Food Research Institute
UNIVERSITY OF WISCONSIN-MADISON

College of Agricultural & Life Sciences
1550 Linden Drive
Madison, WI 53706-1521
Web: fri.wisc.edu.

DATE: February 27, 2025

RE: Mouse Count for 2024

Animal use January 1, 2024 thru December 31, 2024

Date	Mice Ordered	LAR counts
Total Mice	3614	3614
1/8/2024	200	200
1/10/2024	150	150
1/29/2024	200	200
1/31/2024	200	200
2/19/2024	180	180
3/4/2024	150	150
3/6/2024	100	100
3/25/2024	200	200
4/1/2024	150	150
4/15/2024	200	200
4/29/2024	120	120
5/1/2024	180	180
5/29/2024	300	300
6/5/2024	90	90
6/10/2024	150	150
6/17/2024	90	90
6/19/2024	60	60
7/3/2024	30	30
7/29/2024	66	66
8/12/2024	60	60
9/4/2024	48	48
9/9/2024	90	90
9/30/2024	30	30
10/21/2024	150	150
11/11/2024	60	60
11/18/2024	90	90
12/2/2024	120	120
12/16/2024	150	150

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

University of Wisconsin-Madison Institutional Animal Care and Use Committee (IACUC)

Protocol # : M006344-R01

Date Approved : 5/12/2023

Expiration date : 5/11/2026

Protocol Basics

1. Protocol Title

* Give your protocol a title.

> Production of [redacted] in Food

2. Principal Investigator (PI)

If you cannot find the name you want, email arrow_help@rarc.wisc.edu.

* Select the Principal Investigator (PI).

[redacted]

3. PI Status

* Select the current status of the listed PI.

- ○ Faculty
- ○ Emeritus appointment
- ◉ **Other**

4. PI Department

* Enter the PI's department name.

> Food Microbiology & Toxicology

5. Protocol Writers

If you cannot find a name or have other questions, email arrow_help@rarc.wisc.edu

Other than the PI, choose people to help prepare, edit and submit protocols.

Person
[redacted]

6. Email Contacts

If you cannot find the name you want, email arrow_help@rarc.wisc.edu

Along with the PI and protocol writers, add up to two people who should receive pertinent protocol notfications.

Person
[redacted]

7. Emergency Contacts

* Add at least one person authorized to act in an animal emergency if the Principal Investigator is not available. This person must understand the research and be able to answer questions in the PI's absence.

Person
[redacted]

Funding

Identify all funding sources that support your protocol. If you have questions about grant-protocol congruence, email or submit the **Congruence Review Request Form** to **congruence@rarc.wisc.edu**.

1. Funding Administered by UW-Madison

NOTE: All funding proposals or awards in RAMP must be selected here to link this IACUC protocol to funding.

Select any pending or awarded funding that is managed by Research and Sponsored programs (RSP) or has a funding string.

PI Name	Funding ID	Title	Sponsor	Primary Sponsor	Primary Reference #	Sponsor Reference #	Status	Congruence Determination	F
[redacted]		[redacted]	FARTHER FARMS INC			N/A	Active		
[redacted]		[redacted]	CORBION			[redacted]	6-Completed		

2. Other Funding

NOTE: Funding listed below will NOT link this IACUC protocol to funding in RAMP.

If you have any funding that is not listed above, please provide the name of the funder or sponsor (e.g. department) and a brief title or description of the funding.

Project Title	PI Name	Sponsor (Source)	Start Date	End Date
Safety Evaluation of Foods	[redacted]	Food Industry		
Microbiological Safety of low-acid foods	[redacted]	University of Wisconsin Foundation		

3. Public Health Service (PHS), NSF, NASA, DOD Funding

See https://en.wikipedia.org/wiki/United_States_Public_Health_Service for a list of PHS agencies.

* Are any of the funding sources above (RSP Managed or Other Funding) directly from or subawards from NIH (or other PHS agencies), NSF, NASA, or DOD Funding?

Yes **No**

Protocol Type

Biomedical Research, Basic Biology, Teaching and/or Colony Management

For protocols that involve any of the following:

- Basic biological processes, human clinical medicine, or medical trials intended as models of human (not animal) diseases
- Instruction related to topics listed above
- Breeding and colony management practices for animals used in basic biology and biomedical research and teaching
- Wildlife species brought to campus for more than temporary procedures
- The use of horses to teach students veterinary medicine (the prevention, diagnosis and treatment of disease, disorder and injury in non-human animals)

Agricultural Research, Teaching, and/or Herd Management

For protocols that involve any of the following:

- Improving animals' use in production agriculture
- Trials intended to improve animal welfare
- Breeding and herd management practices for animals used in agricultural research and teaching
- The use of horses to study or teach equine science (the study of the reproduction, physiology, behavior and

nutrition of horses)

Wildlife Study with No Housing OR Educational Display Only

Wildlife Study
For protocols that involve:

- Only wildlife
- No Housing

And may also involve:

- Observation or field instruction*
- Modification of animals' environment
- Capture
- Handling
- Use of anesthesia
- Procedures in the field
- Procedures at a campus location for a period lasting NO MORE than 24 hours

Educational Display
For protocols that involve:

- Housing or no housing
- No experimental procedures
- Wildlife and/or domestic/lab animals

*If the study involves no animal handling and no modification of the animals' environment, a protocol requirement may be waived. Contact an **IACUC administrator** for more information.

Other

You must consult with an **IACUC administrator** before selecting.

1. Infectious Disease

* Does this protocol include work with infectious disease?

○ Yes ◉ **No**

2. Protocol Type

For help, email [redacted]@rarc.wisc.edu.

* What type of protocol are you submitting?

◉	**Biomedical Research, Basic Biology, Teaching and/or Colony Management**
○	Agricultural Research, Teaching, and/or Herd Management
○	Wildlife Study with No Housing OR Educational Display Only
○	Other

VA ACORP

VA researchers must complete the entire UW protocol application to provide answers about procedures and/or housing at UW facilities.

1. VA Status

Indicate if any of the following apply to this study or project. Select all that apply.

There are no items to display

2. Veterans Administration ACORP

* Is your work also described in an approved Veterans Administration Animal Component of Research Protocol (ACORP)?

○ Yes ◉ **No**

Significance and Justification

1. Significance of Research

* Using language that a high school student would understand (avoid technical grant application language), briefly describe the goals of your study including an explanation of how your work will advance knowledge, improve human or animal health, or benefit society. At the end of your response, briefly and in nonscientific language describe how you plan to interpret the collected data to meet the goals of the study.

The overall goal of this work is to provide validation of combinations of formulation, processing, and storage temperature-time needed to assure the microbial safety of low-acid foods. Specifically, the objective of this research is to determine if [redacted] will produce its highly potent neurotoxin [redacted] in various commercial food products. This information is essential to identify critical control points to food manufacturers for formulation, processing, and storage conditions. The benefit to humans of these food challenge studies is to ensure the safety of low acid foods from [redacted].

Benefits to society (Public Health Significance of Work): Spores of [redacted] *are* ubiquitous and can be isolated from almost any type of food, including vegetables, fish, meat, and dairy. If contaminated, low acid foods (pH >4.6, that are not canned or formulated for safety) stored at temperatures >4°C (40°F) could support bacterial growth and toxin production. Consumption of these contaminated foods can result in a severe paralytic foodborne disease. The average duration of illness is 52 days for non-hospitalized patients and 128 days for hospitalized patients (Minor et al., 2014).

Commercially-prepared foods are implicated each year with cases of this foodborne disease (e.g. 2017 outbreak related to process cheese sauce; 2011 European outbreak related to stuffed olives; 2010-11 cases associated with refrigerated potato soup; 2014 outbreak in Ohio related to pesto, 2006 multi-state outbreak related to carrot juice etc.). None of these foods contained growth inhibitors and labels advising refrigeration or hot-holding were either ignored or not noticed. Additionally, a survey of home refrigerators in the U.S. (Ecosure, 2008) found 27% of the foods were at temperatures greater than 10°C (50°F) and 3% were greater than 15.6°C (60°F), temperatures which can support growth of the bacteria. Because of these incidents, the food industry is continually reviewing their portfolio for high-risk foods and reformulating to provide an added margin of safety. Changes to historically safe and stable foods are called into question when chemical preservatives (e.g. sodium nitrite) are removed in response to consumers demand for natural (such as uncured meats) or low salt foods (such as low-sodium, shelf-stable cheese spread). The food industry does not conduct their own challenge studies because this type of research requires specially trained microbiologists with expertise in both animals and toxin. Authorized containment facilities are also required for research with the bacteria and toxin.

Predictive models generated using laboratory media are inadequate to identify safe formulations because complex, heterogeneous food matrices may have microenvironments at the interface of various components. The challenge studies conducted in our lab are essential to guide food manufacturers regarding adequate formulation, processing, labeling, and handling procedures. In turn, these modifications

will benefit society (protect public health) by generating foods which can withstand abuse by the consumer and remain safe.

Data has demonstrated that certain natural plant extracts which are cultured to convert indigenous nitrate to nitrite has similar inhibitory properties as chemical sodium nitrite; preliminary data suggest that the lower effectiveness of the "natural" nitrite in a food system may be due to differences in the concentration of in-going nitrite rather than the source of the nitrite. Furthermore, these experiments have identified that the addition of certain commercial organic acid blends and fermentation byproducts which have been shown to control L. monocytogenes also have efficacy against this bacterium in a model meat system (unpublished data). This research will be continued to determine the concentrations of these natural antimicrobials required for efficacy under different conditions of moisture, pH, salt, food substrate, and storage temperatures/times. Studies in laboratory media and pasteurized process cheese products have demonstrated that potassium sorbate, sorbic acid, and sodium and potassium benzoate provide significant delays in time to growth and toxin production compared with earlier predictive models developed in standard processed cheese products. A model developed from additional process cheese research demonstrated that sorbate had a significant inhibitory effect on bacterial growth and that fat and salt substitution had lesser of an effect. Challenge studies during the next three years will collect data to validate the safety of products that are formulations outside the model parameters, and to initiate additional research evaluating the effect of other adjunct ingredients. Furthermore, research will continue to evaluate the effect of clean-label antimicrobial ingredients (such as plant extracts, fermentates, etc.) on extending the margin of safety for refrigerated and shelf-stable foods.

2. Justify Use of Animals

* Explain why you must use live vertebrate animals instead of nonanimal alternatives such as computer simulation or in vitro systems.

> The standard procedure of the U.S. Food and Drug Administration (FDA) uses the mouse lethality test to detect the presence of functional toxin in food samples or cultures (Bacteriological Analytical Manual Online, 2001, updated 10/31/2017; accessed 3/28/2023). To date, the FDA has not accepted an alternative in-vitro toxin test that is reliable or accurate for detection/confirmation of [redacted] for all types of complex food samples. The FDA BAM describes the use of the DIG-ELISA assay for the screening of presumptive toxin containing samples. If in-vitro assays, such as the DIG-ELISA are used, FDA guidelines indicate that positive results are to be confirmed using the mouse bioassay; for research specifically validating the safety of foods, FDA requires that the mouse assay be used to verify any negative results ([redacted] personal communication). Toxin detection assays have recently been reviewed ([redacted] et al., 2019) and the mouse bioassay is still considered the gold standard assay. Furthermore, many in-vitro assays have the drawback of high background, and most measure only one biological property of toxin activity (binding of the toxin to antibody, or proteolytic activity in the endopeptidase assays) and do not involve all stages of intoxication. Some in-vitro toxin tests are not as sensitive as the mouse lethality test, in some cases because specific antibodies against all toxin types have not been developed. Certain in-vitro assays may be sensitive enough to detect lethal levels of toxin but may give false-negative or false-positive results due to loss of biological activity of the toxin or because of interaction with the food substrate, respectively. The use of bacterial (or C. sporogenes as a surrogate) plate counts as alternate indicators of potential toxin production in foods

> has been shown by our laboratory to be unreliable ([redacted]) and correlation between log growth and toxin production has not yet been identified. Culture methods also do not easily differentiate between [redacted] producing species and other nontoxigenic anaerobic sporeformers that can be naturally found in foods. Lastly, the organism but not the toxin is commonly found in foods and even in our intestines; therefore, the presence of toxin and not the organism (such as detected by PCR) is the only true indicator of food safety by formulation inhibition.

Experimental Narrative

1. Experimental Narrative Summary

If you are unsure if your study-specific husbandry practices are different from the standards provided by the vivarium staff, consult with a RARC research animal veterinarian, WNPRC veterinarian, or the supervisor of the animal facility.

* In language that scientific colleagues outside your discipline would understand, provide a global, chronological summary of your experiments that focuses on the experience of the animals from initial assignment to final disposition. Briefly outline all proposed surgeries, non-surgical procedures, and other manipulations. Do Not Include: breeding schemes, blood draws, housing arrangements, complete surgical descriptions, euthanasia methods, drug doses, drug routes, or other standard practices.

Summary of procedure:

For this assay, food samples previously inoculated with spores are extracted using buffer, and the potential [redacted] extracts are injected into the intraperitoneal cavities of adult mice. Mice are routinely and frequently observed for typical symptoms of diseasm (ruffled fur, “wasp-waist”, hind limb paralysis, difficulty breathing, and death) as required in the FDA (Food and Drug Administration) mouse assay (Bacteriological Analytical Manual Online, 2001, updated 10/31/2017; accessed 3/28/2023). Unnecessary suffering is reduced by rapid euthanasia in mice that show symptoms during or after the 48-hour standard assay period, or exhibit non-specific symptoms such as inflammation or distress. Occasionally, symptoms may develop quickly between welfare checks and death may occur prior to being able to euthanize the animals. Death is not the intended endpoint.

If a sample is found to cause symptoms or death in mice within 48 hours, additional mice will be similarly injected with supernatant that heated to 80°C for 15 minutes to inactivate any potential [redacted], and likewise observed for 48 hours. Mice exhibiting typical symptoms or non-specific signs of illness or stress will be euthanized to reduce suffering.

Most mice are injected only once with a food suspected to contain toxin. However, in order to reduce the total number of mice used, certain healthy mice will be used for a second injection whenever possible. For example, certain mice (negative controls) are injected with extracted foods samples that contain no [redacted] and these mice will not

exhibit any disease symptoms, die, or develop immunity. Cages will be marked as "used" and mice will be observed for a minimum of 48 hours post-injection to ensure that they show no signs of stress or illness. If all mice within the negative control injection group survive and are healthy, the negative control mice may be used for a second injection with bacterial cultures that may have high levels of toxin and which typically cause death within 2-4 hours. Negative control mice will be re-used only once. Animals that do not exhibit [redacted] symptoms or die within 48 hours of the second injection will be euthanized in accordance with OLAW (Office of Laboratory Animal Welfare) and AVMA (American Veterinary Medical Association) guidelines.

2. Research Cores

* Some campus Research Cores conduct unique procedures (e.g. breeding or imaging) where ALL OF THE PROCEDURES they conduct are described on the CORE protocol. In these situations, animals are formally transferred to the core protocol for these specialized activities.

Note, if service personnel are conducting procedures described on your IACUC protocol, do not select them here, but rather choose them on the Select Study Team page.

Do you plan to transfer animals for services under a research core protocol?

○ Yes ◉ **No**

3. Supporting Publications or Manuscripts

Do not list standard husbandry references.

List the title/name of manuscripts, abstracts, or other references supporting your research that the IACUC may find helpful in evaluating this protocol.

> Bacteriological Analytical Manual Online, 2001, updated 10/31/2017; accessed 03/28/2023
>
> [redacted] et al., 2017, J. Food Prot. 80:1478-1488
>
> [redacted] et al., 2017, J. Food Prot 80:1252-1258 and 1478-1488
>
> [redacted] et al., 2019. Toxins 11: 418
>
> [redacted] et al., Toxicological Sciences 134(1), 64–72 2013

[redacted]

[redacted] et al., 2020. Anal. Bioanal Chem 412:1385-1393

[redacted] et al., 2017. Nanoscale Res Lett 12:227

National Advisory Committee on Microbiological Criteria for Foods. 2010. J Food Prot 73:140-202

[redacted] et al., 2017 J. Pharm Toxicol Methods

[redacted] et al., 2019. Toxins 11:713

[redacted] et al., Biochem Biophys Res Comm (2011) 404:388–39

[redacted] et al., J Pharmacol Toxicol Methods. 2010 61:304-10

[redacted] et al., 2017. Front Parma 8:796

[redacted] et al., 2010 Appl Environ Micro 76:7653-7657

[redacted] et al., Appl Environ Microbiol. 2006 72:1231–1238

[redacted] et al .2015, Health Security 13:37-44

[redacted] et al, 2018. Toxins 10:476

[redacted] et al., 1986, J. Food Prot. 49:526-531

[redacted] et al., 2019. Scientific Reports 9:5531

[redacted] et al., 2017, ACS applied materials & interfaces

[redacted] et al., 2019. Anal. Bioanal Chem 411:5489-5497

[redacted] et al., 2019. Biosensors Bioelectronics 146:222754

[redacted] et al., Toxicol Sci 126:426-435

[redacted] et al., 2011, Comp Med. 61, 235-242

[redacted] et al., 2017. J. Immunol Sci 415:90-99

[redacted] et al., 2012, Analytical Biochemistry. 430:185-192

4. Summary Files

Attach file(s) with timelines, illustrations, figures, or other supplemental information that provides an overview of the protocol. Do not attach copies of grant applications.

There are no items to display

Duplication

Animal welfare regulations do not allow unnecessary duplication of previous experiments.

1. Experiment Duplication

* Do the proposed activities duplicate previous work?

- (•) **Yes**
- ( ) No
- ( ) Not Applicable - This is a teaching activity involving different student groups

1.1. Duplication Explanation

* Please explain why it is scientifically necessary to replicate the experiment.

> This research does not duplicate existing knowledge, with the exception of the inclusion of certain control treatments to validate the accuracy of results. A literature review is conducted prior to starting each challenge study to identify essential factors to test, including use of any relevant predictive models to help determine optimal incubation times and sampling intervals. These estimates are then used to design the study for the most efficient use of animals and other resources by reducing the number of treatments to the minimum level that will provide accurate and reproducible results.
>
> Accurate assessment of [redacted] in food challenge studies is essential to meet the objectives of our research, which is to provide critical control points to food manufacturers for formulation, processing, and storage conditions to prevent toxin production in foods, and ultimately to protect public health. The standard procedure of the FDA uses the mouse lethality test to detect the presence of toxin in food samples or cultures (Bacteriological Analytical Manual Online, 2001). Currently, there are no validated, alternative assays to this test that will provide equivalent assessment of toxin production in all the foods and all toxin types tested in our challenge studies.

Selected Species

Questions regarding each species can be found in the Species Details section of the protocol.

Click on the Species Details button next to the species you would like to work on. When you are finished answering questions for all species, click Continue or save and exit. You can exit before answering all questions and return later to finish.

1. Species Details

Species	Max. Number	Surgery?	MSS?	Breeding?	GM?	USDA Code	Print	Complete?
Laboratory mouse	28300	no		no	no	Not USDA-covered activity or species		

Select Study Team

1. Study Team

For help, email arrow_help@rarc.wisc.edu.

Do NOT include:
*Rotating Students who will be in the lab for less than 30 days
*RARC Veterinary Staff
*RARC Training Staff

Only include **Animal Facility Supervisors, Animal Care Staff** and **Student Workers** if one of the following applies:
*This is a herd management or ART tracking protocol
*They will be performing procedures specifically listed on your lab protocol

* Add all research personnel, including the PI, who will work with a species under this protocol.

	Name	Office phone	Lab phone	Cell phone	Email
View	[redacted]		[redacted]		[redacted]
View	[redacted]			[redacted]	[redacted]

View [redacted]
View [redacted]
View [redacted]
View [redacted]
View [redacted]

2. Research Service Groups

Please select service provider group(s) that will directly handle live animals on this protocol or conduct any of the procedures listed on your protocol. Study teams must contact service providers to coordinate services and ensure they have the resources to provide the services.

	Name	Protocol Manager
☐	BRMS Research Services	[redacted]
☐	Center for Biomedical Swine Research and Innovation (CBSRI)	[redacted]
☐	RARC Veterinary Analgesia and Anesthesia Services	[redacted]
☐	RARC Veterinary Research Services	[redacted]
☐	Small Animal Imaging & Radiotherapy Facility (SAIRF)	[redacted]
☐	UW Gnotobiotic Shared Resource	[redacted]
☐	Waisman Center Behavioral Testing Services (BTS)	[redacted]
☐	WNPRC Animal Services Division	[redacted]

3. Groups of Supervised Individuals

List groups (not service providers or cores) working under supervision on this protocol (for example, 4th year vet students). Do not name individuals or include any assignments.

> *No Answer Provided*

4. PI Oversight

If the PI (him or herself) will not be handling or working with a live species, explain how the PI will provide the oversight necessary for compliance with animal program regulations and requirements.

> [redacted] maintain weekly contact with laboratory staff who directly handle animals and ensure compliance with animal program regulations and requirements ([redacted]). [redacted] is the primary contacts with vivarium staff, veterinarians, and inspectors.

5. Supervisor/Trainer for Staff

* Please state who will train and supervise study team members.

> [redacted] and [redacted] will train all staff with less than 1 year of experience.

Assignments and Qualifications

1. Study Team Member Assignments

For help email, arrow_help@rarc.wisc.edu

Click 'Add' below to associate each team member with a species and/or a procedure. Each member must be associated with at least one species and each procedure must be associated with at least one member.

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
	UW Microisolator Technique - 2023-01-05

View

RARC Classes	Mouse Training - 2023-01-04 Animal User Orientation - 2021-09-01
EHS/UHS Training	Animal Contact Risk Questionnaire - 10/1/2025 Risk Communication in Animal Facilities - 11/23/2025 Safety for Personnel with Animal Contact - 11/27/2027
Education	**Degrees:** [redacted] [redacted] **Professional Memberships:** International Association for Food Protection
Experience	[redacted]
Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	*No value entered*
Transport Method Assignment	*No value entered*

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
RARC Classes	UW Microisolator Technique - 2022-03-22 Mouse Training - 2022-02-24 Animal User Orientation - 2021-08-31
EHS/UHS Training	Animal Contact Risk Questionnaire - 8/6/2025 Risk Communication in Animal Facilities - 1/14/2028 Safety for Personnel with Animal Contact - 2/11/2027

View

Education	[redacted]
Experience	Mouse handling class completed 2/24/22
Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	*No value entered*
Transport Method Assignment	*No value entered*

View

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
RARC Classes	Mouse Training - 2023-01-23 RARC Animal User Recertification - 2022-12-15 Animal User Orientation - 2021-12-23
EHS/UHS Training	Animal Contact Risk Questionnaire - Expired Risk Communication in Animal Facilities - 11/14/2025 Safety for Personnel with Animal Contact - 11/23/2027
Education	[redacted]
Experience	*No Value Entered*

Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	*No value entered*
Transport Method Assignment	*No value entered*

View

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
RARC Classes	UW Microisolator Technique - 2020-10-07 Animal User Orientation - 2020-05-05 Mouse Training - 2012-08-30 Animal User Orientation - 2012-08-28 Mouse Training - 2009-01-08 Animal User Orientation - 2008-12-08
EHS/UHS Training	Animal Contact Risk Questionnaire - 9/20/2025 Risk Communication in Animal Facilities - 4/6/2026 Safety for Personnel with Animal Contact - 5/6/2025
Education	[redacted]
Experience	*No Value Entered*
Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation	*No value entered*

Assignment	
Transport Method Assignment	*No value entered*

View

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
RARC Classes	UW Microisolator Technique - 2023-06-21 Mouse Training - 2023-05-26 Animal User Orientation - 2023-01-04
EHS/UHS Training	Animal Contact Risk Questionnaire - 6/6/2025 Risk Communication in Animal Facilities - 5/25/2026 Safety for Personnel with Animal Contact - 1/4/2028
Education	*No Value Entered*
Experience	*No Value Entered*
Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	*No value entered*
Transport Method Assignment	*No value entered*

View

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
RARC Classes	RARC Animal User Recertification - 2021-09-14 Animal User Orientation - 2016-09-12 Animal User Orientation - 2011-10-10 Mouse Training - 2009-04-08 Animal User Orientation - 2006-09-29
EHS/UHS Training	Animal Contact Risk Questionnaire - 2/5/2026 Risk Communication in Animal Facilities - 6/26/2027 Safety for Personnel with Animal Contact - 5/27/2025
Education	*No Value Entered*
Experience	*No Value Entered*
Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration

Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	*No value entered*
Transport Method Assignment	*No value entered*

Name	[redacted]
Species	Laboratory Mouse
Surgeries	*No value entered*
RARC Classes	UW Microisolator Technique - 2022-11-02 Mouse Training - 2021-10-22 Animal User Orientation - 2021-08-27
EHS/UHS Training	Animal Contact Risk Questionnaire - 8/14/2025 Risk Communication in Animal Facilities - 8/7/2027 Safety for Personnel with Animal Contact - 9/6/2026
Education	◦ [redacted] ◦ [redacted] ▪ [redacted] ▪ [redacted] ▪ [redacted]
Experience	◦ Mouse ▪ Handling and Restraint ▪ IP Injections ▪ CO2 Euthanasia
Painful nonsurgical procedures	[redacted] and/or [redacted], Supermatant fluid of buffered extract administration
Physical euthanasia	*No value entered*

View

methods	
Anesthesia Analgesia Sedation Assignment	*No value entered*
Transport Method Assignment	*No value entered*

2. Research Service Group Assignments

Assign species, surgeries, and procedures to the service group. At least one species must be assigned to each service group.

There are no items to display

3. Other Relevant Experience or Training

Include any protocol-specific experience and/or relevant training for a given study team member that is not found above.

> *No Answer Provided*

Occupational Health

Use of hazardous materials requires separate review and approval by EH&S. The Principal Investigator is responsible for obtaining all relevant approval(s) prior to initiating work with hazardous materials.

1. Occupational Hazards

If you have any questions regarding this section, visit the Animal Research Safety Protocol Guidance Website.

* Are any of the following used in the research involving live animals

under this application? Check all that apply:

☑	**Biological hazards (zoonotic agents, human or animal pathogens, human cells, prions, etc.)**
☐	Chemical hazards (carcinogens, flammables, highly reactive, corrosives, etc.)
☐	Physical hazards (UV light, magnetic fields, noise, electric shock, temperature, etc.)
☐	Radiation and/or radioactive materials (administration of radionuclides, etc.)
☐	Other hazards (zoonotic agents, BSL1 agents that do not require a biosafety protocol, farm work safety precautions, other.)
☐	NONE. None of the hazards listed above apply to research performed on living animals under this application.

Biological Hazards

Biological hazards or biohazards includes all microorganism and toxins produced by microorganisms that are human pathogens regardless of their transmissibility, invasiveness, virulence or lethality. Include human or primate-derived cells, tissues or other materials, as well as prions, and pathogenic fungi. Also include zoonotic pathogens (i.e., pathogens transmissible from animals to humans).

Note that most uses of biological hazards also require an approved UW-Madison Biosafety Protocol from the Office of Biological Safety (OBS). Contact OBS if assistance is needed to complete this section.

1. Biohazard OBS

* Is this work with biological hazards covered by an approved Biosafety protocol?

Yes

1.1. BH-OBS Number

* Please provide the OBS protocol number(s).

B00000449

2. Biohazard Details

* Add or edit details on any biohazards associated with this protocol listed below.

View

Name	[redacted] and/or [redacted]
Species	Laboratory mouse
Biohazard Risk	Samples potentially containing [redacted] or [redacted] will be handled as Biosafety Level 2 according to Biosafety in Microbiological and Biomedical Laboratories (Centers for Disease Control and Prevention (CDC)). Biological hazard signage is posted outside animal suite. Personnel handling samples have experience working with microbial toxins and hazardous substances, (immunization for [redacted] was discontinued by CDC in 2011, new personnel are not immunized; any exposures or suspected exposures are reported for further medical assessment or prophylactic treatment). Standard practices for the handling of such materials include aseptic technique, proper containment of potentially toxic samples within the laboratory, biocontainment centrifugation, opening of rotor and filling syringes under fume hood or biological safety cabinet, and decontamination of all equipment and surfaces by use of appropriate disinfectants or autoclaving. Non-autoclavable equipment and surfaces are disinfected with an appropriate concentration of Trifectant, Virkon S, or similar solution for a minimum of 10 minutes. Contaminated needles and sharps are disposed in puncture-resistant sharps disposal containers and autoclaved before discarding as per University of Wisconsin policy. All personnel wear lab coats, eye protection, and gloves when handling potentially toxic materials and animals. Lab coats and gloves are autoclaved prior to disposal; eye protection is decontaminated by use of appropriate disinfectant. Special handling of cages or animal waste or use of respirators when handling animals, is not required. Visitors must comply with entry requirements and shall be accompanied by authorized personnel at all times.
PPE needed	Exam gloves - Other, Lab coat or Disposable gown, Safety glasses / Goggles, Exam gloves - Latex, Face shield
Is Recombinant	No

2.1. Biohazard Files

Upload any documents associated with the listed biosafety

hazards.

There are no items to display

3. Biohazard Safety Signage

Upload any biohazard safety signage associated with this protocol.

There are no items to display

Species: Laboratory mouse

Justify Species Choice

Species: Laboratory mouse

1. Species or Group Choice Justification

* Explain why you chose this species or target group.

> Mice are known to be sensitive to specific doses of [redacted]. The standard procedure of the U.S. Food and Drug Administration (FDA) is the mouse lethality test to detect the presence of functional toxin in food samples or cultures. To date, no reliable and accurate in-vitro toxin test has been accepted by FDA for detection/confirmation in all types of complex food samples. If in-vitro assays are used, FDA guidelines indicate that positive results are to be confirmed using the mouse bioassay; for research specifically validating the safety of foods, FDA requires that the mouse assay be used to verify any negative results.

Number of Animals

Species: Laboratory mouse

1. Maximum 3-year Total

* What is the maximum number of this species that you will use during your protocol's three-year period?
Include control and replacement, breeding colony, preweaned, and euthanized animals.

> 28300

2. Animal Number Justification

* Provide a justification for the maximum number of animals requested.
For renewals, provide an updated justification for the animals you require for the next three years.

28300 mice are needed for the next three years of

this protocol. Our laboratory conducts numerous [redacted] food challenge studies (average 90-100 experiments) per year. Each challenge study requires an average of 100 mice to achieve 95% confidence in data. Plate count assays are unreliable in determining whether a treatment has failed at any given time point. Mouse toxicity testing at multiple time points, although censored data (uncertain time to failure; failure may occur between intervals), provides the most reliable assessment of whether or not a formulation/process/storage conditions are safe. The number of sampling intervals and samples assayed per each testing interval complies with those recommended by the National Advisory Committee for Microbiological Criteria for Foods for conducting food challenge studies (http://www.fsis.usda.gov/PDF/NACMCF_JFP_Inoculated_Pack.pdf).

Five to ten samples per time interval may be needed to address potential variability in toxin production among food samples. Using FDA methodology for toxin detection in foods, a minimum of two mice are required for samples which are non-toxic and as many as 12 to 24 mice may be needed for confirmation of positive samples containing non-proteolytic strains (i.e. strains unable to cleave their own toxin and require an external protease for activation). For example, the testing schedule of a shelf-stable product inoculated with only proteolytic strains would be 0-time (as negative growth control), plus 1, 2, 3, 4, 6, 9, 12, and 18 months. If all samples are negative, 90 mice would be injected (9 sampling intervals x 5 samples/interval x 2 mice per sample). For refrigerated food studies that utilize a mix of proteolytic and non-proteolytic strains, additional mice are used to test samples that require trypsin as an external protease to activate the toxin of the non-proteolytic strains. Since it is at a colder temperature, fewer sampling intervals would be used (such as 0-time, 2, 4, 6, 7, and 8 weeks; 6 sampling intervals x 5 samples/intervals x 2 treatments/sample x 2 mice/sample treatment =120 mice). If any samples are presumed positive, those samples are confirmed via reinjection [(1 presumed toxic sample + 1 heat-neutralized subsample) x 2 mice/treatment = additional 4 mice per presumed toxic sample]. Testing of a formulation is discontinued when toxin is detected in the majority of the samples assayed for two consecutive sampling intervals.

In addition to food experiments, screening studies and enumeration of spores in media requires mice to verify toxicity. Because these samples are not used for regulatory compliance, presumptive positive tubes are used for calculation and confirmation as described for food sample toxicity and are not injected in an effort to reduce the total number of mice used.

3. Justifications and/or Experience

See policy UW-4131, Justification of Numbers, for guidance and examples of acceptable justifications.

Provide a statistical justification or cite your past experience.

> See above explanation.

4. Upload Number Documentation

Attach file(s) that support your determination of animal numbers. If

possible, use tables to organize your information.

There are no items to display

Bio Species Source

Species: Laboratory mouse

1. Species Source

Animals arriving from outside the main UW-Madison campus will require a time period of acclimation before use. For details, see policy UW-4106 ,Acclimation After Transport.

* Check all sources that apply for this species.

☑	**Investigator at UW-Madison / including another protocol held by PI (check for maximum flexibility in animal transfers)**
☑	**Approved vendor (e.g. Jackson labs, BRMS Breeding Core, etc.)**
☐	Bred under this protocol
☐	Investigator at non-UW Madison institution (Labcorp, other university)
☐	Unapproved vendor
☐	Capture or collection from wild (free-living) population
☐	Herd, flock, etc
☐	Client/privately owned animals
☐	Other

Prior Use

Species: Laboratory mouse

Animals that have undergone a major surgical procedure, permanent physiologic alteration, or substantial impairment on a previous protocol are not eligible for major surgical procedures on subsequent protocols.

1. Prior Use of Animals

* Were any of these animals used in another protocol?

◉ ○

Yes No

1.1. Prior Use Description

* Describe previous nutritional manipulations, blood draws, administered drugs or other materials, or any other past manipulations, and explain how you determined that the animals' assignment to past projects will not compromise your research or the animals' health.

> Prior use of animals that may be received from other investigators will not compromise my research or the health of the animals.

Breeding and Genetically Modified Y/N

Species: Laboratory mouse

1. Breeding

* Does your protocol design include breeding of this species?

○ Yes ◉ **No**

2. Genetically Modified

* Will any of this species be genetically modified? Include animals modified through breeding schemes, purchase of genetically modified animals, or modified using CRISPR-cas9.

○ Yes ◉ **No**

Substance Administration Checklist

Species: Laboratory mouse

Include delivery of materials to animals via injection, infusion, inhalation, implantation, ingestion of food/water, and other means. Include administration of radionuclides. Include nonstandard diets under all other substances. Refer to **RARC guidance for substance administration**

checklist for additional information.

1. Substance Type Selection

* If you will administer substances, check all purposes that apply.

- [ ] analgesics/anesthetics/sedatives to relieve pain or distress caused by nonsurgical and/or surgical procedures
- [x] **euthanasia substance(s)**
- [x] **all other substances**
- [ ] I will not administer any substances.

Euthanasia Substance

Species: Laboratory mouse

If a substance is used to euthanize this species, it should be entered here. Include CO_2. Refer to **RARC guidance for Euthanasia Methods** for additional information.

1. Euthanasia Substance Details

* Provide details on each euthanasia substance you will use.

View

Name	CO2 for euthanasia
Drugs or Compounds	CO2
Euthanasia Procedure Description	Animal will be placed in a nonprecharged chamber and 100% CO2 will be introduced at the rate of 30-70% of the chamber volume per minute as regulated by a flow meter attached to the CO2 canister.

All Other Substances

Species: Laboratory mouse

For each substance or regimen, click "Add" to answer questions about its administration.

Describe the materials delivered to animals via injection, infusion, inhalation, implantation, ingestion in food or water, nonstandard diets, and by other means. Include administration of radionuclides via injection or in food.

Do not include substances used for **clinical relief** of pain or distress (anesthesia/analgesia) or for euthanasia of this species. See help for additional guidance.

1. Other Substances Details

* Provide details on all other substances you will use. Refer to **RARC guidance for All other Substance Administration** for additional information.

View

Name	[redacted] and/or [redacted]
Drugs or Compounds	None.
Category	Natural Biological Toxin
Dosing Details	Mice (Mus) will be given an intraperitoneal injection of 0.5 ml of supernatant fluid of buffered extract of a food or culture media that was previously inoculated with [redacted] spores.
Purpose of Use/Monitoring	Lab personnel observe mice frequently for typical [redacted] symptoms or death for 48 hours (at least twice daily). Occasionally, symptoms may develop and death may occur between welfare checks. Dead mice are removed immediately upon discovery. If a sample is found to cause symptoms or death in mice within 48 hours, additional mice will be similarly injected with supernatant that is pretreated with antitoxin or heated to 80°C for 15 minutes to inactivate any potential toxin, and likewise observed for 48 hours. Negative control mice will be injected with the food extract in which no [redacted] has been added. These mice will be observed and should be normal and healthy throughout the 48 h. These negative control mice may be injected a second time with samples of suspected high toxicity to reduce the overall number of laboratory animals that need to be utilized. Mice exhibiting symptoms or non-specific signs of illness or stress will be euthanized in accordance with OLAW and AVMA guidelines with carbon dioxide.
Painful/Distressful?	Yes
Anesthesia/Analgesia	

Regimen	*No value entered*

Special Substances Checklist

Species: Laboratory mouse

1. Special Substances Selection

* If you are using any special substances, select all that apply. Refer to the **RARC guidance for Special Substance** page for more information.

- [ ] cells, cell lines, tissues, or tissue products (animal and/or human)
- [ ] complete Freund's adjuvant (CFA)
- [ ] controlled substances (requiring DEA and sometimes SUA registration)
- [ ] nonpharmaceutical-grade compounds
- [ ] paralytic agents
- [x] **none of the above**

Nonsurgical Procedures Checklist

Species: Laboratory mouse

1. Nonsurgical Procedures Selection

* Check all types of nonsurgical procedures that will be performed.

- [ ] **Blood collection**
  Sampling by nonsurgical procedures
- [ ] **Food and/or fluid regulation**
  Applies to scheduled or restricted access to food or fluids for experimental purposes.
  Do NOT check this box for fasting before sedation or use of anesthesia or for standard presurgical fasting or fluid regulation. Presurgical fasting will be described in Surgery Summary.
- [ ]

☐ **Genotyping/identification**

Imaging

☐ CT scans, MRIs, ultrasound examinations, X-rays, and other imaging procedures, including those that expose the animal to small amounts of radiation for the purpose of producing a visual image of bodies or processes.
If a dye is used for imaging, add details about the dye in Substance Administration.

Irradiation

☐ Exposure to gamma irradiation and other ionizing radiation for the purpose of affecting animal tissue or physiology.
Administration of radionuclides via injection or in food should be described in Substance Administration.

Physical restraint

☐ Applies to the use of manual or mechanical means to limit some or all of an animal's movement.
Does NOT apply to brief procedures that are part of normal handling or husbandry.
Does NOT apply to normal wildlife-capturing techniques.

Other nonsurgical procedures

☐ Applies to a wide range of other experimental manipulations of animals such as behavioral assays, gastric lavage, maze trials, oocyte collection, preference tests, and more.

☑ **I will not perform any nonsurgical procedures.**

Surgery Y/N

Species: Laboratory mouse

1. Surgery Performed

Surgical procedures that are initiated on a live animal prior to confirmation of death, such as thoracotomy for terminal perfusion, are considered surgeries.

Not surgery: Fine-needle biopsies, intravitreal or subcutaneous injections, simple catheter insertions. These should be described in Other Nonsurgical Procedures.

* Will major, minor, or nonsurvival surgery be performed on any of this species?

○ Yes ◉ **No**

Alternatives Search

Species: Laboratory mouse

Review the following procedures and genetic modifications (if applicable) you described that cause more than momentary pain or distress. Then answer the questions that follow to explain how you determined that there weren't less painful or distressful alternatives to the procedures.

1. Alternatives Databases

* List one or two databases you searched (e.g., AltWeb, Biological Abstracts, NORINA, PubMed, etc.) to look for alternatives.

> Web of Knowledge, Biological Abstracts, Science Citation Index, CAB abstracts, Medline, Citation Index-Science

2. Alternatives Years Covered

* What years did your search cover? (yyyy-yyyy)

> 1900-present

3. Alternatives Recent Search

* What was the date of your most recent search?

> 3/27/2023

4. Alternatives Other

What methods did you use beyond database searches to look for alternatives to painful or distressful procedures (e.g. conference attendance, professional expertise, journal articles, training)?

conference proceedings

5. Alternatives Search Strategy

* Describe your search strategy, including the scientifically relevant keywords you used.

[redacted], [redacted], detection, animal welfare, alternatives to animal testing, refinement and mouse and bioassay

6. Alternatives Narrative

* Evaluate the information you've gathered and explain any alternatives or refined methods that cannot be used in this research.

[redacted] maintains contact with scientists at FDA (Dr. [redacted]) and the Agriculture Research Service of USDA (Dr. [redacted]) who are working to develop alternative assays for [redacted]. FDA described a nanobiosensor capable of detecting and quantifying active Types A and B; however, this method has not been validated in foods, does not include Types E and F, and the authors suggested that it be used as rapid method to "supplement" the mouse bioassay rather than as a replacement.

Another recent publication used a neuronal cell-based assay, but the method was designed to evaluate a biopharmaceutical (derived from purified toxin) rather than detecting toxin in a complex food matrix. The December 2015 issue of the journal Toxin reported that the mouse phrenic nerve hemidiaphram assay as a sensitive and rapid assay for types A, B and E. However, this functional assay still requires animals. The results of an international proficiency test revealed that immunological assays were not as effective as a functional assay. Research by FDA comparing the mouse bioassay and the most promising ELISA system indicated that the DIG-ELISA cannot be used for confirmatory identification.

Another also reported that the ELISA is an effective preliminary screening method for foods, but did not advocate its use as an alternative to the mouse assay. Use of rat primary spinal cord cells, mouse embryonic stem cells, and a neuron and Schwann cell co-culture model has been recently published, but has not been validated for food samples. Because multiple serotypes (Types A, B, E, and F) may be inoculated into a single food system tested, all [redacted] types must be assayed. Some potentially useful assays detect only one toxin type (frequently Type A); hence, no reduction in animal use is made by using the in vitro assay that cannot detect the mixture of toxin types. Scientists at CDC reported a nonlethal mouse toe-spread reflex model to detect Type A spiked into buffer, serum, and milk samples. The assay corrected identified only 80% of the samples with 1 MLD50/ml and it has not been confirmed with the other toxin types (B, E, F) used in the challenge studies. Recently, simultaneous detection of six serotypes by ELISA has been described. However, it too has not yet be validated with complex food matrices. Therefore, although progress is being made, no alternative in-vitro assay or more humane in vivo assay has been developed, validated through collaborative studies, approved, and published (in J. Official Analytical Chemists) that detects potentially harmful levels of

toxin in complex food matrices as well as the mouse bioassay which is proposed in this protocol. Furthermore, there are no better methods to minimize discomfort or injury to the animals without compromising the accuracy and reliability of the results that are used to protect public health.

Complications

Species: Laboratory mouse

In previous sections, you identified the pain and discomfort animals might experience from each procedure. Now consider your procedures from a broader perspective.

1. Potential Complications

* What are the potential complications animals may experience from any of your procedures (e.g., internal bleeding after liver biopsy, Graft Versus Host Disease (GVHD) with transplant) or from any chronic condition resulting from the procedures (e.g., lameness, disease) and how will the complications be managed?

Mice may experience distress resulting from [redacted]. Symptoms include extremely labored breathing and hind limb paralysis. Most mice die within 12-24 hours and frequently within 2-4 hours after injection. Observation of symptoms is necessary for accurate evaluation of [redacted] presence. Although the endpoint of the experiment may involve the death of a mouse to indicate the presence of a certain minimum amount of toxin, observation of symptoms indicating that death is inevitable may be deemed sufficient for diagnosis and these mice will be euthanized. This is the basis from which comparison of the results of different tests can be made. Moreover, the amount of toxin within the sample is not known; therefore, lethal doses must be included.

Lab personnel will observe mice for development of symptoms or death frequently throughout the day and at a minimum of at least twice daily, including at least once within 2 and 4 hours of injection, prior to leaving for the day, and immediately in the morning. Dead mice will be removed from cages immediately upon discovery.

2. Unrelieved Pain or Distress

* Will treatment for pain or distress be withheld from any animals of this species?

(•) **Yes** ( ) No

2.1. Unrelieved Justification

* Provide scientific justification for why pain or distress will not be relieved.

> No drugs are administered because the goal of the experiment is to determine the presence of [redacted] in foods and the administration of drugs would interfere with the observance of symptoms of [redacted].

USDA Designation

Species: Laboratory mouse

The United States Department of Agriculture (USDA) established the following B-E categories based on levels of pain, discomfort, and distress associated with procedures.

1. USDA Designation Code

* Choose the highest category of pain/distress that this species will experience as part of this protocol.

○	B	Animals bred or held for use in teaching, testing, experiments, research, or surgery but not used for such purposes
○	C	Teaching, research, experiments or tests conducted that involve no pain or distress that require use of analgesics
○	D	Experiments, teaching, research, surgery or tests conducted that involve accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic or tranquilizing drugs or palliative measures are used (including surgery or procedures under anesthesia that without the anesthesia would be painful)
○	E	Teaching, experiments, research, surgery or tests conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic or tranquilizing drugs are not used because they would adversely affect the procedures, results or interpretation of the teaching, research, experiments, surgery or tests
◉	**Not USDA-covered activity or species**	**USDA animal welfare regulations do not apply to the use of this species as described in this protocol. If your research is funded by the Department of Defense (DOD) or the USDA Agricultural Research Service (ARS), do not select this. Instead, select the appropriate pain category above regardless of species.**

Endpoints/Euthanasia Methods

Species: Laboratory mouse

Your euthanasia plans must follow the RARC veterinary staff recommendations (refer to **RARC guidelines for Euthanasia by Species**) unless your alternative method is scientifically justified and approved by your IACUC. Refer to the **Help for Euthanasia Methods** to view the AVMA guidelines and guidance about how to complete this page.

1. Criteria for Anticipated Euthanasia

What are your study endpoints?

> Mice are observed for typical symptoms (ruffled fur, "wasp-waist", hind limb paralysis, difficulty breathing, and death) as required in the FDA mouse assay (Bacteriological Analytical Manual Online, 2001, updated 10/31/2017; accessed 12/11/17). Unnecessary suffering is reduced by rapid euthanasia.
>
> Mice show symptoms during or after the 48-hour standard assay period or exhibit non-specific symptoms such as inflammation or distress. Occasionally, symptoms may develop quickly between welfare checks and death may occur prior to being able to euthanize the animals.
>
> As per standard procedures outlined by FDA Bacteriological Analytical Manual Online (2001, updated 10/31/2017; accessed 12/11/17), mice are to be observed for 48 hours for development of symptoms before they are euthanized. If an animal does not die within the observation period, the animal will be euthanized in accordance with OLAW and AVMA guidelines. Negative control mice that have been re-used one time for detection of [redacted] in cultures will be similarly euthanized at the end of the 48 hour observation period.
>
> Most mice are on study 12-48 hours. Negative controls may be on study up to 96 hours total.

2. Criteria for Unanticipated Euthanasia

* For unanticipated events or nonstudy-related health issues, what criteria or clinical signs will you use to determine an unanticipated endpoint for an animal?

> If mice become ill, but do not exhibit typical symptoms, animals will be euthanized immediately rather than wait for the 48 hour observation period.

3. Plan for Anticipated Euthanasia

Select all applicable euthanasia methods for planned study procedures.

	Regimen/Substance Name	Drugs or Compounds	Species
☑	CO2 for euthanasia	**CO2**	**Laboratory mouse**

4. Plan for Unanticipated Euthanasia

Select all applicable euthanasia methods for unanticipated events or nonstudy-related health issues.

	Regimen/Substance Name	Drugs or Compounds	Species
☑	CO2 for euthanasia	**CO2**	**Laboratory mouse**

5. Plan for Physical Methods of Euthanasia

After discussing with an RARC veterinarian, describe your plan for physical methods of euthanasia.

Name	Description
There are no items to display	

6. Other Euthanasia Methods

Describe other planned and unplanned euthanasia methods not included above, including euthanasia performed by the RARC veterinary staff.

> *No Answer Provided*

7. Nonstandard Euthanasia Justification

For methods of euthanasia described above that are NOT listed in RARC Veterinary Standards for this species, justify the use of this method.

> *No Answer Provided*

8. Ensure Death

* Describe the methods you'll use to ensure death following euthanasia procedures.

> Death will be confirmed by respiratory and cardiac arrest.

Disposition

Species: Laboratory mouse

Indicate the final arrangements for animals assigned to this protocol.

1. Disposition Plan

* At the end of their assignment in this protocol, animals will be:

- [x] **Made available to other investigators.**
- [ ] Returned to a UW colony, herd or flock for other use.
- [ ] Returned to their client-owners.
- [ ] Maintained at a privately owned herd or flock.
- [ ] Made available for adoption. Adoption must be preapproved by a laboratory animal veterinarian.
- [ ] Sold at market.
- [x] **Euthanized.**
- [ ] Other.

2. Consumption

* Is there a possibility that animals or humans will consume your animals or their byproducts at the end of your study?

( ) Yes (•) **No**

Nonstandard Husbandry Checklist

Species: Laboratory mouse

Don't include medically justified, standard pre- or post-anesthetic/surgical exceptions, such as short term withholding of food and water. Describe these in SURGICAL PROCEDURES.

Don't include longer-term food or fluid regulation. Describe these in NONSURGICAL PROCEDURES.

Don't describe the use of wire bottom caging here if non-avian animals will be on wire-bottomed caging for less than 12 hours. That should be included in the EXPERIMENTAL NARRATIVE.

This protocol assumes that social animals (including Nonhuman Primates) may be housed singly for non-experimental reasons (e.g. husbandry management, veterinary clinical management) in accordance with campus policies and SOPs.

Don't check 'Single housing of social species' if the reason for single housing is approved in the **UW-Madison Animal Social Housing and Enrichment Requirements (ASHER)** document. If you are using Nonhuman Primates and are unsure if you should check this box, consult with your research animal veterinarian.

1. Nonstandard Husbandry Selection

* Check ALL non-standard conditions that apply to this species.

- ☐ **Housing animals outside dedicated animal facility**
  Animals will be kept for greater than 12 hours for USDA covered animals, or 24 hours for non-USDA covered animals in any location that is not a dedicated animal facility.
- ☐ **Lab staff provide husbandry in facility**
  Laboratory or research staff, rather than professional facility animal-care staff, will provide animal husbandry for a subset of animals housed in facilities.
- ☐ **Single housing of social species**
  Social species are singly housed for periods longer than 12 hours for experimentally-driven reasons. This does not include: clinical reasons, recovery from anesthesia/surgery, social incompatibility, final animal in an experiment, and female rodents near parturition (see ASHER document).
- ☐ **Enrichment withholding**

- [ ] Animals are not provided with the minimum required enrichment as outlined in the facility SOP.

- [ ] **Exercise withholding for dogs**
Dogs are not provided with the minimum exercise as required by the facility SOP.

- [ ] **Ambient Noise**
Animals will be exposed to white noise that is not part of the standard environmental enrichment for the species.

- [ ] **Nonstandard lighting**
Animals will be exposed to lighting paradigm of non-standard wavelength, intensity, or altered light/dark.

- [ ] **Vibration**
Animals will be exposed to vibrations of an amplitude and or frequency known to cause clinical effect.

- [ ] **Cleaning/sanitation schedule different than facility standard**

- [ ] **Enclosure smaller or denser than standard for species**
Animals will be housed in an enclosure that is smaller than the facility standard or at a density higher than the standard for the cage size.

- [ ] **High velocity air**
Animals will be directly exposed to high velocity air that is not a normal part of their husbandry.

- [ ] **Bare floor (no bedding) with no structure for resting or sleeping**

- [ ] **Wire bottom cage for more than 12 hours (NOT AVIAN)**

- [ ] **Temperature outside recommended range**
Animals will be exposed to temperatures outside of the normal reference ranges for the species.

- [ ] **Other nonstandard housing or husbandry**
Animals are subject to other non-standard housing or husbandry conditions.

- [x] **Not applicable**
There will be no non-standard husbandry for this study.

Select Locations

Species: Laboratory mouse

Add all housing and procedure locations for this species. Use only one of the following three questions to add a location.

Add your location in question 1, if it has been approved by the IACUC.
If you will house animals and perform procedures in the same established animal facility:
Type “vivarium” in the search box and select from the results. To allow flexibility and avoid possible protocol violations, do not select a specific room.

If you will use clinical space in the [redacted] or South do not select a specific room. Choose [redacted] etc.:
Type [redacted] in the search box and select from the results scrolling to find [redacted] buildings.

If you will use a non-vivarium PI laboratory to hold animals and/or perform procedures:
Type the room number in the search box and select from the results.
Include the building module (e.g. K4/123) for the [redacted]. Add each room separately; you cannot add room ranges.

Add your location in question 2, if it is a UW-Madison location that you did not find in the search box for question 1.

Add your location in question 3, if it is not controlled by UW-Madison or its affiliates.

1. Current ACUC Approved Locations

Location Common Name	Room Name	Location Type	Committee	Housing Allowed	Procedure Allowed	Surgery Level
[redacted]	vivarium	facility	SMPH	yes	yes	Most Surgeries Allowed

2. Locations Not Found under Current ACUC Approved Locations

You must request ACUC approval for these locations.

Building Name	Building Address	Room Name
There are no items to display		

3. Locations Not Controlled by UW-Madison or Its

Affiliates

Location	Location Address
There are no items to display	

Select Purpose Of Locations

Species: Laboratory mouse

1. Locations Details

* Click on the name of each selected location. On the pop-up, indicate which of the following procedures and housing will occur at that location. Check all that apply for each location.

Location name	Facility housing	Lab husbandry	Laboratory housing	Nonsurgical Procedures	Surgical Procedures	Euthanasia
[redacted]	yes		no	[redacted] and/or [redacted], CO2 for euthanasia, Supermatant fluid of buffered extract administration	*No value entered*	yes

Transport

Species: Laboratory mouse

See **policy UW-4099**, Campus Transportation of Laboratory Animals, for guidance on transporting laboratory animals outside the animal facility. A minimum acclimation period is not required for animals intended for use after intra-campus transport or in non-survival procedures; it is however strongly recommended animals receive at least 72 hours post-transport acclimation prior to use in a research protocol. See **policy UW-4106**, Acclimation After Transport.

1. Animal Transport

* Animals will NOT be transported.

(•) **True** ( ) False

End of Species Details

Species: Laboratory mouse

You are done answering questions about this species.

Click on "Species Complete." You will be redirected to the Species start page where you can answer questions about additional species in your protocol or continue to the next section.