APPENDIX G.2
MORI SILK MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST

STUDY TITLE

Silk Fibroin:
Mammalian Erythrocyte Micronucleus Test
(Peripheral Blood, Flow Cytometry - Mouse)

DATA REQUIREMENT

US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C.1.d. Mammalian Erythrocyte Micronucleus Test (2000)

AUTHOR

Jayson Chen, PhD

STUDY COMPLETED ON

October 11, 2017

PERFORMING LABORATORY

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

Participating Laboratory:

Litron Laboratories
3500 Winton Pl.
Rochester, NY 14623

LABORATORY STUDY NUMBER

45997

SPONSOR

Tufts University
169 Holland St
Somerville, MA 02144

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Silk Fibroin

This study meets the requirements of U.S. FDA GLP: 21 CFR Part 58, 1987, with the following exception:

Characterization of the positive control substance and verification of concentration of the positive control substance in their carriers during this study were not determined analytically; however, the purity of the material used was certified by the supplier and all preparations were thoroughly documented.

Specific information related to the characterization of the test substance as received and tested is the responsibility of the Study Sponsor (see Test Substance section).

Study Director: [redacted] Date: Oct 11, 2017

Name of Signer: Jayson Chen, PhD

Name of Company: Product Safety Labs

Sponsor: _____ Date: _____

Name of Signer: _____

Name of Company: Tufts University

Submitter: _____ Date: _____

Name of Signer: _____

Name of Company: Tufts University

QUALITY ASSURANCE STATEMENT

The Product Safety Labs' Quality Assurance 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	B. Simms; M. Zakrzewski	Aug 3, 2017[1]; Sep 21, 2017	Aug 3, 2017; Sep 22, 2017
In-process inspection: *Day 2-Initiation of dosing*	B. Simms	Aug 16, 2017	Aug 16, 2017
Raw data audit	M. Zakrzewski	Sep 21 and 22, 2017	Sep 22, 2017
Draft report review	M. Zakrzewski	Sep 21 and 22, 2017	Sep 22, 2017

The QA Statement for the micronucleus analysis phase of the study is in Appendix A.

Final report reviewed by:

[redacted]

Maryann Zakrzewski
Quality Assurance Auditor
Product Safety Labs

October 11, 2017
Date

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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SILK FIBROIN: MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST (PERIPHERAL BLOOD, FLOW CYTOMETRY - MOUSE)

PROTOCOL NO.:	P603.PBF-M
PSL STUDY NUMBER:	45997
SPONSOR:	Tufts University 169 Holland St Somerville, MA 02144
TEST SUBSTANCE IDENTIFICATION:	Silk Fibroin Batch #: 2017_27_07
DATE RECEIVED:	August 2, 2017
PSL REFERENCE NO.:	170802-1D
STUDY INITIATION DATE:	August 4, 2017
IN-LIFE DATES OF TEST:	August 15 – August 18, 2017
NOTEBOOK NO.:	45997: pages 1-157

1. PURPOSE

The objective of this study was to evaluate the potential of Silk Fibroin to cause damage to the chromosomes or mitotic spindle apparatus of erythroblasts, observed as an increase in micronucleated immature erythrocytes (MIE) in mouse peripheral blood.

2. SUMMARY

The *in vivo* mouse erythrocyte micronucleus test was performed to investigate the potential of Silk Fibroin to induce a statistically significant increase in MIE in mouse peripheral blood, which is the genotoxicity endpoint for this study type.

Silk Fibroin was dosed by oral gavage to mice (5 per sex) at 1000 mg/kg/day. Distilled water was used as the negative (vehicle) control and cyclophosphamide monohydrate was used as the positive control. The volume administered was 10 mL/kg *bis in die* (20 mL/kg total) for test substance/ negative control and 5 mL/kg for positive control. Animals were weighed prior to test initiation on Day 1. Animals were dosed with test substance or negative control on Days 1 and 2. The positive control was administered on Day 2 only. Blood samples were collected from all groups for analysis 44-48 hours after treatment. For all groups, a minimum target of 4000 polychromatic erythrocytes per animal was scored for incidence of micronucleated immature erythrocytes.

There were no mortalities in the study. There were no clinical observations noted in any animal during the study.

Silk Fibroin did not induce a statistically significant increase in frequency of micronucleated reticulocytes (%MN-RET or MIE) in male or female mice.

Frequency of reticulocyte (%RET) and micronucleated normochromatic erythrocytes (%MN-NCE) did not differ significantly after treatment with the test substance.

The negative control group animals show MIE values consistent with existing data. The positive control caused a clear, statistically significant increase in MIE with individual and mean values consistent with existing data and outside the historical control range for negative control animals.

Under the conditions of this study, Silk Fibroin at 1000 mg/kg/day did not induce micronucleus formation in the immature erythrocytes of the mouse. In conclusion, Silk Fibroin is not considered to be genotoxic with respect to micronucleus induction in the *In Vivo* Mouse Erythrocyte Micronucleus Test (Flow Cytometry).

3. MATERIALS

A. Test Substance

The test substance, identified as Silk Fibroin, Batch #: 2017_27_07, was received on August 2, 2017 and was further identified with PSL Reference Number 170802-1D. The test substance was stored refrigerated. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by Tufts University Science & Tech Ctr, 4 Colby St, Medford, MA.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Silk fibroin, 5%
Water, 95%

Physical Description: Clear Liquid

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: September 27, 2017

B. Positive Control

The positive control substance, identified as cyclophosphamide monohydrate (Batch #: MKBX1822V), was received on August 16, 2016, and was further identified with PSL Reference Number 160816-11H. The positive control substance was stored refrigerated. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103.

Composition: Cyclophosphamide monohydrate – 100.0%, CAS #6055-19-2

Physical Description: White powder

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: December 2018

4. GENERAL TEST SYSTEM PARAMETERS

A. Animal Requirements

4.A.1 Number of Animals: 30

4.A.2 Number of Groups and Animals per Group:
Test: (1 group): 5 animals per sex per group

Negative (Vehicle) Control (1 group): 5 animals per sex per group
Positive Control (1 group): 5 animals per sex per group

4.A.3 Sex: Male and female; females were nulliparous and non-pregnant.

4.A.4 Species/Strain: Mouse/Swiss albino (ICR)

4.A.5 Age/Body weight: Young adult (approximately 8 weeks old)/males were 35-38 grams and females were 24-28 grams at experimental start.

4.A.6 Supplier: Received from Envigo Laboratories, Inc. on August 9, 2017.

B. Test System Justification

The rodent erythrocyte micronucleus test is the best-validated and most widely used *in vivo* model for investigating genotoxic potential of test agents. Damage to the chromosomes or the spindle apparatus in dividing erythroblasts in the bone marrow results in acentric fragments or lagging chromosomes which condense in the cytoplasm to form micronuclei. As erythrocytes mature, the main nucleus is expelled but micronuclei tend to remain behind. The newly formed (immature) micronucleated erythrocytes migrate to peripheral blood where they are easy to identify and readily quantifiable. Unlike many other species, micronucleated erythrocytes are not efficiently removed from the circulating blood in mice, making mouse peripheral blood suitable for analysis.

C. Husbandry

4.C.1 Housing: The animals were group-housed in solid bottom cages, which conforms to the size recommendations in the latest *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Bedding (bed-o'-cobs®) was used and changed at least once per week.

4.C.2 Animal Room Temperature and Relative Humidity Ranges: 19-22°C and 50-58%, respectively.

4.C.3 Animal Room Air Changes/Hour: 13. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

4.C.4 Photoperiod: 12-hour light/dark cycle

4.C.5 Acclimation Period: The animals were conditioned to the housing facilities for 6 days prior to testing.

4.C.6 Food: Envigo Teklad Global 16% Protein Rodent Diet® #2016. The diet was available *ad libitum*.

4.C.7 Water: Filtered tap water was supplied *ad libitum*.

4.C.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Product Safety Labs.

D. Identification

4.D.1 Cage: Each cage was identified by a cage card indicating the study number, dose level, group assignment, individual animal identifications, and sex of the animals.

4.D.2 Animal: Each animal was given a sequential animal number in addition to being uniquely identified (e.g., ear tag or color marking). This number, together with a sequential animal number assigned to study 45997, constituted unique identification. Only the sequential animal number is presented in this report.

5. EXPERIMENTAL DESIGN

A. Route of Administration

The test, negative (vehicle) control, and positive control substances were administered by oral gavage (PO).

B. Justification of Route of Administration

The oral route of administration was used because it is a common route of human exposure and is widely used in the micronucleus and other safety assessment tests, as noted in the referenced guidelines (Section 10.C).

C. Control of Bias

General procedures associated with the balanced design and conduct of this study were employed to control bias (see Section 6.A).

D. Dose Levels (Main Test)

Five male and five female test animals were assigned to each of the following test groups.

Group	No. Animals/ Group M/F	Dose Level *(mg/kg body weight)*	Dose Volume (mL/kg)[a]	Sampling time (hr) after final treatment
1	5/5	Negative (Vehicle) Control *0*	10 b.i.d.[b]	44-48
2	5/5	Test substance (high dose) *1000*		
3	5/5	Positive Control (Cyclophosphamide monohydrate) *40*	5	

[a] An alternative dose volume was selected due to the stability of the test substance.
[b] *Bis in die.*

E. Justification of Dose Level Selection

The Sponsor, in consultation with the Study Director, selected a limit dose of 1000 mg/kg/day, the maximum achievable dose level of the test substance formulation in this test system. A dose volume of 10 mL/kg *bis in die* (b.i.d.) was selected to reach 20 mL/kg, the maximum dose volume recommended in the referenced guidelines (Section 10.C), without causing potential negative health effects to the study animals. The positive control dose is based on existing data.

6. PROCEDURES

A. Selection of Animals

For all testing, mice were indiscriminately allocated to cages/groups so that there was no statistically significant difference among group body weight means within a sex. Animals were

selected for study on the basis of freedom from clinical signs of disease or injury and a body weight within 20% of the mean within a sex.

B. Dosing and Preparation Procedures

6.B.1 Preparation: The vehicle was distilled water. The test substance was a liquid and was administrated as received (undiluted) at 50 mg/mL. The positive control substance was prepared in distilled water at 8 mg/mL.

6.B.2 Dose Calculation: Individual animal doses were calculated based on the initial body weight to achieve the targeted dose for all animals (i.e., mg/kg/day). All doses for the test substance and negative control groups on the main test were administered at a constant volume of 10 mL/kg b.i.d (20 mL/kg total). The negative control group received distilled water only. The positive control group was treated at 5 mL/kg.

6.B.3 Dosing: The positive control substance was administered via oral gavage on Day 2 only. The negative control and test substance were administered by oral gavage on Days 1 and 2 in divided doses. Oral gavage was conducted using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. The dosing formulations were maintained at room temperature. The first day of administration was considered Day 1 of the study. Dosing was at approximately the same time each day ± 2 hours.

C. 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.

Observations of the test system were conducted at intervals appropriate to assess the onset and termination of adverse effects (if any were observed). The first observation was made approximately 30 minutes following administration. Animals were also observed during the first several hours post-dosing on Day 1 for the negative control and test substance-treated groups.

D. Terminal Sacrifice and Blood Collection

At terminal sacrifice, all animals were anesthetized with carbon dioxide. Timing followed the table in Section 5.D. Whole blood was collected by cardiac puncture while the animals were anesthetized. Mice were then euthanized by exsanguination and discarded without further examination.

E. Sample Processing and Analysis

6.E.1 Blood Fixation and Shipment: Blood was processed according to the instructions in the Litron *In Vivo* Micronucleus Kit (MicroFlow^{BASIC} (Rodent Fixed Blood), Rochester, NY). The sampling target was 100 µL per sample. Duplicate samples were prepared from each mouse.

An additional sample of blood (200-500 µL) was collected for potential toxicokinetic analysis. The additional samples were placed in a tube containing K_2EDTA and mixed by inversion. Plasma was separated by centrifugation, transferred to a separate, labeled tube, and stored frozen (approximately -70°C) until the completion of the study.

Blood for micronucleus analysis was fixed and permeabilized by rapid pipetting in chilled methanol (e.g., using a dry ice box); samples were stored for at least three

days in methanol (storage followed kit instructions). Samples were then centrifuged; the methanol was removed, and the cells were resuspended in shipping buffer provided with the kit. One set of samples was shipped to Litron Laboratories (Section 10.A); shipping conditions followed kit instructions. The other set was held in reserve by PSL, stored in accordance with kit instructions. Retained fixed blood samples were perishable and discarded after study completion.

6.E.2 Staining and Analysis: Cells were stained with dyes to identify immature vs. mature erythrocytes (fluorescent labeled anti-CD71 antibody), platelets (fluorescent labeled anti-CD61 antibody) and DNA (propidium iodide, following RNAse treatment). Stained cells were analyzed by flow cytometry (cf. Section 10.A). The analysis target was a minimum of 4000 immature erythrocytes per animal. Analyses measured DNA content (i.e., propidium iodide staining) in both immature erythrocytes (i.e., CD71+/CD61- cells) and mature erythrocytes (i.e., CD71-/CD61- cells).

7. STATISTICAL ANALYSIS

The data generated (the proportions of immature among total erythrocytes and micronucleated erythrocytes) were analyzed by analysis of variance followed by Bonferroni-corrected multiple comparison test using GraphPad Prism (version 5.03), GraphPad Software, San Diego, CA.

8. ASSAY VALIDITY

The test was considered valid if negative control group animals showed micronucleated immature erythrocyte (MIE) values close to or within the expected range based on published values for this method and laboratory control data. Additionally, the positive control should have caused a clear increase in MIE with individual and mean values outside the historical control range for negative control animals.

9. POSITIVE RESULT EVALUATION

A positive result was defined as a statistically significant increase in MIE compared with the negative control group in the absence of cytotoxicity (reticulocyte fraction less than 5% of vehicle control).

10. STUDY CONDUCT

A. Testing Facility

This in-life portion of this study was conducted at Product Safety Labs' (PSL) test facility at 2394 U.S. Highway 130, Dayton, NJ 08810. The Study Director for this study was Jayson Chen, PhD. The primary scientist was Colleen Wojenski, BS, LATG, with contributions by Aubrey Blue, Lisa Broske-Godin, BS, RLATG, Janet Dell John BA, RLATG, and Katherine Sibly, BS. Flow cytometry procedures were conducted at Litron Laboratories, 3500 Winton Pl, Rochester, NY 14623 (PI: Dorothea Torous).

B. GLP Compliance

This study was conducted in compliance with the following Good Laboratory Practice (GLP) regulations:

- US FDA GLP: 21 CFR 58, 1987.

C. Test Procedure Guideline

The procedures described in this report are based on the following testing guideline:

- US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C.1.d. Mammalian Erythrocyte Micronucleus Test (2000).

11. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards.

In addition, PSL QAU functioned as lead QA for this study and monitored QA activities at Litron Laboratories. For portions of the study conducted by a subcontractor, the QAU for that facility conducted necessary critical phase inspections and audited respective results and reported for the study phase according to the SOPs of that facility.

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

12. AMENDMENTS TO THE PROTOCOL

At the request of the Sponsor, Protocol Sections 6.E Dose Levels (Main Test) and 6.F Justification of Dose Level Selection were updated to run the main test as a limit test with the following changes:

6.E Dose Levels (Main Test)

Group	No. Animals/ Group M/F	Dose Level *(mg/kg body weight)*	Dose Volume (mL/kg body weight)[a]	Sampling time (hr) after final treatment
1	5/5	Negative (Vehicle) Control *0*	10 b.i.d.[b]	44-48
2	5/5	Test substance (high dose) *1000*		
3	5/5	Positive Control (Cyclophosphamide monohydrate) *40*	5	

[a] An alternative dose volume was selected due to the stability of the test substance.
[b] *Bis in die*

E.F Justification of Dose Level Selection

The Sponsor, in consultation with the Study Director, selected a limit dose of 1000 mg/kg/day, the maximum achievable dose level of the test substance formulation in this test system. Dose volume of 10 mL/kg *bis in die* (b.i.d.) was selected to reach 20 mL/kg, the maximum dose volume recommended in the referenced guidelines (Section 10.C), without causing potential negative health effects to the study animals. The positive control dose is based on existing data.

13. DEVIATIONS FROM THE PROTOCOL

None.

14. FINAL REPORT AND RECORDS TO BE MAINTAINED

Information on care of the test system, equipment maintenance and calibration, storage, usage, and disposition of the positive control 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.

The original, final report will be sent to the Sponsor. A copy of the signed report, together with the protocol, associated amendments and/or deviations, if applicable, and all raw data generated at PSL and raw data from Litron Laboratories will be maintained in the PSL 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.

Any electronic raw data generated by the Test Site will be maintained in accordance to the Test Site SOPs.

15. RESULTS

A summary of average % micronucleated reticulocytes, % of reticulocytes, and % micronucleated normochromatic erythrocytes are presented in Table 1. Individual animal bodyweights and cage-side observations are presented in Tables 2 and 3, respectively. The Microflow® Mouse Micronucleus Analysis Report from Litron and PSL's historical control data are presented in Appendices A and B, respectively.

Five male and five female mice per group received oral administration of distilled water (negative/vehicle control, Group 1) or 1000 mg/kg/day of Silk Fibroin (test substance, Group 2) for two days, or 40 mg/kg/day of cyclophosphamide monohydrate (positive control, Group 3) for a single dose.

All animals survived administration of the negative control, test substance, or positive control.

A. Body Weight (Tables 2)

The initial body weights of all animals were within ± 20% of the mean within a sex.

B. Clinical Observations (Tables 3)

There were no clinical observations noted in any animal during the study.

C. Cytotoxicity and Micronucleus Induction (Tables 1, Appendix A)

Silk Fibroin did not induce a statistically significant increase in frequency of micronucleated reticulocytes (%MN-RET or MIE) in male or female mice.

Frequency of reticulocyte (%RET) and micronucleated normochromatic erythrocytes (%MN-NCE) did not differ significantly after treatment with the test substance.

The negative control group animals show MIE values consistent with existing data (Appendix B). The positive control caused a clear, statistically significant increase in MIE with individual and mean values consistent with existing data and outside the historical control range for negative control animals.

16. CONCLUSION

Under the conditions of this study, Silk Fibroin at 1000 mg/kg/day did not induce micronucleus formation in the immature erythrocytes of the mouse. In conclusion, Silk Fibroin is not considered to be genotoxic with respect to micronucleus induction in the In Vivo Mouse Erythrocyte Micronucleus Test (Flow Cytometry).

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]

Jayson Chen, PhD
Study Director
Product Safety Labs

Oct. 11, 2017
Date

TABLE 1: FLOW CYTOMETRY RESULT SUMMARY

% MN-RET

Group	Dose (mg/kg/day)	Mean ± SEM		*p*-value[1]	
		Male	Female	Male	Female
Negative (Vehicle) Control	0	0.12 ± 0.01	0.11 ± 0.01	-	-
Test Substance	1000	0.12 ± 0.02	0.14 ± 0.01	ns	ns
Positive Control	40	2.06 ± 0.35	1.41 ± 0.15	$p < 0.001$	$p < 0.001$

% RET

Group	Dose (mg/kg/day)	Mean ± SEM		*p*-value[1]	
		Male	Female	Male	Female
Negative (Vehicle) Control	0	1.88 ± 0.12	1.52 ± 0.10	-	-
Test Substance	1000	1.55 ± 0.14	1.42 ± 0.21	ns	ns
Positive Control	40	0.62 ± 0.08	0.42 ± 0.08	$p < 0.001$	$p < 0.001$

% MN-NCE

Group	Dose (mg/kg/day)	Mean ± SEM		*p*-value[1]	
		Male	Female	Male	Female
Negative (Vehicle) Control	0	0.11 ± 0.01	0.09 ± 0.01	-	-
Test Substance	1000	0.11 ± 0.01	0.13 ± 0.01	ns	ns
Positive Control	40	0.14 ± 0.02	0.13 ± 0.01	ns	ns

ns = not significant ($p > 0.05$)
% MN-RET = frequency (%) of positive CD71 micronucleated reticulocytes (i.e., micronucleated immature erythrocytes [MIEs])
% RET = frequency (%) of CD71 positive reticulocytes
% MN-NCE = frequency (%) of micronucleated normochromatic erythrocytes

[1] Bonferroni-corrected multiple comparison test.

TABLE 2: INDIVIDUAL ANIMAL BODY WEIGHTS

Individual Animal Body Weights
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Male Bodyweight (g)

0 mg/kg/day Group 1	Day(s) Relative to Start
	1
5201	37
5202	38
5203	38
5204	36
5205	36
Mean	37.0
SD	1.0
N	5

Individual Animal Body Weights
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Male Bodyweight (g)

1000 mg/kg/day Group 2	Day(s) Relative to Start
	1
5211	37
5212	38
5213	36
5214	37
5215	37
Mean	37.0
SD	0.7
N	5

Individual Animal Body Weights
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Male Bodyweight (g)

40 mg/kg/day Group 3	Day(s) Relative to Start 1
5221	35
5222	36
5223	37
5224	37
5225	35
Mean	36.0
SD	1.0
N	5

Individual Animal Body Weights
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Female Bodyweight (g)

0 mg/kg/day Group 1	Day(s) Relative to Start
	1
5206	26
5207	26
5208	25
5209	27
5210	26
Mean	26.0
SD	0.7
N	5

Individual Animal Body Weights
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Female Bodyweight (g)

1000 mg/kg/day Group 2	Day(s) Relative to Start
	1
5216	27
5217	26
5218	28
5219	26
5220	28
Mean	27.0
SD	1.0
N	5

Individual Animal Body Weights
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Female Bodyweight (g)

40 mg/kg/day Group 3	Day(s) Relative to Start
	1
5226	28
5227	27
5228	26
5229	26
5230	24
Mean	26.2
SD	1.5
N	5

TABLE 3: INDIVIDUAL ANIMAL CAGE-SIDE OBSERVATIONS

Individual Animal Clinical Observations
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Male	Animal	Observation Type: All Types	From Day -9999 (Start Date) to 9999 (Start Date)
Control	5201	Normal	1 to 3
	5202	Normal	1 to 3
	5203	Normal	1 to 3
	5204	Normal	1 to 3
	5205	Normal	1 to 3

Values = Clin Obs Range

Individual Animal Clinical Observations
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Male	Animal	Observation Type: All Types	From Day -9999 (Start Date) to 9999 (Start Date)
High dose-1000	5211	Normal	1 to 3
	5212	Normal	1 to 3
	5213	Normal	1 to 3
	5214	Normal	1 to 3
	5215	Normal	1 to 3

Values = Clin Obs Range

Individual Animal Clinical Observations
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Male	Animal	Observation Type: All Types	From Day -9999 (Start Date) to 9999 (Start Date)
Cyclophosphamide	5221	Normal	1 to 3
	5222	Normal	1 to 3
	5223	Normal	1 to 3
	5224	Normal	1 to 3
	5225	Normal	1 to 3

Values = Clin Obs Range

Individual Animal Clinical Observations
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Female	Animal	Observation Type: All Types	From Day -9999 (Start Date) to 9999 (Start Date)
Control	5206	Normal	1 to 3
	5207	Normal	1 to 3
	5208	Normal	1 to 3
	5209	Normal	1 to 3
	5210	Normal	1 to 3

Values = Clin Obs Range

Individual Animal Clinical Observations
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, FlowCytometry-4/ouse)

Sex: Female	Animal	Observation Type: All Types	From Day -9999 (Start Date) to 9999 (Start Date)
High dose-1000	5216	Normal	1 to 3
	5217	Normal	1 to 3
	5218	Normal	1 to 3
	5219	Normal	1 to 3
	5220	Normal	1 to 3

Values = Clin Obs Range

Individual Animal Clinical Observations
PSL Study Number 45997
Mammalian Erythrocyte Micronucleus Test (Peripheral Blood, Flow Cytometry-Mouse)

Sex: Female	Animal	Observation Type: All Types	From Day -9999 (Start Date) to 9999 (Start Date)
Cyclophosphamide	5226	Normal	1 to 3
	5227	Normal	1 to 3
	5228	Normal	1 to 3
	5229	Normal	1 to 3
	5230	Normal	1 to 3

APPENDIX A: MICROFLOW® MOUSE MICRONUCLEUS ANALYSIS REPORT

MicroFlow® Mouse Micronucleus Analysis Report

Study Identification:	45997
Good Laboratory Practice Number:	GLP-2017-45997PSL
Date Samples Received:	August 25, 2017
Experimental Start Date:	August 28, 2017
Experimental End Date:	August 29, 2017
Date of Report:	October 6, 2017
Study Phase Plan:	MS1MFv15
Principal Investigator:	Dorothea K. Torous
Test Site:	Litron Laboratories 3500 Winton Place Suite 1B Rochester, New York 14623
Study Director:	Dr. Jayson Chen Study Director
Test Facility:	Product Safety Labs 2394 US Highway 130, Suite E Dayton, New Jersey 08810

Litron Laboratories

Table of Contents

Libron Laboratories

1. List of Figures and Tables

Libum Laboratories

2. Good Laboratory Practice Compliance Statement

This phase of the study was conducted in compliance with Good Laboratory Practice (GLP) regulations for non-clinical laboratory studies by the FDA (21 CFR 58), current version as of April 2016.

The study phase data have been reviewed by the Principal Investigator, who certifies that the information contained in this report accurately reflects and is supported by the study phase raw data and represents an appropriate conclusion within the context of the study phase design and evaluation criteria. Methods relating to the receipt and flow cytometric analysis of blood samples specified in the test facility's protocol and the MicroFlow Study Phase Plan were followed.

Principal Investigator _____ Date 4 Aug 2017
Dorothea K. Torous, B.S.

3. Other Scientists Involved in the Study

Not applicable.

Litron Laboratories

4. Quality Assurance Statement

This study has been subjected to inspection and the report has been audited by the Quality Assurance (QA) Unit of Litron Laboratories in accordance with GLP regulations for non-clinical laboratory studies by the FDA (21 CFR 58), current version as of April 2016. The report describes the methods and procedures used in the study phase and the reported results accurately reflect the raw data of this study phase.

The following inspections were performed by Nikki E. Hall, B.S., RQAP-GLP:

Date	Phase Inspected	Date Reported to PI and Test Site Management	Date Reported to Test Facility SD, QA and Management*
August 25, 2017	Sample Receipt	August 28, 2017	August 29, 2017
August 28, 2017	Sample Washing	August 28, 2017	August 29, 2017
August 28, 2017	Sample Staining	August 28, 2017	August 29, 2017
August 28, 2017	Flow Cytometer Calibration	August 28, 2017	August 29, 2017
August 28, 2017	Sample Analysis	August 28, 2017	August 29, 2017
August 30, 2017	Check Training	August 31, 2017	September 5, 2017
August 30, 2017	Check Data Sheets for Completion	August 31, 2017	September 5, 2017
August 30, 2017	Raw Data versus Report	August 31, 2017	September 5, 2017
August 30, 2017	Draft Report Check	August 31, 2017	September 5, 2017
October 6, 2017	MicroFlow Report Check	October 5, 2017	October 6, 2017

Quality Assurance Auditor _____ Date 06 Oct 2017
Nikki E. Hall, B.S., RQAP-GLP

* Indicates the date that the inspection reports were sent to the Test Facility.

5. Summary

On August 25, 2017, 30 fixed mouse blood samples were received from the test facility for this study. Flow cytometric analysis was performed on all samples according to Litron's Standard Operating Procedures.

The frequency of reticulocytes (% RET) was determined and used to provide an indication of bone marrow toxicity (i.e., a decrease in the % RET for a treatment group as compared to the Negative Control group). The frequency of micronucleated reticulocytes (% MN-RET) was determined to provide an indication of genotoxicity. Statistical tests will be performed by the test facility.

Dose Level (mg/kg body weight)	Sex	Average % RET	% RET Decrease*	% MN-RET
Negative (Vehicle) Control 0	Male	1.88	N/A	0.12
Test Substance (high dose) 1000	Male	1.55	18%	0.12
Negative (Vehicle) Control 0	Female	1.52	N/A	0.11
Test Substance (high dose) 1000	Female	1.42	7%	0.14
Negative (Vehicle) Control 0	Male	1.88	N/A	0.12
Positive Control (CP) 40	Male	0.62	67%	2.08
Negative (Vehicle) Control 0	Female	1.52	N/A	0.11
Positive Control (CP) 40	Female	0.42	72%	1.41

* Comparisons of each treatment group with the corresponding negative control. A negative value indicates increases to the % RET.
N/A = Not applicable. CP = Cyclophosphamide monohydrate.

6. Objective

The objective of this analysis was to evaluate test facility prepared mouse peripheral blood samples for the presence of micronuclei (MN) using the MicroFlow procedure. Micronuclei were analyzed in both the reticulocyte and normochromatic erythrocyte (NCE) populations. Micronucleus measurements made in the RET population provide an indication of genotoxicity associated with an acute dosing regimen, while those made in the NCE population are appropriate when subchronic or chronic exposure regimens have been utilized. The frequency of RETs among total red blood cells was measured to provide an indication of bone marrow toxicity.

7. Materials and Methods

7.1. Experimental Procedures (performed by Test Facility)

The test facility was responsible for following the procedures detailed in the MicroFlow kit manual supplied to the test facility. The supplies and reagents were provided as a MicroFlow kit by the test site. No deviations from the manual were noted.

7.2. Blood Cell Receipt

On August 25, 2017, Litron Laboratories received 30 fixed mouse blood samples from Study 45997. All fixed blood samples were stored in a freezer (–90 °C to –80 °C) until analysis.

7.3. Blood Cell Preparation

The fixed blood samples were thawed and then washed by adding each aliquot to tubes containing 5 ± 1 ml of cold Hank's Balanced Salt Solution (HBSS) + 1% fetal bovine serum. Cells were isolated by centrifugation, and the cell pellets were stored on ice until staining and then at 2 °C to 10 °C. After analysis of each stained sample, the remaining cell pellets were discarded.

7.4. Staining for Identification of Cell Populations

An aliquot (20 µl) of each washed blood sample was added to 80 µl of a solution containing RNase (to degrade RNA, 1 mg/ml), a fluorescently labeled (fluorescein isothiocyanate; FITC) antibody to the transferrin receptor to label RETs (anti-CD71-FITC, 10 µl/ml), and a fluorescently labeled antibody (phycoerythrin; PE) to label platelets (anti-CD61-PE, 5 µl/ml) in a base of HBSS. The samples were incubated in the labeling solution for 30 ± 10 minutes at 2 °C to 10 °C and 30 ± 10 minutes at room temperature. After incubation, the cells were kept at 2 °C to 10 °C until analysis. A propidium iodide (PI) solution (2 ml ± 0.5 ml) was added to each sample immediately before flow cytometric analysis to stain all DNA, including MN in the cells.

7.5. Flow Cytometer Calibration

Methanol-fixed blood from mice infected with *Plasmodium berghei* and methanol-fixed blood from uninfected rats were used to configure the flow cytometer before analysis. Whereas MN are relatively rare and exhibit a heterogeneous DNA content, parasitized cells are prevalent and have a homogenous DNA content. These characteristics make them ideal for calibrating the flow cytometer for the micronucleus scoring application.

7.6. Analysis of Blood Samples

Each blood sample was analyzed by high-speed flow cytometry using CellQuest software, version 5.2 (Becton Dickinson, San Jose, CA). The stained cells were moved at a high velocity past an argon laser set to provide 488 nm excitation. Photomultiplier tubes collected the fluorescence emitted by each cell. Using the previously described staining procedure, the PI-stained DNA of the MN emitted a red fluorescence, the anti-CD71-FITC antibody emitted a high green fluorescent signal, and platelets were excluded based on their anti-CD61-PE fluorescence. Upon successful analysis of the stained samples, each was discarded.

7.7. Number of Cells Analyzed

For test facility samples, at least 5,000 RETs (CD71+) were evaluated for the presence of MN.

7.8. Data Provided

The number of NCEs, MN-NCEs, RETs and MN-RETs are provided for each sample analyzed. The frequency of MN-RETs and MN-NCEs were calculated as an indication of genotoxic potential. The % RET was determined to provide an indication of bone marrow toxicity. For each group, the average and standard deviations for % RET, % MN-RET and % MN-NCE are provided for males and females separately.

7.9. Criteria for a Valid Assay

The test facility will establish if the criteria for determining whether an analysis is valid were met.

7.10. Statistical Analysis of the Data

The test facility provided the following information regarding test article treatment groups.

Male Animal Numbers	Female Animal Numbers	Group Dose Level (mg/kg body weight)	Sampling Time (hr) after Final Treatment
5201, 5202, 5203, 5204 and 5205	5206, 5207, 5208, 5209 and 5210	1 Negative (Vehicle) Control 0	44-48
5211, 5212, 5213, 5214 and 5215	5216, 5217, 5218, 5219 and 5220	2 Test Substance (high dose) 1000	44-48
5221, 5222, 5223, 5224 and 5225	5226, 5227, 5228, 5229 and 5230	3 Positive Control (Cyclophosphamide monohydrate) 40	44-48

Five samples/sex/group were submitted (see table, above) for all groups. No statistical analyses were performed on the data, other than the calculations indicated in this report. The test facility is responsible for the evaluation and interpretation of results.

7.11. Determining a Positive Response

A test article is considered genotoxic if the average % MN-RET for the treated animals is significantly higher than the average % MN-RET values for the negative (vehicle) control animals ($p < 0.05$). A test article is considered negative if statistical significance is not demonstrated ($p \geq 0.05$). Biological relevance of results is also considered in the final determination of genotoxicity. Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgment about the activity of the test article. These results are considered equivocal and are very rare. Equivocal results should be clarified by further testing preferably using a modification of experimental conditions.

8. Discussion of Results

All statistical evaluations will be performed by Product Safety Labs.

9. Conclusions

The genotoxic potential of the test article will be evaluated by Product Safety Labs.

10. Historical Data

None available at time of analysis, but a historical control database is being developed.

11. Records Maintained

The study phase plan, MicroFlow report, and study-specific records (copies, if applicable) will be transferred to the test facility at the completion of the study phase. Litron will maintain copies of the report, protocol, study phase plan, and other study-specific records for two years following completion of the study. After the retention period, Litron will contact the Test facility and study-specific records will either be discarded or sent to a location designated by the Test facility. Electronic copies of some records will be stored off-site (ESL Federal Credit Union, Brighton Henrietta Branch, Rochester, NY) in addition to storage at Litron Laboratories.

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- Section 4 of the OECD Guidelines for the Testing of Chemicals: Mammalian Erythrocyte Micronucleus Test, Guideline 474 (Adopted 26th September 2014).
- Where applicable, GLP regulations for non-clinical laboratory studies as developed by the FDA (21 CFR 58). Please note that the computerized systems utilized for data acquisition, data analysis and report generation have undergone an internal validation guided by FDA GLP regulations. Litron is working towards 21 CFR part 11 compliance.
- Where applicable, ISO 10993-3: Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicology (2009-10-15).
- Where applicable, ICH Harmonised Tripartite Guideline: Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, S2(R1), current Step 4 version dated 9 November 2011.

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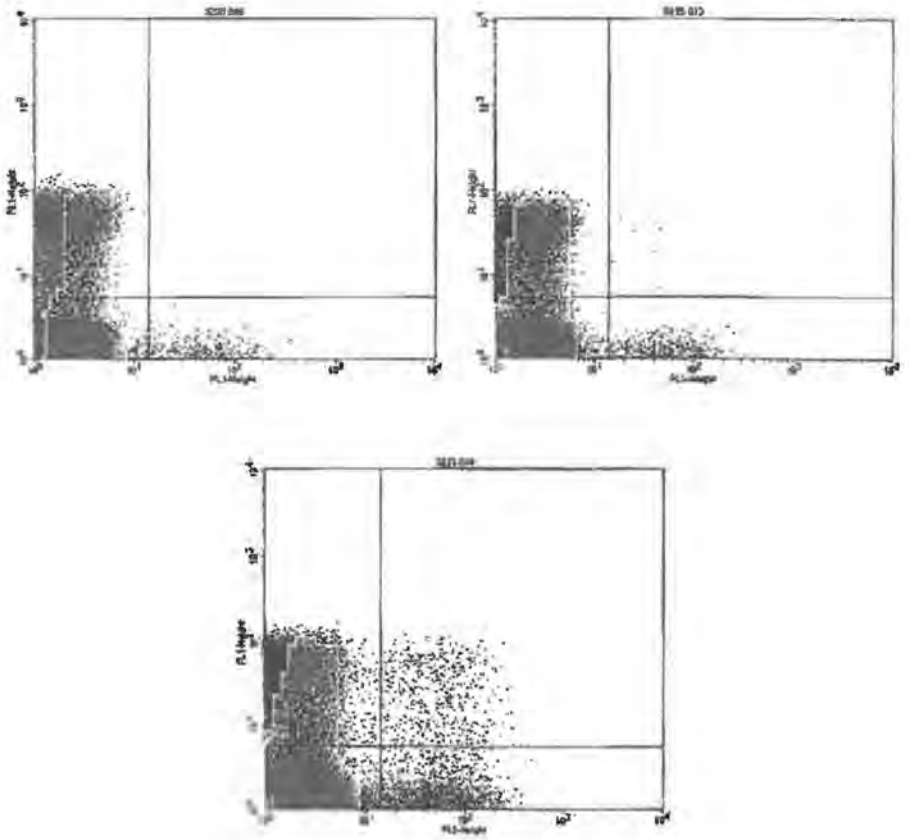
Figures 1-3: Representative Bivariates - Males

These representative bivariates of test facility submitted Samples 5205, 5215 and 5225 illustrate the resolution of the various erythrocyte populations in mouse peripheral blood: Lower Left quadrant = NCE [cells which are low in green and red fluorescence]; Lower Right = MN-NCE [cells high in red (PI) fluorescence]; Upper Left = CD71 positive RET [cells with green (CD71) fluorescence]; Upper Right = CD71 positive MN-RET [cells with both red and green fluorescence; the population of primary interest for this analysis].



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Figures 4-6: Representative Bivariates - Females

These representative bivariates of test facility submitted Samples 5206, 5216 and 5226 illustrate the resolution of the various erythrocyte populations in mouse peripheral blood: Lower Left quadrant = NCE [cells which are low in green and red fluorescence]; Lower Right = MN-NCE [cells high in red (PI) fluorescence]; Upper Left = CD71 positive RET [cells with green (CD71) fluorescence]; Upper Right = CD71 positive MN-RET [cells with both red and green fluorescence; the population of primary interest for this analysis].

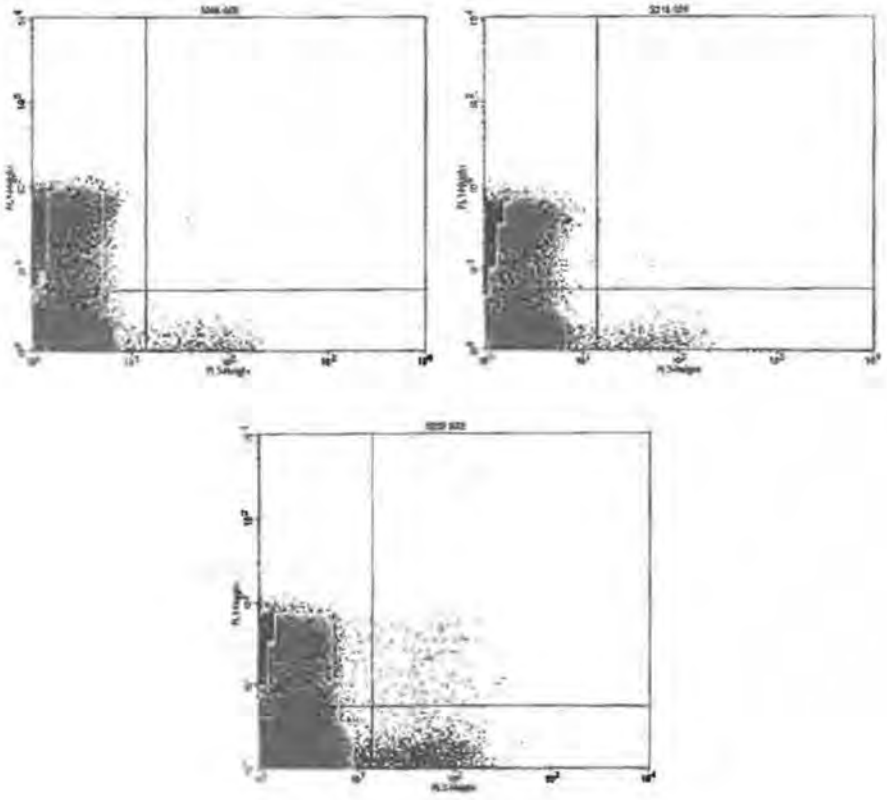

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Table 1: Individual Animal Data – Raw Data

Animal No.	Group	Sex	Dose Level (mg/kg body weight)	No. NCE[1]	No. MN-NCE[2]	No. RET[3]	No. MN-RET[4]
5201	1	Male	Negative (Vehicle) Control 0	1123789	1374	19970	30
5202	1	Male	Negative (Vehicle) Control 0	1051210	1275	19972	28
5203	1	Male	Negative (Vehicle) Control 0	1003595	1271	19976	24
5204	1	Male	Negative (Vehicle) Control 0	847800	675	19983	17
5205	1	Male	Negative (Vehicle) Control 0	1271744	1086	19985	15
5206	1	Female	Negative (Vehicle) Control 0	1496832	1190	19972	28
5207	1	Female	Negative (Vehicle) Control 0	1085645	1107	19979	21
5208	1	Female	Negative (Vehicle) Control 0	1220816	902	19980	19
5209	1	Female	Negative (Vehicle) Control 0	1488874	1681	19975	25
5210	1	Female	Negative (Vehicle) Control 0	1283811	1048	19982	18
5211	2	Male	Test Substance (high dose) 1000	1103763	1289	19980	20
5212	2	Male	Test Substance (high dose) 1000	1095103	1202	19980	20
5213	2	Male	Test Substance (high dose) 1000	1233241	1038	19978	22
5214	2	Male	Test Substance (high dose) 1000	1984335	1674	19981	19
5215	2	Male	Test Substance (high dose) 1000	1241507	1921	19964	36
5216	2	Female	Test Substance (high dose) 1000	994214	1286	19963	36
5217	2	Female	Test Substance (high dose) 1000	1145942	1304	19976	24
5218	2	Female	Test Substance (high dose) 1000	1347030	1489	19979	21
5219	2	Female	Test Substance (high dose) 1000	2525095	4087	19969	31
5220	2	Female	Test Substance (high dose) 1000	1679145	1946	19973	27
5221	3	Male	Positive Control (CP) 40	2606393	1887	9890	110
5222	3	Male	Positive Control (CP) 40	2639650	3469	19499	501
5223	3	Male	Positive Control (CP) 40	3966214	5409	19669	331
5224	3	Male	Positive Control (CP) 40	3165193	5722	19619	381
5225	3	Male	Positive Control (CP) 40	2392784	4613	19378	622
5226	3	Female	Positive Control (CP) 40	3493568	4108	19637	363
5227	3	Female	Positive Control (CP) 40	4575952	5807	19754	246
5228	3	Female	Positive Control (CP) 40	4849524	7320	9840	160
5229	3	Female	Positive Control (CP) 40	3482604	4952	9856	144
5230	3	Female	Positive Control (CP) 40	3268788	3186	19807	193

[1] NCE = normochromatic erythrocytes; [2] MN-NCE = micronucleated normochromatic erythrocytes;
[3] RET = CD71 positive reticulocytes; [4] MN-RET = CD71 positive micronucleated reticulocytes;
CP = Cyclophosphamide monohydrate

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Table 2: Individual Animal Data – Calculated Data

Animal No.	Group	Sex	Dose Level (mg/kg body weight)	%RET[a]	%MN-NCE[b]	%MN-RET[c]
5201	1	Male	Negative (Vehicle) Control 0	1.75	0.12	0.15
5202	1	Male	Negative (Vehicle) Control 0	1.86	0.12	0.14
5203	1	Male	Negative (Vehicle) Control 0	1.95	0.13	0.12
5204	1	Male	Negative (Vehicle) Control 0	2.30	0.08	0.09
5205	1	Male	Negative (Vehicle) Control 0	1.55	0.09	0.08
			Average	1.88	0.11	0.12
			StDev	0.28	0.02	0.03
5206	1	Female	Negative (Vehicle) Control 0	1.32	0.08	0.14
5207	1	Female	Negative (Vehicle) Control 0	1.84	0.10	0.11
5208	1	Female	Negative (Vehicle) Control 0	1.61	0.07	0.10
5209	1	Female	Negative (Vehicle) Control 0	1.32	0.11	0.13
5210	1	Female	Negative (Vehicle) Control 0	1.53	0.08	0.09
			Average	1.52	0.09	0.11
			StDev	0.22	0.02	0.02
5211	2	Male	Test Substance (high dose) 1000	1.78	0.12	0.10
5212	2	Male	Test Substance (high dose) 1000	1.79	0.11	0.10
5213	2	Male	Test Substance (high dose) 1000	1.59	0.08	0.11
5214	2	Male	Test Substance (high dose) 1000	1.00	0.06	0.10
5215	2	Male	Test Substance (high dose) 1000	1.58	0.16	0.18
			Average	1.55	0.11	0.12
			StDev	0.32	0.03	0.03
5216	2	Female	Test Substance (high dose) 1000	1.97	0.13	0.18
5217	2	Female	Test Substance (high dose) 1000	1.71	0.11	0.12
5218	2	Female	Test Substance (high dose) 1000	1.46	0.11	0.11
5219	2	Female	Test Substance (high dose) 1000	0.78	0.16	0.16
5220	2	Female	Test Substance (high dose) 1000	1.18	0.12	0.14
			Average	1.42	0.13	0.14
			StDev	0.46	0.02	0.03
5221	3	Male	Positive Control (CP) 40	0.38	0.07	1.10
5222	3	Male	Positive Control (CP) 40	0.75	0.13	2.51
5223	3	Male	Positive Control (CP) 40	0.50	0.14	1.66
5224	3	Male	Positive Control (CP) 40	0.63	0.18	1.91
5225	3	Male	Positive Control (CP) 40	0.83	0.19	3.11
			Average	0.62	0.14	2.08
			StDev	0.18	0.05	0.76
5226	3	Female	Positive Control (CP) 40	0.57	0.12	1.82
5227	3	Female	Positive Control (CP) 40	0.43	0.12	1.23
5228	3	Female	Positive Control (CP) 40	0.21	0.15	1.60
5229	3	Female	Positive Control (CP) 40	0.29	0.14	1.44
5230	3	Female	Positive Control (CP) 40	0.61	0.10	0.97
			Average	0.42	0.13	1.41
			StDev	0.17	0.02	0.33

[a] % RET = frequency (%) of CD71 positive reticulocytes
[b] % MN-NCE = frequency (%) of micronucleated normochromatic erythrocytes
[c] % MN-RET = frequency (%) of CD71 positive micronucleated reticulocytes
CP = Cyclophosphamide monohydrate

APPENDIX B: PSL's HISTORICAL CONTROL DATA

Validation data: cyclophosphamide

		%MN-RET		%RET		%MN-NCE	
Sex	Treatment	Result	Significance	Result	Significance	Result	Significance
Male	Vehicle	0.17 ± 0.02	n/a (ref.)	1.74 ± 0.10	n/a (ref.)	0.12 ± 0.01	n/a (ref.)
Male	1.5 mg/kg	0.23 ± 0.03	ns	2.36 ± 0.37	ns	0.11 ± 0.02	ns
Male	5 mg/kg	0.28 ± 0.03	$p < 0.05$	1.39 ± 0.15	ns	0.10 ± 0.01	ns
Male	15 mg/kg	0.57 ± 0.03	$p < 0.001$	1.48 ± 0.15	ns	0.15 ± 0.00	ns
Male	40 mg/kg	1.33 ± 0.07	$p < 0.001$	0.81 ± 0.18	$p < 0.05$	0.12 ± 0.01	ns
Female	Vehicle	0.17 ± 0.02	n/a (ref.)	1.79 ± 0.17	n/a (ref.)	0.11 ± 0.01	n/a (ref.)
Female	1.5 mg/kg	0.22 ± 0.04	ns	1.83 ± 0.23	ns	0.11 ± 0.01	ns
Female	5 mg/kg	0.29 ± 0.02	$p < 0.05$	1.77 ± 0.13	ns	0.10 ± 0.01	ns
Female	15 mg/kg	0.46 ± 0.05	$p < 0.001$	1.15 ± 0.14	ns	0.11 ± 0.01	ns
Female	40 mg/kg	1.36 ± 0.29	$p < 0.001$	1.15 ± 0.36	ns	0.14 ± 0.02	ns

Cylcophosphamide reference publications: De Boeck, M. et al. (2005) Flow cytometric analysis of micronucleated reticulocytes: Time- and dose-dependent response of known mutagens in mice, using multiple blood sampling. Environmental and Molecular Mutagenesis (46) 30-42. LeBaron, M. J. et al. (2012) Influence of counting methodology on erythrocyte ratios in the mouse micronucleus test. Environmental and Molecular Mutagenesis (54) 222-8. Witt, K. L. et al. (2008) Comparison of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. Mutation Research (649) 101-13.

Historical control data

		%MN-RET		%RET		%MN-NCE	
Sex	Treatment	Mean ± SEM	Control lim.	Mean ± SEM	Control lim.	Mean ± SEM	Control lim.
Male	Vehicle	0.16 ± 0.01	-0.01 to 0.34	2.21 ± 0.15	-0.80 to 5.22	0.12 ± 0.00	0.03 to 0.22
Male	CP 40	1.56 ± 0.08	-0.12 to 3.24	0.69 ± 0.05	-0.29 to 1.68	0.12 ± 0.00	0.02 to 0.23
Female	Vehicle	0.17 ± 0.01	-0.07 to 0.41	1.90 ± 0.08	0.27 to 3.54	0.11 ± 0.01	-0.04 to 0.26
Female	CP 40	1.49 ± 0.12	-1.04 to 4.02	0.81 ± 0.06	-0.54 to 2.17	0.13 ± 0.00	0.04 to 0.22

APPENDIX G.3
MORI SILK 14-DAY REPEATED DOSE ORAL GAVAGE RANGE-FINDING STUDY IN RATS

STUDY TITLE

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

PRODUCT IDENTIFICATION

Silk Fibroin

TESTING 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)

STUDY NUMBER

50725

PERFORMING LABORATORY

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

STUDY COMPLETION DATE

January 24, 2020

STUDY DIRECTOR

Raghavendra Gowda, PhD

SPONSOR

Cambridge Crops Inc
444 Somerville Ave
Somerville, MA 02143

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]

Raghavendra Gowda, PhD
Study Director
Product Safety Labs

01 | 24 | 2020
Date

[redacted]

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

01/24/2020
Date

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STUDY INFORMATION

Protocol No.:	P710.01 CMR
Test Substance:	Silk Fibroin Batch #: 128
Physical Description:	Slightly yellow liquid
Date Test Substances Received:	1) June 14, 2019 2) June 28, 2019
PSL IDs:	1) 190614-1D 2) 190628-1D
PSL Study Number:	50725
Sponsor:	Cambridge Crops Inc 444 Somerville Ave Somerville, MA 02143
Study Initiated-Completed:	June 20, 2019 – (see report cover page)
In-Life Study Initiated-Completed:	June 26 – July 8, 2019

KEY PERSONNEL

Product Safety Labs:	
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Technical Writing Supervisor:	Celeste Dunn, AS

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 14 days. These data will be used, along with existing data, to select dose levels for a subsequent longer toxicity study in rats.

2. SUMMARY

Forty adult Crl: Sprague-Dawley CD® IGS rats (20 males, 20 females) were equally distributed into four dose groups (5/sex/group). Dose levels of 0, 125, 250, and 500 mg/kg/day (Groups 1-4, respectively) were selected for the study.

An appropriate amount of the test substance or vehicle control (distilled water) was administered daily via oral intubation to each rat for 14 days. The test substance was prepared at concentrations of 12.5 (low dose), 25 (intermediate dose), and 50 (high dose) mg/mL, w/v in the vehicle (distilled water). The test substance preparations were administered at a dose volume of 10 mL/kg/day.

The animals were observed at least once daily for viability, signs of gross toxicity, and behavioral changes, and weekly for a battery of detailed observations. Body weights were recorded two times during the acclimation period (including prior to initial dose administration on Day 1), on Days 8 and 15, and immediately prior to sacrifice. Individual food consumption was also recorded in conjunction with scheduled body weights. Food efficiency was calculated. All animals were subjected to a gross necropsy at study termination on Day 15.

There were no mortalities, clinical observations, body weight, body weight gain, food consumption, or food efficiency changes attributable to Silk Fibroin administration. There were no macroscopic findings at necropsy.

Under the conditions of the study and based on the toxicological endpoints evaluated, male and female Sprague Dawley rats are expected to tolerate dose levels of 500 mg/kg/day Silk Fibroin in a study of longer duration.

3. TEST SUBSTANCE

A. Source

The test substance was received from the Sponsor.

B. Identification

The test substance was received on June 14, 2019, and identified using the following information provided by the Sponsor and PSL identification number.

Test Substance (Amendment 2): Silk Fibroin
Batch #: 128; Batch #: 136 (Amendment 1)
PSL IDs: 190614-1D; 190628-1D (Amendment 1)
Physical Description: Slightly yellow liquid
Composition (Amendment 3): 5.0 % Silk Fibroin (CAS# 9007-76-5) & 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.

C. Analysis

The test substance, as received, was expected to be stable for the duration of the study.

D. Hazards

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

4. GENERAL TEST SYSTEM PARAMETERS

A. Animal Requirements

4.A.1 Number of Animals: 40

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: 10 (5 males, 5 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: Seven to 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 June 18, 2019, forty-four (44) CRL Sprague-Dawley CD® IGS rats (22/sex) arrived from Charles River Laboratories, Raleigh, NC, with an assigned birth date of May 3, 2019. The rats were designated by the supplier to be six to seven weeks of age upon arrival.

B. Test System Justification

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

C. Animal Husbandry

4.C.1 Housing

The animals were housed in regularly cleaned cages which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). The animal room had a 12-hour light/dark cycle and was kept clean and vermin free.

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

Temperature and humidity was 19-22°C and 50-67%, respectively.

4.C.3 Acclimation

The animals were conditioned to the housing facilities for six days prior to testing. Body weights and clinical observations were recorded at least 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, except when animals were fasted for terminal sacrifice.

4.C.5 Water

Filtered tap water was 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.

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).

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 the 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.

E. Dose Levels

Five male and five female rats were 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] Distilled water, Fox Ledge; Lot #: 19000510, Exp. Date: 05/10/21.

F. 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. As provided by the Sponsor, the highest achievable level of a visually homogenous solution was 50 mg/mL. 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.

6. GENERAL PROCEDURES

A. Selection of Animals

After acclimating to the laboratory environment for six 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 were selected for test. Forty (40) healthy rats (20 males; 20 females) were selected for test. The animals weighed 210-264 grams (males) and 130-172 grams (females) and were approximately seven to eight weeks of age at initiation of dosing. The rats that were used on test were randomly distributed, stratified by body weight, among the groups on the day of study start.

B. Dose Preparation and Procedures

6.B.1 Test Substance Preparation

The test substance was mixed weight to volume (w/v) in distilled water. Group 1 received 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 were prepared once a week. The formulations were vortexed, if necessary, at ambient temperature 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/day). All doses

were administered volumetrically at 10 mL/kg. The control group received 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 at least 14 days. 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). Residual dose mixtures were properly discarded following daily administration and sampling (as required).

C. Sampling of Test Substance and Dose Preparations

6.C.1 Sample Collections

The neat test substance and dose preparations were sampled in duplicate.

6.C.2 Test Substance and Dose Preparation Stability

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

6.C.3 Dose Preparation Homogeneity

At the beginning of the study, formulation of each concentration was 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. Sample of the vehicle control was collected from the middle of the container only.

6.C.4 Dose Preparation Concentration Verification

Samples for concentration verification were collected from dose preparations at the beginning of the study (as part of the homogeneity assessment, Section 6.C.3).

6.C.5 Sample Preservation

Upon sampling, dose preparations and neat test substance 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.6 Sample Analysis

Samples collected above were not analyzed. Samples are kept for possible future analysis of dose preparations (i.e., high and low doses) and/or neat test substance. If necessary, the concentration verifications can be identified via BCA assay with Silk Fibroin as the standard. Alternatively, gravimetrical methods are also applicable (i.e., TGA, moisture analyzer).

D. Clinical Observations

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

Prior to the first treatment with the test substance on Day 1, and approximately a week after, 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.

E. Body Weight and Body Weight Gain

Individual body weights were recorded at least two times during acclimation. All animals were weighed on Day 1 (prior to study start) and weekly thereafter (intervals of 7 days ±1). The animals were also weighed prior to sacrifice. Body weight gain was calculated for selected intervals and for the study overall.

F. Food Consumption and Food Efficiency

Individual food consumption was measured and recorded to coincide with body weight measurements. Food efficiency was also calculated and reported. Due to a system error, the food consumption and food efficiency for males and females is not reported for Days 1-8.

G. Terminal 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.

7. STATISTICAL ANALYSIS

In-Life Data

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

Statistical analysis was conducted by Provantis® version 9, Tables and Statistics, Instem LSS, Staffordshire UK; INSTAT.

7.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 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 and non-significant findings 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.

8. STUDY CONDUCT

A. Laboratories

Testing Facility

In-life	Product Safety Labs 2394 US Highway 130 Dayton, NJ 08810

B. GLP Compliance

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

C. Test Procedure Guidelines

This study design conformed to 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).

9. FINAL REPORT AND RECORDS TO BE MAINTAINED

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

The following records are maintained:

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

Storage	Disposition
Usage	

B. Information on animals includes but was not limited to the following:

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

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

10. PROTOCOL, PROTOCOL AMENDMENTS, AND PROTOCOL DEVIATION

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

11. RESULTS

A. Mortality and Clinical Observations (Tables 1-2; Appendices C-E and J)

All animals survived test substance administration. There were no clinical signs considered related to test substance administration.

The fate of all animals is presented in Appendix J.

Males

Incidental clinical signs included: superficial eschar on the face of 1/5 Group 2 males and a lesion on the tail of 1/5 Group 4 males which correspond to the following detailed clinical observations: eschar on the face of 1/5 Group 2 males and a lesion on the tail of 1/5 Group 4 males. Neither of these findings was deemed to be test substance-related.

Females

All females appeared active and healthy throughout the entire study.

B. Body Weight and Body Weight Gain (Tables 3-4; Appendices F-G)

Overall and mean weekly body weights and mean daily body weight gain for treated male and female rats in Groups 2-4 were comparable to their respective control Group 1 values throughout the study.

C. Food Consumption and Food Efficiency (Tables 5-6; Appendices H-I)

Due to a recording error, the food consumption for Days 1-8 could not be determined. Mean daily food consumption and mean food efficiency for treated male and female rats in Groups 2-4 were comparable to control Group 1 values on Days 8-15.

D. Necropsy Observations (Appendices J-K)

There were no macroscopic findings at necropsy.

12. CONCLUSION

Under the conditions of the study and based on the toxicological endpoints evaluated, male and female Sprague Dawley rats are expected to well tolerate dose levels of 500 mg/kg/day of Silk Fibroin in a study of longer duration.

13. REFERENCES

Dunnett, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics*, 20(3), 482-491.

Dunnett, C.W. (1980). Pairwise multiple comparisons in the unequal variance case. *J. Amer. Statist. Assoc.* 75, 796-800.

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

National Research Council of the National Academies. (2011). *Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Research, Division of Earth and Life Studies.* National Academy Press, Washington, D.C.

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 [R1]	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 = Mean Daily Body Weight Gain
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	0.178I,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

Raghavendra Gowda, PhD

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**

Raghavendra Gowda, PhD
Study Director
Tel: 732-438-5100 x1542
Email: RaghavendraGowda@ProductSafetyLabs.com

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

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

5. **SPONSOR**

Cambridge Crops Inc
444 Somerville Ave
Somerville, MA 02143

6. **SPONSOR REPRESENTATIVE**

Adam Behrens
Cambridge Crops Inc
444 Somerville Ave
Somerville, MA 02143
Tel: 301.580.3965
Email: adam@cambridge-crops.com

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 vortex 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): David Sinning, BS William D. Gravelle, MS Catherine Wo, PhD Lyla Ansah-Johnson, BS Peter Zehr, PhD Jessica Bellizio, MS

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.

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[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

18. PROTOCOL APPROVAL

Signature: [redacted]
Adam Behrens
Sponsor Representative
Cambridge Crops, Inc
Date: 6/20/2019

Signature: [redacted]
Raghavendra Gowda, PhD
Study Director
Product Safety Labs
Date: 6/20/2019

Signature: [redacted]
Dan Merkel, BS, MBA
President
Product Safety Labs
Date: 6/20/19

'roduct 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]

Raghavendra Gowda Ph.D
Study Director
Product Safety Labs

7/12/2019
Date

Product Safety Labs

PROTOCOL AMENDMENT

6-Day Oral Toxicity Study

PROTOCOL NO.:

AMENDMENT NO.:

STUDY NO.:

PSL NO.:

Section: Study Title

Change from:

Change to:

REASON:

[redacted]

Study Director
Product Safety

Product Safety Labs

PROTOCOL AMENDMENT

[illegible]-Day Oral Toxicity Study

PROTOCOL NO.: P[illegible] CMR **AMENDMENT NO.:** [illegible]

STUDY NO.: 50[illegible] **PSL NO.:** [illegible]

Section 8 [illegible] Identification

Change from: Composition: [illegible] Silk [illegible] (CAS [illegible]) & 95% Water

Change to: Composition: [illegible] (CAS [illegible]) & [illegible] Water

REASON:

The [illegible] [illegible] [illegible]. This amendment [illegible] adverse [illegible] on the study.

[redacted]

[illegible] Ph.D. 7/6/2[illegible]
Study Director
Product Safety Labs

'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]

Raghavendra Gowda, PhD
Study Director
Product Safety Labs

07/24/2019
Date

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] 2019.04.29 11:24:17 -05'00'

Analysis	Result (%)
Protein	16.90
Fat	3.66
Fiber	3.09
Moisture	12.31
Ash	4.81
Calcium	0.65
Phosphorus	0.66

Analysis	Result	Units	Established Maximum Concentration
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
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
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	< 0.06	ppm	0.50
Thirmel	< 0.16	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Diaultaton	< 0.16	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.

[redacted]
2019.04.08 19:23:49 -05'00'

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

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 Hydrocarbon			
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
Organophosphate			
Thimet	< 0.16	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.16	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
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)													
	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

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)											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

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)													
	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

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)				
	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 G.4
MORI SILK 28-DAY ORAL TOXICITY STUDY

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

Raghavendra Gowda, PhD

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: Raghavendra Gowda, PhD

Name of Company: Product Safety Labs

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

Name of Signer: Adam Behrens

Name of Company: Cambridge Crops, Inc.

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

Name of Signer: Laith Abu-Taleb

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

[redacted]

Maryann Zakrzewski
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]

Raghavendra Goyda, PhD
Study Director
Product Safety Labs

04/02/2020
Date

[redacted]

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

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:	Daniel J. Merkel, BS, MBA
Laboratory Director:	Daniel Merrill, BS, MBA
Study Director:	Raghavendra Gowda, PhD
Primary Scientist:	Aubrey Blue
Contributing Personnel:	Cindy Bodnar Jamie Boulet, RLATR Amber Burke, BS Janet Dell John, BA, RLATG Sydney Gavinelli, BS Mallory Kolar Harry Maselli, ALAT Samantha Matricardi, BS Mary Matthews, BS Amber Norton, BS Xiomara Portuguez, BS Mark Schooley Matthew Sorber, BS Colleen Wojenski, BS, LATG Morgan Woods, BS
Director of Quality Assurance:	Rhonda S. Krick, BS
Technical Writing Supervisor:	Celeste Dunn, AS

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:	Dr. Jayachandra, K.C. MVSc, DABT

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:	Victor Ansah-Johnson, BS

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:	Christine E. Watson, MS, BVMS, MRCVS, DACVP

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 -80°C 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 -80°C 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

6.1.2 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 P.I.: Victor Ansah-Johnson, BS

Test Site

Clinical pathology data evaluation	Eurofins Advinus 21 & 22 Phase II, Peenya Industrial Area Bengaluru, 560 058, India Primary Investigator (P.I.): Dr. Jayachandra, K.C., M.V.Sc., DABT
Test Site QA for Clinical Pathology Evaluation	Muktha Bhagavan, M.Sc., RQAP-GLP
Test Site Management for Clinical Pathology Evaluation	Rajiv Malik
Histological slide preparation	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 P.I. (histology): Craig Zook
Histological slide evaluation (Amendment 6)	Histo-Scientific Research Laboratories (HSRL) 5930 Main Street Mount Jackson, VA 22842 P.I. (pathology): Christine E. Watson, MS, BVMS, MRCVS, DACVP

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	411.1	416.5	428.2
	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	8.01 **
	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 **	5.33 **	5.36 **	6.51
	SD	1.55	0.81	0.93	1.42
	N	10	10	10	10
1 → 29	Mean	7.03 *2,3	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	1.55
	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	2.30	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	2.20	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: / To:	1 / 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: Need 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	0.1151	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	2.968	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	11.960	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	8.569	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	0.0058	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	5.488
		SD	0.327	0.283	0.302	0.209
		N	10	10	10	10
Epididymides /TBW (Ratio)	Day 30	Mean	2.6177	2.7204	2.6529	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	32.749	32.159
		SD	2.900	2.283	3.416	4.055
		N	10	10	10	10
SV&CG /TBW (Ratio)	Day 30	Mean	3.556	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	0.65952
		SD	0.16255	0.13100	0.13645	0.10037
		N	10	10	10	10
Ventral Prostate /TBW (Ratio)	Day 30	Mean	1.662	1.950	1.816	1.971
		SD	0.323	0.261	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	0.3205	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	34.256	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	5.479	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	1.517	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	0.288	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	0.0575	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	0.266
		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	0.139	0.185	0.129
		N	10	10	10	10