

STUDY TITLE

Mother ferment. All purpose whole house cleaner (1:12 Dilution):
Acute Oral Toxicity – Up-And-Down Procedure in Rats

TEST GUIDELINE(S)

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)
OECD Guidelines for the Testing of Chemicals, Test No. 425 (2022)

AUTHOR

Samantha Bloom, BS

STUDY COMPLETED ON**PERFORMING LABORATORY**

Product Safety Labs

LABORATORY STUDY NUMBER

71721

SPONSOR

Bioferment Technology
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Sanford, Florida 32771

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NO CLAIM OF CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter: _____

Date: _____

Name of Signer: _____

Name of Company: Bioferment Technology _____

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Mother ferment. All purpose whole house cleaner (1:12 Dilution)

This study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), which are compatible with OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17, OECD, Paris, 1998, JMAFF GLP: Japanese Ministry of Agriculture, Forestry and Fisheries: No. 76, 30 November 2018, EC Directive 2004/10/EC, Official Journal of the European Union, L50/44, Feb. 20, 2004 with the following exception:

The test solution, prepared by PSL in accordance with Sponsor instructions, was not analyzed for concentration, uniformity or stability.

Specific information related to the determination of the stability, identity, strength, purity, and composition of the concentrated test substance is the responsibility of the study Sponsor (see Test Substance section).

Samantha Bloom, BS
Study Director
Product Safety Labs

Sponsor: _____

Date: _____

Name of Signer: _____

Name of Company: Bioferment Technology

Submitter: _____

Date: _____

Name of Signer: _____

Name of Company: Bioferment Technology

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	E. Hyman; K. Rea	Sep 29, 2025; Jan 8, 2026	Sep 29, 2025; Jan 8, 2026
Critical phase inspection: <i>Initiation of dosing for Animal #3102 and 3103</i>	K. Rea	Jan 8, 2026	Jan 8, 2026
Raw data audit	A. Villagran	Jan 26, 2026	Jan 26, 2026
Draft report review	A. Villagran	Jan 26, 2026	Jan 26, 2026

Final report reviewed by:

Quality Assurance
 Product Safety Labs

SIGNATURE

Mother ferment. All purpose whole house cleaner (1:12 Dilution)

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.

Samantha Bloom, BS
Study Director
Product Safety Labs

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KEY PERSONNEL

Study Director:	Samantha Bloom, BS
President:	Daniel J. Merkel, BS, MBA
Laboratory Director:	Daniel Merrill, BS, MBA
Quality Assurance Director:	Edward Hyman, BA, MBA, RQAP-GLP
Laboratory Manager:	Jamie Boulet, LAT
Technician Manager:	Mark Schooley
Primary Scientist:	Harry Maselli, ALAT

**MOTHER FERMENT. ALL PURPOSE WHOLE HOUSE CLEANER (1:12 DILUTION):
ACUTE ORAL TOXICITY – UP-AND-DOWN PROCEDURE IN RATS**

PROTOCOL NO.: P320.UDP
STUDY NUMBER: 71721
SPONSOR: Bioferment Technology
2660 Jewett Lane
Sanford, Florida 32771
TEST SUBSTANCE IDENTIFICATION: Mother ferment. All purpose whole house cleaner (1:12 Dilution)
DATE RECEIVED: December 23, 2025
PSL REFERENCE NO.: 251223-1H
STUDY INITIATION DATE: December 29, 2025
DATE(S) OF TEST: January 7 - January 22, 2026
NOTEBOOK NO.: 71721: pages 1-27

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Mother ferment. All purpose whole house cleaner (1:12 Dilution) by the oral route.

2. SUMMARY

An acute oral toxicity test was conducted with rats to determine the potential for Mother ferment. All purpose whole house cleaner (1:12 Dilution) to produce toxicity from a single dose via the oral route. Under the conditions of this study, the acute oral LD₅₀ of the test substance is greater than 5000 mg/kg of body weight in female rats.

An initial limit dose of 5000 mg/kg was administered to one healthy female rat by oral gavage. Due to the absence of mortality in this animal, two additional females received the same dose level, simultaneously. Since these animals survived, no additional animals were tested. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration (initial) and again on Days 7 and 14 (terminal) following dosing. Necropsies were performed on all animals at terminal sacrifice.

All animals survived test substance administration, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse clinical effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

The concentrated test substance, identified as Mother ferment. All purpose whole house cleaner, MF: 12122026, was received on December 23, 2025, and was further identified with PSL Reference Number 251223-1H. The concentrated test substance was stored at room temperature. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

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

Composition: Active no# 1 is 0.864% - 0.864%, CAS #869062-42-0
Active no# 2 is 0.648% - 0.648%, CAS #148409-20-5

Physical Description: Light amber, liquid.

pH: 4.25

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

Expiration Date: December 20, 2026

B. Animals

- B.1. Number of Animals: 3
- B.2. Sex: Female, nulliparous and non-pregnant.
- B.3. Species/Strain: Rat/Sprague-Dawley derived, albino.
- B.4. Age/Body Weight: Young adult (11 weeks)/195-207 grams at experimental start.
- B.5. Source: Received from Envigo RMS Inc. on December 16, 2025.

4. METHODS

A. Husbandry

- A.1. Housing: The animals were housed in caging which conforms to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Animals were group housed, except on the day of administration, at which time they were single housed and until the animals were deemed acceptable, based on observations, to return to group housing. Enrichment (e.g., toy) was placed in each cage and litter was changed at least once per week.
- A.2. Animal Room Temperature and Relative Humidity Ranges: Environmental controls were set to maintain temperature and relative humidity ranges of $21\pm 2^{\circ}\text{C}$ and 30-70%, respectively. The temperature and relative humidity were monitored daily. There were no significant temperature or relative humidity excursions that adversely affected the health of the animals.
- A.3. Animal Room Air Changes/Hour: Animals were maintained in rooms with at least 10 air changes per hour. Airflow measurements are evaluated regularly, and the records are kept on file at Product Safety Labs.
- A.4. Photoperiod: 12-hour light/dark cycle
- A.5. Acclimation Period: 22 or 23 days

- A.6. Food: Inotiv Global 16% Protein Rodent Diet® #2016. The diet was available *ad libitum*, except during fasting.
- A.7. Water: Filtered tap water was supplied *ad libitum*.
- A.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.

B. Identification

- B.1. Cage: Each cage was identified with a cage card indicating at least the study number, dose level, identification, and sex of the animal.
- B.2. Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study number 71721, constituted unique identification. Only the sequential animal number is presented in this report.

5. PROCEDURE

A. Selection of Animals

Prior to each dosing, experimentally naive rats were fasted overnight by removing the feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Three healthy, naive female rats (not previously tested) were selected for test.

B. Preparation of Test Substance

The concentrated test substance as received was a liquid. The test substance was prepared following the Sponsor's instructions; 59.1 mL (2 oz) of the test substance was mixed with 650.6 mL (22 oz) of distilled water to create a solution for dosing. The prepared test substance was mixed well prior to each use.

C. Dose Calculations

Individual doses were calculated based on the initial body weights, taking into account the density (determined by PSL) and concentration of the test mixture.

D. Dosing

The prepared test mixture was administered to the stomach using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

Individual animals were dosed as follows:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5000	S	S
2	3102		S	S
3	3103		S	S

S – Survival

E. In-life Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes approximately 30 minutes post-dosing, during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma.

F. Body Weights

Individual body weights of the animals were recorded prior to test substance administration (initial) and again on Days 7 and 14 (terminal) following dosing.

G. Necropsy

All rats were euthanized via CO₂ inhalation at the end of the 14-day observation period. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

6. STATISTICAL ANALYSIS

Statistical analysis was limited to the calculation of the mean density value for dosing.

7. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

The testing conducted under this protocol was approved by the PSL Institutional Animal Care and Use Committee (IACUC) and is subject to regular review. Any significant modifications to the protocol were also reviewed and approved by the IACUC prior to implementation.

8. STUDY CONDUCT

This study was conducted at Product Safety Labs' (PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- U.S. EPA GLP (FIFRA): 40 CFR Part 160, 1989

which are compatible with:

- OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17, OECD, Paris, 1998
- JMAFF GLP: Japanese Ministry of Agriculture, Forestry and Fisheries: No. 76, 30 November 2018
- EC Directive 2004/10/EC, Official Journal of the European Union, L50/44, Feb. 20, 2004

and based on the following testing guidelines:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100 (2002)
- OECD Guidelines for the Testing of Chemicals, Test No. 425 (2022)

9. 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. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

10. AMENDMENTS TO THE PROTOCOL

None.

11. DEVIATIONS FROM THE PROTOCOL

None.

12. 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 original version of the signed final report and the original protocol, raw data, and associated documents will be sent to the Sponsor. An electronic version of the signed final report and electronic copies of the original protocol, raw data, and associated documents will be maintained in the PSL Archives.

Any electronic raw data generated will be maintained on-site in accordance with GLP archiving procedures.

13. RESULTS

Individual body weights and doses are presented in Table 1. Individual in-life and necropsy observations are presented in Tables 2 and 3, respectively.

All animals survived test substance administration, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse clinical effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

14. CONCLUSION

Under the conditions of this study, the acute oral LD₅₀ of Mother ferment. All purpose whole house cleaner (1:12 Dilution) is greater than 5000 mg/kg of body weight in female rats.

15. REFERENCES

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

TABLE 1: INDIVIDUAL BODY WEIGHTS AND DOSES

Animal No.	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose
			Initial	Day 7	Day 14	mL
3101	F	5000	207	217	233	1.0
3102	F		195	209	233	0.98
3103	F		201	212	242	1.0

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal	Animal	Dose Level (mg/kg)	Organ / Tissue	Observation
3101	F	5000	All tissues and organs	No gross abnormalities
3102	F	5000	All tissues and organs	No gross abnormalities
3103	F	5000	All tissues and organs	No gross abnormalities