Institutional Animal Care and Use Committee

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National Park Service
Natural Resource Stewardship and Science
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Standard Operating Procedures for Small Mammal Field Studies

The purpose of this Standard Operating Procedure (SOP) is to describe the National Park Service Institutional Animal Care and Use Committee (NPS IACUC) approved methods for routine procedures used in field research involving small mammals. This SOP covers the capture, handling, marking, sampling, and collection of small mammals. It is not comprehensive, and methods not described herein may be approved by the NPS IACUC upon further review and justification. This document will be reviewed every three years and updated as needed to reflect current procedures and policy. Last update: August 2025

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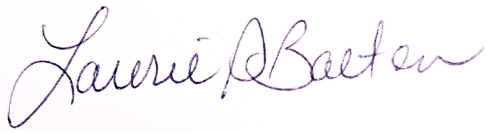
IACUC Chair and Attending Veterinarian Signature:



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I: Introduction

The objective of this SOP is to provide a suite of NPS IACUC approved methods for use in small mammal field research activities. It adheres to the guidelines approved by the American Society of Mammologists for the use of mammals in research (Sikes et al. 2016). It may be referenced in individual project plans and NPS IACUC project submission forms to help speed up the review process for those submissions. It does not preclude the use of other methods that are not described in this SOP. However, methods not covered by this SOP will require additional NPS IACUC review and approval.

II: Definitions

Job Hazard Analysis: A Job Hazard Analysis (JHA) is a multi-step process designed to study and analyze a job or order to identify hazards and eliminate or minimize their risks.

Non-target species: Any non-study animals directly or indirectly affected by the research project. Examples include unplanned live capture (e.g., incidental trapping of non-study species), loss of non-target individuals (e.g., death of offspring from taking of one or both parents or parental orphaning after release from capture), or the disturbance/harassment of other species during the research activity (e.g., environmental disturbance during the use of airplanes, drones or airboats, alteration of breeding activity/behavior during nest site visits, etc.).

Opportunistic species: A sub-category of non-target animals whose capture is unplanned, but which can lead to the collection of valuable information. Examples include non-target species of small mammals which, if captured, may be marked, and released.

Small mammal: Any mammal that is non-volant, forages predominantly aboveground, and weighs between 5g and 5 kg. Small mammals found in North America include cricetids, heteromyids, small sciurids, and introduced murids (Paull et al. 2023). Bats, prairie dogs, mustelids, and lagomorphs are excluded from this document.

Zoonoses: Infectious diseases that can be transferred between domestic or wild animals and humans.

III: Human Health Precautions/Job Hazard Analysis

All field personnel should maintain up-to-date tetanus immunizations and practice common sense personal hygiene during small mammal studies, including handwashing and prevention of contamination of food. A Job Hazard Analysis (JHA) should be conducted before work commences and should consider the following potential hazards, personal protective equipment, training, and personnel considerations (NPS 4.15).

A. Potential Hazards

1. Zoonotic Diseases

All persons handling small mammals must be aware of zoonotic diseases, most notably Hantavirus and plague, in their specific study area(s). The United States Geological Survey's (USGS) Safe Practices for Working with Wildlife chapter provides guidance for personnel working in the field (Taylor and Buttke 2020). NPS also has Safe Practices to Avoid Zoonotic Disease from Wildlife for additional guidance (NPS 2024). For the most recent zoonotic disease information, the NPS IACUC attending veterinarian is available for consultation.

a. Hantavirus

Although small mammals and their ectoparasites are critical to the lifecycle of many disease-causing organisms, there is no evidence that the handling of small mammals significantly increases risk of exposure to many of the diseases beyond other types of field work (Paull et al. 2023). One possible exception to this is Hantavirus Pulmonary Syndrome (HPS), which is believed to be transmitted via bites or the inhalation of aerosolized urine, feces, or tissues contaminated with the virus. It is important to note that most known cases of HPS resulted from inhalation of aerosolized virus present in cabins and other buildings in which small mammals were nesting (Kelt et al. 2007, 2010).

There are multiple genera in the Hantavirus family that circulate in North America, and most of the North American cricetid rodents appear to be competent reservoirs (Pauell et al. 2020). Peromyscus maniculatus, a member of the cricetid family, is the primary reservoir for the Sin Nombre virus, the hantavirus most often linked to HPS. Despite the distribution of P. maniculatus including much of North America, the Centers for Disease Control and Prevention (CDC) reports that HPS cases are more frequently diagnosed in western states, including California, Arizona, New Mexico, and Colorado (Pauell et al. 2020). The American Society of Mammologists has published a set of practical guidelines modified from CDC guidelines that incorporate new information about HPS risk and better match the level of personal protection to the level of risk associated with common field activities (Kelt et al. 2010). These guidelines should be reviewed prior to conducting field research on small mammals.

b. Plague

Plague is a bacterial disease caused by infection with *Yersinia pestis*. It causes disease in mammals, including humans. Plague is typically transmitted through the bite of an infected flea, but it can also be transmitted following direct contact with an infected live animal or carcass. The bacteria normally circulate between fleas and rodents (e.g., squirrels, chipmunks, woodrats) but occasionally other susceptible mammals become infected. In humans, infection with plague results in serious and life-threatening disease with high risk of mortality in untreated or

unrecognized cases. Human cases are seen most commonly in western states including Arizona, California, Colorado, and New Mexico. The use of insect repellent with 20 – 30% DEET, other EPA-approved repellents, permethrin-treated clothing, and proper PPE when handling animal carcasses reduces the risk of plague transmission.

c. ***SARS-CoV-2***

The risk of SARS-CoV-2 spillback from humans to wildlife or spillover from wildlife to humans is unknown at this time but precautions are prudent. The NPS IACUC encourages researchers to review CDC Recommendations to reduce the risk of SARS-CoV-2 spreading between people and wildlife and to incorporate biosecurity controls as appropriate into their field activities (NPS 2020).

2. **Injury from Animals and Equipment**

Persons handling small mammals may be bitten or scratched. This risk may be minimized by wearing proper PPE. If personnel are injured by animals or equipment during handling, apply first aid in the field (e.g., wound flushing, infection prevention measures). Inform the injured person's supervisor of the injury. Monitor the injury and determine if medical treatment from a health care provider is necessary.

3. **Exposure to Isoflurane**

Isoflurane, an anesthetic gas, is often used for anesthesia and/or euthanasia of small mammal species. Personnel using isoflurane should review the manufacturer's materials safety data sheet (MSDS) for this chemical. Isoflurane may cause irritation to the skin, drowsiness, and/or headaches. It is not safe for persons who are pregnant or could possibly become pregnant. If exposed to isoflurane, the following measures should be followed (Cayman Chemical Safety Data Sheet 2022):

a. ***Inhalation***

Move the exposed individual to fresh air. If the exposed individual is not breathing, administer artificial respiration or provide oxygen (trained personnel only). Seek immediate medical attention.

b. ***Skin Contact***

Immediately wash skin with soap and plenty of water for at least 15 minutes. Remove contaminated clothing. Seek medical attention if symptoms occur. Wash clothing before reuse.

c. ***Eye Contact***

Hold eyelids apart and flush eyes with plenty of clean water or eye wash solution for at least 15 minutes. Seek medical attention as soon as possible.

d. ***Ingestion***

Wash out mouth with clean water, provided the person is conscious. Never give anything by mouth to an unconscious person. Seek immediate medical attention. Do NOT induce vomiting unless directed to do so by medical personnel.

B. Personal Protective Equipment

Personal protective equipment (PPE) should be worn to prevent injury and infection from animals or tools used to capture animals. Non-latex disposable exam gloves are required for all handling of small mammals and traps. Leather gloves may be worn under disposable gloves to protect from bites or injury from equipment. The NPS IACUC requires that researchers wear a face mask (preferably a fit-tested N95 respirator but a surgical mask is also acceptable) when working within 6 feet of any mammal.

The use of N95 respirators requires medical evaluation, training, and fit testing prior to use in order to comply with Occupational Safety and Health Administration (OSHA) regulations. For additional information, refer to the 3M Particulate Respirator N95 User Instructions (3M 2019). PPE should also be used when cleaning and disinfecting traps and other field equipment to minimize contact with potentially infectious material.

C. Training and Personnel

1. Human Health

All personnel handling animals during the study period should receive training on the risks of potential zoonoses, including signs, symptoms, and prevention techniques. Personnel should be aware of zoonotic diseases in the specific project area. It is advisable for all personnel to have basic first aid skills and training.

2. Sample Collection

All personnel directly involved in field activities should be trained in all appropriate sampling and handling techniques that they will be assisting with, including trap placement, baiting, operation, appropriate restraint, monitoring, and marking (as needed). Sample collection should be performed only by individuals trained on the specific small mammal sample collection technique they will be performing (e.g., blood, oral/buccal swab, hair collection) or those who have received classroom training and performed the procedure(s) under the supervision of someone experienced in these procedures.

3. Environmental Factors

Personnel should be aware of and prepared for environmental conditions associated with working with small mammals (e.g., weather, terrain, noxious vegetation).

IV: Methods of Remote Observation

A. Remote Cameras

Motion, heat-triggered, or timed interval trail cameras (hereafter called camera traps) can be used to study small mammal species presence or absence, diversity, community composition, activity patterns, habitat selection, and behavior with minimal disturbance to the landscape and without handling animals (McCleery et al. 2014). Cameras do not need to be checked or monitored on a regular basis and can detect animals in a manner that greatly reduces stress and harm to both animals and researchers (McCleery et al. 2014). Wearn and Glover-Kapfer (2017) provides best practices for use of cameras for monitoring. Personnel should consult this or similar guides when considering using cameras for monitoring. The NPS Field Study Protocol should be consulted to determine if activities involving remote cameras qualify as a field study or a general submission.

Minimal disturbance to target and non-target animals should occur from camera traps or associated installation. Cameras must be placed securely to ensure they will not fall on or injure an animal due to interference from animals, gravity, or weather events. Cameras should be set or modified so that the camera flash and sensor lights will not cause harm or disturbance to light-sensitive species and will not potentially disrupt the vision of any animal. Use of the white flash on camera traps requires justification to be provided to the IACUC. Depending on the purpose of the project, bait or scent lures may be used to attract animals to camera traps. However, bait should be placed in a manner that would not attract unwanted, non-target species (e.g., predators, non-study species) to the area.

B. Track Tubes

Track tubes are used to identify small mammals by their tracks. Animals are lured to the tube via bait and an ink pad is placed at the front of the tube. Animals that are small enough to fit in the tube must walk over the ink pad and onto cardstock paper when traversing the tube, thereby leaving their footprints. Results of continuous, seasonal track tube monitoring can be used to assess annual emergence and torpor, season activity associated with reproduction, recruitment, and localized colonization and extinction events (Brehme et al. 2019). Minimal disturbance to target and non-target animals should occur from use of track tubes.

C. Unmanned Aircraft Systems/Drones

Unmanned aircraft systems (UAS) are considered "aircraft operated without the possibility of direct human intervention from within or on the aircraft" by the Federal Aviation Administration (FAA) (14 CFR §1.1). UAS are also known as remotely piloted aerial vehicles (RPAV), drones, unmanned aircraft (UA), and uncommanded aerial vehicles (UCAVs).

The NPS is increasingly using UAS for research, monitoring, and visitor and resource protection. In general, launching, landing, or operating unmanned aircraft in NPS units is prohibited. However, there are exceptions where use is allowed, subject to NPS approval (i.e., UAS may be used for scientific study after approval by the NPS Regional Director [DOI 2015]). A researcher is advised to contact a park or regional aviation manager for more information and guidance.

Of concern to the IACUC is the disturbance or harassment of wildlife of both target and non-target species by drone activities. In a particular, drones can disrupt avifauna during the breeding season. Precautions should be taken to stay away from any known nests, particularly that of raptors, and an observer should always be present watching for birds during a flight. If a drone comes within 75 feet (about two school bus lengths) of birds, it should take evasive action even if that means returning to base. Drones should also maintain 75 feet from any medium to large sized mammals and 25 feet from smaller animals. When monitoring small mammals, operators should be aware of where their shadow will fall and try to avoid shadowing (i.e., having the shadow fall across) wildlife where possible. A drone that alters the behavior of wildlife is considered harassment and it is the responsibility of the operator to remove the drone to a further distance.

V: Methods of Capture

Methods and procedures for capturing animals should follow Guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2016.) These guidelines combine information from multiple sources, including the Animal Welfare Act (AWA), American Veterinary Medical Association (AVMA), National Research Council (NRC), and other governments and professional organizations.

A. **Live Traps**

When performing live capture, utilization of methods that are assessed to be humane for both target and non-target species is essential. Lethal take is highly discouraged but, if required, requires extensive justification, and is considered on a case-by-case basis. If the end goal of trapping is death, kill traps or other lethal methods that provide swift, humane kill should be considered over live traps *unless* fresh tissues are required to meet study objectives (American Veterinary Medical Association 2020).

The type and number of traps being utilized should be based on the number of persons available to check them in a timely manner, the environmental conditions of the study site, the anticipated weather, and the species of mammal that may be trapped (Animal Care and Use Committee 1998). In addition, trap type should be chosen based on factors such as relative size, efficacy of a trap to capture the species of interest and mortality rates associated with a trap (Stephens and Anderson 2014). All traps should be individually inspected and tested for functionality prior to deployment. There are a variety of effective techniques for trap distribution within sampling sites, including distributing traps along transects, grids, or webs. Varying placement of individual traps within these constructs to account for terrain constraints and efforts to place traps in a sheltered location may be beneficial.

Additional precautions should be taken to protect the captured animal from injury, stress, predation, and exposure to harsh environmental conditions. The number of traps set at a particular time and location should not exceed the ability of the researchers to monitor them at reasonable intervals (Sikes 2016). Traps should be set in a manner that provides protection from the elements (e.g., using natural features or covering traps with branches, vegetation, bedding

material, or canvas/waterproof covers [e.g. Torre et al 2023]) and predators that may tamper with the traps. Strategies to reduce predator interference will vary by predator species and should be targeted toward the species suspected to be tampering with traps. When placing traps next to animal runways (e.g., rocks, downed logs), set the trap parallel to the runway. Be aware of drainage issues that may cause flooding of the localized area. Foot traffic within the trapping site should be minimized and restricted to the perimeter of the site whenever possible.

1. **Trap Types**

   a. ***Box Traps***

   Box traps, oftentimes referred to as Sherman traps, consist of a single, enclosed box (primarily metal) and a trap door that closes when an animal enters the trap and steps on the treadle (Jung 2016). These traps are available in multiple sizes and styles to cater to the target species. Styles include folding and non-folding models. Foldable models typically facilitate transport and storage. Tunnel and nest box models have been found to be effective for live capture of shrews (Stromgren and Sullivan 2013). Traps with a counterweighted door allow for capture of multiple animals in one trap (Jung 2016). In addition to providing food, hydrating material or foodstuffs, and nesting material, traps can be insulated to guard against hypo- and hyperthermia (Sikes 2016). To prevent accidental capture of limbs or tails, ensure the complete closure of doors after placing materials into the trap.

   b. ***Cage Traps***

   Cage traps consist of a single, wire mesh cage and either one or two doors. They come in a variety of sizes and mesh sizes which allow for ventilation, rendering trapped animals less susceptible to heat stress (Petit and Waudby 2013). The mesh of the traps should be small enough to prevent animals from climbing partway through and becoming caught, either while inside the trap and trying to escape or when outside the trap and reaching in to access the bait. They may be wired to logs and along tree trunks/limbs where possible to target tree-dwelling squirrel species. Use of cage coverings is recommended to reduce stress and exposure to the elements (Mantor et al. 2014). As with box traps, care should be taken to ensure the complete closure of doors after placing bait and/or nesting material into the trap to prevent accidental entrapment and possible injury to limb(s) or tail.

   c. ***Pitfall Traps***

   Pitfall traps consist of a container with smooth, vertical walls that is placed in the ground, designed such that the animal falls into the trap. Pitfall traps should be checked at least once per day to avoid opportunistic predation and multiple captures, which can be stressful for certain species. Depending upon the anticipated non-target species and anticipated environmental conditions, traps may need to be checked more frequently than once per day. For live trapping, appropriate monitoring and care must be taken to avoid injury or death of animals. To keep water from accumulating at the bottom of traps and causing animals to

drown, consider the use of small holes to drain water or small sections of polyvinyl chloride pipe to allow animals to escape. Raised covers over traps (Figure 1) can minimize capture of non-target species, as well as provide cover from the elements and reduce risk of predation.

Photo credit: https://www.sciencefriday.com/educational-resources/protected-pitfall-traps/

Photo credit: https://mississippientomologicalmuseum.org.msstate.edu/collecting.preparation.methods/Pitfalls.htm

Figure 1. Examples of pitfall trap coverings.

d. ***Glue Traps***

Glue traps are not an approved method for small mammal trapping. Glue traps cause unacceptable pain and distress to both target and non-target species. Glue traps are prohibited for sale and use in New Zealand, Australia, and England (Animal Welfare (Glueboard Traps) Order 2009, Prevention of Cruelty to Animals Regulations 2019, Glue Traps (Offences) Act 2022), and bans have been proposed in some areas of the United States (City of West Hollywood Municipal Code, 2023).

2. **Timing of trapping**

The scheduling of trapping efforts should consider the status of the target species, the behavior of the species, and the risk of separating mothers from dependent offspring.

3. **Baiting**

Bait is used to attract target animals into traps as well as to provide for metabolic needs for the duration of the time they are trapped (Petit and Waudby 2013). Traps may be pre-baited and left open prior to setting the traps to increase trap success rates (Rodas et al. 2009). Traps should be baited with foods appropriate for the species of interest, such as sterilized seed mixes, oats, jam, or peanut butter. When animals with higher metabolic rates may be captured as either target or non-target species, additional bait sources such as mealworms or blowfly pupae should be added to traps to reduce mortality events (Stomgren and Sullivan 2013). Efforts should be made to avoid scattering bait on the ground. Extra bait

should be added to traps on nights when temperatures are anticipated to be <7°C (45°F) (Paull et al. 2023).

4. **Trap Monitoring**

It is essential that the exact location and the number of traps deployed in an area be accurately recorded to ensure that no traps are missed or skipped during trap checks. Traps can be visually marked or flagged to ensure prompt and accurate trap checking if neither humans nor predators will be drawn to the flagging. If visual marking is not permitted, GPS mapping can accomplish the same objective.

Traps should be checked frequently. The interval between trap checks will depend upon the type of trap used, the species, metabolism, and activity of the mammals to be trapped, configuration of the traps, climate, and season (Animal Care and Use Committee 1998). Thermoregulatory demands on small mammals can induce stress, even if trapped for short periods of time (Sikes et al. 2016), contributing to the need for a more frequent trap check interval. Metabolic demands may further necessitate more frequent trap checks, most notably for members of the Order Insectivora. Traps set for shrews should be checked every 1.5 - 2 hours to minimize mortality (Hawes 1977, Ministry of Environment, Lands and Park 1998). Providing rationale to the IACUC for the frequency of trap checks in relation to the metabolism of the species is important to determine the minimum length of time between trap checks. To further aid in meeting thermoregulatory and metabolic requirements, appropriate and adequate amounts of food and nesting material should be placed in all traps. Animals should be provided with both food *and* hydrating material or foodstuffs if they are expected to be in traps longer than the time that the animal can be sustained metabolically or if ambient temperatures are expected to be cold or excessively hot.

All traps should be checked for both captured animals and feces. Target species will be retained for further processing and non-target individuals should be immediately released. The trap location should be recorded for each animal moved to a central processing area (both targets and non-targets) to ensure that animals are released at the same site after processing. All captured animals should be assessed for injury prior to release. All traps containing animals, feces, urine, or other debris should be cleaned, disinfected, and inspected for proper function prior to re-use.

When capturing nocturnal species, traps should be set no more than 3 hours before sundown and checked shortly before sunrise (Sikes 2016). More frequent monitoring may be necessitated by the behavior and physiology of the target and potential non-target species that may be captured or by adverse environmental and weather conditions. Traps should be checked the following morning, and all traps should be checked prior to processing captured animals. Traps should be closed during daylight hours to prevent unintentional capture of diurnal animals.

When capturing diurnal species, traps should be placed in the shade and checked at least twice per day (Petit and Waudby 2013). Species considerations (e.g., metabolic requirements, size, possibility of dependent young) and weather conditions may necessitate

more frequent trap checking. During extremely hot or cold weather, or if traps are not able to be shaded, traps should be checked more often or closed entirely (Petit and Waudby 2013) Traps should be removed or closed at dusk to prevent unintentional capture of nocturnal animals.

5. **Potential Hazards**

Injuries to animals using the methods described should be rare when trap selection and monitoring are appropriate for the anticipated species and environmental conditions.

a. ***Weather***

Careful planning that considers the trapping location, target species, and anticipated weather should be reflected in the protocol. *In general,* trapping should not occur on nights when temperatures are expected to be <5.5°C (42°F) *and* either rain is expected (>20% chance at sites with bedding and >5% chance in sites with no bedding) or dew is expected (i.e., humidity is >75% and the projected minimum temperature is below the dew point) (Paull et al. 2023). When ambient temperatures are expected to exceed 27°C (80°F) by 10:00 AM, extra effort should be made to ensure that traps are checked more frequently, placed in the shade, and animals are processed as soon as possible (Paull et al. 2023).

Adverse weather conditions may increase the thermoregulatory stress placed on animals in traps. The demands placed on an animal and its tolerance to adverse weather varies by species and local environmental conditions. Trapping procedures should be modified accordingly to minimize stress to captured animals. For example, to protect from heat, wind, and precipitation, care should be taken to place traps in sheltered locations. If excessive temperature, wind, and/or precipitation are forecasted, traps should be closed or removed from the landscape. Insulation (e.g., polyester or wool batting, leaves, twigs) should be added to traps when capturing occurs at anticipated temperatures of less than 18° C (65° F) (Paull et al. 2023). Insulated traps, covers, and addition of bedding materials all can help improve the situation the animals during cooler weather trapping (Torre et al 2023). These parameters should be carefully considered, clearly explained in the project proposal, and strictly adhered to by the research team.

b. ***Adverse Event***

If predators or some other animal(s) destroy (i.e., damage beyond repair) or disturb (e.g., close trap doors, move traps, remove the bait) a trap, operations should be paused and the veterinarian of record consulted. Traps could be closed and removed from the site, moved to a new location, reinforced, or monitored overnight. Alternatively, traps or trap placement could be modified to avoid being destroyed by predators or other animal(s) (e.g., placed in a box, moved to a higher, less accessible level).

c. ***Mortality***

Thresholds for reporting mortalities to the project veterinarian and to the IACUC should be included in the protocol to be reviewed and approved by the Committee. The proposed thresholds should take into account species differences, selected study sites, and method of trapping.

B. Kill Traps

The AVMA (2020) states that properly deployed kill traps often do not meet criteria for euthanasia, and thus are better classified as a method of humane killing, but recognizes that kill traps "…can be practical and effective for scientific animal collection or pest control when used in a manner that ensures selectivity, a swift kill, and no damage to body parts needed for field research". If lethal trapping is required for the purpose(s) of a project, scientific justification must be provided and consideration of any further use after the primary purpose should be discussed (e.g. archiving of the animal and their parasites [Galbreath et. al. 2019]). Kill traps should render ≥70% of animals caught, with 95% confidence, irreversibly unconscious in ≤3 minutes (Powell and Prolux 2003).

1. Trap Types

a. ***Snap Traps***

Snap traps are spring loaded devices with a quick trigger mechanism designed to severe the spinal cord or rapidly crush the skull or rib cage (Figure 2). Sizes vary to allow for species-specific capture. Bait, if necessary, should reflect the preferences of the target species. Care should be taken to ensure that the ground around the trap is not contaminated with bait. Some traps are pre-baited with a scent.

Photo credit: https://www.wtamu.edu/~rmatlack/Mammalogy/snap_traps.htm

Figure 2. Three examples of snap traps: Victor rat trap, "Museum Special", and mouse snap trap (from left to right).

b. ***Macabee Gopher Trap***

The Macabee Gopher Trap is a spring action kill trap devised on the practical principle that a pocket gopher pushes dirt while tunneling which keeps it from noticing the trap (Figure 3). The dirt triggers the trip lever, and the trap catches the gopher around the neck or chest. Death by suffocation from the pressure or by stabbing from the prongs is reported. Due to insufficient information on the exact mechanism of death and the time to render the animal irreversibly unconscious, the Macabee Gopher trap is not recommended for use to trap small mammals.

Photo credit: https://www.wildlifecontrolsupplies.com/animal/NWSMAC.html

Figure 3. Example of the Macabee Gopher Trap.

c. ***Captive bolt***

The Goodnature® A24 rat trap is a self-resetting captive bolt trap powered by a CO_2 canister (Ryan et al. 2022) (Figure 4). Humane death to both mice and rats is caused by a pressured bolt strike to the rodent's head or neck after activation of the trigger (Shiels et al. 2022). The trap meets the New Zealand National Animal Welfare Advisory Committee's kill trap testing guidelines for humaneness for ship rats and stoats (National Animal Welfare Advisory Committee 2019). Twenty-four bolt strikes may occur before the canister is depleted. Additional studies are needed regarding the effectiveness of the Goodnature® A24 on wild rodents (Shiels et al. 2022) and potential risk to non-target animals (Ryan et al. 2022) but it is hypothesized that this trap may be advantageous in remote sites or ecologically sensitive areas where repeated human presence should be avoided (Ryan et al. 2022). Blockers are recommended when deploying Goodnature® A24 traps to increase specificity.

d. ***Pitfall Traps***

Pitfall traps are not a recommended lethal trapping method. Pitfall traps may only be considered when more effective, selective, and humane methods are not possible.

Photo credit: https://goodnature.co.nz/

Figure 4. Example of the captive bolt Goodnature® A24 traps without a blocker (left, not recommended) and with a blocker (right).

2. **Trap Monitoring**

All kill traps should be checked at least once a day (AVMA 2020). When trapping nocturnal animals, traps should only be set during the evening and checked and closed in the morning to minimize capture of non-targets.

3. **Potential Hazards**

Traps may only injure animals or may not render a trapped individual irreversibly unconscious in ≤3 minutes". If injured animals (either target or non-target) are found in a kill trap, they must be euthanized using an AVMA-approved method (AVMA 2020).

4. **Alternatives**

If fresh tissues are required to meet the objectives of the project, use of live traps followed by euthanasia is the recommended course of action. If fresh tissues are not required, whichever method (use live traps and subsequent euthanasia or use of kill traps) results in the least amount of stress to the target species should be utilized (AVMA 2020).

C. Alternatives to Trapping

Indirect monitoring techniques, such as using camera trapping, non-invasive DNA collection (e.g., feces, hair) or field sign indexes (e.g., feces, tracks, nest counts, hair traps), may be appropriate alternatives to capture in some instances. However, these methods may not provide the same data points or a comparable level of detailed information that can be acquired when animals are trapped, such as animal number and/or density, sex, age class, and weight (Flowerdew et al. 2003). Capture may also be necessary if certain tissue samples need to be collected.

VI: Transport, Temporary Holding, and Release

A. **Transport**

Personnel should limit disturbance at the trapping site by walking on the periphery as much as possible and entering the site only when necessary to check, collect, or return a trap or animal. A flag, marker, or GPS waypoint should be placed at the site of each trap that is removed to further ensure that the captured animal is returned and released at the same location (or reasonable proximity based on the species' movement and social ecology) where it was trapped.

Once live trapped, target animals need to be processed. The potential or anticipated costs (e.g., stress, disease transmission) versus the potential or anticipated benefits (e.g., less equipment needed, improved consistency of methods used) of transporting animals to a central processing as compared to processing them at the site of capture should be considered. If animals are moved to a central or consolidated processing location on the periphery of the trapping site, the location of the trap should be noted prior to transport to ensure that the animal is released at the trap site. Traps can be individually hand carried from the trapping site to the processing site or plastic sleds can be used to transport multiple traps simultaneously, thereby limiting the time animals spend in traps. Depending upon the species and situation, reducing visibility from the traps may be necessary to reduce stress from forced social interaction. This can be achieved by placing solid, opaque barriers between traps or using a large sheet to cover both the tops and sides of traps.

On rare occasions, animals may need to be transported by motor vehicle. Projects that necessitate vehicular transport of animals will require inclusion of specific details in the protocol submission form that describe caging used, vehicle type and size, appropriate temperature range and precautions taken to maintain that temperature range during transport, measures taken to minimize direct exposure to the sun, anticipated maximum length of transport time, remote monitoring devices to be used during transport, and clinical signs suggestive of stress or distress that will be monitored for during transport. Animals transported in motor vehicles should be assessed at least hourly for evidence of distress. Food and water should be offered if transit time is greater than 4 hours. If animals are to be anesthetized, food should be removed at least two hours prior to administration of anesthesia.

B. **Temporary Holding**

Once at a central processing location, traps should be placed under a shade canopy and protected from moisture and temperature extremes until processing. Captured animals should be left in the traps until processing. The length of time animals remain in the trap will vary by project and effort should be made to reduce this time as much as possible. If animals are held for more than 1 hour, their physical condition and respiratory rate should be assessed regularly, as appropriate for the species and environmental conditions. Food and water should be provided for the duration of their time in holding, if compatible with the research project. If animals are to be anesthetized, food should be removed at least two hours prior to administration of anesthesia. If at any time an animal is exhibiting signs of distress (i.e., unusual vocalizations, respiratory distress, change in

alertness, extreme lethargy), they should not be processed and should be released as appropriate and as soon as possible. If the number of animals needing to be processed exceeds the threshold where animals can all be processed in a reasonable period of time that is not expected to jeopardize welfare, “extra” animals should be released rather than risk harm by holding them for too long.

C. **Release**

All trapped individuals (target species and non-target species) should be released as close to their capture location as possible. If at any point it is confirmed that the captured animal has already been processed during the same trapping period, the animal should be put back in the trap and returned to its original location for release. See the Marking and Tagging section below for marking options. If anesthetized, animals should be recovered in a dry, clean trap or holding container for a minimum of 30 minutes or until completely awake and recovered (if recovery takes longer than 30 minutes). Once fully awake, recovered animals should be released as close to their capture location as possible.

VII: Handling and Restraint

Animals may need to be handled for a variety of research, teaching, training, or management purposes. These include species identification, determining reproductive condition and age class, obtaining morphologic measurements, marking for individual identification, and collection of biological samples for genetic assessment, pathogen surveillance, and/or other purposes.

A. **Removal From the Capture Device**

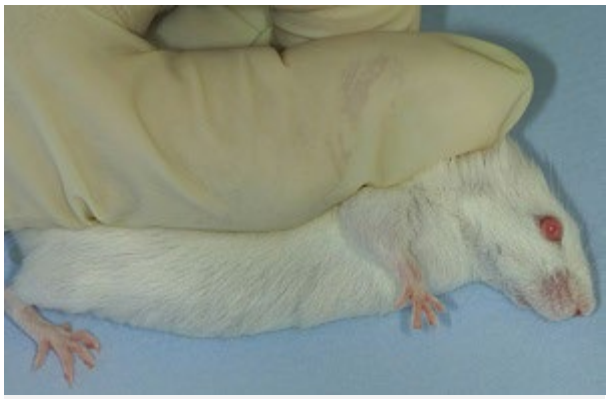
Prior to handling, each trap number should be recorded on the data sheet, along with the processing start time. To verify there is an animal in the trap, the trap should be held vertically to ensure that the animal is as far from the trap door as possible and the door opened briefly. Any prior markings made should be recorded, if present. After confirming that the animal has not already been processed during the same trapping period, gravity shall be utilized to carefully slide the animal from the trap into a clean plastic or cloth bag or onto a clean work surface. After removal from trap, the animal should be briefly examined to confirm that it is healthy enough to undergo anesthesia and/or processing and to re-confirm that it has not been previously processed. If the animal is exhibiting any signs of distress, is in poor body condition, or is too small/young to process, the animal should be returned to the trap and released as close to its original location as possible. Rationale for not processing the animal should be recorded on the data sheet.

B. **Restraint**

Restraint techniques for small mammals vary by species and individual project data requirements. Handling should be such that movement is controlled, breathing is unrestricted, and stress is minimized. Handling time should be kept to a minimum to decrease stress. All handlers should possess or obtain experience handling target species they are likely to encounter.

For many species of small mammals, an effective technique is to scruff the animal, grasping the skin behind the shoulders with two or three fingers (Figure 5). A scruff hold should effectively immobilize an animal's head and forelimbs. For some species, the tail and hind limbs can also be secured with the same hand that is being used to scruff the animal. For other species, such as chipmunks and squirrels, a "bander's grip" may be used (Figure 6). This grip involves holding the animal's back against the palm of one hand, with the neck restrained between index and middle fingers (Paull et al. 2023).

For most species, covering an animal's eyes during restraint has been suggested to minimize stress (Mantor et al. 2014). Use of canvas cone handling bags has proven especially effective in minimizing stress to squirrels (Koprowski 2002).



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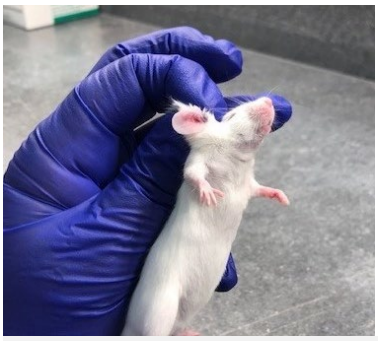


Photo credit: https://www.research.psu.edu/sites/default/files/imported/images/three%20finger%20grip.jpg

Figure 5. Two examples of scruffing. Scruffing is accomplished by grasping the skin behind the shoulders.

Photo credit: https://vt.audubon.org/sites/default/files/styles/wysiwyg_slide/public/fiona_banding_robin.jpg?itok=ulsOxHyo

Figure 6. "Bander's grip" modeled in a bird. The same concepts apply when restraining small mammals using this technique. Note that gloves should be worn when working with wildlife whenever possible.

1. **Potential Hazards**

Hazards include stress from handling, hypo- and hyperthermia, and trap-related injury if the trap door is triggered prematurely on a body part or if predators/other animal(s) damage or disturb a trap while an animal is inside, leading to distress or injury. Injuries to small mammals handled by the described methods should be rare. However, capturing and handling can be very stressful for small mammals, and they may go into shock or die if subjected to forceful handling (Petit and Waudby 2013). If an injury occurs that renders an animal unlikely to survive in the wild, the individual should be euthanatized using an AVMA-approved method (AVMA 2020).

C. **Anesthesia**

The application of anesthesia for most of the procedures described in this SOP is not usually necessary. However, it may be judged useful and appropriate to apply anesthesia on occasions when multiple procedures are performed on an individual animal. Procedures for anesthesia are described in this SOP, but additional detail will be needed in specific protocol submissions. Use of anesthesia will be required for any procedures that are considered painful or distressful, unless scientifically justified by the researcher. All potential candidates for anesthesia must be assessed by visual examination (at minimum) to be healthy enough to survive anesthesia.

Isoflurane is a highly volatile anesthetic that is commonly used to anesthetize small mammals. The open drop method is the most common route of isoflurane administration in the field. This method will typically deliver 30 – 45 seconds of deep anesthesia during which rapid procedures, such as blood collection or ear tag placement, may be completed (University of Montana 2020). Direct contact between mucus membranes and isoflurane is highly irritating to mammals and precautions should be taken to avoid the risk. See Appendix 1 for additional details on how to conduct the open drop method of anesthesia in small mammals. A vaporizer provides a more controlled administration of isoflurane and is a safer option; its use should be considered if planning to anesthetize a small mammal.

VIII: Marking and Tagging

Identifying animals may be necessary to meet the objectives of the study or to prevent duplicate data collection from the same animal if multiple rounds of trapping are conducted in the same study site.

A. **Temporary Methods**

1. **Paints and Dyes**

Non-toxic paint and dye or colored pens (if just a few days of identification is required) can be easily applied directly to the hair, fur, or skin and is a highly visible form of marking. The longevity of this type of marking is a few weeks to several months and depends on hair shedding (i.e., molting), wear (e.g., rubbing), and fade tendencies of the marking used (e.g., dyes). Application most often involves painting the mark

directly onto the animal by hand using stencils and brushes, paint pens, liquid paper, or nail polish. The mark can also be applied remotely using a brush-tipped pole. Apply paint or dye to the area of the animal that will be most visible to the observer (e.g., both flanks for ground observation, the back for aerial observation). The use of stencils is recommended if it is a requirement of the project to identify individual animals. The dye should be dry before releasing the animal. Recently reported successful applications include injecting UV fluorescent tattoo pigment subcutaneously into the tail of eight small mammal species (Petit et al. 2012) and the use of Muromachi hair dye on southern red-backed voles (*Clethrionomys gapperi*) and white-footed mice (*Peromyscus leucopus*) (Haines et al. 2018).

a. ***Potential Hazards***

Only non-toxic paints/inks/dyes should be used. Paint should not be used on animals with thick fur, as grooming will cause ingestion of the paint. Even if the mark persists, fur matting may cause fur loss or skin irritation. Paint or dye markings have the potential to alter the animal's behavior (e.g., increased time spent grooming) or may increase its visibility to predators or prey; particularly if bright colors not prevalent in nature are used. Use of projectiles to apply paint or dyes is not considered a safe or appropriate method of marking small mammals.

b. ***Restraint and Anesthesia***

When using temporary marking handling and restraint time of the animal should be kept to a minimum and no anesthesia may be needed.

2. **Fur Removal**

This method of marking should only be used on animals with sufficient hair/fur. The longevity of this type of marking is a few weeks to several months and is dependent on the timing of the next molting. Fur can be clipped or shorn from sections of the animal's body that will be easily visible to the observer, while minimizing the visibility of the animal to predators or prey. For larger animals, unique marks (e.g., numbers or combinations of letters and numbers) may be applied to improve individual animal identification.

Blunt-ended, curved blade scissors are recommended to clip hair from the area of the animal that will be most visible to the observer and most appropriate for the purposes of the project. Electric shears or clippers should only be used on calm animals that do not exhibit signs of distress while being clipped.

a. ***Potential Hazards***

Removal of extensive amounts of fur should be avoided to reduce the possibility of sunburn or hypothermia. Powered clippers should only be used on low-stress individuals of larger species as the noise is a significant stressor to many if not most species. Care must be taken to avoid nicking the skin when using clippers or shears.

b. ***Restraint and Anesthesia***

Handling and restraint time of the animal should be kept to a minimum. No anesthesia is likely needed.

B. Semi-Permanent Methods

Semi-permanent markers last for months to years (but not for the expected lifetime of the animal) or may be removed if the individual is recaptured.

1. Ear Tags, Punches, and Snips

Captured and processed animals can be marked by placement of a metal ear tag. Care must be taken to ensure that the tag is of an appropriate size, shape, and color to permit normal behavior of the marked animal. Ear tag weight should not exceed 5% of the animal's body weight. Although larger tags may be easier to read, there is generally a trade-off between tag visibility and risk of negative impacts to the animal, such as snagging the tag on vegetation, impaired grooming capability, reduced auditory perception, or making them more conspicuous to predators.

Prior to tag placement, the pinna should be briefly inspected, and any dirt or debris should be removed with an alcohol wipe. Although the exact location of tag placement will vary depending upon the differing anatomy among species, in general, tags should be placed close to the head and with as little space between the tag and the pinna as possible (i.e., closer to the base of the ear than the edge) to minimize the chance of the tag snagging on something and ripping out. The tagging pliers that are supplied with the ear tags should be used to place the tag. Once placed, the tag should be examined to ensure it does not impede movement of the ear and to check for bleeding. If bleeding occurs, direct pressure should be applied. If bleeding continues, the biopsy site should be cauterized using a silver nitrate stick until the bleeding stops.

If a skin biopsy is necessary for study purposes, an ear punch biopsy or ear snip can serve the dual purpose of marking an animal (Figures 7 and 8). An ear punch can be collected from the caudal half of the pinna, distal to the base of the ear. This method of marking is appropriate when tissue collection is required, when the objectives of the project do not require tracking of individual animal movements, and when animals that have already been processed need to be marked.

Alternately, a small (less than 2mm) piece of tissue can be snipped from the edge of the pinna in the same location described above by sterile, sharp, surgical scissors or an ear punch tool (if a tissue sample is required). The incision site should be monitored for bleeding following the procedure. If bleeding occurs, direct pressure should be applied. If bleeding continues, the biopsy site should be cauterized using direct pressure, Kwik Stop, or a silver nitrate stick, until the bleeding stops.

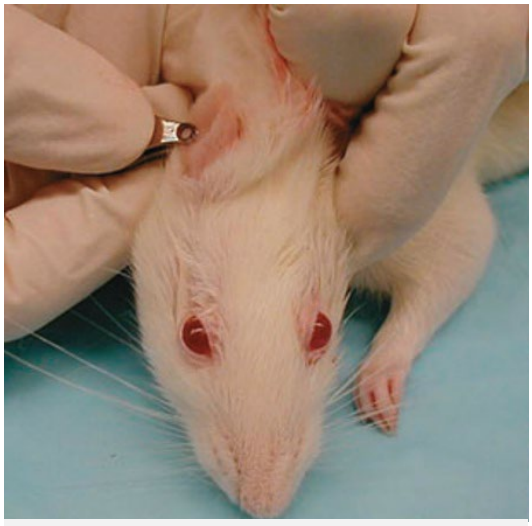


Photo credit: Wang (2005)

Figure 7. Use of an ear punch to mark an animal.

Photo credit: Wang (2005)

Figure 8. Ear punchers are available in two styles: thumb (top) and scissor (bottom).

a. ***Potential Hazards***

Ear tag placement is a standard procedure that has been completed in the field for decades with relatively few complications, provided it is placed appropriately and only in animals where the ear is fully developed. Tags placed in inappropriate locations (i.e., anterior surface of the ear or not close to the base of the ear) can become entangled in vegetation or removed traumatically during intraspecific interactions, thereby permanently damaging the animal's pinna. Tags placed on the anterior side of the pinna at the base of the ear run a higher risk of bleeding, injuring the ear canal, or creating an obstruction in the ear canal by catching debris. Ear tags may also inhibit grooming and lead to infestations of mites and ticks (Ostfeld et al. 1996). Careful and appropriate ear tag placement should minimize the chance of these adverse impacts to the animal.

b. ***Restraint and Anesthesia***

If ear tag placement is the only procedure being performed, general anesthesia is not necessary. If other procedures necessitate the use of anesthesia, the ear tag should be placed while the animal is anesthetized.

C. Permanent Methods

1. Passive Integrated Transponders (PIT tags)

PIT tags are used to permanently mark animals, including captive wildlife, pets, wild fish and free-ranging wildlife involved in ecological research. They have been used with

minimal harm on a variety of terrestrial small mammals. PIT tags are injectable microchips (8-12 mm in size) that do not emit signals except momentarily (less than 0.04 seconds) when activated by an electronic reader. The chip then emits a unique identification code at a frequency of 125 or 134.2 kHz. PIT tags are quick to place, long lasting, reliable, and provide unequivocal identification. Their small size, minimal weight, and subcutaneous placement do not normally alter the behavior or appearance of the animals.

Tags are implanted subcutaneously using a large bore needle/syringe. PIT weight should not exceed 5% of the animal's body weight. PIT tags should be implanted in areas of low movement, such as at the base of the ears or between the shoulder blades. Whatever site is chosen, it should be used consistently to ensure ease of location at reading. The entire animal should be scanned prior to application to ensure it has not already been implanted with a PIT tag. Before implanting, the tag should be scanned while still within its single-use needle to ensure that it is functioning and that the scanned number matches the number shown on the packaging. PIT tags should be sterile, and instruments used to implant the PIT tag should be single use (i.e., disposable), sterilized, or changed between animals. Skin and surrounding fur should be cleaned with antiseptic prior to implantation of PIT tag. Surgical glue can be used to seal the skin. The insertion site should be scanned to ensure the PIT tag has been applied correctly and is functioning.

Disadvantages of PIT tags include the fact that animals usually must be recaptured to identify marked individuals. However, PIT tags may be detectable by the scanner while the animal is still in a trap, eliminating the need to restrain the animal. If a PIT tag is not detected while the animal is in the trap, only minimal, short-term restraint is typically required in order to complete a scan. In some cases, scanning may occur passively as the animal walks past or through a scanner, eliminating the need for handling. Additional disadvantages of PIT tags are the expense associated with the equipment required to read the PIT tag after application and the chance that the tags may migrate, making them more difficult to locate in larger species. Due to migration, the whole animal may need to be scanned to detect the PIT tag. Lastly, there is a small possibility for chip failure.

a. Potential Hazards

Application is briefly painful, but the period of pain is less than that for tattooing (see the following section). Poor technique may result in prolonged pain and/or infection. The use of sterile instruments and aseptic skin preparation (e.g. disinfect the site with alcohol or chlorhexidine) minimizes the potential for infection and for transmission of disease when multiple animals are tagged. There is reported potential for tumors to develop adjacent to or around the site of implantation of the PIT tag. Several papers have reported malignant tumor development around or adjacent to implanted PIT tag in laboratory mice, rats, and cats, as well as microchip-related cancer in dogs (Albrecht 2010, Daly et. al. 2008).

b. ***Restraint and Anesthesia***

Anesthesia is usually not required. Temporary restraint is usually sufficient, and pain is momentary and minimal.

2. Tattooing

Tattooing is considered a permanent method for marking wildlife, but the longevity of this type of marking depends on the species and age of the marked animal and the quality and location of the tattoo. Lindner and Fuelling (2002) used ear tattoos on voles and reported a recapture rate of 90%.

Animals of all sizes can be tattooed. Advantages include no weight added to the animal, no alteration in behavior, and no increase in conspicuousness to predators. The best results are achieved by tattooing any lightly pigmented area that is clean and relatively hairless. The most common site for tattoo application is the inside surface of the ear pinna. All tools and dyes used for tattoo application should be sterile.

i. *Piercing Method of Tattoo Application*

Sterile forceps, a lancet, tattoo needles, or hammer instruments are used to pierce the skin in the required pattern, and a highly contrasting tattoo pigment, dye, ink, or paste (e.g. red, green, or black for non-pigmented skin) is then rubbed into the puncture wounds (Figures 9 – 12). Forceps, lancets, or pliers pierce the skin in patterns of letters and numbers, while hammer systems are 'slapped' onto larger animals to create a pattern of pinpricks. Before applying the tattoo, check that the instrument has been loaded properly (i.e., numbers are placed in reverse to normal viewing). A piece of cardboard is a good aid for checking this. After application, rub the ink rigorously with a cotton-tipped applicator to ensure a permanent mark. Wipe off the excess ink and clean the ear.

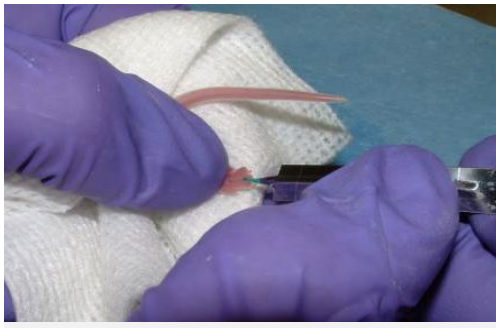


Photo credit: https://medipoint.com/tattooing-laboratory-animals/

Figure 9. Lancet used to pierce a mouse foot for application of a tattoo.

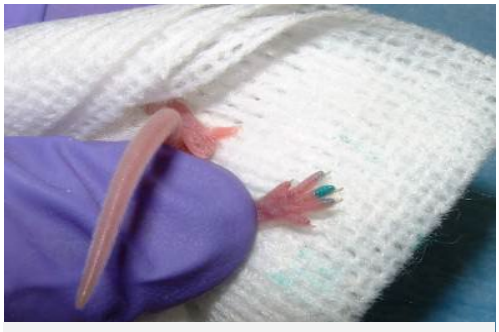


Photo credit: https://medipoint.com/tattooing-laboratory-animals/

Figure 10. Application of dye paste post-lancet piercing in a single toe.

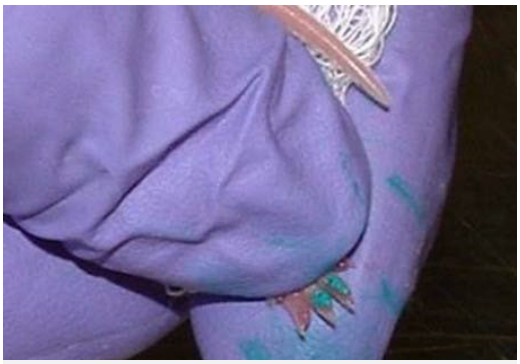


Photo credit: https://medipoint.com/tattooing-laboratory-animals/

Figure 11. Example of a double toe tattoo.

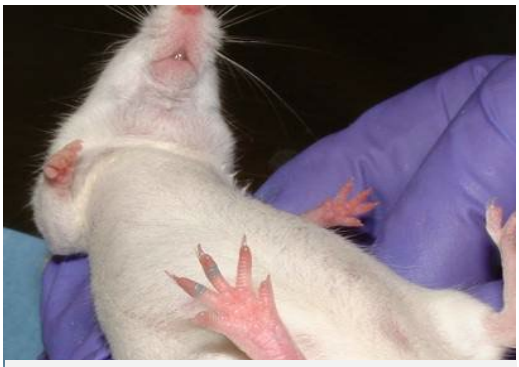


Photo credit: https://medipoint.com/tattooing-laboratory-animals/

Figure 12. Double toe post-tattoo application after 16-weeks.

ii. Electro-Vibrator Method of Tattoo Application

Electro-vibrator systems or needles both pierce the skin and inject the pigment and can be used to simply 'write' an identifying code into the skin. Excess pigment should be removed and the ear should be cleaned after application.

a. ***Potential Hazards***

Tattooing requires prolonged restraint during application. The application process is painful and may result in infection. Care should be taken ensure tools and skin have been sterilized prior to use. When injecting pigment it is important to avoid hair, blood vessels, or bones in the tattoo area. Disadvantages of tattoos include necessary restraint when the tattoo is read after recapture and tattoos can fade or become otherwise illegible over time.

b. ***Restraint and Anesthesia***

Animals should be restrained during tattoo application to immobilize the head. General or local anesthesia may be required, depending upon the species.

3. **Visible Implant Elastomer (VIE)**

VIEs are a recently employed marking method that was originally formulated in the marine industry for individually marking fish (Figure 13). A visual elastomer compound is injected into the ventral surface of an animal, just under the skin, with a small-gauged needle and syringe. VIE is injected as a liquid and cures into a pliable, non-bioactive, florescent solid (Rentz 2014). VIE tags are designed to be visible in daylight conditions but also fluoresce under ultraviolet light. Elastomer markings can be highly visual to the naked eye in some species (e.g., geckos). In other species, a LED or UV light will enhance visual

identification. VIEs are used primarily in fish and reptiles (e.g., squamates and frogs), though very limited use has been reported in small mammals as compared to ear tags and PIT tags (Jung et al. 2020). Marking success using VIE will likely be species-specific. Rentz (2014) describes the use of three fluorescent colors injected subcutaneously into the underside of the tails of red-backed voles and compared rates of tag loss and retention for VIEs and standard ear tags. VIE tags lasted considerably longer than ear tags in voles and were lost less frequently. However, broadly speaking, VIE can be relatively expensive, challenging to use in the field, and its suitability for mammals has not been established (Petit et al. 2012). If this technique is proposed, provide justification for selection and discuss what other alternatives were considered for marking small mammals.

Photo credit: https://www.nmt.us/visible-implant-elastomer/

Figure 13. Application of a visible elastomer implant tag. Note that gloves should be worn whenever possible when working with wildlife.

a. ***Potential Hazards***

VIEs may lead to higher predation rates by species who can detect UV. The risk of infection also exists.

b. ***Restraint and Anesthesia***

Restraint is necessary. General or local anesthesia may be required.

4. **Freeze Branding**

Freeze branding, or cryobranding, is not a recommended method in small mammals due to their small size.

IX: Biological Sampling

A. **Blood Collection**

Blood collection should only be performed by trained and proficient individuals. Blood should only be collected from animals weighing more than 10 grams. Blood should not be collected from animals that are observed to be clinically dehydrated, have pronounced injuries, or are exhibiting clinical signs of illness. When selecting a venipuncture technique there are several factors to be considered, including study species, health status of the animal, animal size and total blood volume, quantity of blood required, and sampling frequency. In general, it is

recommended to draw no more blood than 1% of the animal's body weight at a given time (NIH 2022).

When selecting a method and site for blood collection, care should be taken to consider the normal behavior and activity of the species and the potential impacts the blood collection procedure may have on the individual animal. For example, tail clipping could affect survival of individuals that are dependent on their tails for balance or locomotion.

Venipuncture Techniques

i. Submandibular Method

For most species of small mammals, the submandibular method of blood collection is effective and appropriate (Golde et al. 2005). It is commonly used to collect larger volumes of blood (UCSF 2022). In fact, while collecting blood using the submandibular method is relatively easy, from a technical perspective, the ease of access may pose a significant risk of inadvertently collecting too much blood (Queen's University 2022a). Additionally, this method can be highly stressful for mice and may result in damage to the inner ear and major masticatory muscles (Teilmann et al. 2014), therefore its use requires that personnel be highly trained. Submandibular blood collection is typically done under general anesthesia. If it is necessary to use this method without anesthesia, scientific justification is required.

To restrain, the animal is scruffed and held on its side (see section VII B). The point of insertion for the lancet is identified by digitally palpating the posterior border of the lower jaw (i.e., mandible) and finding the intersection point between a line drawn from the lateral (i.e., outside) corner of the eye to the point of the shoulder and a line drawn from the bottom of the mandible to the posterior aspect of the ear (University of California San Francisco 2022) (Figure 14). A disposable 4 – 6 mm lancet is used to puncture the cheek behind the mandible but in front of the ear canal at the intersection of these two lines. Blood is collected into a capillary tube or other blood collection device (Figure 15). To stop the bleeding, release the scruff and apply direct pressure.

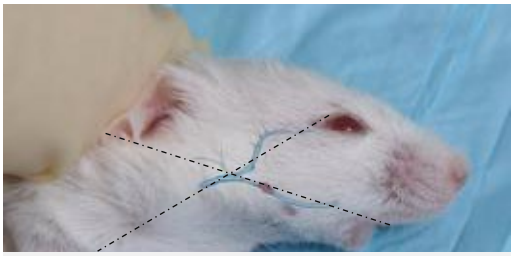


Photo credit: https://medipoint.com/for-use-on-mice/

Figure 14. The intersection point between a line drawn from the lateral corner of the eye to the point of the shoulder and a line drawn from the bottom on the mandible to the posterior aspect of the ear represents the location where the lancet should be inserted.

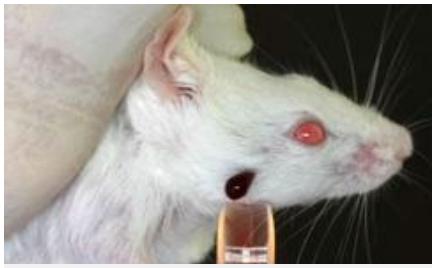


Photo credit: https://medipoint.com/for-use-on-mice/

Figure 15. A blood collection device can be used to collect blood after a lancet is used to puncture the vascular bundle.

ii. Saphenous Vein

Blood collection from the saphenous vein has proven to be an effective method for wild small mammals, with rapid healing and minimal effects on recapture and survival (Flaherty et al. 2013). This method is not appropriate if large volumes of blood are needed. For this method, a small patch of fur is shaved from the medial aspect of the animal's hind leg, which allows for visualization of the medial saphenous vein (Figure 16). The size of the vessel may be slightly increased by applying a small amount of alcohol to the skin overlying the vein. The vein is either lanced with a small needle or lancet and pooled blood is collected into a capillary tube or other blood collection device, or blood is drawn directly from the vein using a small gauge needle and ≤1 cc syringe (Figure 17). Brief anesthesia may facilitate success of the procedure in addition to minimizing stress to the animal.

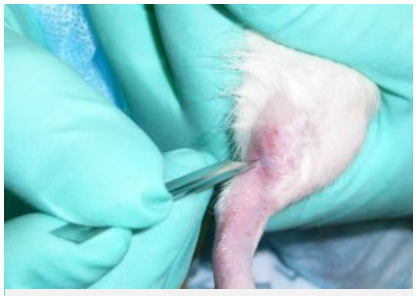


Photo credit: https://medipoint.com/for-use-on-rats/

Figure 16. The saphenous vein is isolated by shaving the area and occluding the vessel.

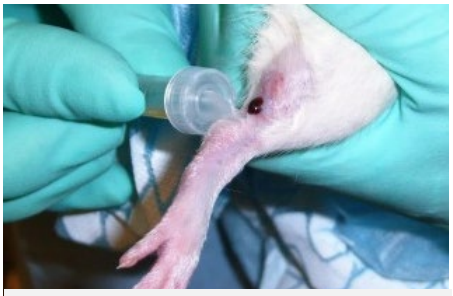
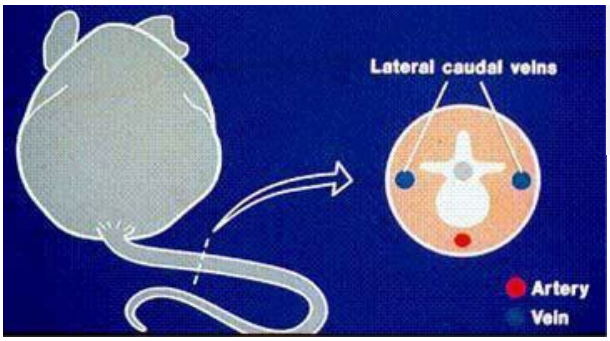


Photo credit: https://medipoint.com/for-use-on-rats/

Figure 17. After lancing, the blood is collected into a blood collection or capillary tube.

iii. Tail Vein and Artery

Multiple methods exist for collecting blood from the tail of an animal. Venipuncture of the lateral tail vein or ventral tail artery can be performed after aseptic preparation of the tail (Figure 18; Queen's University 2022b). Laceration of the tail vein and collection of blood via a capillary tube is also effective.



Phot credit: https://www.queensu.ca/animals-in-science/policies-procedures/sop/rats/10-10-1

Figure 18. Note the location of the tail veins (i.e., at the 3 and 9 positions on a clockface) and the tail artery (i.e., at the 6 position).

Alternatively, in certain small rodents, a tail tip amputation may be performed. This method involves amputating a small (~1-2 mm) section of the distal tail with a scalpel blade (Joslin 2009). Impacts to the individual animal should be considered, as amputating the tip of the tail can have deleterious effects to animal behavior, potentially affecting grooming and ambulation. Tail tip amputation should be performed with the animal under general anesthesia. Justification on the use of a tail tip amputation over blood collection must be provided in the project proposal.

iv. Retroorbital Sinus

Historically, retroorbital sinus blood collection was commonly practiced on small mammals under general anesthesia in the laboratory. However, this method has fallen out of preference over time. As such, unless extensively justified, it is not considered a recommended method for collection of blood from small mammals in the field.

1. **Potential Hazards**

The most common potential hazards of blood collection include development of a hematoma and excessive bleeding. Applying direct pressure to the site following the procedure can help minimize both risks. In the event of excessive bleeding, silver nitrate or coagulating powder should be used to help slow the bleeding. Disease transmission and infection are also risks that should be mitigated by using sterile equipment and proper aseptic procedures (e.g. disinfect the site with alcohol or chlorhexidine).

2. **Restraint and Anesthesia**

For all types of blood draws, animals should be restrained by scruffing the animal by hand, as described in section VII B. When bleeding via the submandibular method, scruffing by holding the skin above the shoulder blades has proven more effective than scruffing the skin behind the ears. Anesthesia is required when using the submandibular method. Depending upon the experience and proficiency of the individual collecting blood, anesthesia should be considered when collecting blood from either the saphenous vein or the tail.

3. **Alternatives**

There are no known non-invasive methods for obtaining blood. The collection method should be chosen carefully based upon experience, training, and the species and age of the mammal being sampled.

B. **Skin Biopsy**

A skin biopsy may be necessary for bio-banking/long-term cryopreservation. A single, 4 mm or less-sized biopsy is adequate for small mammals (Arenivas et al. 2023). Aseptic technique must be utilized when preparing the biopsy site (i.e., disinfect the site with alcohol or chlorhexidine) and when collecting the biopsy (i.e., use new sterile instruments for each animal or sterilize the instruments between individuals).

1. **Potential Hazards**

When possible, a local anesthetic should be used to minimize pain at the biopsy site. Aseptic technique must be used to minimize the risk of infection at the biopsy site. Direct pressure with clean gauze should be used to avoid excessive bleeding. Proper wound care should be employed after the biopsy is collected to minimize risk of infection. Consultation with the bio-banking lab prior to sample collection is recommended to confirm proper sample handling, storage, and shipping methods to ensure the cells remain viable for cell culture and cryobanking.

2. **Restraint and Anesthesia**

No anesthesia is required but will make the procedure easier. The animal must be properly restrained during the procedure if not anesthetized.

3. **Alternatives**

Ear punches are suitable for cryopreservation and are less invasive than skin biopsies. This is an especially useful technique if an ear tag is concurrently being placed. Less-invasive samples, such as saliva, blood, and hair, should be utilized for genetic analysis or measures of relatedness of individuals.

C. **Hair Sampling**

Hair sampling may be necessary for studies of genetics, hair structure, contaminants, dietary habits, geographic origins, and assessment of migratory pathways using isotope analyses. In many cases, previously preserved specimens can be used, but it may be necessary to obtain hair from live animals in some cases. Typically, only one or a few (up to 12) hairs are needed for these analyses. Hair from the mid-dorsal region is usually used. The follicle must be present for analysis so the hair should be plucked from the body.

1. **Potential Hazards**

This technique presents no known hazards to the animal. Relative to the total number of hairs in the pelage a miniscule sample is removed for these purposes.

2. **Restraint and Anesthesia**

No anesthesia is required. Small mammals are held by hand briefly and the hair is plucked.

3. **Alternatives**

Other samples, such as tissue, saliva, blood, and feces, may be used for analysis of genetics or relatedness of individuals. Degradation of these types of samples can occur and the scope of information provided by the analyses may vary so consultation with reference laboratories on preferred samples and storage requirements is recommended to meet research objectives.

D. Saliva/Buccal Swab

Saliva or buccal swabs may be useful for genetic analysis or targeted pathogen testing. It is performed by scruffing the neck of the animal (see section VII B). Through the act of scruffing, the corners of the mouth are retracted/opened and a miniature, cotton-tipped (approximately 17 mm length by 1 mm depth tip) applicator with an aluminum shaft (approximately 150 mm length) can be inserted into the mouth and gently wiped along the inside of the cheek (Mitrecic et al. 2008).

1. **Potential Hazards**

The risk of biting off the swab exists but is mitigated by use of an aluminum-shafted swab. Trauma to the oral cavity may occur. Care should be taken to gently swab the oral cavity.

2. **Restraint and Anesthesia**

No anesthesia is required. Proper restraint is necessary for the safety of the animal and the sample collector and to ensure access to the oral cavity.

3. **Alternatives**

Collection of a hair sample for genetic analysis is less invasive than saliva collection.

E. External Parasites

It is possible to remove ectoparasites from small mammals during handling by using fine forceps and/or a flea comb (Figure 19). Ticks are usually found on the head or underbody of small mammals, fleas and lice are usually found on the lower part of the abdomen and underbody, and chiggers are typically found in the ears (Figure 20) (Herbreteau et al. 2011). Proper PPE should be worn when collecting ectoparasites.

1. **Potential Hazards**

Collection of ectoparasites poses a risk of ectoparasite exposure. Care should be taken to prevent ectoparasites from transferring from the small mammal to the holder or sample collector. Risk of injury from the animal during sample collection is low if it is properly restrained during sample collection.

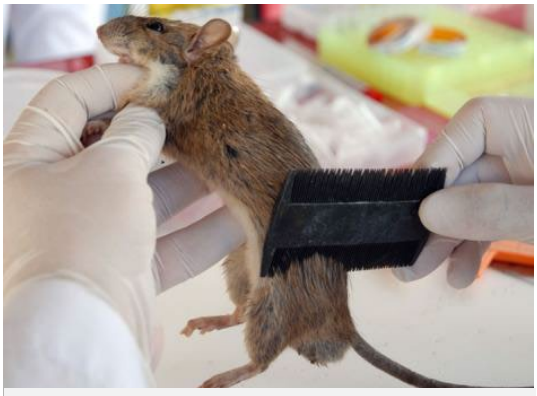


Photo credit: Herbreteau et al. 2011

Figure 19. Use of a flea comb to collect ectoparasites.

Photo credit:
Herbreteau et al. 2011

Figure 20. Ticks attached to the ear.

2. **Restraint and Anesthesia**

No anesthesia is required. Animals must be restrained as they are combed or examined for ectoparasites.

3. **Alternatives**

There are no known less-invasive alternatives to ectoparasite collection.

F. Feces

The capture and handling of small mammals provides opportunities to collect additional materials that may be useful in some directed studies. For example, small mammals may defecate and/or urinate while in the trap or during handling. Fecal pellets, which can provide information on diet, certain endoparasites, some pathogens, and genetics may be opportunistically collected. Fecal pellets may be extracted from the trap with gloves and disinfected tweezers while the animal is removed for processing. Pellets are transferred to ethanol vials or plastic bags, labeled with the pertinent data, and conveniently stored until analysis. Forceps should be cleaned and rinsed with ethanol immediately after fecal sampling is complete.

1. **Potential Hazards**

Fecal collection may pose a risk of pathogen exposure. Gloves must be worn during collection to minimize exposure.

2. **Restraint and Anesthesia**

No anesthesia or restraint is necessary for fecal collection.

3. **Alternatives**

There are no known less-invasive alternatives for fecal collection.

X: Animal Disposition, Lethal Collection, and Euthanasia

A. Animal Disposition After Completion of Project

Animals that are captured for species identification and morphological inspection, biopsy sampling, and/or marking and tagging are released at the point of capture after handling as described elsewhere in this document. Animals are not typically recaptured for removal of ear tags. Carcasses of animals that die during capture or processing or that are intentionally euthanized for project purposes should be disposed of appropriately and according to local regulations and permitting. Incineration at a veterinary facility may be required if drugs have been administered or disease concerns exist. Carcasses or tissues may be frozen in sealed plastic bags prior to disposal, ensuring the bag is properly labeled to identify the carcass and any potential contaminants.

B. Lethal Collection of Small Mammals

Animals may be collected and euthanized for project purposes, such as whole body or organ analysis for contaminants and/or pathological, physiological, or morphological study. Sample size requirements and methods of sample preparation (see Galbreath et. al. 2019 for a protocol on collecting and preserving sample materials) for such collections should be provided by the researcher in adequate detail.

C. Euthanasia in the Field

The NPS IACUC requires all investigators to submit a euthanasia plan even when death as an endpoint is not a planned component of the project. Investigators and field staff assisting with the project should be prepared to euthanize an animal if unexpected injury occurs. Euthanasia methods should conform to the guidelines published by the AVMA (2020) and any deviations must be scientifically justified. Investigators and field staff should be trained and deemed competent in euthanasia techniques prior to implementing them.

Any unplanned mortality due to capture or handling procedures employed by the investigator should be reported to the NPS IACUC within 48 hours. Field work should be immediately halted if two or more unplanned mortalities occur during any 24-hour period.

1. **Inhalant Anesthetics**

   a. ***Isoflurane***

      See Appendix 1 for information on how to use isoflurane to euthanize small mammals.

   b. ***Carbon Dioxide***

      Exposure to high concentrations of carbon dioxide has an initial depressant and anesthetic effect (i.e., induces a state of narcosis), which is followed by death via asphyxiation while the animal is unconscious. High concentrations of carbon dioxide may be painful to the respiratory tract, therefore a gradually rising concentration of carbon dioxide that induces a state of narcosis prior to death is recommended. If animals are observed to exhibit significant or prolonged signs of distress prior to death (e.g., hyperactivity, jumping, vocalizing), the flow rate should be reduced. While a reduced flow rate may increase the time to death, it should be less stressful and painful to the animal by inducing a state of narcosis prior to asphyxiation. The AVMA (2020) recommends an optimal flow rate that displaces 30 – 70% of the chamber volume per minute. In order to achieve this precise flow rate, the administration of carbon dioxide requires the use of a regulator and a flow meter or a combination device (University of Texas at Austin 2020). See Appendix 2 for additional information on the use of carbon dioxide to euthanize small mammals.

      The NPS wildlife veterinarians have developed a portable carbon dioxide chamber that can be used to euthanize animals in the field. Contact the NPS Wildlife Health Branch for more information.

2. **Physical Methods**

   a. ***Cervical Dislocation***

      When performed properly, cervical dislocation causes disarticulation of cervical vertebrae and loss of consciousness that is shortly followed by death. Cervical dislocation is most commonly used to ensure death after administration of an overdose of anesthetic inhalant in small mammals weighing <200 grams. If cervical dislocation is proposed as the primary method of euthanasia, researchers must provide justification as to why primary inhalant overdose has been discounted.

      Cervical dislocation on conscious animals should only be performed by trained staff that conduct the procedure regularly and are occasionally

observed/monitored to ensure proficiency (AVMA 2020). This technique should never be employed on conscious animals by untrained individuals.

To perform the procedure, secure the head between the thumb and index finger at the base of the skull (Figure 21). Alternatively, a metal rod (e.g., scissors, forceps) can be firmly placed across the neck at the base of the skull (Figure 22). Grasp the base of the tail with the other hand and tug sharply away from the body. This method should not be used for animals that are being submitted for rabies testing.

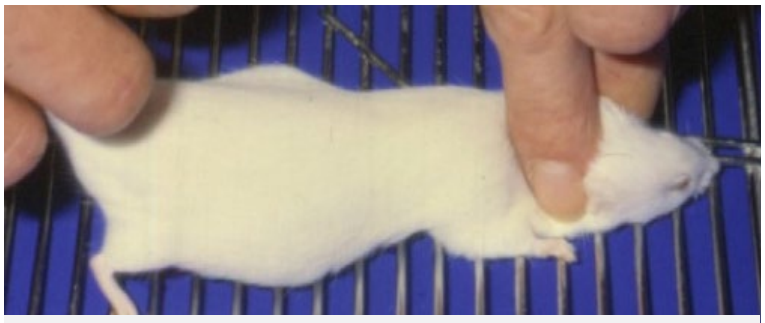


Photo credit: https://www.bu.edu/research/ethics-compliance/animal-subjects/animal-care/euthanasia/carbon-dioxide-euthanasia-for-rats-and-mice/

Figure 21. Cervical dislocation performed in a mouse. Note that gloves should be worn whenever possible when working with wildlife.

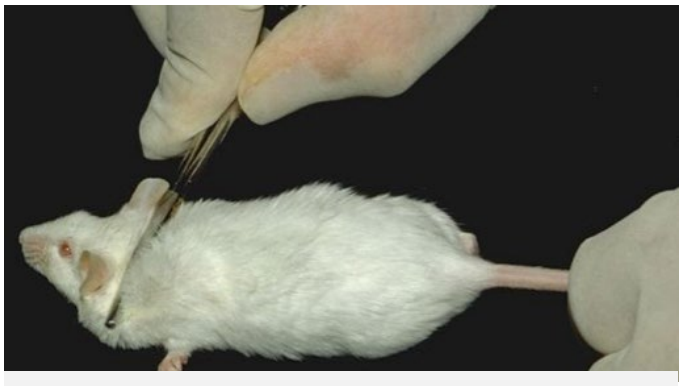


Photo credit: https://www.bu.edu/research/files/2016/08/Slide-20.png

Figure 22. Cervical dislocation performed in a mouse using scissors to stabilize the head.

b. ***Thoracic Compression***

Also known as cardiopulmonary or cardiac compression. Thoracic compression is the application of pressure to an animal's chest to prevent respiration and/or cardiac movements with the intent of causing death (AVMA 2011). Use of this technique can be also used to verify death after administration of inhalant aesthetic in small mammals. Thoracic compression is an unacceptable means of euthanizing animals that are not deeply anesthetized (AVMA 2020).

D. Verification of death

Death must be verified after an animal is euthanized or presumed dead. A combination of criteria is most reliable in confirming death, including lack of breathing, absence of a palpable heartbeat, lack of response to a firm toe pinch, graying of the mucous membranes, and rigor mortis (UC Davis 2024).

XI. Additional Considerations

A. Taxa Specific Considerations

There are a vast number of small mammal species which differ greatly in their biology. Investigators should familiarize themselves with the species-specific differences regarding behavior, physiology, handling, and husbandry and seek refinements specific to these noted species differences.

B. Regulation of Animal Activities

1. IACUC Review and Approval

Investigators proposing small mammal research, teaching, or training activities within NPS units must request NPS IACUC review and approval prior to beginning of field work, even if they have approval from other IACUCs. To initiate NPS IACUC review contact.

2. Permits

Activities within NPS units require research permits and compliance, which is independent from NPS IACUC review. To initiate NPS permitting and compliance review visit https://irma.nps.gov/RPRS/.

The U.S. Fish and Wildlife Service (USFWS) may require additional permits if target or anticipated non-target species are included on a federal threatened and endangered species list. To check if species are federally listed visit https://www.fws.gov/endangered/. To initiate USFWS permit review visit https://fwsepermits.servicenowservices.com/fws.

If your project will also occur outside NPS units, check to see if state, other federal, or tribal research permits are required.

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Appendices

Appendix 1. Open drop method of isoflurane administration.

Anesthesia is administered according to approved protocols and agency guidelines. It is only administered by veterinarians experienced in these techniques or field staff that have been trained in these techniques by a veterinarian and judged competent.

Open drop method of isoflurane administration

1. Use a clear, sealable chamber (e.g., Rubbermaid or similar airtight container) that is at least 2-4 times the volume of the animal.

2. Use a syringe or pipette to apply 0.25 – 0.5 mL isoflurane per 500 mL container volume to a cotton ball or gauze (University of Michigan 2025, University of Montana 2023).
    a. The most effective isoflurane dose varies depending upon the species, age, size of the animal, and size of the container.

3. Place the isoflurane-soaked cotton in a tea infuser (or other permeable barrier) to prevent direct contact between the animal and the liquid anesthetic.

4. Place the animal in the chamber, add the tea-diffuser and isoflurane-soaked cotton, and close the lid.

5. Monitor the respirations continuously during induction by visualizing chest/abdominal movements.

6. Refer to steps 8a – 11a if isoflurane is used for general anesthesia.

7. Refer to steps 8b – 10b if isoflurane is used for euthanasia.

Use of the open drop method for general anesthesia

8a. As soon as the animal appears to have lost consciousness, is unable to right itself, and is non-response to gently rocking the container back and forth, leave the animal in the induction chamber for 10 more seconds before removing from the chamber (University of Montana 2023).

9a. Remove the animal from the chamber. If the animal does not respond to physical stimuli (e.g., removal from the chamber) or noxious stimuli (e.g., toe pinch), processing can be initiated. If the animal responds to either physical or noxious stimuli, replace the animal in the induction chamber and continue to monitor as described above until a deep plane of anesthesia is reached.

10a. Immediately replace the lid on the induction chamber after the animal is removed.

11a. Animals should be anesthetized in less than 1 minute, but induction times will vary amongst individuals and species. 10a. Induction time, total length of anesthesia, and quality/depth of anesthesia should be recorded to help assess if the dose is appropriate.

Use of the open drop method for euthanasia

8b. Keep the animal in the container until the animal stops breathing. At this time, a physical method of euthanasia may be utilized to ensure death.

9b. If a secondary method is not used, keep the animal in the chamber for an additional 5 minutes.

10b. Remove the animal from the chamber and confirm death (i.e., lack of a heartbeat, unresponsive to all painful stimuli, no response when the surface of the eye is touched, dilated and non-responsive pupils).

Appendix 2. Use of carbon dioxide for small mammal euthanasia.

Supply List

- Commercially supplied compressed 100% CO_2 tank
- Pressure regulator (i.e., gauge showing pounds per square inch (PSI), indicates how much gas remains in the tank)
- CO_2 flowmeter (i.e., gauge showing liters per minute (LPM), represents the flow rate)
- Hose with fittings to connect to the regulator OR swivel barb connector and gas line tubing
- Chamber (e.g., large clear plastic container, cooler with a plexiglass window, aluminum box with plexiglass window)
- For non-airtight chambers: materials to seal the edges of the chamber (e.g., garbage bag and bungee cord)

Flow Rate Calculations

Calculating CO_2 Flow Rates - Converting Displacement Rate (%) to Flow Rate (L/minute)

- Determine the volume of the chamber in liters (L)
    - Measure the internal length (l), width (w), and height (h) of the chamber in inches
        - w x l x h = volume in cubic inches
        - Divide the volume in cubic inches by 61 to convert to liters
    - 1 QT ~ 1 L
- Multiply the volume of the cage (L) by % displacement
    - Example: 60% displacement rate for a 15 L chamber
        - Example: 15 L X 0.6 = 9 L/min

Operational Instructions

1. Drill an inflow hole large enough to fit the CO_2 hose in one corner of the top of the chamber, about 2" from the edge.
2. If using an airtight container, drill an outflow port approximately the same size as the inflow hole in the opposite corner, about 2" from the edge. Containers that do not have an airtight lid do not need an outflow port.
3. If using a cooler or other non-transparent container, cut a rectangular window in the top or side that is large enough to allow the animals to be monitored. Use a scroll saw to cut a piece of plexiglass large enough to cover the rectangular window hole. Screw or glue the window of plexiglass over the hole. Apply silicone sealant to the edges of the plexiglass to ensure a tight fit and prevent gas leakage.
4. Connect the pressure regulator to the CO_2 tank. Use a wrench if necessary but do not overtighten.
5. If using a hose, connect the hose to the flowmeter. Use a wrench if necessary but do not overtighten. If using gas tubing, connect the swivel barb connector to the flowmeter. Use a wrench if necessary but do not overtighten. Soften the tubing with warm water if it is

difficult to insert the tubing onto the barb. If the tubing inserts easily onto the barb, ensure a tight seal by using a hose clamp.

6. Insert the other end of the tubing or hose into the inflow hole.
7. Ensure the chamber is not pre-filled with CO_2 prior to placement of the animal(s) in the chamber.
8. Place the animal(s) in the chamber. Do not overcrowd the chamber.
9. Turn on the CO_2 to the desired flow rate (see above for instructions on how to calculate flow rate). Start at the lowest recommended flow rate (30- 70% for rodents). Because high levels of CO_2 can be painful, if the animals are observed to exhibit significant or prolonged signs of distress prior to death, reduce the flow rate. A reduced flow will take longer to cause death but will cause less stress and pain to the animal by inducing a state of narcosis prior to asphyxiation. Juvenile mammals typically require extended exposure to higher flow rates
10. Leave the CO_2 running for at least 1 minute after the animal(s) have stopped breathing.
11. Turn the CO_2 off and do not remove the cover.
    a. Remove the CO_2 hose and seal the inflow hole.
    b. Seal the outflow port or create an air-tight seal around the lid if using a non-airtight container that does not have an outflow port.
    c. Leave the animal(s) in the chamber for at least 10 additional minutes (see below for an alternative method).
12. After at least 10 minutes, remove the animal from the chamber. Confirm death.
    a. If unable to confirm death, perform cervical dislocation, thoracic compression, or decapitation.
13. **Alternative to leaving the animal in the chamber for 10 additional minutes:** Remove the animal 1 minute after turning off the regulator (i.e., at least 2 minutes since the animal stopped breathing). Immediately perform cervical dislocation, thoracic compression, or decapitation.
14. Turn the chamber on its side and flush the chamber with room air for at least 1 – 2 minutes between euthanasia events.