15.A).

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, Staffordshire UK; Pristima® version 7.2.0, Statistical Analysis, Xybion Corporation, Lawrenceville, NJ; INSTAT or Prism Biostatistics,

GraphPad Software, San Diego, CA; Statview, version 5, SAS Institute Inc., Cary, NC; Pheonix 8.1, Certara USA, Inc., Princeton, NJ; 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, and 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.

[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.

13.B Statistical Methods (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[1] 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).

[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.

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

14. FINAL REPORT

A report of the results of this study will accurately describe all methods used for generation and analysis of the data. This report will include, but not be limited to, the following information:

- individual animal data (and averages where appropriate) for actual concentration of test substance received; time of observation of each abnormal sign and its subsequent course;
- body weights food consumption, and food efficiency values (if applicable);
- hematology, clinical chemistry, coagulation, and urinalysis results;
- organ weights, organ to body weight and organ to brain weight ratios;
- necropsy and pathology findings;
- dose preparation analysis;
- a compliance statement signed by the Study Director that states whether or not 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 states whether or not the report accurately reflects the raw data obtained during the performance of the study.

[1] Shapiro, S.S. & Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4), 591–611.

15. STUDY CONDUCT

15.A Laboratory

Test Facility

In-life portion	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810
Clinical pathology and Dose analysis (clinical chemistry, hematology, coagulation, and urinalysis), and dose formulation analysis	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810

Test Site

Clinical pathology data evaluation	Eurofins Advinus 21 & 22, Phase II, Peenya Industrial Area Bengaluru, 560 058, India Prospective P.I.(s):
Test Site QA for Clinical Pathology Evaluation	
Test Site Management for Clinical Pathology Evaluation	
Histological slide preparation	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 P.I. (histology):
Histological slide evaluation	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 Prospective P.I.(s) (pathology):

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.

15.C Test Procedure Guidelines

This study design is based on the following guidelines:

- OECD Guidelines for Testing of Chemicals, Section 4, Test No. 407: Health Effects, Repeated Dose 28-Day Oral Toxicity Study in Rodents (adopted 1995; updated October 2008). US EPA Health Effects Test Guidelines: OPPTS 870.3050 Repeated Dose 28-day Oral Toxicity Study in Rodents (2000).
- US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, Revised 2007 IV.C. 4. a. *Subchronic Toxicity Studies with Rodents* (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 phase report, clinical pathology phase report, and final report 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 HSRL and Eurofins Advinus Ltd. 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 Unit from HSRL and Eurofins Advinus Ltd 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

An electronic signed copy of the report, will be sent to the Sponsor. The original signed report, together with the protocol and all raw data generated at Product Safety Labs, 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 substance will include but not be limited to the following:

Storage	Dose preparation analysis
Usage	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. Prepared slides and pathology data will be maintained by Product Safety Labs and/or by HSRL, 5930 Main Street, Mount Jackson, VA, 22842. Dose preparation analysis data will be maintained by Product Safety Labs, 2394 US Highway 130, Dayton, NJ 08810.

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, and described in the final report.

19. DISPOSITION OF TEST SUBSTANCE

A reserve sample of the test substance and records of sample disposition will be maintained at Product Safety Labs. All remaining test substance will be retained for at least one year from receipt, unless otherwise specified by the Sponsor. All remaining test substance will be returned to the Sponsor unless otherwise directed.

Product Safety Labs

28-Day Oral Toxicity Study
Protocol # P713.01 CMR
PSL ID: 191015-2D
Study No: 51651

20. PROTOCOL APPROVAL

Signature: [redacted]
Sponsor Representative
Cambridge Crops, Inc.

Date: November 1, 2019

Signature [redacted]
Study Director
Product Safety Labs

Date: Nov 1, 2019

Signature: [redacted]
President
Product Safety Labs

Date: Nov. 1, 2019

21. PROTOCOL REVIEW:

Signature [redacted]
Director, Quality Assurance
Product Safety Labs

Date: Nov 1, 2019

Product Safety Labs

PROTOCOL AMENDMENT

SILK FIBROIN: A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 CMR **AMENDMENT NO.:** 1 - 3

STUDY NO.: 51651 **PSL NO.:** 191015-2D

Amendment # 1

Protocol Section: 9.C Analysis

Change from: The test substance, as received, is expected to be stable for the duration of the study. The stability of the test substance and verification of the test substance in the dose preparations will be determined as part of this study (Section 10.C).

Change to: The test substance, as received, is expected to be stable for the duration of the study. Verification of the test substance concentration in the dose preparations will be determined as part of this study (Section 12.C).

Amendment # 2

Protocol Section: 10.C.0 Housing

Change from: The animals will be housed in cages which conform to the size recommendations in the latest Guide for the Care and Use of Laboratory Animal1. Litter paper placed beneath the cage will be changed at least three times/week. 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 23 ± 3°C and 30-70%, respectively. Observed ranges will be documented in the raw data

Change to: The animals will be housed in regularly cleaned cages which conform to the size recommendations in the latest Guide for the Care and Use of Laboratory Animals. 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. Observed 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.

Amendment # 3

Protocol Section: 12.I.1 Scheduled Sacrifice

Change from: At respective terminal sacrifice, all survivors will be euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals in the study (including decedents) will be subjected to a gross necropsy, which will include examination of the external surface of the body, all orifices, musculoskeletal system, and the cranial, thoracic, abdominal, and pelvic cavities, with their associated organs and tissues. All gross lesions will be recorded.

The following tissues (of all animals sacrificed by design) will be weighed wet as soon as possible after dissection to avoid drying:

adrenals (combined)	kidneys (combined)	spleen
brain	liver	thymus
epididymides (combined)	heart	testes (combined)
Uterus	**Ovaries w/o oviducts**	

Product Safety Labs

Change to: At respective terminal sacrifice, all survivors will be euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals in the study (including decedents) will be subjected to a gross necropsy, which will include examination of the external surface of the body, all orifices, musculoskeletal system, and the cranial, thoracic, abdominal, and pelvic cavities, with their associated organs and tissues. All gross lesions will be recorded.

The following tissues (of all animals sacrificed by design) will be weighed wet as soon as possible after dissection to avoid drying:

adrenals (combined)	kidneys (combined)	spleen
brain	liver	thymus
epididymides (combined)	heart	testes (combined)
Uterus	**Ovaries with oviducts**	

REASON: Clarification of Protocol and study requirements

Effective Date: November 5th, 2019

[redacted]

Study Director
Product Safety Labs

11/05/2019
Date

Product Safety Labs

PROTOCOL AMENDMENT

SILK FIBROIN: A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 CMR AMENDMENT NO.: 4

STUDY NO.: 51651 PSL NO.: 191015-2D

PROTOCOL SECTION: **10.C.6 Viral Screen**

Change from
Serum samples from naïve 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).

Change to: (change in bold)
Serum samples from **few representative control 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).

Reason:

Use serum from control animals for viral screen.

EFFECTIVE DATE: 2/17/2020

[redacted] 2/17/2020

Study Director
Product Safety Laboratories

Date

Product Safety Labs

PROTOCOL AMENDMENT

SILK FIBROIN: A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RATS

PROTOCOL NO.: P713.01 CMR **AMENDMENT NO.:** 5

STUDY NO.: 51651 **PSL NO.:** 191015-2D

AMENDMENT (various sections): Additional method validation will be done to improve upon the method as it currently exists. Method validation will include samples of unused test substance to evaluate differences specific to the test substance use with this kit. Other modifications may be made as deemed necessary and will be documented. If improvements in the method can be achieved, the additional set of samples that were collected ("B" samples) will be analyzed and the results will be used to replace the original results ("A" Samples).

REASON: The results of the original analysis were below the concentrations expected by the Sponsor based on qualification of the samples prior to being shipped to PSL. As a result, it is believed that the original method validation may not have been sufficiently robust and therefore additional method validation is expected to improve upon the method being used and to yield more accurate results.

Effective Date: March 04, 2020

[redacted]

03|04|2020
Date

Study Director
Product Safety Labs

Product Safety Labs

PROTOCOL AMENDMENT

SILK FIBROIN: A 28-DAY ORAL GAVAGE TOXICITY STUDY IN RAT'S

PROTOCOL NO.: P713.01 CMR **AMENDMENT NO**: 6

STUDY NO.: 51651 **PSL NO.:** 191015-2D

PROTOCOL SECTION: 15 Study Conduct

Change From:

Histological slide evaluation	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 Prospective P.I.(s) (pathology) [redacted]

Change To:

Histological slide evaluation	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 Prospective P.I.(s) (pathology): [redacted]

REASON: Addition of a Prospective P.I and clarification of responsibilities.

Effective Date: March 30, 2020

[redacted] 03/30/2020

Study Director
Product Safety Labs

Date

APPENDIX B: FEED, WATER, AND SEROLOGY ANALYSES

PRODUCT IDENTIFICATION

Silk Fibroin

APPENDIX B: FEED

+++ ENVIGO

Teklad Certified Global 16% Protein Rodent Diet

Lot Number	2016C-090619MA
Date of Manufacture	06Sep2019
Report Date	16Sep2019

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.

2019.09.16 13:40:02 -05'00'

Analysis	Result (%)
Proximate Analysis	
Protein	16.80
Fat	3.62
Fiber	3.67
Moisture	11.38
Ash	5.11
Calcium	0.94
Phosphorus	0.79

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	0.29	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 December 2019, 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.

PRECISION ANALYTICAL SERVICES, INC.
NJDEP Lab Cert # 15001
2181 WHITESVILLE ROAD TOMS RIVER, NJ 08755 PHONE 732-914-1515 FAX 732-914-1616

CERTIFICATE OF ANALYSIS

Customer: Product Safety Labs
2394 Route 130
Dayton, NJ 08810

Project Info: Quarterly Sampling - 4th Quarter
PAS Project ID: P19-11584
Purpose of Test: Regulatory Samples
Conditioner: Canister

Matrix: Drinking Water
Report Date: 12/24/2019

PAS Sample ID	Sample Location	Analysis	Results	Units	DF	PQL	MRL	MCL	Method	Date Sampled	Date Analyzed
P19-11584-01	Cage Wash Sink	Copper	0.159	mg/L	1	0.050	0.0226	1.30*	SM 3111 B	12/17/19 11:25	12/18/19 15:28
P19-11584-01	Cage Wash Sink	Zinc	0.0067 J	mg/L	1	0.025	0.0036	5.00	SM 3111 B	12/17/19 11:25	12/18/19 11:04
P19-11584-01	Cage Wash Sink	Lead	ND	mg/L	1	0.002	0.0009	0.015*	SM 3113 B	12/17/19 11:25	12/20/19 12:17
P19-11584-01	Cage Wash Sink	E. Coli / Colilert	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9223 B	12/17/19 11:25	12/17/19 17:40
P19-11584-01	Cage Wash Sink	Total Coliform / Colilert	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9223 B	12/17/19 11:25	12/17/19 17:40
P19-11584-02	Room 13 Sink	Copper	ND	mg/L	1	0.050	0.0226	1.30*	SM 3111 B	12/17/19 11:35	12/18/19 15:24
P19-11584-02	Room 13 Sink	Zinc	ND	mg/L	1	0.025	0.0036	5.00	SM 3111 B	12/17/19 11:35	12/18/19 11:08
P19-11584-02	Room 13 Sink	Lead	ND	mg/L	1	0.002	0.0009	0.015*	SM 3113 B	12/17/19 11:35	12/20/19 12:29
P19-11584-02	Room 13 Sink	E. Coli / Colilert	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9223 B	12/17/19 11:35	12/17/19 17:40
P19-11584-02	Room 13 Sink	Total Coliform / Colilert	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9223 B	12/17/19 11:35	12/17/19 17:40
P19-11584-03	Room 29 Pressure Sta Hose	Copper	0.0917	mg/L	1	0.050	0.0226	1.30*	SM 3111 B	12/17/19 11:45	12/18/19 15:28
P19-11584-03	Room 29 Pressure Sta Hose	Zinc	0.0128 J	mg/L	1	0.025	0.0036	5.00	SM 3111 B	12/17/19 11:45	12/18/19 11:09
P19-11584-03	Room 29 Pressure Sta Hose	Lead	ND	mg/L	1	0.002	0.0009	0.015*	SM 3113 B	12/17/19 11:45	12/20/19 12:55
P19-11584-03	Room 29 Pressure Sta Hose	E. Coli / Colilert	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9223 B	12/17/19 11:45	12/17/19 17:40
P19-11584-03	Room 29 Pressure Sta Hose	Total Coliform / Colilert	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9223 B	12/17/19 11:45	12/17/19 17:40
P19-11584-04	Sipper Bottle	E. Coli	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9221 E + MUG	12/17/19 11:50	12/18/19 12:40
P19-11584-04	Sipper Bottle	Total Coliform	Neg Col/100 mL	Col/100mL	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9222 B	12/17/19 11:50	12/18/19 12:40
P19-11584-05	Sipper Top	E. Coli	Absent	Pres/Abs	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9221 E + MUG	12/17/19 11:50	12/18/19 12:40
P19-11584-05	Sipper Top	Total Coliform	Neg Col/100 mL	Col/100mL	1	1 Col/100mL	1 Col/100mL	0 Col/100mL	SM 9222 B	12/17/19 11:50	12/18/19 12:40

Except for the parameters tested, PAS makes no representation as to the fitness or quality of the water sample taken.

DF = Dilution Factor
MCL = Maximum Contaminant Level
PQL = Practical Quantitation Limit
MRL = Minimum Reporting Limit
ND = Analyte(s) Not Detected
J = Estimated Result
* = Federal Action Limit

All samples are analyzed in accordance with New Jersey Department of Environmental Protection protocols.

APPENDIX B (cont.): SEROLOGY

In December 2019, serology from representative control animals residing in Room #48, was obtained from collected blood serum for a battery of common viral and microbiologic pathogens.

The representative control animals were in Room #48 from November 6 – December 6, 2019, for the duration of the study. Blood samples were collected on December 6, 2019.

LABORATORY: IDEXX BioAnalytics
4011 Discovery Drive
Columbia, MO 65201

Results of the serology analyses for the representative control animals corresponding with this study are reported as samples 7004M 9/13/19, 7005M 9/13/19, and 7009M 9/13/19. 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 # 6643-2020

Received: 2/20/2020
Completed: 2/21/2020

Submitted By

[redacted]
Product Safety Labs
2394 US Highway 130
Dayton, NJ 08810

Phone: 732-438-5100 ext [redacted]
Email: [redacted]

Specimen Description

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

Purchase Order #: US003456567

Client ID	Investigator	Room #	Species	Strain /Breed	Sex	Study #	Age
7004M 9.13.19	Raghu Gowda	48	rat	CD/CRL	M	51651	3M
7005M 9.13.19	Raghu Gowda	48	rat	CD/CRL	M	51651	3M
7009M 9.13.19	Raghu Gowda	48	rat	CD/CRL	M	51651	3M

Services/Tests Performed: Primary Serology Profile (1-3)

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

Summary: All test results were negative.

SEROLOGY SUMMARY

	7004M 2.19.20	7005M 2.19.20	7009M 2.19.20
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

IDEXX BioAnalytics

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 # 6643-2020

Received: 2/20/2020
Completed: 2/21/2020

SEROLOGY DETAILS

	Baseline	7004M 2.19.20	7005M 2.19.20	7009M 2.19.20
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 > 3,250	-	-	-
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: CHEMICAL ANALYSIS

PRODUCT IDENTIFICATION

Silk Fibroin

Product Safety Labs

Project Title:

Analysis of Samples from Study
Silk Fibroin
A 28-Day Oral Gavage Toxicity Study in Rats

SPONSOR

Cambridge Crops, Inc
444 Somerville Ave
Somerville, MA 02143

ANALYTICAL REPORT

TEST SUBSTANCE

Silk Fibroin

AUTHOR

[redacted]

REPORT COMPLETION DATE

April 2, 2020

PERFORMING LABORATORY

Analytical Services
Product Safety Labs
2394 US Highway 130
Dayton, NJ 08810

PROJECT IDENTIFICATION NUMBER

Product Safety Labs Study Number 51651

Product Safety Labs

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Silk Fibroin

The analytical phase of this study meets the requirements of 21 CFR Part 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.

Principal Investigator: [redacted] Date: 4/6/2020

Name of Signer: [redacted]

Name of Company: Product Safety Labs

Product Safety Labs

QUALITY ASSURANCE STATEMENT

The Product Safety Labs' Quality Assurance Unit has reviewed this analytical 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	[redacted]	Oct 31, 2019; Mar 20, 2020;	Oct 31, 2019; Mar 23, 2020;
Critical phase inspection: *Standard and sample preparation for BCA Protein Assay*	[redacted]	Feb 14, 2020	Feb 14, 2020
Raw data audit	[redacted]	Mar 20 & 23, 2020	Mar 23, 2020
Draft report review	[redacted]	Mar 20 & 23, 2020	Mar 23, 2020

Final Analytical report reviewed by:

[redacted]
Quality Assurance Auditor
Product Safety Labs

April 2, 2020
Date

Product Safety Labs

SIGNATURE

Silk Fibroin

I, 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.

[redacted]

Principal Investigator
Product Safety Labs

4/9/2020
Date

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STUDY INFORMATION

Protocol No:	P713.01 CMR
Test Substance:	Silk Fibroin
Physical Description:	Slightly yellow liquid
Dates Test Substance Received:	October 15, 2019
PSL IDs:	191015-2D
PSL Study Number:	51651
Sponsor:	Cambridge Crops, Inc 444 Somerville Ave. Somerville, MA 02143
Dates of Analysis:	February 18 - March 6, 2020
Analytical Principal Investigator:	[redacted]
Primary Chemist:	
Study Director	

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1. SUMMARY

This report presents the dose analysis phase of PSL Study Number 51651: Silk Fibroin: A 28-Day Oral Gavage Toxicity Study in Rats. Dose mixture samples were collected from all dose concentrations for homogeneity (HO) on Day 1. Concentration verification (CV) samples were collected on Days 16 and 30. All samples were transferred to the Analytical Services laboratory of Product Safety Labs. The active ingredients (as % or mg/mL) were determined in each of the samples using the BCA protein assay. This method was validated in terms of linearity. All samples were stored refrigerated prior to testing and thawed just prior to analysis.

Samples:

Samples for Homogeneity (HO; T = top; M = middle, B = bottom; Day 1)

HO 1 BM
HO 2 BT
HO 3 BM
HO 4 BB
HO 5 BT
HO 6 BM
HO 7 BB
HO 8 BT
HO 9 BM
HO 10 BB

Samples Concentration Verification (CV; Days 16 and 30):

CV 1 A
CV 2 A
CV 3 A
CV 4 A
CV 5 A
CV 5 A
CV 7 A
CV 8 A

2. PROCEDURE

A. Reference Standard(s)

Name: Silk
PSL ID: 191015-2D
Batch #: 215
Concentration: 50 mg/mL, aqueous
Physical Description: Slightly yellow liquid
Expiration Date: Stable at 4 °C for one month. Upon thawing, please use in one month.

Note: The neat test substance was used as the reference standard. No purity correction was applied in order to report results as percent test substance (versus percent active ingredient).

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B. Chemical Analysis

2.B.1 Standard Preparation

Three sets of 6 microcentrifuge tubes were labeled from #1 to #6 and prepared as noted below (for all three replicates). The tubes were vortexed five times in 1 second intervals to mix well. The standards were used for linearity determination.

- Tube #1, 50 µL of silk + 450 µL of deionized.
- Tube #2, 100 µL from Tube #1 + 400 µL of deionized water.
- Tube #3, 375 µL from Tube #2 + 225 µL of deionized water.
- Tube #4, 400 µL from Tube #3 + 100 µL of deionized water.
- Tube #5, 200 µL from Tube #4 + 300 µL of deionized water.
- Tube #6, 125 µL from Tube #5 + 375 µL of deionized water.

Final Silk Concentrations Prepared from Serial Dilutions

Tube Number	1	2	3	4	5	6
Silk Concentration mg/mL	5.0	1.0	0.625	0.5	0.2	0.05

2.B.2 QC Preparation

QCs were prepared using Albumin Standard provided in the Pierce BCA Protein Assay Kit (Lot #UI289347; Ref: 23225) in a 1: 5 dilution.

- 3 microcentrifuge tubes were labeled QC-1, QC-2, QC-3
- 100 µL of the Albumin Standard was added to 400 µL of deionized water in tube QC-1 and repeated for QC-2 and QC-3. The tubes were vortexed.

2.B.3 BCA Protein Assay

All dose mixture samples were brought to room temperature and prepared for testing using the below two steps. Results from the initial and repeat samples were averaged and reported.

A 1:10 dilution was prepared using a deep well plate. One line of wells was labeled "1:10". A volume of 450 µL of deionized water was added to 3 wells. The samples were inverted 5 times and 50 µL was added to the 3 wells. The plate was covered and vortex five times 2-second intervals.

A 1:100 Dilution was prepared from the stock. The next line of wells was labeled "1:100". A volume of 450 µL of deionized water was added to 3 wells and 50 µL was added from 1:10 to 1:100. The plate was covered and vortex five times 2-second intervals.

Working Reagent (WR) Preparation

The following formula was used to determine the total volume of WR required.

0.3 mL x 96 x # of Plates Needed = Total WR Required

Round to nearest whole number

Total WR Required / 50 = Volume of Reagent B

Total WR Required – Volume of Reagent B = Volume of Reagent A

The volume of BCA Reagent A was added to a centrifuge tube of appropriate size. The volume of BCA Reagent B was added to the same centrifuge tube. The WR was gently inverted 10 times or until well mixed.

Assay

A volume of 9 µL of each silk standard, deionized water, and 1:100 dilutions were pipetted into the appropriate microplate wells and 260 µL of WR was added to each well. The plates were placed into an incubator set to 37°C for 30 minutes on a slow shaker. The plates were allowed to cool to room temperature for 5 minutes. The absorbance was then measured on a plate reader at 562 nm.

2.B.4 Method Performance

Linearity. In triplicate, reference standards 1-6 were assayed using the BCA assay and the wavelengths were recorded. The correlation coefficient, r, was ≥ 0.995 for all three linearity curves.

2.B.5 Calculations

All calculations were performed using Excel with full precision. Minor differences may be found between the values reported and those obtained if calculated manually.

Calc. Conc. (mg/mL) = (Value - Intercept) / Slope

Note: Intercept and Slope being specific to the standard curve of each plate

Dose Conc. (mg/mL) = Calc. Conc.*(Final Vol. First/Dilution First)*(Final Vol. Second/Dilution Second)

% Target = Average (mg/mL) / Dose level (mg/mL) *100

3. RESULTS

Summaries of results are presented in Tables 1A-1B. Instrument conditions are presented in Table 2. Linearity results are reported in Table 3. Results of homogeneity and concentration verification of the test substance in the dosing solutions are presented in Tables 4 and 5, respectively.

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TABLE 1A: CHEMICAL ANALYSIS RESULTS[1]

Results for Homogeneity

Group	Target Dose Level (mg/mL)	Sampling Location	Average Conc. (mg/mL)	Overall Average Conc. (mg/mL)	% of Target[2]	Average % of Target	%RSD
1	0	Middle	0	NA	NA	NA	NA
2	12.5	Top	12.7	13.1	101.5	104.4	10.4
		Middle	11.9		95.3		
		Bottom	14.6		116.4		
3	25	Top	22.1	22.8	88.2	91.0	2.7
		Middle	23.1		92.3		
		Bottom	23.1		92.6		
4	50	Top	42.5	42.7	85.0	85.5	3.6
		Middle	41.3		82.7		
		Bottom	44.4		88.7		

NA = Not Applicable

ND = None Detected

[1] See Amendment 5.

[2] % of Target = [Average Test Substance in dose (mg/mL)/Target Dose Concentration (mg/mL)] x 100.

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TABLE 1B: CHEMICAL ANALYSIS RESULTS[1]

Results for Concentration Verification

Study Day	Group	Dose Level (mg/mL)	Average Conc. (mg/mL)	% of Target
1	1	0	0	NA
	2	12.5	13.1	104.4
	3	25	22.8	91.0
	4	50	42.7	85.5
16	1	0	0	NA
	2	12.5	12.0	96.2
	3	25	24.9	99.8
	4	50	45.1	90.2
30	1	0	0	NA
	2	12.5	16.1	128.9
	3	25	22.7	90.7
	4	50	44.8	89.6

[1] See Amendment 3.

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TABLE 2: INSTRUMENT PARAMETERS

Instrument	Molecular Devices Spectramax M3
UV Wavelength (nm)	562

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TABLE 3: LINEARITY

Plate B1

	Concentration	Mean Value
STD2	1	0.994
STD3	0.625	0.655
STD4	0.5	0.521
STD5	0.2	0.225
STD6	0.05	0.053

Intercept	0.0000
Slope	1.0169

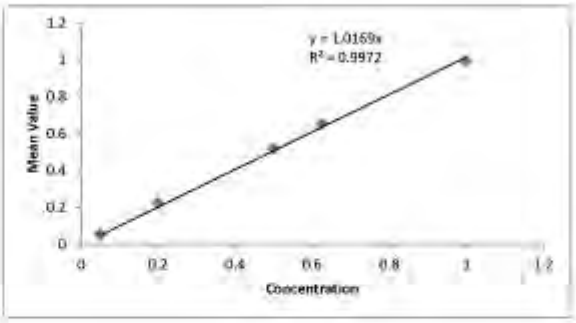

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TABLE 3 (cont.): LINEARITY

Plate B2

	Concentration	Mean Value
STD2	1	0.942
STD3	0.625	0.613
STD4	0.5	0.505
STD5	0.2	0.211
STD6	0.05	0.045

Intercept	0.0000
Slope	0.9714

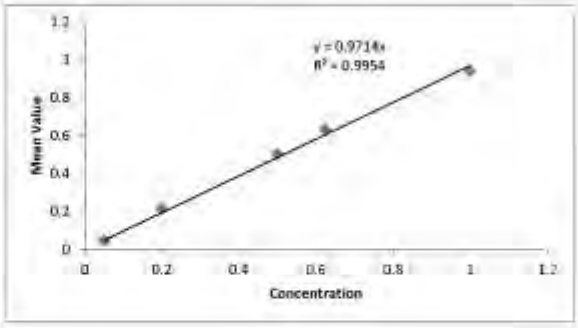

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TABLE 3 (cont.): LINEARITY

Plate B3

	Concentration	Mean Value
STD2	1	0.945
STD3	0.625	0.628
STD4	0.5	0.508
STD5	0.2	0.21
STD6	0.05	0.042

Intercept	0.0000
Slope	0.9718

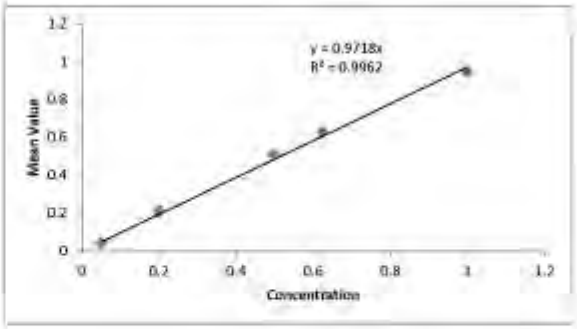

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TABLE 4: RESULTS OF HOMOGENEITY SAMPLES

Plate	Sample ID	Value	Calc. Conc. (mg/mL)	Dose Concentration (mg/mL)	Average (mg/mL)	STDEV	%RSD	% Target	Average % Target (if sample tested)	%Target between the strata
Plate B1	HO-1 BM-1	-0.002	-0.002	-0.20	-0.2	–	–	–	–	–
	HO-1 BM-2	-0.003	-0.003	-0.30						
	HO-1 BM-3	-0.002	-0.002	-0.20						
Plate B1	HO-2 BT-1	0.135	0.135	13.28	11.6	1.46	9.06	95.5	101.5	Average 104.8
	HO-2 BT-2	0.115	0.113	11.51						
	HO-2 BT-3	0.114	0.112	11.23						
Plate B2	HO-2 BT-1	0.127	0.131	13.07	13.1	0.84	6.27	107.6		
	HO-2 BT-2	0.125	0.129	12.87						
	HO-2 BT-3	0.14	0.144	14.41						
Plate B1	**HO-3 BM-1**	**0.101**	**0.099**	**9.93**	9.7	0.25	2.56	77.4	95.3	%RSD 13.89
	HO-3 BM-2	**0.098**	**0.096**	**9.64**						
	HO-3 BM-3	**0.096**	**0.094**	**9.44**						
Plate B2	**HO-3 BM-1**	**0.142**	**0.146**	**14.62**	13.9	0.65	4.72	110.9		
	HO-3 BM-2	**0.131**	**0.135**	**13.49**						
	HO-3 BM-3	**0.131**	**0.135**	**13.49**						
Plate B3	**HO-3 BM-1**	**0.115**	**0.118**	**11.83**	12.2	0.93	7.65	97.7		
	HO-3 BM-2	**0.129**	**0.133**	**13.27**						
	HO-3 BM-3	**0.112**	**0.115**	**11.53**						
Plate B1	**HO-4 BB-1**	**0.178**	**0.175**	**17.50**	15.6	2.71	17.40	124.7	116.4	
	HO-4 BB-2	**0.139**	**0.137**	**13.67**						
	HO-4 BB-3	[1]	–	–						
Plate B2	**HO-4 BB-1**	**0.143**	**0.147**	**14.72**	14.6	0.33	2.27	116.7		
	HO-4 BB-2	**0.144**	**0.148**	**14.82**						
	HO-4 BB-3	**0.138**	**0.142**	**14.21**						

[1] Result is not reported because it was an outlier.

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Plate	Sample ID	Value	Calc. Conc. (mg/mL)	Dose Concentration (mg/mL)	Average (mg/mL)	STDEV	%RSD	% Target	Average % Target (if sample reran)	%Target between the strain
Plate B3	**HO 4 BB-1**	**0.144**	**0.148**	**14.82**						
	HO 4 BB-2	**0.123**	**0.127**	**12.66**	**13.5**	**1.17**	**8.67**	**107.8**		
	HO 4 BB-3	**0.126**	**0.130**	**12.97**						
Plate B1	HO 5 HT-1	0.224	0.220	22.03						
	HO 5 HT-2	0.208	0.205	20.45	22.1	1.62	7.36	88.2	–	
	HO 5 HT-3	0.241	0.237	23.70						Average 91.0
Plate B1	HO 6 BM-1	0.223	0.219	21.93						
	HO 6 BM-2	0.243	0.239	23.90	23.1	1.02	4.44	92.3	–	
	HO 6 BM-3	0.238	0.234	23.40						% RSD 2.66
Plate B1	HO 7 BB-1	0.257	0.253	25.27						
	HO 7 BB-2	0.246	0.242	24.19	23.1	2.84	12.19	92.6	–	
	HO 7 BB-3	0.203	0.200	19.90						
Plate B1	HO 8 HT-1	0.443	0.439	43.86						
	HO 8 HT-2	0.422	0.418	41.80	42.5	1.03	2.43	85.0		
	HO 8 HT-3	0.432	0.428	42.85						Average 85.5
Plate B1	HO 9 BM-1	0.433	0.429	42.58						
	HO 9 BM-2	0.402	0.398	39.51	41.1	1.57	3.79	82.3		
	HO 9 BM-3	0.428	0.424	42.09						% RSD 3.56
Plate B1	HO 10 BB-1	0.442	0.438	43.47						
	HO 10 BB-2	0.477	0.469	46.91	44.4	2.25	5.07	88.7	–	
	HO 10 BB-3	0.434	0.427	42.68						

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TABLE 5: RESULTS OF CONCENTRATION VERIFICATION SAMPLES

Plate	Sample ID	Value	Calc. Conc (ug/mL)	Dose Concentration (mg/mL)	Average (mg/mL)	STDEV	%RSD	% Target	Average % Target (if sample reran)
Plate 01	CV-1B-1	-0.001	-0.001	-0.10					
	CV-1B-2	-0.003	-0.003	-0.30	-0.1	-	-	-	-
	CV-1B-3	-0.002	-0.002	-0.20					
Plate 01	CV-2B-1	0.138	0.136	13.62					
	CV-2B-2	0.126	0.124	12.39	12.9	0.80	6.20	98.2	-
	CV-2B-3	0.123	0.121	12.10					
Plate 01	CV-3B-1	0.253	0.249	24.88					
	CV-3B-2	0.245	0.241	24.09	24.5	0.80	3.26	98.0	-
	CV-3B-3	0.263	0.259	25.86					
Plate 01	CV-4B-1	0.453	0.445	44.55					
	CV-4B-2	0.476	0.468	46.84	45.3	1.51	3.34	90.2	
	CV-4B-3	0.447	0.440	43.96					
Plate 01	CV-5B-1	-0.004	-0.004	-0.39					
	CV-5B-2	-0.003	-0.003	-0.30	-0.3	-	-	-	-
	CV-5B-3	-0.003	-0.003	-0.30					
Plate 01	**CV-6B-1**	**0.154**	**0.151**	**15.14**					
	CV-6B-2	**0.158**	**0.155**	**15.54**	**15.4**	**0.26**	**1.69**	**123.5**	**129.9**
	CV-6B-3	**0.159**	**0.156**	**15.64**					
Plate 02	**CV-6B-1**	**0.163**	**0.168**	**16.78**					
	CV-6B-2	**0.156**	**0.161**	**16.06**	**16.8**	**0.72**	**4.29**	**134.2**	
	CV-6B-3	**0.170**	**0.175**	**17.50**					
Plate 03	CV-7B-1	0.231	0.227	22.72					
	CV-7B-2	0.228	0.224	22.42	22.7	0.25	1.09	90.7	-
	CV-7B-3	0.233	0.229	22.91					
Plate 03	CV-8B-1	0.45	0.445	44.53					
	CV-8B-2	0.464	0.456	45.61	45.5	0.74	1.66	89.6	-
	CV-8B-3	0.452	0.444	44.45					

APPENDIX D: INDIVIDUAL ANIMAL IN-LIFE CLINICAL OBSERVATIONS[1]

PRODUCT IDENTIFICATION

Silk Fibroin

[1] If no observations are listed on the day the animals were necropsied, animals were normal upon observation.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Male	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
Vehicle	7001	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7002	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7003	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7004	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7005	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7006	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7007	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7008	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7009	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7010	Normal	1 to 29
		Scheduled Removal (Terminal)	30

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled recovery, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Male	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
Low Dose	7021	Normal	1 to 29
From Day 1 (Start Date) to 31 (Start Date)		Scheduled Removal (Terminal)	30
	7022	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7023	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7024	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7025	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7026	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7027	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7028	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7029	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7030	Normal	1 to 29
		Scheduled Removal (Terminal)	30

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled recovery, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Male	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
Mid Dose	7041	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7042	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7043	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7044	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7045	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7046	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7047	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7048	Normal	1 to 26
		Scheduled Removal (Terminal)	30
		Eschar: Head: Superficial	27 to 30
	7049	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7050	Normal	1 to 29
		Scheduled Removal (Terminal)	30

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled recovery, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Male	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
High Dose	7061	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7062	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7063	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7064	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7065	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7066	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7067	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7068	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7069	Normal	1 to 29
		Scheduled Removal (Terminal)	30
	7070	Normal	1 to 29
		Scheduled Removal (Terminal)	30

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled recovery, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Female	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
Vehicle	7011	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7012	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7013	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7014	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7015	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7016	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7017	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7018	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7019	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7020	Normal	1 to 30
		Scheduled Removal (Terminal)	31

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled necropsy, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Female	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
Low Dose	7031	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7032	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7033	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7034	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7035	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7036	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7037	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7038	Normal	1 to 2,4 to 30
		Scheduled Removal (Terminal)	31
		Swelling, Right Eye: Slight	3
	7039	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7040	Normal	1 to 30
		Scheduled Removal (Terminal)	31

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled necropsy, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Female	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
Mid Dose	7051	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7052	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7053	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7054	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7055	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7056	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7057	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7058	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7059	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7060	Normal	1 to 30
		Scheduled Removal (Terminal)	31

Values = Clin Obs Range

[1] If no observations are present for an animal on the day of scheduled necropsy, the animal was normal upon examination.

Individual Animal In-Life Clinical Observations
PSL Study Number 51651 / A 28-Day Oral Gavage Toxicity Study in Rats

Sex: Female	Animal	Observation Type: All Types	From Day 1 (Start Date) to 31 (Start Date)
High Dose	7071	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7072	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7073	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7074	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7075	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7076	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7077	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7078	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7079	Normal	1 to 30
		Scheduled Removal (Terminal)	31
	7080	Normal	1 to 30
		Scheduled Removal (Terminal)	31

[1] If no observations are present for an animal on the day of scheduled necropsy, the animal was normal upon examination.