جامعة الملك عبدالله
للعلوم والتقنية
King Abdullah University of
Science and Technology

IACUC User Manual

Rodents

Rev I - Approval Date: 25 November 2020

Revision History

Revision Number	Approval Date	Summary
I	25 November 2020	Creation of the manual

IACUC User Manual - Rodents

Topic Index

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Role and Responsibilities of the IACUC

The Institutional Animal Care and Use Committee (IACUC) is a faculty-led committee charged with ensuring the humane and ethical care and use of vertebrate and invertebrate animals in research. Consistent with the IACUC's purpose, the committee has oversight over all KAUST research activities involving animal subjects occurring both on the academic campus and in the field. Ethical approval for collaborative research conducted at other institutions is the responsibility of the host organization.

KAUST's Institutional Biosafety and Bioethics Committee (IBEC) serves as the registered local committee for all National Committee of Bioethics (NCBE)-regulated activities. The review of animal-related activities is delegated to IACUC. The outcome of such reviews is periodically reported to IBEC.

1. **IACUC Responsibilities**
    - Approve standard procedures and forms
    - Approve and review activities before the initiation of any research involving animal subjects
    - Review modifications to approved research activities involving animal subjects
    - Establish training procedures and criteria for research on animal subjects
    - Conduct continuous research proposal reviews at intervals appropriate to the degree of risk and as required by the standards established by Saudi Arabia's National Committee of Bioethics (NCBE)
    - Review the animal care and use program at least once per year
    - Inspect the animal facility and all satellite facilities at least once per year
    - Prepare reports on program reviews and facility inspections for submission to Research Operations and Compliance and VPR; such reports include recommendations to ensure the effectiveness of the animal care and use program

2. **IACUC Protocol**

Form & Tools
IACUC Protocol Form

- No work with live vertebrates and/or invertebrates can be initiated without IACUC approval.
- It is important to be aware that only procedures approved by the IACUC can be performed.
- Approval must be obtained in advance of performing the work. Failure to comply with this requirement can have serious implications for the Principal Investigator, such as loss of the privilege to work with animals.
- Protocol(s) involving the use of biological agents, hazardous chemicals/drugs, or radiation must also be approved by the Institutional Biosafety and Ethics Committee and/or the Institutional Radiation Safety Committee.
- For further information regarding approval processes for IACUC and other relevant committees, please check the Research Compliance website.

3. **Training**
   - Evaluate the experience of the Principal Investigator and his/her team to conduct research involving animal subjects
   - Ensure that the Principal Investigator and his/her research team have completed all committee-required training

4. **Non-compliance**
   - Investigate alleged non-compliance involving animal subjects
   - Review any concerns raised involving the care and use of animals, including complaints from the public, facility personnel, and users
   - Suspend non-compliant research involving animal subjects
   - Provide a written statement outlining the reasons for the suspension of non-compliant research addressed to the Principal Investigator, respective Dean, and Research Operations and Compliance

5. **Inspections**
   - The IACUC will monitor animal research activities for compliance with the approved IACUC protocol(s). The goal is to:
   - Inform the IACUC of the current status of the project
   - Ensure continued compliance with institutional requirements
   - Provide for a re-evaluation of the animal activities
   - As part of the IACUC monitoring program, the IACUC will conduct inspections at least once a year to KAUST's animal facility (and satellite facilities) using the Guide for the Care and Use of Laboratory Animals as the basis for evaluation.

Reporting and Investigating Non-compliance Concerns Involving Animal Care and Use

"Safeguarding animal welfare is the responsibility of every individual associated with the Program." (The Guide, 2011). The IACUC is charged with reviewing concerns involving the care and use of animals and has the authority to suspend research that is not conducted in accordance with the committee's requirements.

1. **Investigation**
   - The Head of Research Compliance will receive all allegations of non-compliance or mistreatment of animals.
   - All allegations and findings of non-compliance, whether these reports arise internally (i.e., from faculty, staff, ORRP, the IACUC, or investigator self- reports) or from outside the University (e.g., regulators, anonymous reports) will be investigated.

2. **Initial Assessment**
   - When a non-compliance allegation is received, the IACUC will be notified, and an initial assessment will be conducted by the Head of Research Compliance and the IACUC Chair (or Vice-Chair).
   - After the initial assessment is concluded, the potential outcomes will be:
     - **Dismissal** where non-compliance allegations have been unsubstantiated.
     - **Referral** to other appropriate University process (i.e., misconduct review)
     - **No further action** required where the issue has already been corrected.
     - **Further investigation** required by an Investigation Team.
   - The IACUC will be informed of the initial assessment.

3. **Formal Investigation**
   - **Investigation Team**
     - The IACUC Chair and the Head of Research Compliance may impanel an Investigation Team.
     - The team will be led by a Research Compliance staff member and may include members of the IACUC, the Research Safety Team, and/or others with specialized expertise.
   - **Procedure**
     - The Investigation Team will consider witness/Principal Investigator (PI) statements, interviews, audits of research records, any other supporting evidences.
     - During the course of the investigation, the PI will have the opportunity to respond to all the allegations raised.
   - **Investigation Team Report**
     - The Investigation team will prepare for the IACUC a summary report that includes:
       - Allegations
       - Summary of findings

- Conclusions
- Recommendations and/or corrective actions

4. **IACUC Determination**

- **Procedure**
  - The outcome of the Initial Assessment and, where applicable, the Investigation Team Report, will be reviewed at a convened IACUC meeting. Relevant materials will be distributed to all members in advance of the meeting.
  - IACUC will review the information provided and determine corrective action(s) based on the nature of the non-compliance, the extent to which animals were placed at risk, and previous non-compliance history.

- **Outcome**
  Potential outcomes may include:
  - **Modification(s)** of the IACUC protocol
  - **Monitoring** of animal use activity, including audits or assessments of technical abilities
  - **Training** for the research staff involved
  - **Further investigation** required to make a determination
  - **Periodic reporting** from research staff
  - **Restriction** on the use of research facilities or the use of data
  - **Suspension** of all or part of the IACUC protocol or individual personnel

- **Reporting of outcomes**
  - The Principal Investigator will be notified by the IACUC Chair or the RO of the investigation outcome.
  - The notification will include an outcome description and any required corrective actions.
  - The Dean may be copied at the discretion of the RO.
  - In the case of a suspension, a written statement of the reasons for suspension shall be reported promptly to the PI, the Dean, and the RO.

5. **Appeal process**

- The PI may appeal to the IACUC decision in writing.
- The appeal will be considered during a convened IACUC meeting.
- The PI may be invited to or may request to attend the IACUC meeting.

6. **Continuing non-compliance**
   If the PI does not comply with the required corrective action(s) within the specified time, additional action(s) may be taken; including suspension of IACUC approved activities.

Experimental Design

Summary of the overall number of animals required for an experiment. It describes an animal's procedures and experiences in each unique study group and may be repeated for every unique experimental design or project aim.

1. **Functional title:** a short descriptive title which captures the aim of the experiment

2. **Summary of the number of animals per experiment:** mathematical description of the expected numbers of animals based on the experimental variables.
   A. number of animals per group (n) x variable 1 (e.g. drug dose range) x variable 2 (max number of time points) = Total
   B. number of animals per group (n) (target + non-target species) x variable 1 (max number of locations) x variable 2 (max number of collections per locations) = Total
   NOTE: If the overall procedures are the same between groups of animals with only minor variation such as the use of a different drug, cell line, dose, or time point, it may be beneficial to use a descriptive category or to treat this difference as a variable rather than a new experiment.

3. **Justification for the animal group size (n):** based on **power analysis**, reference to an existing **publication**, the **quantity** of tissue used, or the need for **pilot data**.
   - The use of pilot projects will result in the approval of a small number of animals to obtain pilot data.
   - For **Field Studies**, justifications may be based on historical or published sample size data.

4. **Pilot studies:** for studies with unknown experimental outcomes.
   - According to The Guide (page 26), “when novel studies are proposed, or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC, and veterinarian. A system for communication with the IACUC should be in place both during and after such studies.”
   - Pilot studies are used for **new, unknown studies** that propose innovative methods for which data has not yet been published at KAUST or in the rest of the scientific community (i.e., New line of cell line).
   - This may be used to initially assess the effects on the animals involved in the proposed studies and/or to determine whether to go ahead with larger studies based on the results obtained.
   - After the conclusion of the pilot study, the results will be reviewed and assessed by IACUC, and approval for further studies will be appropriately granted.

5. **Model development studies:** for studies with unknown experimental techniques
   - Model development studies will be used to evaluate the feasibility and establish the reliability of the model.
   - This will may also be used if users need to be trained, or validation between individuals performing the model is required. In this case, please consider adding extra number of animals in the protocol.

6. **Description of Endpoints**: refer to Endpoint Section

7. **Pain Classification:** reflects the maximum pain and distress the animal could potentially experience during the experiments.
   - Pain or distress will be mild or acute (example: IP injection)
   - Moderate to severe pain/distress could occur, but will be relieved by therapeutic drugs or euthanasia
   - Moderate to severe pain/distress could occur, but relief measures would compromise the scientific aims of this study

8. **Example:**

Functional Title:	Practical training
Summarize the number of animals per experiment: Examples: A. number of animals per group (n) x variable 1 *(e.g. drug dose range)* x variable 2 *(max number of time points)* = Total B. number of animals per group (n) *(target + non-target species)* x variable 1 *(max number of locations)* x variable 2 *(max number of collections per locations)* = Total	
Practical training: 6 mice (per trainee/instructor per training day) * 6 participants (trainee + instructors) * 10 training days (per year) * 3 years = 1080	
For each group of animals (n), please provide a justification for the animal group size: • Justification should be based on **power analysis**, reference to an existing **publication**, the **quantity** of tissue used, or the need for **pilot data.** • Use of pilot project will result in the approval of a small number of animals to obtain pilot data. • For **Field Studies**, justifications may be based on historical or published sample size data.	
3 to 6 animals per trainee/instructor are necessary to conduct the one day practical training on basic procedures described above. The minimum number is 3 but the number can increase if the trainee has none or limited experience with the procedures. A maximum of 4 participants and 2 instructors per training session, leading to a maximum of 36 animals/1 day of training session and an expected 10 training sessions per year (360 animals/year).	
Chronological summary of the procedures an individual animal will undergo during this experiment. • This could be in the form of a flowchart • reference the procedure name from question 2 and part II – Common Procedure • Do not repeat specific information such as dosing, or detailed procedural description in this question	
Practical training include one or more of the following procedure: - Main mice restraint techniques - Intra-peritoneal / intramuscular /subcutaneous / intravenous injections: how to calculate a dose / administration volumes / the maximum volumes/dose to be administered - Blood collection techniques, including facial and submandibular vein bleeding, cardiac puncture. - Anesthesia - Euthanasia All animals are euthanized at the end of the training and are not re-used for training purposes.	
Describe the Humane endpoints: (point at which pain or distress in an experimental animal is prevented, terminated or relieved)	All animals are euthanized at the end of the training session or earlier if sign of major pain and/or distress are recognized.
Describe the Scientific endpoints: (point at which the scientific aims and objectives have been reached)	N.A.
Pain/distress classification	Moderate to severe pain/distress could occur, but will be relieved by therapeutic drugs or euthanasia.
When moderate to severe pain/distress cannot be relieved, provide a scientific justification. (State methods or means used to determine that pain and distress relief would interfere with research results.)	Those training are needed for ARCL users.

Harm-Benefit Analysis

IACUC will weigh the potential adverse effects of an animal proposal study against the potential benefits that are likely to accrue as a result of the research.

Harm-benefit will be evaluated in the context of the five freedoms:

- **Freedom from hunger or thirst**: by ready access to fresh water and a diet to maintain full health and vigor.
- **Freedom from discomfort**: by providing an appropriate environment including shelter and a comfortable resting area.
- **Freedom from pain, injury or disease**: by prevention or rapid diagnosis and treatment.
- **Freedom to express (most) normal behavior**: by providing sufficient space, proper facilities and the company of the animal's own kind.
- **Freedom from fear and distress**: by ensuring conditions and treatment, which avoid mental suffering.

Implementation of the 3R's – Refinement, Replacement, Reduction

"In 1959, W.M.S. Russell and R.L. Burch published a practical strategy of replacement, refinement, and reduction—referred to as the Three Rs—for researchers to apply when considering experimental design in laboratory animal research (Russell and Burch 1959). Over the years, the Three Rs have become an internationally accepted approach for researchers to apply when deciding to use animals in research and in designing humane animal research studies." (The Guide, 2011)

1. **Refinement**

Modifications of husbandry or experimental procedures to enhance animal well-being and minimize pain and distress. i.e., early endpoints (body scoring), pain and management relief methods, telemetry, use of minimally invasive techniques.

2. **Replacement**

Full or partial replacement with in vitro models, simulations, or less-sentient species, i.e., use of inanimate systems (as computer programs), test on single-cell/tissue type, use of animal lower on the phylogenetic scale in part of the project.

3. **Reduction**

Steps taken to reduce the number of animals i.e., precise experimental design, use of tier testing (sequential design), serial imaging, refined statistical methods.

Pain and distress

Management of pain and distress levels is an ethical and scientific imperative. Pain prevention or alleviation associated with procedural and surgical protocols is one of the main components of veterinary medical care in order to reduce or eliminate an unacceptable level of stress and distress in animals.

1. **Pain**

An unpleasant sensory and emotional experience associated with actual, or potential tissue damage, or described in terms of such damage. Clinical signs of pain are different depending on the animal species (i.e., hunched posture, reduced activity); see the table below.

2. **Distress**

"An aversive state in which an animal fails to cope or adjust to various stressors with which it is presented." (The Guide, 2011). Signs of distress is not always clearly observable, so during invasive periods of experimentation, it is mandatory to implement humane experimental endpoints for animals

3. **Common clinical and behavioral signs** of pain or distress in rodents:

Clinical sign	Definition	Clinical description	Detailed reporting	Background information
Kyphosis (Hunched posture)	Abnormally increased convexity in the curvature of the thoracic and lumbar spine.	Hunched back. An animal in kyphotic posture keeps its head down and limbs underneath the body.	Indicate the pattern of the condition (episodic or continuous) and the chronicity.	It is a sign of a painful and stressful condition. Animals may appear apathetic or hyperkinetic and may vocalize.
Poor coat condition	Ungroomed hair coat.	Normally, animals will groom by licking, wiping, scratching, or allogrooming. Lack of grooming will result in a fur in disarray and lacking natural gloss. It may also look greasy.	Indicate location (or generalized pattern) and chronicity of the condition.	Poor grooming may have many reasons: cold (piloerection), pain, illness, animal too fat to groom, aged animal.
Hyperkinesia (Increase movement)	Abnormally increased motor function or activity	Animal is constantly moving around in its cage, pen, or other enclosure, in excess of normal level of activity.	Indicate type and frequency of movement and any repetitive pattern or specific avoidance behavior (e.g., away from another more dominant animal).	Sign of boredom, anxiety, or pain. The sign itself is not pathological, but the cause may be.

Clinical sign	Definition	Clinical description	Detailed reporting	Background information
Hypokinesia (Reduced movement)	Abnormally diminished motor function or activity.	Reduction of spontaneous movements, but conservation of muscular tone and alertness.	Indicate type and frequency of inactivity and any specific pattern, e.g., the place when the animal spends inactive time.	Inactivity may be a sign of pain, disease, or boredom. Typically, older animals are less active than young ones, and singly housed animals tend to be less active.
Dyskinesia (Uncontrolled movement)	Impairment of the power of voluntary movement.	The animal shows an intention to move but doesn't succeed properly. Dyskinesia is usually a sign of pain or generalized weakness.	Indicate type and frequency of hampered movement and any specific pattern, e.g., the place where the animal spends inactive time.	Dyskinesia is usually a sign of pain or generalized weakness.
Aggression aggressive	An angry and destructive behavior against other animals (e.g., cage mates) or the handler.	It may be manifested by overt attacking or by more passive attitudes of hostility and obstructionism. Antagonistic behavior may also occur in self-defense.	Indicate against what/whom is the animal aggressive, and under what circumstances. The capacity for aggressive behavior is physiological for the ranking of animals in a group or for the defense against danger.	It may be a sign of fear, related to neurological conditions (e.g., in rabies) or occur because of a painful condition
Vocalization	Emission of sounds from the larynx.	Animals will vocalize in social interaction or in response to other stimuli.	Excess vocalization must be reported if associated with a pathological condition.	Vocalization is a behavioral feature that differs between species. Abnormal vocalization may express pain.
Self-mutilation	A self-inflicted wound.	Animals may over lick, bite and injure a localized painful area of their body. This happens especially with limbs and tail.	Must be differentiated from erosion, ulcer, or fight wounds.	Self-mutilation can be pain related.
Time to integrate into the nest	The ability to rebuild the nest and to populate it.	Healthy animals will quickly restore the nesting material after cage change.	Pain severity can be evaluated according to nesting activity: absent (severe pain) or delayed (moderate to severe).	Pain perception negatively impacts on nest-building activity.
Facial Expressions	Changes in a number of facial expressions.	Animals in pain can display the following: orbital tightening, nose bulge, chick bulge, ears pulled back, whiskers change.	Abnormal facial expressions should be promptly reported. Pain severity can be scored based on numbers/severity and frequency of findings.	Check grimace scale posters available within the facility for appropriate scoring.

Adapted from GLOSSARY OF CLINICAL SIGNS IN LABORATORY ANIMALS - The reporting of clinical signs in laboratory animals, FELASA Working Group Report JM Fentener van Vlissingen, M Borrens, A Girod, P Lelovas, F Morrison and Y Saavedra Torres, Lab Anim OnlineFirst, published on May 8, 2015, doi:10.1177/0023677215584249 Issue 1/2015

Analgesia Use

It is the ethical and legal obligation of all personnel involved with the use of animals in research to reduce or eliminate pain and distress in research animals whenever such actions do not interfere with the research objectives.

Safety Considerations
• Avoid spills and exposures when handling hazardous substances. • Avoid using sharps or use safety-engineered sharps whenever possible. • Do not recap needles or use the “scoop method” if absolutely necessary. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Substance Administration and Sharps Safety resources for additional details.

Forms & Tools
Procedure/Surgery Card

1. **Pain assessment criteria:** clear criteria should be defined in the IACUC protocol.

2. **Selection of analgesia** should be based on the following factors: species, age, strain or stock of the animal, type, and degree of pain, potential impact on the study results, the nature and length of the surgical or pain-inducing procedure, safety of the agent.

Analgesic name	Dose	Route	Frequency
Carpofen	5-10 mg/kg	Orally or Intramuscularly	Once a day
Buprenorphine	0.05 to 0.10 mg/kg	Subcutaneously	Twice/three times a day
Meloxicam	2-5 mg/kg	Orally	Twice a day

3. **Pre-emptive analgesics:** It is important to provide analgesics prior to painful procedures (i.e., surgery) in order to avoid the “wind-up effect”. The wind-up effect is a phenomenon in which central pain sensitization results in a pain response to otherwise non-painful stimuli (allodynia; Joshi and Ogunnaike 2005).

4. **Post-operative/procedural analgesics:** Animals must be observed at least once daily following a surgery or pain-producing procedure.

5. **Recordkeeping:** Observations and dosing will be recorded in the Procedure/Surgery Card, and be available for inspection.

Anesthesia Use

It is the ethical and legal obligation of all personnel involved with the use of animals in research to reduce or eliminate pain and distress in research animals whenever such actions do not interfere with the research objectives.

Safety Considerations
• Avoid spills and exposures when handling hazardous substances. • Avoid using sharps or use safety-engineered sharps whenever possible. • Do not recap needles or use the “scoop method” if absolutely necessary. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Substance Administration and Sharps Safety resources for additional details.

Forms & Tools
Procedure/Surgery Card

1. **Supportive care**
    - Ophthalmic ointment should be applied to both eyes to prevent desiccation from any anesthesia longer than 5 minutes. Re-apply regularly in case of prolonged surgery.
    - Maintain normal body temperature using a warm circulating water blanket or thermal pads.
    - Provide warm fluids (e.g., IV, IP, SQ) to animals during prolonged anesthesia to maintain adequate hydration.

2. **Monitor and assessment**
    - Monitor respiratory rate and effort, the color of mucous membranes, and reflected eye color (in albino animals) at regular intervals (no longer than 15-minute intervals).
    - Assess the level of anesthesia by pedal reflex (firm toe pinch) and adjust anesthetic delivery as appropriate to maintain the surgical plane.
    - Depth of anesthesia is considered adequate when the animal shows:
        - Decreased respiratory frequency;
        - Deep breathing;
        - Absence of withdrawal reflex evoked by pinching toenails.
    - The adequate level of anesthesia must be maintained during the whole procedure.

3. **Recovery**
    - Place rodent in a warm, clean, dry, quiet environment away from other animals.
    - Cover or replace bedding material with toweling material (Bedding can stick to eyes or be inhaled while animals are recovering from anesthesia).
    - Provide warmth during recovery.
    - Warm sterile saline can be administered to replace body fluids lost during surgery.
    - Post-anesthesia monitoring should be performed until maintaining an upright posture and walking normally before return to the animal housing room.

Isoflurane Use

Isoflurane provides rapid anesthesia and recovery for surgeries and techniques requiring complete immobilization of the animal.

Safety Considerations
• Isoflurane is an inhalation hazard and must be used with adequate ventilation and/or scavenging equipment. You should not smell isoflurane while using it. If you do, contact ARCL staff immediately. • Weigh the fluosorber canister prior to use. If it weighs more than 1400 g, contact ARCL staff. • The oxygen supply cylinder is a high-pressure vessel, exercise caution when handling the cylinder and its regulator. • Refer to Anesthesia Using Isoflurane System and Guidelines for Working with Compressed Gases resources for additional details.

Forms & Tools
Isoflurane vaporizer operation: Contact ARCL Staff

1. **Administration of Isoflurane:** Use a precision vaporizer and adjust the dosing level accordingly to the table below.

Anesthetic Agent	Induction	Maintenance
Isoflurane	3-5%	1-3%
O2 Flow	2.5L/min	1L/min

2. **Induction Chamber:**
   - Close the induction chamber and ensure the lid is tightly closed and latched when not moving or replacing mice.
   - Flush the induction chamber with oxygen prior to opening the chamber to transfer animals.

3. **Nose Cone:**
   - Ensure a tight seal around the animal's nose cone and mouth.
   - Allow time for the anesthesia gas to reach the manifold nose cones prior to removing the mice from the induction chamber. This can take from 2 minutes.

Blood Collection

Safety Considerations
• Avoid using sharps or use safety-engineered sharps whenever possible. • Do not recap needles or use the "scoop method" if absolutely necessary. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Sharps Safety resources for additional details. • If administering anesthesia, refer to Safety Considerations of the Anesthesia Use and Isoflurane Use subchapters.

1. **Approximate blood sample volumes** for a range of body weights in mice

Body weight (g)	Circulating Blood Volume (CBV)			
	CBV (mL)	1% CBV every 24hrs†	7.5% CBV every 7 days†	10% CBV every 2 – 4 wks†
20	1.10 – 1.40	11 – 14 µL	90 – 105 µL	110 – 140 µL
25	1.37 – 1.75	14 – 18 µL	102 – 131 µL	140 – 180 µL
30	1.65 – 2.10	17 – 21 µL	124 – 158 µL	170 – 210 µL
35	1.93 – 2.45	19 – 25 µL	145 – 184 µL	190 – 250 µL
40	2.20 – 2.80	22 – 28 µL	165 – 210 µL	220 – 280 µL
† Maximum sample volume for that sampling frequency				
• Mouse total blood volume = 0.058 ml per g (approx.) • For in vivo bleeds <10 % of an animal's total blood volume can be taken in a single collection. • Up to 15% of an animal's total blood volume may be collected over a 4 week period. • For the maximum sample volume in survival procedures, use the table below				

Adapted from NIH Guidelines for Survival Bleeding of Mice and Rats

2. **Non-terminal blood collection methods**

	Lateral tail vein	Submandibular vein puncture	Retro-orbital puncture
Anesthesia	Not required	Not required	Required
Procedure	- Warm the animal for up to 2 to 3 minutes to allow vasodilation - Restrain the animal in a Plexiglas tube - Pinch the lateral tail vein using a dedicated lancet or needle tip as close as possible to the tail tip - Collect blood and apply pressure to allow hemostasis - Recommended methods for small volume serial sampling.	- Animal is restrained firmly - Submandibular vein punctured using a dedicated lancet - After blood collection: gentle pressure with gauze until hemostasis is achieved - Place the animal inside the cage and check for recovery. - Recommended intervals between samples collection at least 2 weeks	- Stabilize the head of the animal between the forefinger and thumb of one hand - Insert the tip of a microhematocrit tube or Pasteur pipette into the medial canthus of the eye passing to the side of the globe of the eye - Using a rotating motion, insert the tube into the retro-orbital sinus and collect the blood - Recommended intervals between sample collection is 1 month.
Total collectible volume	Up to 0.1 ml	Up to 0.2 ml	Up to 0.2 ml

3. **Terminal blood collection methods**

	Retro-orbital puncture	Cardiac puncture
Anesthesia	Required	Required
Procedure	- Stabilize the head of the animal between the forefinger and thumb of one hand - Insert the tip of a microhematocrit tube or Pasteur pipette into the medial canthus of the eye passing to the side of the globe of the eye - Using a rotating motion, insert the tube into the retro-orbital sinus and collect the blood - Euthanize the animal	- Place the animal in dorsal recumbency - Insert a 22-25 ga needle at a 30-degree angle under the xiphoid process or access thoracic cavity from the lateral side, inserting the needle between the ribs. - Apply light pressure on the plunger of the syringe until blood collected - Euthanize the animal.
Total collectible volume	Up to 1 ml	Up to 1 ml

Biological materials/cells administration

Tissues and biological materials may be infected with a variety of agents that can affect the health of humans or animals and may act as a confounding variable on research results. Murine viruses can be transmitted by cell culture products and additives or by the cells themselves.

Safety Considerations
• Human cell lines and unfixed tissues may carry blood borne pathogens. • If handling such materials, enroll into Bloodborne Pathogens Safety Program and follow its requirements. • Administration of human biological materials/cells must be conducted under Handling of Samples Requiring Biosafety Level 2 Containment.

1. **Biological materials that require testing**:
   - Any that have originated from rodents or which have been exposed to rodents directly (in vivo passage) or indirectly (e.g., via tissue culture media additives).
   - The Principal Investigator is responsible for the testing which can be performed by:
     - Charles River Laboratories (CRL) for screening according to the Rodent Infectious Panel Agent – Mouse Essential Panel
     - KAUST Bioscience Core Lab (BCL). (BCL test will screen according to the CRL Mouse Essential Panel with the exemption of the Vesivirus and Reovirus 1).

2. **Biological materials exempted from testing**:
   - Cells harvested from animals housed within the ARCL do not require testing.
   - Veterinary staff and ARCL Manager will review and approved grounds for exemptions.

Expired veterinarian drugs and medical materials

The use of expired pharmaceuticals, biologics, and supplies is not consistent with an acceptable veterinary practice or adequate veterinary care and may negatively impact animal welfare or compromise the validity of the study.

- Expired drugs and medical materials must either discarded or segregated physically from non-expired drugs/medical materials and labeled as “EXPIRED”
- Expiration date should be verified prior to use
- Expired drugs and medical materials must not be used in live animals without explicit IACUC approval.

Use of non-pharmaceutical grade compounds

Pharmaceutical-grade chemicals and other substances must be used whenever possible to ensure efficiency and limit toxic and unwanted side effects, which may compromise the research outcomes or animal welfare.

1. **Pharmaceutical grade compounds** are any active or inactive drug, biologic or reagent compound that has been:
   - Manufactured under Good Manufacturing Practices (GMP) and recognized by the Saudi Food and Drug Administration (SFDA) or international equivalent
   - Chemical purity standard has been written or established by a recognized compendia (e.g., a national Pharmacopeia or National Formulary)

2. **Non-Pharmaceutical grade compounds** are all that are not recognized as pharmaceutical grade compound, and could be:
   - Routine care drugs (RCD): for the clinical treatment of animals and to prevent or reduce/eliminate animal pain or distress (i.e., anesthetics, analgesics, antibiotics)
   - Experimental compounds (EC): to accomplish the scientific aims of the study (i.e., drugs, chemicals, cells)

The use of non-pharmaceutical-grade compounds must be based on scientific necessity and must be scientifically justified in the approved IACUC protocol prior to the use of the drug.

Examples of appropriate justifications to use non-pharmaceutical compounds include:

- The pharmaceutical-grade compound is not available in the appropriate concentration or formulation, or the appropriate vehicle control is unavailable
- The compound is required to generate data that are part of an ongoing study or that are comparable to previous work
- Cost savings alone is not an adequate justification for using non-pharmaceutical compounds in animal

3. **Preparation of non-pharmaceutical grade compounds:**
   - Chemical properties of the compound must be appropriate for the study and the route of administration (e.g., the purity, grade, stability in and out of solution, solution vehicle properties, pH, osmolality, and compatibility of the solvent and other components of final preparation)
   - Where possible, the dilutant should be pharmaceutical grade

4. **Dilution of pharmaceutical-grade compounds:**
    - Sterile dilutions or mixtures of drugs may result in a shorter effective expiration date than the expiration date of the individual components due to the risk of contamination and dilution of preservatives
    - The smallest amount of agent suspension/dilution/mixture should be used to minimize storage time prior to administration
    - Expiration date from the date of preparation should follow the manufacturer's instructions or the earliest expiration date for any single component. However, dilutions or mixtures of drugs should be stored for no longer than 6 weeks from the date of preparation

5. **Storage in secondary containers**:
    - Consideration should be given to the administration and storage of formulations to ensure drug stability and quality (i.e., to prevent inadvertent co-administration of infectious agents or contaminants)
    - Labeling: name of the drug(s) contained; concentration of the drug; date of expiration
    - New, sterile containers with a septum must be used (e.g., red-topped blood tubes)

Substance administration methods

Methods of administration must be appropriate for the type of substances and study aims. Considerations should be given to volume of the substances, Ph., and desired onset of effect.

Safety Considerations
• Cages containing animals treated with hazardous drugs should be labeled with a yellow cage card template. • Avoid spills and exposures when handling hazardous substances. • Avoid using sharps or use safety-engineered sharps whenever possible. • Do not recap needles or use the “scoop method” if absolutely necessary. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Substance Administration and Sharps Safety resources for additional details.

Forms & Tools
Yellow cage card

1. **Considerations for maintaining drug sterility and stability:**
   - The rubber injection port/cap should be swabbed with alcohol prior to insertion of the needle
   - Use new sterile needles for each entry into a sterile container
   - Examine multiple-dose injection vials/tubes prior to use for evidence of physical or chemical contamination and discard any substance meeting any of the following criteria:
     - Particulate matter
     - Precipitation of solids
     - Turbid or discolored appearance
     - Mislabeled or unlabeled container
     - Damage to the rubber stopper compromising integrity

2. **Substances administrations methods:**

Procedure name	Procedure	Administration route (Max Volume in mL/kg of body weight)	Needle size (gauge)
Medicated food or water	- When additives are placed in the drinking water or in the food it is responsibility of the investigator to monitor the animal(s) and assure that adequate fluid and food intake occurs. - Cages with medicated water or diet must be properly identified.	N/A	N/A
Oral gavage	- Restrain the mouse using the scruff method; - Head and body vertically aligned with the esophagus; - Insert feeding needle behind the incisors towards the back of the throat and slightly hyperextend the mouse's head back; - Insert into the esophagus allowing the mouse to swallow the feeding needle down without forcing; - Inject substance slowly; - Pull the needle straight out; - Observe the animal for any adverse signs (cyanosis, struggling/gasping, fluid coming out the nose may occur in case of tracheal dosing or esophagus rupture).	10	18-24 Needle length (cm): 2.5-7.5 Ball tip diameter (mm): 1.25-2.25 -Appropriate size determined by measuring the needle to the last rib of the mouse to the incisors.
Intraperitoneal (IP) Injection	- Restrain mouse using one-handed restraint method; - Expose ventral (abdomen) side of the mouse; - Tilt head slightly down; - Locate the animal's midline and mentally divide the abdomen into quadrants using the stifles as a reference point for the transverse plane; - Insert the needle at about a 30° degree angle towards the head, into either the lower right or left quadrant and aspirate the syringe; - Inject substance and remove the needle.	10	25-29
Subcutaneous (SC) Injection	- Restrain the mouse using the scruff method. Make a tent of the skin at the scruff; insert the needle at a 45° angle between the fingers (careful not to push the needle through the other side); - Alternatively, perform the injection in the lateral side of the thorax, and insert the needle parallel to the ribs and gently lift the need to check proper positioning; - Aspirate to check the needle is properly positioned and inject substance: a small bleb in the subcutaneous space should be noted; - Remove the needle and apply gentle pressure to the site to prevent the backflow of the fluids.	5	25
Intramuscular (IM) Injection	- Restrain the mouse using a scruff restraint or restraining device, position the mouse so that its body is parallel to the table in a lateral position or exteriorize one hind leg from the restrainer; - Aim the needle towards the cranial thigh of the quadriceps; - Insert the needle into the cranial portion of the muscle at a 45° degree angle; - Aspirate and inject; - Remove the needle and apply a gentle pressure to the muscle to aid the distribution of the substance.	0.05	27-29

Procedure name	Procedure	Administration route (Max Volume in mL/kg of body weight)	Needle size (gauge)
Intradermal (ID) Injection	**- Anesthesia required** - Shave mouse; - Pinch and lift the skin gently from the back; - Insert the needle at a slight angle bevel up, just under the epithelium; - Slowly inject substance (a small bleb will appear at the injection site); - Remove the needle and apply gentle pressure to the site to ensure the fluid does not leak back out; - Place the animal in the cage and check for recovery after anesthesia.	0.1	25-27
Intravenous (IV) Injection	- Restrain mouse using the mechanical devices available at ARCL choosing the appropriate size and type; - Place the tail under a lamp or heating pad (if the mouse has not been previously warmed under a lamp before restraining); - Holding the tail, rotate it ¼ degree, placing the lateral vein at the top of the tail; - Start distally (about middle or lower tail) and approach the tail at a 30° angle; - Inject slowly, if successful the vein will blanch, and fluid will flow easily; - Remove the needle and apply direct pressure to the tail after the injection for hemostasis.	5	29-25
Intrafemural (IF) Injection	- **Anesthesia required** - Apply ophthalmic ointment to both eyes to prevent desiccation for any anesthesia longer than 5 minutes; - Proceed only when the animal is properly anesthetize and shows no reaction to painful stimuli (toe-nail pinching); - Shave the leg area around the patella (see drawing below) on the site that will be injected (left or right) and disinfect the area with a 70% Ethanol solution (or equivalent disinfectant solution); - Hold with the tip of the thumb and index finger the patella of the leg to be injected in a stable position; - Approach the center of the patella keeping the needle perpendicular to the femur; - Insert a 28 ga needle; - When on the patella, apply some light pressure and rotation of the needle. If successful, you will feel resistance when moving the needle; - Check for the needle to be in the proper position within the femur cavity by moving the needle in any direction: if entry is successful, the operator will not feel the tip of the needle on fingers; - Inject slowly; the volume should be less than 50 microliters; - Gently remove the needle and let the animal recover from the anesthesia.	0.05	28

Surgery

"Successful surgical outcomes require appropriate attention to pre-surgical planning, personnel training, anesthesia, aseptic and surgical technique, assessment of animal well-being, appropriate use of analgesics, and animal physiologic status during all phases of a protocol involving surgery and postoperative care." (The Guide, 2011)

Safety Considerations
• For administering anesthetics and analgesics, refer to Safety Considerations of the Anaesthesia Use Section. • Avoid using sharps or use safety-engineered sharps whenever possible. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Substance Administration and Sharps Safety resources for additional details.

Forms & Tools
Procedure/Surgery Card

1. **Definitions**

- **Minor surgery**: does not penetrate a body cavity or cause permanent physical impairment to the animal.
- **Major surgery**: involves penetrating a body cavity, causing permanent physical impairment or tissue dissection/transection (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation). Depending on the level of trauma and side effects associated, there are types of laparoscopic surgery considered as minor (e.g., avian sexing and oocyte collection) rather than major surgery (e.g., hepatic lobectomy and cholecystectomy).
- **Recovery surgery**: the animal is expected to awaken from anesthesia, including those in which the expected survival time is minimum.
- **Non-recovery (non-survival) surgery:** the animal is euthanized while still under anesthesia.
- **Multiple surgery:** More than one recovery surgery (major or minor) on a single animal.

2. **Aseptic technique**

Aseptic techniques are used to reduce the risk of microbial infection as part of a surgical process. Requirements are listed below:

Surgery type	Recovery	Non-Recovery
Surgeon attire	Sterile gloves, face mask, and clean lab coat/gown	Non-sterile gloves and lab-appropriate attire
Surgical site	Hair removal followed by alternate scrubs of iodophor/chlorhexidine and 70% alcohol repeated 3 times. Surgical draping is recommended	Hair removal only
Instruments	Sterile One sterile surgical pack may be used for no more than 5 batch surgeries without reserialization provided they are maintained in a sterile field.	Clean

3. **Steps of surgery**

Pre-operative

- Surgery must be conducted in a clean, uncluttered, minimal-traffic portion of the lab dedicated for surgery.
- Pre-emptive analgesics must be provided (unless scientifically justified), and documented on the Procedure/Surgery Card.
- Follow aseptic technique requirements from the table above.

Intra-operative

- The animal must remain in a surgical plane of anesthesia throughout the procedure.
- Monitor the animal's vital signs (i.e., increase respiratory rate) throughout surgery.
- Close surgical wounds using appropriate techniques and materials.

Post-operative

- Post-operative monitoring is the responsibility of the Principal Investigator and his/her staff.
- After surgery, move the animal to a warm, dry area and monitor it during recovery.
- Post-operative analgesics must be provided (unless scientifically justified), and documented on the Procedure/Surgery Card.
- Post-operative monitoring should be performed until the animal is able to stand and walk.
- Animals must be evaluated post-operatively, and any abnormalities must be promptly reported to the Animal facility/Vet staff.
- The following frequency of post-operative observations are recommended:
    - Facility staff will check animals for general health once per day.
    - The Principal Investigator and his/her staff should observe the surgical site for signs of surgical complications daily for the first **5 days** (including weekends and holidays).
- Surgical sutures and/or staples are to be removed **14 days** after surgery unless approved by the IACUC.

4. **Records**

Records for all surgeries must be documented on the Procedure/Surgery Card.

Euthanasia

Act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort, or distress.

Safety Considerations
• If administering anesthesia prior to euthanasia, refer to Safety Considerations of the Anaesthesia Use Section and Isoflurane Use Section. • CO_2 supply cylinder is a high-pressure vessel, exercise caution when handling the cylinder and its regulator. • While abnormal release of CO_2 may present an asphyxiation hazard, its risk assessment in ARCL concluded that the occupational exposure limits are much higher than any plausible scenarios. • Refer to Guidelines for Working with Compressed Gases resources for additional details. • Decapitation devices present increased sharps injury risk. Individuals responsible for the use of method shall be trained. Use of a decapicone is encouraged when using a guillotine. • Surgical scissors and guillotine must be regularly maintained and checked to ensure sharpness and proper function before each use.

Forms & Tools
Euthanasia form

1. **Training:** euthanasia and confirmation of death must be performed by qualified personnel only.

2. **Special Considerations** when euthanizing animals.
   - Euthanasia should not be performed when other animals are present in the area, unless in the case of euthanasia of a badly injured animal where additional suffering may be caused by moving the animal.
   - Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function.

3. **Guillotine or scissors use:** check to ensure sharpness and proper function before each use.
   - A maintenance records must be maintained for guillotines and scissors.
   - Maintenance should be tailored on the frequency of use.

Physical methods (non-reversible)	
Procedure name	**Procedure**
Cervical dislocation without anesthesia Animals over 10 days age	• Place the mouse on a steel grid that it can grip securely and hold the animal with one hand at the base of the tail. • Using the other hand, press a pen longitudinally at the base of the skull. Alternatively, the thumb and index finger can be placed on either side of the neck at the base of the skull. • Slightly elevate the hindquarters (no more than 20-30 degrees) by lifting the tail base. • In a quick motion, firmly pull back hindquarters by the tail away from the head and neck while simultaneously pressing forward and down with the pen behind the base of the skull. This motion separates the cervical vertebrae from the skull and causes a rapid loss of consciousness. • Confirm cervical vertebrae separation by palpating the neck and death by cessation of vital signs.
Cervical dislocation under anesthesia Animals over 10 days age	• Anesthetize the animal (i.e., by inhalation of carbon dioxide gas or by injectable or inhalational anesthetics) • Hold the animal with one hand at the base of the tail. • Using the other hand, press a pen longitudinally at the base of the skull. Alternatively, the thumb and index finger can be placed on either side of the neck at the base of the skull. • Slightly elevate the hindquarters (no more than 20-30 degrees) by lifting the tail base. • In a quick motion, firmly pull back hindquarters by the tail away from the head and neck while simultaneously pressing forward and down with the pen behind the base of the skull. This motion separates the cervical vertebrae from the skull and causes a rapid loss of consciousness. • Confirm cervical vertebrae separation by palpating the neck and death by cessation of vital signs.
Decapitation without anesthesia (Guillotine) Animals over 10 days age	• Use an appropriately sized guillotine. • Use of a DecapiCone or other similar restraint devices is recommended. • Make sure the animal is calm. When a plastic restrainer is used, gently allow the animal to enter the restrainer and position it properly. Hold the animal securely, and place it on the stage of the guillotine. Advance the head into the guillotine opening. • Once checked for correct positioning and absence of any obstruction to the blades, quickly and smoothly depress the guillotine lever, removing the head from the animal with a single clean movement. Check that the animal's head has been cut entirely off.
Decapitation without anesthesia (Scissors) Neonatal: under 10 days age	• Decapitation using scissors is acceptable, but the use of guillotine is also possible. (In case of guillotine use, please follow directions above as for mice older than 10 days old). • Hold the neonate with one hand using the thumb and forefingers positioned in the middle of the animal's body. • Position the mouse's neck beneath the scissors' and separate the head from the body at the cervical level in one motion by firmly closing the scissors.
Decapitation under anesthesia (Guillotine) Animals over 10 days age	• Anesthetize the animal (i.e., by inhalation of carbon dioxide gas or by injectable or inhalational anesthetics) • Use an appropriately sized guillotine. • Use of a DecapiCone or other similar restraint devices is recommended. • Make sure the animal is calm. When a plastic restrainer is used, gently allow the animal to enter the restrainer and position it properly. Hold the animal securely, and place it on the stage of the guillotine. Advance the head into the guillotine opening. • Once checked for correct positioning and absence of any obstruction to the blades, quickly and smoothly depress the guillotine lever, removing the head from the animal with a single clean movement. Check that the animal's head has been cut entirely off.
Perfusion with fixative under anesthesia	• Anesthetize the animal (i.e., by inhalation of carbon dioxide gas or by injectable anesthetics) • Open the thoracic cavity to expose the heart. This will result in an irreversible collapse of the lungs and the death of the animal. • The animal is then perfused with fixative.

Potentially reversible methods	
Procedure name	**Procedure**
Carbon dioxide (compressed gas source) Animals over 10 days age	• Remove the animals from the cage, place them in an empty cage, and bring them to the euthanasia chamber. If all the animals inside of a cage must be euthanized, remove the cage from the rack and place it directly into the chamber. • Animals should be exposed to CO_2 according to the operational procedure for the type of chamber used. (Minimal flow rate of 30% per minute up to a maximum of 70% per minute) • Unweaned animals over ten days of age must be separated from adults prior to euthanasia. • Do not mix adult males from different cages. • Do not exceed twice the approved cage housing density. • Confirm animal death by looking for absence of respiratory movements, heartbeats, mucos cynaocis, and mily eyes.
Anesthetic Overdose	• **Injectable anesthetic** should be given at 2-3 times the recommended anesthetic dose. • **Isoflurane** should be administered at a flow rate or concentration of 5% or greater until one minute after breathing stops. • Confirm animal death by looking for absence of respiratory movements, heartbeats, mucos cynaocis, and mily eyes.
For the use of potentially reversible methods, the use of a physical method is required to ensure death	

Disposal of animals

Animal tissues must be disposed according to the safety practices below in order to minimize potential occupational hazards.

Safety Considerations	Forms & Tools
• When procedures are conducted outside the Animal Resources Core Lab, assure the "Incinerate Only" label is affixed to the biohazard bags containing carcasses. • Refer to Biohazard, Cytotoxic Hazard and Regular Waste Disposal guidelines for additional details.	Incinerate Only label

1. **Animal Resources Core Lab (ARCL):**
   - For disposal of animals in ARCL follow Biohazard, Cytotoxic Hazard, and Regular Waste Disposal guidelines.

2. **Research Labs:**
   - For disposal of animals in PI maintained research labs, place carcasses and tissues into a small biohazard bag.
   - Biohazard bags must be stored in a container placed into the dedicated freezer.
   - Carcasses collection for incineration will be coordinated with the ARCL personnel.

Prolonged Physical Restraint

Using a manual or device-facilitated method to limit the animal's normal movement, for periods lasting longer than 5 minutes at a time, is defined by the IACUC as prolonged physical restraint. The degree of restraint needed (head only, arms and head, whole body, etc.) will guide the researcher with the restrain method and the type of equipment to consider. Less restrictive methods must always be considered.

1. **Refinement considerations for restraint**
   - Use alternatives compatible with the research objectives (e.g., subcutaneous implantation of osmotic pumps in rodents or backpack-fitted instrumentation)
   - Minimize the restrain period in line with the research objectives
   - Animals to be placed in restraint devices should be given training (with positive reinforcement) to adapt to the equipment and personnel. Animals that fail to adapt should be removed from the study
   - Observations should be frequent enough to ensure the well-being of the animals.

2. **Food and water restrictions during restraint**

   If the restraint limits the ability of the animal to access food and water for more than 6 hours, procedures for ensuring nutrition and hydration should be implemented.

Food and fluids Restrictions

"Regulation of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioral research protocols. The regulation process may entail scheduled access to food or fluid sources, so an animal consumes as much as desired at regular intervals, or restriction, in which the total volume of food or fluid consumed is strictly monitored and controlled (NRC 2003b)." (The Guide, 2011)

Forms & Tools
Restriction Alert Card

1. **Refinement considerations for food and fluids restrictions**
   - Use the least duration and amount of restriction necessary to achieve the scientific objective while maintaining animal well-being.
   - Consideration should be given to minimize potential adverse events.
   - Access to food or fluids intake must be closely monitored and controlled to ensure the animals' nutritional needs are met.
   - Food and fluids provisions to animals will be based on: species, strain, or stock, gender, and age of the animals; thermoregulatory demand; type of housing; time of feeding, nutritive value, and fiber content of the diet. (Heiderstadt et al. 2000; Rowland 2007)

2. **Observation requirement**
   - Daily for animal under food restriction
   - Twice a day for animal under water restriction.
   - Body weights should be recorded at least weekly

3. **Caloric restriction for husbandry**
   Caloric restriction is accepted for rodents as part of the research and does not require IACUC approval. Benefits from caloric restriction may be the reduction on obesity, cancer rated, neurogenerative disorder, or increase in longevity and reproduction.

4. **Special diet use**
   - **Nutritionally complete diets** (i.e., Breeder diet) should be used under veterinary supervision and do not require IACUC approval.
   - **Nutrient deficient diets** (i.e., Low sodium) require scientific justification and IACUC approval.

Transportation

When transporting animals within KAUST, care must be taken to protect animals from extreme conditions, prevent animal escape, and reduce occupational exposure to animals allergens. Additional safety requirements may be needed for animals used in biological, chemical, or radiological studies.

Safety Considerations
• Live animals must be transported in a closable shatterproof and leak-proof container. • Transport container must be clearly labeled. A template for the campus transportation label can be found here. • Please refer to Chapter 6 of the Biosafety Manual for other provisions for transporting biological materials on campus.

1. **General requirements**
   - Containers or cages used for transport should:
     - be clean
     - be leakproof
     - limit exposure to animal allergens
     - provide adequate ventilation
     - include the animal identification
     - prevent animal escape
     - be transported on a cart
   - Animals should be protected from direct sunlight or extreme temperatures.
   - Service elevators must be used where available.
   - Upon arrival at the destination, animals should have access to food and water unless approved in the IACUC protocol.

2. **Transport vehicle requirements**
   - Transport vehicles must be inspected and approved by IACUC prior to use.
   - The heating/cooling system of the vehicle must maintain the inside temperature of the vehicle at an appropriate temperature (based on species) prior to loading the animals.
   - Animals should not be left in the vehicle any longer than what is necessary to transport them to their destination.

Cage Identification

Cage Identification is essential to identify ownership, procedures animals may have undergone, and to facilitate provisions of veterinary care. Cages must be identified at all times.

1. ARCL staff provides **printed cage cards**, and the research staff must ensure that information is up-to-date, and animals are identified at all time.

2. The **cage card must include** the following information:

    - Source of the animal
    - Investigator's name and contact information
    - IACUC protocol number
    - Gender
    - Strain or stock
    - Date of birth
    - Date of arrival
    - Genotype information, when applicable

Social Housing

"Single housing of social species should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being. In these cases, it should be limited to the minimum period necessary." (The Guide, 2011)

1. **Social animals** should be housed in pairs/groups of compatible individuals.

2. **Animals may be individually housed for the following reasons:**
   - Animals that showed aggression or fighting behavior,
   - Animals known to be prone to fight (e.g., adult male mice)
   - Breeders not currently in use according to specific strains breeding programs
   - When a companion animal is not available (e.g., the last animal remaining in an experimental cohort)
   - Animals recovering from surgery or invasive procedures during the period of recovery
   - Scientific necessity as reviewed and approved by the IACUC.

3. **Social well-being:**
   - When single housing occurs, methods to ensure social well-being (enrichment) must be implemented under the direction of the veterinary staff
   - Methods for ensuring social well-being include:
     - Additional enrichment items or addition of a companion animal in the room or housing area
     - Visual, auditory, olfactory, and tactile contact with compatible conspecifics
     - Positive interaction with the animal care staff

4. **Documentation**:
   - Cage must be labeled "Single"
   - Reason and the duration are maintained in the ARCL management software.

Environmental Enrichment

Animals must be housed with appropriate space, supplementary structures, and resources to meet their physical, physiologic, and behavioral needs. Inappropriate housing can compromise the animal wellbeing and the success of the research study.

1. **Standard enrichment**
   Animals must be provided with a flat enrichment sheet (Innorichment®) that engages rodents in complex foraging and nesting activities.

2. **Single housing**
   Animals will be provided with disposable cardboard tunnels and paper huts.

3. **Enrichment selection**
   - Not every structure added to the animal housing will improve their wellbeing; only those that reduce the impact from external stressors are considered environmental enrichment.
   - Veterinary and ARCL staff, in cooperation with the Principal Investigator, will evaluate the appropriateness of enrichment resources to ensure the positive impact on animal wellbeing and compatibility with the research goals.

Satellite housing in PI laboratories

Satellite housing is any area other than CoreLabs in which animals are held for over 24 hours.

Safety Considerations	Forms & Tools
• Authorized staff and users must meet the minimum training and competency requirements. • Allergies to laboratory animals may develop as a result of repeated exposure to allergens derived from animal secretions and excretions.	Terrestrial Husbandry Log example

1. **Approval of location**
   The IACUC must inspect and approve any satellite housing before housing the animals and conduct annual inspections of the space.

2. **Observation requirements**
   When animals are present in the lab (including weekends and holidays), daily animal observations and husbandry must be performed by research staff listed in the IACUC protocol.

3. **Facility requirements**
   - Access control: access to the animal area should be restricted to authorized staff and users.
   - Temperature and humidity monitoring: using a high/low thermometer with a hygrometer.
   - Light cycle control: diurnal light cycle (12:12, 10:14) maintained using an automatic light timer. (Inverted or alternate light cycle requires IACUC approval.)
   - Sanitation: surfaces should be easily disinfected. (Porous surfaces such as unpainted/untreated wood are not appropriate. Metal surfaces should be free from rust or corrosion.)

4. **Attire**
   Appropriate PPE must be worn in animal housing areas.

5. **Documentation**
   Terrestrial Husbandry Log must be maintained by the PI at all times.

Genetically Modified Animals

Safety Considerations
• Avoid using sharps or use safety-engineered sharps whenever possible. • Do not recap needles or use the “scoop method” if absolutely necessary. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Sharps Safety resources for additional details. • If administering anesthesia, refer to Safety Considerations of the Anesthesia Use and Isoflurane Use subchapters.

1. **Breeding**
   Non-standard breeding schemes not included in the Breeding and weaning section must be listed in the IACUC protocol.

2. **Adverse phenotypes**
   Adverse effects, distress, or pain related to the phenotype must be considered when using genetically engineered animals.
   For animals with physiological deficits, supportive care and human endpoints must be described in the IACUC protocol.

3. **Genotyping**
   Where possible, genotyping should be performed using the tissue sample resulting from ear punch identification.

4. **New stains**
   Creating a new, genetically modified animal must be approved by the Institutional Biosafety and bioEthics Committee (IBEC).

5. **Nomenclature**
   “Accurate recording, with standardized nomenclature, when available, of both the strain and sub-strain or of the genetic background of animals used in a research project is important. The International Committee on Standardized Genetic Nomenclature for Mice and the Rat Genome and Nomenclature Committee maintain online guidelines for these species.” (The Guide, 2011)

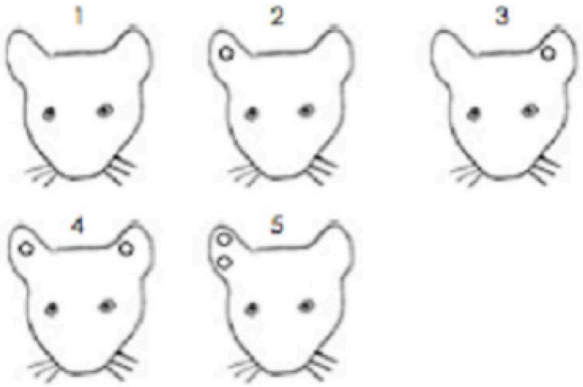
Animal identification

Identification of individual animal may be necessary for genotyping or study purposes. While temporary identification is possible, permanent methods of identification are preferred.

Safety Considerations
• Avoid using sharps or use safety-engineered sharps whenever possible. • Do not recap needles or use the “scoop method” if absolutely necessary. • Dispose of sharps in appropriate sharps containers kept within close reach. • Refer to Sharps Safety resources for additional details.

Forms & Tools
Body Condition Scoring Sheet

1. **Ear punch**: is the veterinarian recommended identification technique.
   - Ear Punch Scheme:



2. **Other identifications methods**: require veterinarian consultation and may require IACUC approval.

Endpoint

In studies where there is a potential for animal to experience pain and distress, well defined endpoints help to minimize the negative experience of the animal. Strong scientific justification is required when animals may experience unrelieved pain and distress in order to achieve the aims of the study.

1. **Definitions**

Term	Definition
Experimental Endpoints	Occurs when the scientific aims and objectives of the study have been reached
Death as an endpoint	A study that requires any animal to die without humane euthanasia in order to meet specific scientific objectives. These studies require strong scientific justification and should only be considered when no other alternatives are available.
	The following are not considered to be death as an endpoint: • longevity studies in which animals are held until they are aged, clinical signs of disease are treated according to recommendations by veterinary staff, and animals are euthanized when they become moribund • spontaneous mortality, when death is not the intended outcome • any study in which animals are euthanized when experimental or humane endpoints are reached as described on the protocol
	Study requirements: • Animals should be monitored daily, or more frequently during the period of the study in which mortality is likely. Consideration will be given to moving animals to individual cages when their condition deteriorates • Dead animals must be promptly removed • Surrogate endpoints, which are clinical symptoms that are predictive of death, should be considered whenever possible
Human endpoints	The earliest point in the study at which pain or distress can be minimized, terminated, or relieved, while still meeting the scientific aims and objectives of the study
	When the identification of humane endpoints is challenging (e.g., internal orthotopic cancers or metastatic disease), pilot experiments using small numbers of animals should be considered to predict clinical signs and to define humane endpoints

2. **Endpoints criteria**

Clinical signs necessitating immediate intervention:

- Reduction in food and water intakes over a 24- to 48-h period resulting in emaciation or dehydration;
- Consistent or rapid bodyweight loss reaching > 20% compared with the pre-treatment weight of adult mice. Note: In some experiments (e.g., tumors), bodyweight is a very poor indicator - muscle atrophy or emaciation is more useful. Body condition scoring provides a very useful indication of muscle loss (See below);
- Persistent hypothermia;
- Bloodstained or mucopurulent discharge from any orifice;
- Labored respiration, particularly if accompanied by nasal discharge and/or cyanosis;
- Hind-limb paralysis or inability to move and react;
- Anemia as indicated by symptoms such as severe pale feet or markedly altered hematological parameters;
- Significant abdominal distension or where ascites burden exceeds 10% of the bodyweight of age-matched controls. Accurate determination is difficult, but body girth is useful, and a 20% increase should be the maximum normally allowed; similar to the appearance of a pregnant mouse;
- Severe and prolonged incontinence or diarrhea;
- Tumors with volume that exceed 1.5 cm3 (approximately the size of the mouse's head);
- Tumors ulceration/necrosis;
- Tumors that interfere with locomotion or cause abnormal vocalization, behavior, or bodily functions.

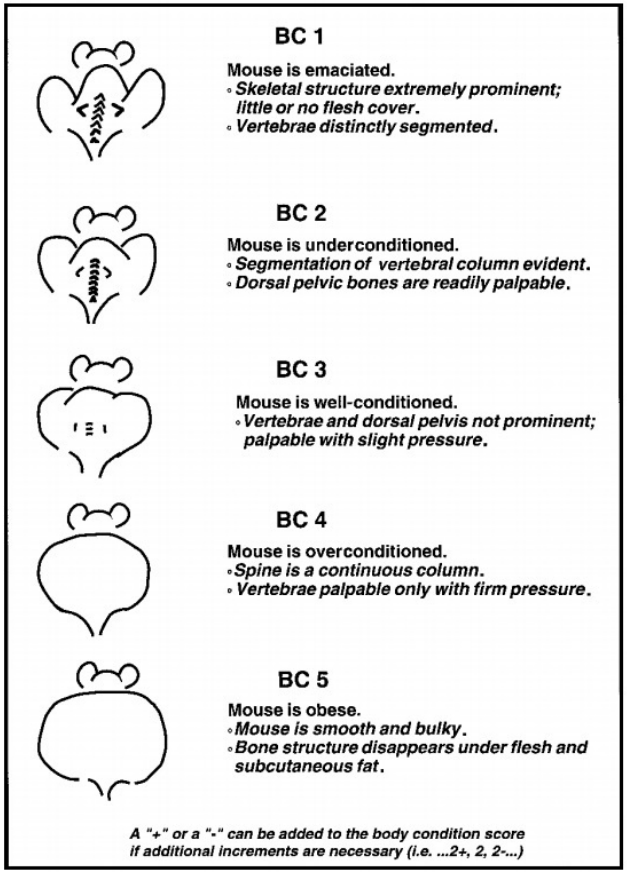
3. **Adverse clinical conditions**

Veterinarian must be informed when adverse clinical conditions occur either due to an approved experimental manipulation or "spontaneously". The veterinarian is authorized to implement treatment, including euthanasia, in order to prevent unnecessary pain and distress in animals.

- **Anticipated outcomes** are clinical conditions that may be allowed to persist for research purposes if scientifically justified in the IACUC protocol.
    - The PI and veterinarian must agree on clearly defined endpoint criteria and actions to take when criteria are reached.
    - Ultimate authority for determining when the endpoint criteria is reached, resides with the veterinarian.

- **Unanticipated outcomes** are clinical conditions not defined in the IACUC protocol.
    - The veterinary staff will determine if treatment is feasible and consult with the PI. If treatment would affect the experimental results, the animals may be euthanized.
    - The IACUC protocol must be modified to include endpoints for any newly identified unexpected outcomes.

4. **Body scoring condition (BC) Evaluation**
   - Method used to assess health and establish endpoints for adults where body weight is not a viable monitoring tool (e.g., tumor models).
   - Gently hold the mouse by the base of the tail and pass a finger over the sacroiliac bones.
   - Compare with the diagram to determine a score.
   - Document scores for each animal.
   - Scores of BC 1 or BC 2 should result in euthanasia unless justified and approved by the IACUC.



5. **Documentation**

   Adverse clinical outcomes, monitoring, and treatment must be recorded. These records must be available for inspection.

Animal irradiation

Safety Considerations
• X-Strahl RS320 Cabinet Irradiator generates ionizing radiation, which can cause injury or death if recommendations are not adhered to. • All authorized users must wear a personal dosimeters issued to them at all times when operating the irradiator. • Please follow the X-ray Irradiator manual, the R320 Cabinet Dose Look Up Tables, and "Local rules: radiation-producing equipment" as well as Guidelines for Working with Electrical Equipment. • Training requirements: X-Ray Analysis Equipment Safety training and Practical Operation Training for the Irradiator. The latter is administered by ARCL. • Upon completion of these training, users must be added to the authorized user's list. Please contact the ARCL manager.

Forms & Tools
Irradiation scoring sheet

1. **Irradiation dosage**

- While dosing varies by strain and age of the mice, a typical dose for bone marrow transplantation study is 9.5 GY in a single dose or two doses of 4.5 GY four hours apart.
- If unfamiliar with the strain, the mice age, or the radiation source to be used, it is advisable to consult the literature or the veterinarian staff.

2. **Supportive care**

- The animals should then be placed in the home cage with DietGel Recovery, which should be provided during the first 14 days after irradiation. As the DietGel becomes contaminated with fecal material, it must be replaced regularly.

3. **Monitoring**

Animals must be carefully monitored daily for the first 14 days following irradiation.

- Findings must be recorded with the help of the Irradiation scoring table.
- If animals experience morbidity or mortality, the ARCL staff and/or the veterinarian must be promptly alerted.

IRRADIATION SCORING TABLE		
Clinical Sign	**Score**	**Symptoms description**
Body loss	0 1 2	No Body loss/BCS 3 to 2 Weight loss up to 20% BW/BCS 2; signs of recovery within 2 weeks post-irradiation Weight loss up to 25% BW/BCS 2; without signs of recovery within week 3 post-irradiation or BCS 1
Anemia	0 1 2	Absent Mild anemia without other relevant signs – mild paleness of mucous membranes. Persistent Anemia/associated with bleeding GI tract - paleness of limbs, tail, and ear pads.
Intestinal Bleeding	0 1 2	Absent Mild to moderate without general signs of anemia – normal mucous membranes, no paleness. Persistent anemia associated with bleeding GI tract – paleness of limbs, tail and era pads, anal bleeding/bloody stools.
Infection	0 1 2	Absent Mild signs of infection without impairment of the animal capacity to move and access food and water autonomously – mild skin infection, mild ocular or nasal discharge without impairment of visual and breathing capacity. General signs of infections with impairment of the animal capacity to move and access food and water autonomously – severe skin infection, severe ocular discharge with eyelids close, nasal discharge with impairment of respiratory frequency, enteritis and dehydration, vestibular syndrome.
Teeth issues	0 1 2	Absent Mild to moderate incisors lesions without impairment of the animal capacity to access food and eat autonomously Severe incisors lesions with impairment of the animal capacity to access food and eat autonomously – weight loss.
Graying of hair coat	0 1 2	Absent/Present without signs of skin ulcers Mild skin damage – mild dermatitis/ulcers without bleeding Severe skin damage – ulcers that do not recover, evident bleeding.

Notes:

- If animals are not recovering by day 14 post-irradiation, considerations should be given to euthanasia. If not recovered by day 21 post-irradiation, animals must be euthanized.
- Consultation with animal care and veterinary staff is highly recommended for any scoring between 2 and 3.
- Animals scoring a value equal or higher than 4, as well as moribund animals, must be promptly euthanized.

4. **Therapeutic interventions**
   - Recovery gel on cage floor
   - Soft food on cage floor
   - Antibiotic Therapy

5. **Humane endpoints**

   Please refer to Endpoints section while evaluating animals.
   - If animals are not recovering by day 14 post-irradiation, considerations should be given to euthanasia. If not recovered by day 21 post-irradiation, animals must be euthanized.
   - Consultation with animal care and veterinary staff is highly recommended for any scoring between 2 and 3.
   - Animals scoring a value equal or higher than 4, as well as moribund animals, must be promptly euthanized.

Breeding and weaning

1. **Breeding**
   - The male and female may remain together continuously or may be separated at pregnancy diagnosis based on the need for additional litters.
   - Standard breeding scheme:
     - 1 male:1 female per cage
     - 1 male:2 females per cage

2. **Weaning**
   - Litters are routinely weaned at 21 days of age. Exceptions can be made for:
     - Pups that appear too small to survive alone, veterinary approval is required
     - Scientific reasons; IACUC approval is required
   - If weaning results in the single housing of an animal, efforts will be made to identify a socially compatible cage-mate. If unsuccessful, the animal will be singly housed according to Social Housing section or euthanized.
   - If weaning is delayed, it must be documented on the cage card.

3. **Cage density**
   - Adult mice: up to 4 per cage
   - Breeders with litters: up to 10 pups per cage;
   - Trios with more than 10 pups: relocate one mother with part of the nest and half of the pups of the same age to a new cage.

Reporting of sick/injured animals: Responsibilities & Vet assistance

1. In case of a **moribund state**, the animal will be immediately euthanized and reported as described below.
2. In case of **emergency veterinary care** is needed, including after working hours, weekends or holidays, contact the ARCL staff through the emergency phone **+966 568984609**. The ARCL staff has the responsibility to inform the veterinarians in this case.
3. To report any **sick/injured animals,** please contact the Animal Facility Manager/designated or the attending veterinarians (Dr. Sara Fuochi: sara.fuochi@crl.com and Dr. Giuseppe Mesiti: Giuseppe.Mesiti@crl.com) within 24h of identifying the animal sickness/injury.
4. Please report any **incident** resulting in the harm or death of animals to iacuc@kaust.edu.sa.

5. **Veterinary care:**
   - The consultant veterinarian (or alternate) is available onsite at regular intervals in order to monitor the in vivo activities, to provide guidance for animal care and welfare issues, and to provide medical veterinary assistance.
   - The veterinarian (or alternate) also provides remote medical veterinary assistance.

APPENDIX

SAFETY ADVISORIES

I. Biohazard, Cytotoxic Hazard and Regular Waste Disposal

Purpose

The procedure identify the different categories of waste inside the Animal Resources Core Lab (ARCL) and define how to manage collection and disposal.

Personnel involved

The activities **must be performed only** by the ARCL staff and by waste management employees who are adequately trained.

Categories of waste potentially contaminated with chemicals and/or biologicals

- Complete cages (lid, bottom, feeder, enrichment) filled with dirty bedding
- Regular waste
- Animal carcasses
- Bedding
- Sharps (hypodermic needles, syringes, scalpels, and blood lancets; all materials designed for use in biological, etiological, bacteriological, or tissue culture work)
- Glassware

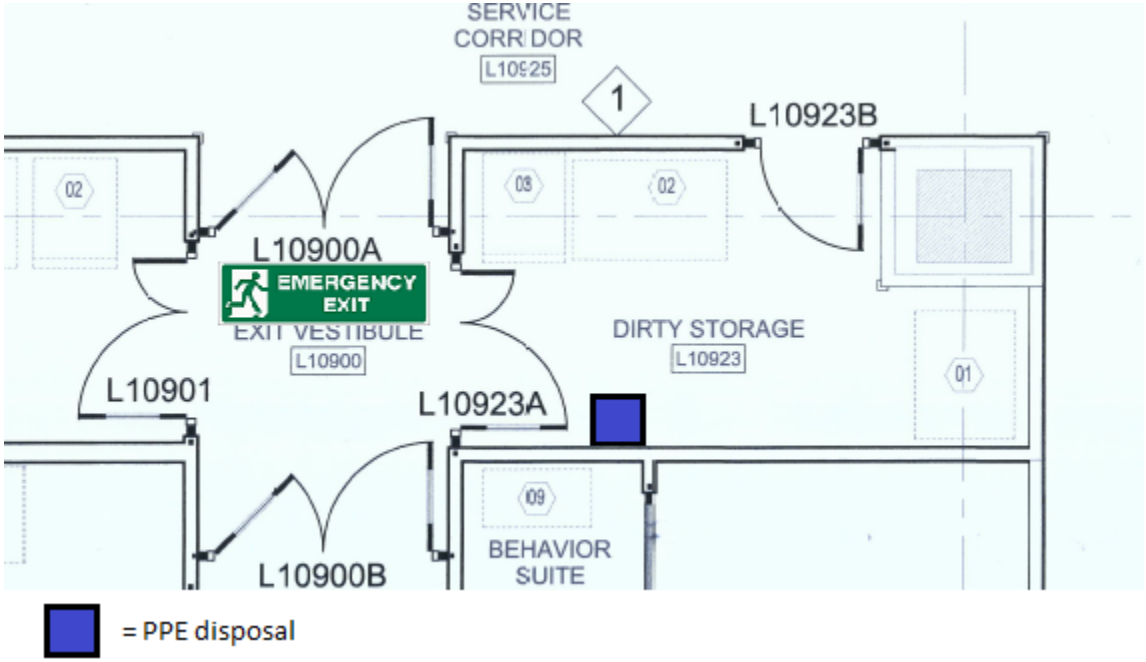
Necessary equipment

- Biohazard bin with pedal and lid
- Yellow biohazard bins (240 L)
- Yellow biohazard bags
- Yellow chemotherapy & biohazard bags for bio-cytotoxic waste
- INCINERATE ONLY labels
- Regular waste bins with pedal and lid
- Sharps containers
- Cardboard containers
- Tape

Biohazard waste collection

1. Collect the waste bags from the bins with a daily routine.
2. Close the bag.

3. Tag with INCINERATE ONLY label.
4. Put the bags inside the biohazard bins located in the waste storage area.
5. Oversized crates containing animals from certified suppliers (e.g., Charles River, Jackson Laboratories) must be emptied from bedding in a biohazard bag and closed by using tape in order to avoid leakage when collected.
6. Plastic boxes containing animals can be stuck up and placed in one biohazard bag.
7. Before leaving the waste storage to re-enter in the clean area, change gloves and shoe covers. PPE disposal map:



Biohazard waste disposal

1. Enter in the waste storage area from outside the facility and accordingly, to the Laboratory Coordinator of Campus Support, place the waste outside the facility for the minimum time required for collection.

Bio-cytotoxic waste collection

1. Collect the waste bags from the bins with a daily routine.
2. Close the yellow bag with biohazard and chemotherapy waste indication.
3. Tag with INCINERATE ONLY Label.

4. Put the bags inside the yellow biohazard & cytotoxic bin located in the waste storage area.
5. Bags containing animals treated with hazardous chemicals should be disposed of in the yellow biohazard and chemotherapy bag, and closed by using tape in order to avoid leakage when collected.
6. Before leaving the waste storage to re-enter in the clean area, change gloves and shoe covers.

Bio-cytotoxic waste disposal

At the scheduled time, enter in the waste storage area from outside the facility, and accordingly to the Laboratory Coordinator of Campus Support, place the bins outside the facility for the minimum time required for collection.

Regular waste collection

1. Collect the waste bags from the bins with a daily routine and close the bag.
2. Put the bags in the waste storage area.
3. Before leaving the waste storage to re-enter in the clean area, change gloves and shoe covers.

Regular waste disposal

1. Accordingly, to the laboratory coordinator of Campus Support, collect the bags from the dirty storage area entering from outside the facility.
2. Place the waste outside the facility for the minimum time required for collection.

Complete cages with dirty bedding collection and disposal (for cage changing procedure, see ARCL_SOP 005)

1. After a complete cage change, stack up the dirty cages inside trash biohazard waste bags. Take care of using more bags to avoid overfilling. Put the lids and the feeders in bags, too.
2. Close the last cage with a lid to avoid leak of the dirt in the environment.
3. Tag with INCINERATE ONLY label.
4. Put the bags inside the dedicated biohazard bins placed in the dirty storage. Each bin should not contain more than 20 cages, lids, feeders.
5. At the scheduled time, enter in the dirty storage area, close the door and place the bins outside the facility for the minimum time required for collection.

Carcasses storage

1. After euthanasia, carcasses should be placed in dedicated bags, and each bag should be labeled with:
   - Date
   - IACUC #
   - Number of animals
   - Strain(s)

2. Bags can be temporarily (< 24h) stored in the ARCL fridge present in the experimental rooms. Bags with carcasses are then collected by ARCL staff to be stored in the dedicated freezer placed in the dirty storage area.
3. All carcass disposal is manually recorded on the dedicated sheet posted on the dedicated freezer.
4. Before leaving the waste storage to re-enter in the clean area, change gloves and shoe covers. If the exit is required, re-enter into the facility from the main entrance according to the personnel entrance procedure.

Carcasses disposal

1. At the scheduled time, collect the carcasses from the dedicated freezer placed in the dirty storage area.
2. Put the biohazard carcasses inside the dedicated biohazard bags, close them, and put the bags in the dedicated biohazard bins.
3. Identify the bin containing the carcasses by placing the dedicated BIOHAZARD CARCASSES label on it.
4. Put bio-cytotoxic carcasses inside the dedicated biohazard & chemotherapy bags, close them, and put the bags in the dedicated bin for bio-cytotoxic waste.
5. Tag with INCINERATE ONLY label.
6. Accordingly to the Laboratory Coordinator with Campus Support, deliver the bins to the Waste Management Team, collecting the bins from inside the facility OR place the tray outside the facility for the minimum time required for collection, only after coordinating with Waste Management Team (rm.siteservices@kaust.edu.sa) for on Tuesdays or Wednesdays pick up.

Biohazard sharps/ glassware collection and disposal

1. Place sharps in the biohazard sharp container.
2. When containers are ¾ full, close them and properly seal with tape.
3. Put the container(s) on a tray in the waste storage area.
4. Accordingly to KAUST Research Materials Site Services, deliver the bins to their Waste Management Team, collecting the bins from outside the facility.
5. Place the tray outside the facility for the minimum time required for collection, only after coordinating with Waste Management Team (rm.siteservices@kaust.edu.sa) for on Tuesdays or Wednesdays pick up.

Bio-cytotoxic sharps/ glassware collection and disposal

1. Place sharps in the sharps container labeled with biohazard & cytotoxic or with biohazard & INCINERATE ONLY
2. When containers are ¾ full, close them and properly seal with tape.
3. Put the container(s) inside a transparent bag in the waste storage area and apply the INCINERATE ONLY label.
4. Place the bags outside the facility for the minimum time required for collection, only after coordinating with Waste Management Team (rm.siteservices@kaust.edu.sa) for on Tuesdays or Wednesdays pick up.

Uncontaminated glassware collection and disposal

1. Place sharps into the cardboard box.
2. When boxes are full, close them and properly seal with tape.
3. Place the box into the waste storage area.
4. Accordingly to the Laboratory Coordinator with Campus Support, deliver the bins to the Waste Management Team, collecting the bins from outside the facility.
5. Place the waste outside the facility for the minimum time required for collection.

Every 3 months, the exterior part of the biohazard bins, particularly the lids and handles, should be wiped with disinfectant wipes (Sanicloth).

Common waste containers in Animal Resources Core Lab

A: Biohazard bin located in dirty storage area

B: Biohazard bin located within the holding rooms
C: Bin for general waste
D: Uncontaminated glassware
E: Bio-cytotoxic sharps/contaminated glassware
F: Bio-cytotoxic bag
G: Carcasses bin located in dirty storage area
H: Bio-cytotoxic bin located in dirty storage area
I: Incinerate only label

II. Anesthesia using isoflurane system

Potential Health Hazards

- Isoflurane is an eye and skin irritant and central nervous system toxicant. The substance can be absorbed into the body by inhalation of its vapor and by ingestion. Long-term exposure may cause chronic or adverse health effects, including nausea, dizziness, fatigue, headache, irritability, reduced mental performance, liver and kidney disease, and possible reproductive effects (sterility, infertility, miscarriages, and birth defects).
- WARNING: Isoflurane may be release in the BSC; thus, it is important to maintain the BSC sash in the correct position to minimize isoflurane exposure. As opening of the induction chamber is associated with picks of isoflurane exposures (Johnstone et al. 2017), it is also particularly important to close the induction chamber soon after the mouse has been removed from the chamber to reduce isoflurane release in the BSC.
- Activities associated with an increased risk of isoflurane exposure:
  - Performing multiple animal surgeries or multiple imaging sessions, during which anesthesia is delivered for an extended length of time;
  - Not ensuring a tight seal around the animal's nose cone and mouth;
  - Failing to flush the induction chamber with oxygen prior to opening the chamber to transfer animals.
- Signs and symptoms of isoflurane exposure can include:
  - Acute Exposure: nausea, vomiting, skin and respiratory irritation, including the nose and throat, headache, dizziness, red and painful eyes and drowsiness**;**
  - Chronic Exposure: hypotension, tachycardia, respiratory depression, elevated blood glucose.
  - If any of these symptoms appear, seek immediate help from KAUST Health.
- HSE can evaluate staff exposure by monitoring laboratory workers while they perform work with isoflurane. Please contact HSE Research Safety Team (researchsafety@kaust.edu.sa).
- The isoflurane (Floran) Safety Data Sheet is available here.

Signs

- Rooms where isoflurane exposure may occur should post the following sign:

ISOFLURANE WARNING! HARMFUL IF INHALED CONTINUOUSLY Use with adequate ventilation and/or scavenging equipment.

III. Handling of Samples Requiring Biosafety Level 2 Containment

- In KAUST, all laboratory personnel working with BSL-2 samples must receive Biosafety training offered by the Health, Safety, and Environment (HSE) Department. In addition, if handling human blood or other potentially infectious materials (e.g., human cell lines, certain body fluids, etc.), personnel must take Bloodborne Pathogens Safety training and enroll in Bloodborne Pathogens Safety Program.
- The principal investigator is responsible for conducting an appropriate risk assessment for obtaining IBEC approval (or certificate of exemption) and for ensuring that the personnel is informed & trained for handling any biohazard materials.
- Biohazardous samples should NOT be brought into ARCL without approval from the ARCL Manager.

Instructions

- All biohazard material must be transported in secure, robust containers, and all containers must be clearly labeled with the type of specimen, Principal Investigator contact information, date, and biohazard logo. Do not use abbreviations. A template for the campus transportation label can be found here.
- Proper personal protective equipment (PPE) must be worn at all times.
- Post the relevant door sign to clarify that procedures requiring BSL-2 containment are conducted.
- Let the BSC run at least 10 minutes before starting so that the laminar airflow at all is set, and only sterile air is blown in the cabinet & check that there is sufficient airflow (green LED).
- **Before each use**, spray with 70% ethanol (contact time 60 s) or use disinfectant wipes (contact time 60 s) to wipe down the hood's working surface (taking care of the grids at the front and the back) and all items placed in the hood. To wipe the hood's working surface, do not use a circular motion but a straight motion from the back to the front of the hood working surface.
- All bench and work areas should be kept with as few items and pieces of equipment as possible.
- Collect the materials used for the work needed in advance and avoid disruptions to airflow.
- Do not place any material on the front air grids or close to the back grids. This will interfere with the airflow.
- Be extremely careful in all situations that involve sharps (needles, scalpels, glassware, coverslip, etc.) and minimize their use as possible.
- Dispose of gloves when overtly contaminated, when working with infectious materials is completed or when the integrity of the glove is compromised.
- Minimize the creation of splashes or aerosols.
- Mouthing pipetting is prohibited.
- **After each use**, wipe down the hood's working surface (taking care of the grids at the front and the back) and all items placed in the hood wipe with 70% ethanol followed by disinfectant wipes (contact time 60 s), using a straight motion from the back to the front of the hood working surface.

- Personnel handling BSL-2 samples must wash their hands after handling biohazard materials, after removing gloves, and before leaving the laboratory.
- Remove the door sign for BSL-2 containment.

In case of a serious emergency or life-threatening emergency, please contact 911 from lab landline or 012 808 0911 from mobiles.

Procedures in case of a minor spill

- If you are not comfortable tackling a spill on your own, then let other users in the area know that a spill has occurred and contact the ARCL staff.
- Notify everyone else who is working in the room that there has been a spill and not to walk through the contaminated area.
- If a biohazardous material spills on you, wash any exposed body parts.
- Using materials from your spill kit:
  - Put on eye protection.
  - Cover the spill with absorbent material.
  - To decontaminate the area, pour freshly prepared 10% bleach solution over the entire area, contact time 30 min.
  - Wipe with paper towels, and discard towels into the bio-hazardous container.
  - As bleach is corrosive, after the decontamination, the BSC shall be wiped with water from the Milli-Q system available in the ARCL.
- Decontaminate surrounding floor and work surface areas where splashes or larger aerosols may have settled around the spill.
- Repeat the decontamination procedure.
- Remove contaminated clothing and place in the biohazard waste bin.
- Wash your hands thoroughly.

IV. Substance administration

Personnel safety

- Medical Emergencies
  - For injury, call 911 from a landline or 012-808-0911 from a cell phone or seek treatment by going to the KAUST Health (KH)
  - All incident must be reported to the Report It system (http://reportit.kaust.edu.sa)
  - Incident involving sharps will be logged through HSE incident investigation process.
- Personal Protective Equipment (PPE)

The following PPE must be used while performing these procedures in addition to standard ARCL required PPE:

- Eye protection in case of use and/or manipulation of hazardous chemicals.

Hazardous substances

Hazardous drug safety and health guidelines

- Employees can be exposed to hazardous drugs through inhalation of drug dust or droplets, absorption through the skin directly, or injection through the skin.
- Care must be taken when performing these procedures or handling spills and sharps to reduce employee exposures to hazardous agents.
- The ARCL staff or the research staff should make available substance-specific SOP and Material Safety Data Sheet to the users.
- Personnel working with hazardous drugs are required to report accidents, possible overexposures, or unsafe conditions to the ARCL Manager and to HSE (via ReportIt website).

General guidelines for working with hazardous drugs include:

- Read and understand the hazards of the materials being used before work begins. Hazardous drugs should be stored in an area that is limited to authorized personnel. If possible, only amounts for daily use should be brought in the ARCL. Depending on the drug, additional PPE may be required. Therefore, personnel working with hazardous drugs must referrer to the specific SOP. Gloves should be changed regularly and immediately if torn, punctured, or contaminated. Syringes and IV sets with Luer-lock fittings should be preferred.
- Compounds that are administered on an emergency basis as part of veterinary care do not require prior IACUC approval.
- Cages containing animals treated with hazardous drugs should be labeled with a **yellow cage card template** for hazardous substances.
- Relevant hazard information should be indicated on the yellow cage card, including warning information and date of treatment(s).
- For most hazardous drugs, it is acceptable to avoid cage change procedures for the first three days after the last administration.

Decontamination from hazardous drugs:

- Should consist of surface cleaning with water and detergent followed by thorough rinsing. The use of detergent is recommended because there is no single accepted method of chemical deactivation for all agents involved.
- Alcohol vapor build-up is a concern. 70% alcohol-based solution should be used only after cleaning.

- Overt contamination of gloves or gowns, or direct skin or eye contact, should be treated immediately by:
  - Removing gloves and/or gown.
  - Wash the affected skin area with detergent and water.
  - If eye is affected flood with water for fifteen minutes.
  - Seek medical attention.

TEMPLATES

I. *Procedure/Surgery Card*

Procedure/Surgery Card			
Procedure Name			
Animal ID			
IACUC #		PI Name	
Performed by (initials)		Date	
Surgery	Y/N	Analgesia Provided	Y/N

Initial	Date	Comments/Therapy provided

Pain Management				
C= Carprofen B= Buprenorphine M=Meloxicam		Other, please specify		
Date	Time	Initials	Analgesia Code	Score
1: normal mouse posture, activity, incision; 2: abnormal mouse posture, activity, incision; 3: euthanized				

NOTE: Records must be available for inspection and must contain the following information: Date, Time, IACUC protocol number, and Principal Investigator's name; Agent (s) used, dosage, route administration (for each administration).

II. Restriction Alert Card

Restriction Alert Card	
Restriction	☐ Food ☐ Water
IACUC #	
PI Name and contact	
Animal ID	
Withhold Start Date	
Withhold End Date	
Specification of restriction	

NOTE: Records must be available for inspection and must contain the following information: Date, Time, IACUC protocol number, and Principal Investigator's name; Agent (s) used, dosage, route administration (for each administration).

III. Yellow cage card template for hazardous substances

Signal Word (check one) ☐ DANGER ☐ WARNING **MICE TREATED WITH (insert chemical name)** _____ **HAZARD STATEMENTS (insert statements)** _____ **DATE OF TREATMENT(s)** _____ **NOTE: For more information regarding chemical(s) used, please consult the SDS**	**GHS PICTOGRAMS (check as applicable)** ☐ ☐ ☐ ☐ ☐ ☐ ☐

IV. "Incinerate Only" label for biohazardous waste

V. Terrestrial Husbandry Log Example

Month:_____ Year:_____

Terrestrial Husbandry Log

Building /Room_____/_____ protocol#:_____

Date (day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Perform daily																															
Initials (ABC)																															
ANIMALS NOT PRESENT ☒ Leave rows below blank																															
Animal Health check ☒																															
Current Temparature (°C)																															
High Temparature (°C)																															
Low Temparature (°C)																															
Humidity (% RH)																															
Food check ☒																															
Water check ☒																															
HVAC check ☒																															
Perform weekly																															
Light timer function ☒																															
Cage change ☒																															
Sweep/Mop																															

Date	Notes

VI. Euthanasia Form

EUTHANASIA FORM								
Month/ Year								
Animals			Date D/M/Y	Method of Euthanasia	IACUC #	PI	Operator	Comments
#	M/F	Strain						

VII. Body condition scoring sheet

Month:_____ Year:_____

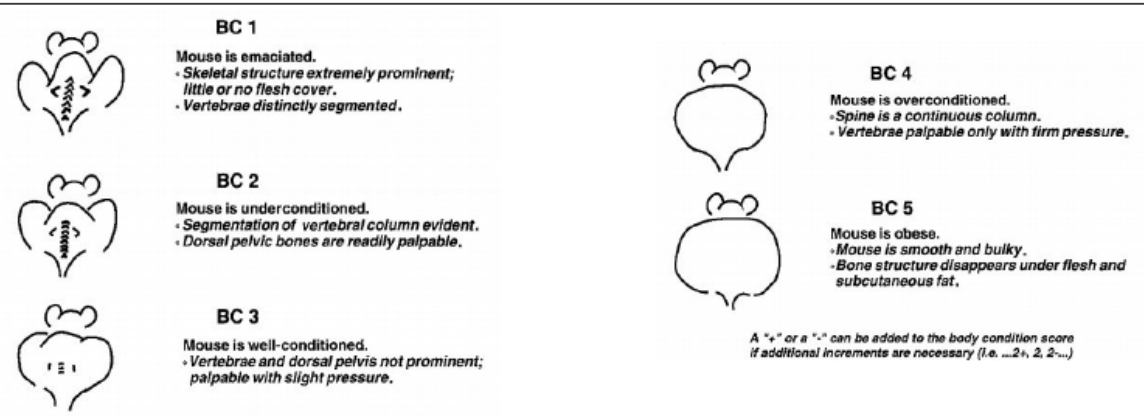
Body Condition Scoring Sheet

Building /Room_____/_____ protocol#:_____

Date (day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Animal ID Number																															
Initials (ABC)																															
Body Condition Score (1-5)																															

Method use to assess health and establish endpoints for adults

1. Gently hold the mouse by the base of the tail and pass a finger over the sacroiliac bones
2. Compare with the diagram to determine a score
3. Document Scores for each animal
4. Scores of BC 1 or BC 2 should result in euthanasia, unless justified and approved by the IACUC



Date	Notes

Template can be download on [Research Compliance website](#).

VIII. Animal irradiation scoring sheet

Month:_____ Year:_____
Animal ID Number:_____

Animal Irradiation Scoring Sheet

Building /Room_____/_____ protocol#:_____

Date (day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Initials (ABC)																															
Body Loss (0-2)																															
Anemia (0-2)																															
Intestinal Bleeding (0-2)																															
Infection (0-2)																															
Teeth issues (0-2)																															
Graying of hair coat (0-2)																															
TOTAL																															

IRRADIATION SCORING TABLE		
Clinical Sign	**Score**	**Symptoms description**
Body loss	0 1 2	No Body loss/BCS 3 to 2 Weight loss up to 20% BW/BCS 2; signs of recovery within 2 weeks post-irradiation Weight loss up to 25% BW/BCS 2; without signs of recovery within week 3 post-irradiation or BCS 1
Anemia	0 1 2	Absent Mild anemia without other relevant signs – mild paleness of mucous membranes. Persistent Anemia/associated with bleeding GI tract - paleness of limbs, tail, and ear pads.
Intestinal Bleeding	0 1 2	Absent Mild to moderate without general signs of anemia – normal mucous membranes, no paleness. Persistent anemia associated with bleeding GI tract – paleness of limbs, tail and era pads, anal bleeding/bloody stools.
Infection	0 1 2	Absent Mild signs of infection without impairment of the animal capacity to move and access food and water autonomously – mild skin infection, mild ocular or nasal discharge without impairment of visual and breathing capacity. General signs of infections with impairment of the animal capacity to move and access food and water autonomously – severe skin infection, severe ocular discharge with eyelids close, nasal discharge with impairment of respiratory frequency, enteritis and dehydration, vestibular syndrome.
Teeth issues	0 1 2	Absent Mild to moderate incisors lesions without impairment of the animal capacity to access food and eat autonomously Severe incisors lesions with impairment of the animal capacity to access food and eat autonomously – weight loss.
Graying of hair coat	0 1 2	Absent/Present without signs of skin ulcers Mild skin damage – mild dermatitis/ulcers without bleeding Severe skin damage – ulcers that do not recover, evident bleeding.

Notes
- If animals are not recovering by day 14 post irradiation, considerations should be given to euthanasia. If not recovered by day 21 post-irradiation, animals must be euthanized.
- Consultation with animal care and veterinary staff is highly recommended for any scoring between 2 and 3.
- Animals scoring a value equal or higher than 4 as well as moribund animals must be promptly euthanized.

Date	Notes

Template can be download on [Research Compliance website](#).

IX. General scoring sheet

General Scoring Sheet

Month:_____ Year:_____ Building /Room_____/_____ protocol#:_____

Date (day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Animal ID Number																															
Initials (ABC)																															
Condtion 1 (specifications)																															
Condtion 2 (specifications)																															
Condtion 3 (specifications)																															
Condtion 4 (specifications)																															

- Condition 1 parameter/Scoring criteria 1 and specifications description
- Condition 2 parameter/Scoring criteria 2 and specifications description
- Condition 3 parameter/Scoring criteria 3 and specifications description
- Condition 4 parameter/Scoring criteria 4 and specifications description

Date	Notes

Template can be download on [Research Compliance website](#).

References

Guide for the care and use of laboratory animals, Current version.
Guidelines for the Care and Use of Mammals. Neuroscience Research Council, Current version.
NIH Guidelines for Survival Bleeding of Mice and Rats, Current version.
AVMA Guidelines for the euthanasia of animals, Current version.