**Laboratory Checklist for Animal Research**

This is a list of basic requirements of the University of Virginia Animal Care and Use Committee (ACUC) for performing animal research. Review the requirements to ensure compliance in preparation for laboratory audits, ACUC semi-annual inspections, or AAALAC site visits. These requirements are based on ACUC policies, PHS Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals, USDA Animal Welfare Act, and AVMA Guidelines on Euthanasia. See end of document for additional references.

### Protocol and Personnel

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Details</th>
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<tbody>
<tr>
<td>The animal use protocol must be approved and accurately describe all of the procedures performed on animals.</td>
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<tr>
<td>A current version of the approved protocol must be available as a reference for all animal handlers. A hard copy must be in the laboratory or animal handlers must know how to easily access the protocol online.</td>
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<tr>
<td>Animal handlers must read the protocol and be aware of the procedures listed on the approved protocol and understand that those are the only procedures that can be conducted without a protocol modification.</td>
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<tr>
<td>Personnel should be aware of the roles of the ACUC (protocol modifications/approval), OAW (animal handler training/compliance), and CCM (animal procurement/housing/husbandry/technical support).</td>
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<tr>
<td>Personnel are responsible for adhering to all ACUC Policies and have an understanding of where to locate them (<a href="https://researchcompliance.web.virginia.edu/acuc/pi/policy/acuc_policies.html">https://researchcompliance.web.virginia.edu/acuc/pi/policy/acuc_policies.html</a>).</td>
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<tr>
<td>Report observed or suspected animal abuse, mistreatment, or non-compliance with approved protocols, University policy, local, state, or federal regulations. Concerns can also be made anonymously. Concerns should be reported to OAW, ACUC, or CCM (<a href="http://www.virginia.edu/vpr/iacuc/concerns.html">http://www.virginia.edu/vpr/iacuc/concerns.html</a>).</td>
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<tr>
<td>Personnel must be listed on an approved protocol as an animal handler before working with animals. Unapproved personnel are not permitted to touch animals or enter the vivarium (unless accompanied by an approved animal handler or PI).</td>
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<td>New animal handlers must attend the Animal Research Orientation Seminar and complete all online training modules.</td>
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<td>Animal handlers should be thoroughly trained in each procedure they will be performing, and be able to demonstrate proficiency upon request.</td>
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<td>Animal handlers must enroll in the Occupational Health Medical Surveillance program and be currently “OK FOR WORK”.</td>
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<td>Ensure strict adherence to occupational health and safety practices when animal use occurs in the laboratory setting and educate those individuals (non-animal handlers) with indirect exposure of the potential risks (allergens associated with indirect exposure to animals or animal products; hazardous chemicals associated with anesthetic gasses and disinfectants; infectious disease transmissible between animals and humans (zoonoses)).</td>
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<tr>
<td>Individuals who directly participate in an animal use activity, but who by the nature of their employment status or involvement in a project, are not listed as an animal handler must complete and submit a Risk Acknowledgment Form to the ACUC prior to beginning work on animal study.</td>
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<td>Any and all rooms (outside of the vivarium) where LIVE animals are taken must be listed in the protocol.</td>
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<td>When a PI plans to be absent for 3 or more months, PI must officially designate, to the ACUC Office, the individual who will be responsible for the laboratory in their absence.</td>
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<tr>
<td>Animal transfers between protocols or PIs must be approved by the Director of CCM. Sharing of live animals is not permitted without protocol animal transfer form and approval.</td>
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<tr>
<td>Pets/non-research animals are prohibited in labs designated as animal research areas. Personnel who have exposure to rodents outside of the workplace should be aware of the potential transfer of pathogens to/from research animals and follow designated procedures to minimize risks.</td>
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<tr>
<td>Visual images of research animals are restricted to educational/instructional and research purposes only. Posting of animal images on a publicly accessible website requires ACUC approval. Posting of any information regarding animal use at the University on any social media website is strictly prohibited. No filming or photographing by the news media may take place without prior approval and guidance from University Public Relations and ACUC.</td>
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### Safety

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<tr>
<td>All animal handlers should know how to report any animal bites or work related injuries to their immediate supervisor and how to contact UVA WorkMed (243-0075) or Student Health (924-5362) for treatment.</td>
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<tr>
<td>NO food or drink in proximity to areas where animals are used or housed.</td>
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**Gloves and protective clothing** must be used at all times when handling animals.

Rodent cages should have **filter tops** on them at all times for protection of the animals and to minimize allergen exposure to personnel. Alternatively, cages can be kept in a biosafety cabinet or chemical fume hood.

All work involving ABSL-2, ABSL-3, human-derived material, radioactive material, and hazardous chemicals must have **prior approval by the ACUC** and the respective safety committee.

Personnel working with hazardous materials must undergo appropriate **training as required by EHS**.

Personnel working with hazardous materials within animals must undergo appropriate training as required by **CCM – Animal Biosafety Level Policies and Procedures training**.

Proper **PPE** (personal protective equipment) must be worn when working with hazardous animals or materials.

All forms of **hazardous communication** must be employed for animal cages with biohazards, hazardous chemicals, or radioactivity – notify the vivarium supervisor prior to experiment; individually label each cage with hazard sticker; post appropriate door signs. Remove hazardous communication in the vivarium when hazard no longer exists.

Follow appropriate **disposal guidelines** for hazardous **materials and carcasses**
- Chemical - [http://ehs.virginia.edu/ehs/ehs.chemicalsafety.cwc.html](http://ehs.virginia.edu/ehs/ehs.chemicalsafety.cwc.html)
- Radioactive - [http://ehs.virginia.edu/ehs/ehs.rs/rs.waste.html](http://ehs.virginia.edu/ehs/ehs.rs/rs.waste.html)

Biosafety cabinets and fume hoods should be **certified** annually or if relocated/repaired.

Precision anesthetic gas vaporizers (or gas analyzers) must be validated at least **once every three years** or anytime a vaporizer is placed back into service after a year of no use. Maintenance record (sticker) should document validation.

Anesthetic gases (e.g. isoflurane, methoxyflurane) must be properly **scavenged** or used in a fume hood.

Activated charcoal canisters (F/AIR, VAPORGUARD) must be **weighed regularly** and within specs (dispose after 50 grams). **Charcoal canister vents cannot be blocked** (F/AIR canisters must be hung in order to keep vents uncovered on the bottom of the canister). Soda-sorb should not have color changes.

Gas cylinders must be adequately **secured**.

Disinfectants must be labeled and maintained in accordance with manufacture instructions to maintain potency.

Sharps boxes must be replaced when **3/4th full**. Contact EHS for pickup (2-4911).

Eyewash station testing/flushing should be performed regularly and documented on eyewash station tag.

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**ANIMAL WELFARE**

Animal handlers should know how to contact veterinary staff during regular business hours, after hours, weekends, and holidays [https://www.virginia.edu/vpr/ccm/staff.html](https://www.virginia.edu/vpr/ccm/staff.html).

Animal handlers **must know the clinical signs of pain** for the species to which they are working.

**Analgesia (or euthanasia) must be promptly provided** to all animals demonstrating pain or suffering, unless specifically exempted as a Pain Category E experiment.

Sick or injured animal(s) with weight loss of 20% or more must be noted on the **vivarium health report** or euthanized.

Animal handlers **must know the humane endpoints (criteria for euthanasia)** approved in the protocol and in accordance to ACUC policy and euthanize animals appropriately.

**Clinical monitoring must be documented** for Pain Category E experiments. Frequency of observations and assessment criteria must be followed in accordance with protocol.

Experimental endpoints, approved in the protocol, **must** be followed.

Animal handlers must follow the **approved methods of euthanasia** included in the protocol.

**Death must be confirmed** in all euthanized animals before disposal (i.e. by cervical dislocation, removal of vital organ, bilateral thoracotomy, exsanguination, decapitation).

Cervical dislocation or decapitation **without anesthesia must be approved** in protocol except for neonatal mice less than 5 days of age.

Cervical dislocation is **not** permitted in rodents larger than 200gm, even to confirm death.

**Guillotines**, when used on live animals (even anesthetized ones), are required to be sharpened annually **OR** after 250 animals. Annual sharpening must be documented with a “Guillotine Maintenance Record” **sticker or stamped** (month/year) on the blade. When sharpening is based on animal use (after 250 animals), then a **usage log** must be kept designating species and animal use numbers.

Guillotine weight limit for rats is 400gm. Decapitation using scissors or sharp blades is only acceptable for altricial neonates <5 days of age.
Carbon dioxide (CO₂) can only be used to euthanize small rodents (mice, rats, hamsters, gerbils, guinea pigs, chipmunks, and squirrels), insectivores, and birds. Flow must be controlled by a flow meter at 10-30% displacement. Maximum number of animals euthanized in a mouse box is limited to ≤10 adult mice or ≤15 pre-weanling mice per mouse cage. No more than 2 rats (200-450g) or one rat >450g per rat cage.

DRUGS

Only the specific drugs, methods, and materials approved in the protocol may be used on animals, unless written documentation is provided from a veterinarian permitting and justifying their immediate use.

Pharmaceutical grade drugs must be used if commercially available. Non-pharmaceutical grade drugs must be scientifically justified and approved in protocol.

Rodents should not come in physical contact with inhalational anesthetic solutions.

Controlled drugs must be securely stored in a locked drawer, cabinet, or safe that is not easily movable. (A locked cash box outside of a locked drawer or file cabinet can be easily removed from the lab and is not considered “secured” by DEA standards. Cash boxes must be locked inside of locked drawer/file cabinet). Block visibility through glass panels to obscure view or relocate controlled drugs.

An up-to-date controlled drug record/log of inventory, use, and balance of each DEA controlled substance is required.

Anesthetic, analgesic, and euthanasia drugs must be used before expiration date for all procedures.

Compounding/diluting anesthetic or analgesic drugs must be performed in a manner that maintains sterility and potency. Vial must contain name of drugs in vial, mix/diluting date, and expiration date (based on component due to expire earliest). Inventory, use, and balance of controlled substances must be maintained.

Tribromoethanol (Avertin), as a non-pharmaceutical grade anesthetic, must be scientifically justified and approved in protocol. Solution must be protected from light, refrigerated, and expires two weeks after dilution.

Controlled drug logs from emptied vials or expired vials must be returned to CCM for reconciliation.

Expired controlled drugs should be properly discarded by returning vials to CCM. Empty drug vials should be disposed of as glass waste.

All secure storage locations of DEA controlled drugs (stock and working) must be included in the protocol.

VIVARIUM

All animal handlers requiring access to the animal facility must complete the CCM – Animal Facility Rules and Procedures (barrier) Training with appropriate vivarium supervisor. Access will not be granted until training has been completed.

Barrier procedures must be adhered to by everyone entering a barrier facility/room, including wearing of protective clothing, disinfection of equipment, and proper handling of animals/cages.

Species-specific physical and psychological environmental enrichment will be provided for all animals. Exemptions for providing environmental enrichment must be scientifically justified in the protocol.

Housing social animals in pairs or groups is the standard method of housing. Single housing of any animal must be based on social incompatibility, veterinary-related concerns, scientific justification (approved in protocol), or end of study animals/singly housed breeders.

Animal researchers are responsible for labeling the cage card of singly housed rodents to signify the cause for separation: fighting/social incompatibility (I), veterinary care reason (V), or for scientific justification (S).

Cage/tank cards must be present on all cages and contain the following information: PI, protocol number, source of animal, strain/stock, DOB or DOA (acquisition), CCM billing code, breeding information. Other useful information: sex, age, number in cage. CCM must be contacted for additional cage card labels when warranted.

Newly received animals can not be experimentally manipulated during the acclimation period. Rodents/fish/frog – 2 days; large animals – 3 days.

Non-standard husbandry/care must be approved in the protocol. Non-standard housing and care may include: alternate light cycle, alternate temperature/humidity, alternate bedding or caging type, alternate cage change schedule, and cage changes performed by research personnel. Cages or entire room must be clearly marked.

Non-standard provision of food and/or water must be approved in protocol. Special provisions may include a special diet or medicated/treated water. Clearly label any special food/water provision on cage with specialized quarter cards indicating type, start date, and end date.

Animals with restricted or scheduled access to food and/or water must be approved in the protocol. Daily written

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**BREEDING AND WEANING**

Unauthorized breeding of animals is not permitted. All breeding must be approved in the protocol.

Date of Birth (DOB) and projected Date of Weaning (DOW) must be listed on the cage card for all litters.

Approved housing densities must be adhered to according to animal age and weight. **Overcrowding is not permitted.**

Preferably only **one** litter per cage. This reduces the chance of overcrowding. Two females with two litters, **less than a week apart in age**, may be housed together provided that when the oldest litter reaches **12 days of age, there are no more than 12 pups in the cage (combined)**. One litter must be removed with the corresponding female as needed. A single female having >12 pups is acceptable if the female is housed in a separate cage (with or without the male).

No more than **one litter at a time per female** per cage with a maximum of two females in the cage. The previous litter must be weaned prior to delivery of the next litter for a single female.

Mouse and rat litters must be **weaned by Day 23** after birth or by Day 21 if a new litter is imminent.

One time exceptions to the weaning policy can be granted by the vivarium supervisor and must be documented on exception log. **Ongoing exceptions must be approved** in the protocol.

CCM husbandry staff will perform the following functions if the laboratory fails to do so and a punitive technical charge will be applied: will wean animals by Day 25 if the laboratory fails to wean; will separate females and litters if two litters with greater than 7 days between DOB; will remove pregnant female if three females are present in single cage; and will separate females with litters if at 12 days of age there are greater than 12 pups in the cage. OAW/ACUC will be notified of repeated violations of overcrowding.

Research personnel **must report animal production information** into the CCM database monthly. Animals should be counted at weaning. Rodents used prior to weaning are reported at time of genotyping. Amphibians are counted after metamorphosis.

**NON-SURGICAL TECHNICAL PROCEDURES (all methods must be described in protocol)**

**Genotyping** – Mice should not be older than **28 days and no more than 5mm of tail should be biopsied**. Mice older than 28 days must receive analgesia either topically or systemically. When >5mm is required or when a single mouse is biopsied more than once, local or general anesthesia is required. Disinfect the tail with 70% ethanol prior to biopsy and monitor for hemostasis after biopsy.

**Toe-clipping** – **Must be scientifically justified** in protocol. Mouse neonates must be no more than 10 days old. For rodents, lizards, and amphibians, a **maximum of one digit per foot is permitted, and local anesthesia is required**.
**Footpad Injections** – Pilot study required prior to antibody production experiments. Freund’s Complete Adjuvant (FCA) is only allowed if alternative adjuvants do not result in satisfactory results. *Only one hind footpad is permitted for use.* Animals should be monitored for pain/distress daily for a minimum of one week. Rodents found in pain/distress (swelling of injected foot, non-weight bearing on foot, limping, self-mutilation of foot, etc.) must be placed on soft bedding; administered analgesic; document analgesic administration on Protocol Treatment Card; and monitored daily until animal can bear weight on injected foot. If lesions develop, animal must be monitored daily until lesion has healed. Lesions must be treated with oral or topical antibiotic.

**Prolonged Restraint** – Procedures where animals are placed into a restraint device for longer than 5 minutes must be described fully in protocol.

Testing equipment, euthanasia chambers, anesthesia chambers, and guillotine/scissors should be cleaned of blood, fur, and excrements after every use.

**TUMOR STUDIES**

<table>
<thead>
<tr>
<th>Tumor diameter must never exceed 20mm (mice - approx. 25g) or 40 mm (rat – approx. 250g) regardless of BCS for single tumors. Calibration curves depicting typical tumor volume increases over time for a specific model are recommended. Volumetric calculations from 2 or 3 dimensions (W, L, and H) provide more stringent data.</th>
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<tbody>
<tr>
<td>In a mouse with two tumors, neither tumor may measure &gt;10mm or have BCS&lt;2. For rats with two tumors, neither tumor may measure &gt;20mm or have a BCS &lt;2 or as otherwise approved in protocol.</td>
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<tr>
<td>In rodents, baseline weights must be recorded and weight must be measured weekly.</td>
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<tr>
<td>Tumor measurements and animal condition assessments (body condition score – BCS) must be performed at least 1X/week after initial injection, 2X/week once tumor growth occurs, then daily when tumor reaches 10mm for mice or 20mm for rats. Written records/documentation must be kept.</td>
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<tr>
<td>Body condition score (BCS) must be calculated for all tumor studies. BSC&lt;2 requires euthanasia.</td>
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<tr>
<td>Body Condition Score (BCS) based on mobility restrictions, inability to access food/water, pressure on internal organs (ascites production), or sensitive regions of the body. Animals exhibiting these types of signs must be euthanized even if the maximum tumor size has not been reached.</td>
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<tr>
<td>Visible or palpable tumors must be evaluated using calipers.</td>
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<tr>
<td>Tumor ulceration ≤1mm must be monitored daily. Rodents with ulcers &gt;1mm must be euthanized.</td>
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<tr>
<td>Bone, brain, lung or other internal solid tumors and metastasis (occult tumors) - the BCS and clinical evaluation of the animal take priority over the measured size of the tumor. Expected clinical signs and humane endpoints of those signs must include a BCS and be clearly described in protocol. Imaging may be performed to measure width, but must be performed at the same frequency as single or multiple tumor requirements.</td>
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<tr>
<td>Tumor records must include: tumor type and number of cells given, date of inoculation, animal number, site of administration, tumor size/volume measurement, body weight, and animal condition (BSC).</td>
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<tr>
<td>Rodents must be euthanized if the following occur: tumor ulceration (&gt;1mm), inability to access food/water, severe dehydration, anemic and lethargic, hunched, 20% weight loss, or moribund.</td>
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<tr>
<td>Humane endpoints must be established to minimize pain and/or distress, or scientific justification provided to do otherwise (Category E). Protocol must include a detailed plan for monitoring animals and defined criteria for euthanasia.</td>
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<td>Death cannot be used as an endpoint.</td>
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**NON-SURVIVAL SURGERY/PROCEDURES**

<table>
<thead>
<tr>
<th>Area should be clean for surgeries – no dirt, blood, fur, used syringes, etc.</th>
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<tr>
<td>Surgical area should be free of unnecessary equipment and supplies.</td>
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<tr>
<td>Surgeon must wear gloves and protective clothing during any animal procedure or surgery.</td>
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<tr>
<td>Surgical instruments and equipment should be cleaned before use and between animals.</td>
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<tr>
<td>External heat source should be applied to maintain the animal’s body temperature during surgery if the procedure lasts more than 1 hour.</td>
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<tr>
<td>Drugs, suture, and surgical supplies should be used before their expiration dates, unless used for terminal experiments, where they should be clearly labeled “for acute use only.” Anesthetic, analgesic, and euthanasia drugs must be used before expiration date for all procedures.</td>
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</tbody>
</table>
Surgeon must **monitor** the depth of anesthesia regularly and adjust as needed to maintain appropriate depth of anesthesia.

Anesthetized animals must **never be left unattended**.

Method of **euthanasia** used must be in protocol.

**RODENT SURVIVAL SURGERY**

- There must be a dedicated area for survival surgery in the laboratory or vivarium.
- The surgery area must be clean (no blood, fur, used syringes, etc.), sanitizable, and free of unnecessary equipment and supplies.
- Surgeon must be properly trained in aseptic technique, surgical skills, and particular procedure being performed.
- Surgeon must always wear sterile surgical gloves, mask, head cover, and protective clothing (over street clothes).
- Surgical instruments must be initially sterilized (autoclave or cold sterilants).
- Sterilized instruments must be used within six months of sterilization date (date packs).
- Surgical instruments need to be cleaned of organic material and placed in a hot bead sterilizer or soaked in 70% alcohol between rodent surgeries performed consecutively on the same day.
- Surgical instrument packs must be replaced with a newly sterilized pack or re-sterilize initial pack after five consecutive animal surgeries performed on the same day.
- Surgical site must be sufficiently clipped, plucked, or depilated (for sensitive skin only) to remove hair around surgical site. Animal hair should not be present within the wound closure.
- Surgical site must be prepped by scrubbing with 3 alternating rounds of antiseptic scrub and 70% alcohol. Final paint of antiseptic solution is optional. Antiseptic and alcohol solutions must be used before expiration date. Do not over-wet the animal with scrubbing solutions.
- Draping rodent surgical sites is recommended.
- Drugs, fluids, suture, and surgical supplies must be within their expiration dates.
- Depth of anesthesia must be monitored regularly and adjusted as needed to maintain appropriate depth of anesthesia.
- An external heat source should be applied to maintain animal’s body temperature during surgery and recovery.
- **Ophthalmic ointment** should be applied.
- Each tissue layer should be closed individually.
- The analgesic dose listed in the protocol must be administered either pre-op, intra-operatively, or immediately post-op, and repeated as listed in the protocol (or as recommended by veterinarian).
- Animals must be observed or monitored regularly until recovered from anesthesia (conscious and at least sternal recumbent). Anesthetized animals must never be left unattended.
- Surgery cage cards must be completely filled out and placed on cages of all post-op animals.
- Animals must be checked at least once daily until the incision is completely healed (at least three days post-op).
- Post-operative health problems must be reported to the veterinary staff.
- Non-absorbable sutures or wound clips must be removed within 10-14 days following surgery (unless justified in protocol).
- The method of euthanasia used must match the method listed in the protocol.
- **Written records** must be maintained of anesthetic administration, surgery performed, post-op care, drugs administered, and any complications. Records must be kept for a minimum of one year after the disposition of the animal. Surgery cage cards may be kept to fulfill this requirement or a separate log may be kept if surgery cage cards are discarded.

**LARGE ANIMAL SURGERY/PROCEDURES/GENERAL ANESTHESIA**

*includes all USDA Regulated Non-Rodent Species*

All items listed for rodent survival surgery above apply to larger species, and additional items listed below. Surgery cage cards are not used and new surgical instrument packs should be prepared for each large animal surgery.

- Mandatory **pre-planning meeting** required for – new species, new technique/surgery, or new protocol.
- Survival surgery must be performed in a dedicated surgical suite with separate anesthetic induction/prep, surgical...
**TEMPORARY (SATELLITE) HOUSING FACILITIES**

ACUC sub-committee must inspect proposed temporary/satellite space prior to approval in protocol.

Any area in which rats, mice, fish, or amphibians are maintained for 24 or more hours outside of the vivarium must be listed as a temporary/satellite housing location on an approved protocol.

Any area in which USDA regulated species (hamsters, gerbils, guinea pigs, rabbits) are maintained for 12 or more hours outside of the vivarium must be listed as a temporary/satellite housing location on an approved protocol.

Animals must be observed and monitored daily. This includes weekends and holidays.

Appropriate light cycles must be provided.

Daily observations must be recorded on a husbandry log and available for inspection. Log must include: daily temperature and humidity; daily health observations; feeding and watering; and cage changing. Animal observation/husbandry record must be sent to CCM weekly. Records must be maintained for one year.

Food must be stored in a container with a tight fitting lid (e.g. Tupperware) and labeled with the food type and milling date or expiration date.

Sick animals or animals experiencing unanticipated pain/distress must be reported to the veterinary staff or euthanized in accordance with approved methods in protocol. Sick animals should be reported on daily observation log.
Routine **cage cleaning** and changing must be performed and documented. Rodents – change bottoms at least twice per week (single housed – once a week); water bottles change at least once per week; wire lids and filter tops change every two weeks. Clean cage components must be obtained from CCM. Soiled components must be returned promptly to CCM for processing. Do not dump cage bedding in lab. Rodents receiving non-standard care (standard listed above) or any non-rodent species must have an **approved SOP** for husbandry practices.

The room, or secondary enclosure, containing the animals and the surfaces harboring the cages must be wiped down as needed and **sanitized** with disinfectant solution at least every two weeks, and documented on logs. Sanitation should be monitored in some manner for efficacy (RODAC plate testing).

Environmental enrichment must be provided to animals while in temporary/satellite housing locations.

**PI-MANAGED PERMANENT ANIMAL HOUSING FACILITIES**

*All items listed for temporary/satellite housing facilities above apply and additional items listed below*

- Area is scientifically justified and approved in protocol. Maybe within confines of vivarium or outside of the vivarium; however, PI/laboratory provides all husbandry and maintenance of the space. Animals are never returned to a vivarium.
- Husbandry SOPs, Disaster Plan, and Environmental Enrichment Plan are approved by the ACUC initially and every three years. Recently approved versions should be available in the space for reference for animal handlers and inspectors/visitors.
- All animals **must** be maintained in accordance with Guide, Animal Welfare Act, UVA PHS Assurance, ACUC policies/procedures, and PI SOPs.
- Daily **logs** of animal health observations, husbandry, and mortality must be maintained similar to temporary/satellite housing requirements above.
- Proper **quarantine** procedures must be used for sick animals. Records of treatment should be available.
- Report animal use to OAW semi-annually for reporting purposes.

**AQUATIC SPECIES** *(additional requirements)*

- Proper **water quality** must be maintained and recorded. Un-ionized ammonia must be calculated.
- Chlorine, chloramines, chemical and reactive bi-products must be removed or neutralized prior to use in aquatic system.
- **Bio-filter** must be of sufficient size to process bio-load.
- GFI electrical outlets must be installed and potential electrical hazards must be eliminated (no extension cords on wet floors or over open tanks of water).
- **Nets** must be cleaned, disinfected and managed to avoid contamination of system.
- **Aseptic technique** must be observed for all survival surgeries, including proper attire, equipment sterilization, and animal prep.
- Proper anesthesia, routine analgesic administration, animal recovery, post-op monitoring, and euthanasia procedures must be followed with animals undergoing survival surgery.
- No more than 6 oophorectomies are permitted on any single frog - with sufficient recovery time between surgeries. Individual animal identification and surgical records must be available for inspection.

**Resources:**

- PHS Policy on Humane Care and Use of Laboratory Animals - [http://grants.nih.gov/grants/daw references/phspol.htm](http://grants.nih.gov/grants/daw references/phspol.htm)