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1.0 SCOPE AND APPLICATION

This Standard Operating Procedure (SOP) describes procedures for sampling and processing small mammal populations. Due to their trophic position as consumers, small mammals can act as indicators of the effects of contamination on terrestrial and wetland communities (McBee and Bickham 1990, U.S. EPA 1997). Small mammals may be used to determine (1) contaminant levels in body tissues, (2) histopathological effects of contaminants, (3) effects of contaminants on body condition, growth, and reproduction, and (4) potential impacts of contaminants on population density and demographics. These data may be incorporated into an ecological risk assessment for the purpose of predicting risk to endangered or protected species, or species that may not be practical to sample (e.g., raptors or mink). This SOP also includes information about personal protective measures that should be taken to reduce the risk of infection by Hantavirus and other diseases that can be transmitted from rodents to humans when trapping, handling, or processing small mammals.

A Quality Assurance Project Plan (QAPP) in Uniform Federal Policy (UFP) format describing the project objectives must be prepared prior to deploying for a sampling event. The sampler needs to ensure that the methods used are adequate to satisfy the data quality objectives listed in the QAPP for a particular site.

The procedures in this SOP may be modified, dependent on site conditions, equipment limitations or other procedural limitations. In all instances, the procedures employed must be documented on a Field Change Form and attached to the QAPP. These changes must be documented in the final deliverable.

2.0 METHOD SUMMARY

Before trapping, the area(s) of impact should be identified and notes on terrain, vegetation type and cover, and land use should be recorded. A reference area should be identified that is similar to the area being trapped, but not impacted by site contaminants. Permission from the property owner(s), if available, must be obtained for access to the reference area. In addition, if applicable, a scientific collection permit may need to be obtained from the appropriate state or federal agency prior to trapping.

The type(s) of traps selected should be based on the target species, types of analyses needed, and number of animals needed to meet the study objectives. For example, live traps (e.g., Sherman and Havahart traps) are preferable for the collection of animals for histopathological analysis. Kill traps (e.g., Museum Special or snap traps) may be used to collect small mammals for residue analysis. Pitfall or Longworth traps may be used to capture smaller species, such as shrews, that are difficult to trap by alternative means.

Trapping locations should be selected based on the availability of suitable habitat and evidence of small mammal presence (DeBlase and Martin 1981). For example, runs are often present in areas of dense grass. Grid orientation and the number of traps should be consistent among areas assuming similar conditions. The location of each trap line and trap should be marked in the field notebook and on a corresponding map or aerial photo. Locations of individual traps, end of trap lines, or perimeter of the trapping area(s) may also be recorded by global positioning system (GPS). Traps should ideally be checked twice daily, early in the morning and late in the day. If live traps are used the animal should be humanely euthanized (see Section 3.0) if needed for analyses else set free. Captured animals should be transferred to individual plastic bags labeled with the trap location number, time of day, lowest taxonomic level known, date, and collector's affiliation and initials. If the species is unknown at the time of collection it may be identified later in the day or back in the laboratory. Bags containing dead animals should be placed on ice for transfer to the laboratory or processing area. Samples should be kept on ice while processing and placed on dry ice for shipment to the laboratory.

Field biologists and other personnel who are exposed to small mammal body fluids and excreta are particularly at risk of Hantavirus infection (Mills *et al.* 1995). Employees who plan to trap, handle, process, or otherwise be involved in any activities related to small mammals should be educated about the risks of such activities, as well as ways to minimize those risks.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

Animals caught live are generally euthanized in the field by cervical dislocation. Animals may also be euthanized by asphyxiation with a chemical inhalant (e.g., carbon dioxide [CO_2]). Cervical dislocation and chemical inhalants meet the criteria of the United States Department of Agriculture (U.S.D.A.) Animal and Plant Health Inspection Service for methods of euthanization for small mammals. The American Veterinary Medical Association's (AVMA) 2013 guidance offers the following which are applicable here: (1) ability to induce loss of consciousness and death without causing pain, distress, anxiety, or apprehension; (2) time required to induce loss of consciousness; (3) reliability; (4) safety of personnel; (5) irreversibility; (6) compatibility with requirement and purpose; (7) emotional effect on observers or operators; (8) compatibility with subsequent evaluation, examination, or use of tissue; (9) drug availability and human abuse potential; (10) compatibility with species, age, and health status; (11) ability to maintain equipment in proper working order; and (12) safety for predators/scavengers should the carcass be consumed. Field setting and study goals impose limits and restrictions on euthanizing the small mammals. Dead animals should be transferred to individual re-sealable plastic bags labeled with the trap location number, genus, species, and date, time of day, and collector's affiliation and initials. Bags should be placed on wet ice for transfer to the laboratory or processing area.

In the laboratory or processing area, small mammal data sheets (Appendix A) are completed. Information entered should include the location and conditions under which each specimen was collected, sex, approximate age, reproductive condition, body mass, total length, and tail, foot, ear, and total lengths if needed. Each animal is kept in its corresponding bag to avoid mixing up sample information. Bags are kept on wet ice whenever possible.

Depending upon the types of analyses to be done (e.g., histopathology, tissue burden, or dietary assessment), specimens may be need to be dissected and organs such as kidneys, livers, stomachs, etc. removed and preserved using appropriate procedures prior to tissue homogenization. Frozen tissue is homogenized with dry ice using a variable speed laboratory blender. After homogenization is complete, the whole contents of the blender (tissue and dry ice) are transferred to clean jars and the dry ice is allowed to sublime in a freezer below -10 degrees Centigrade (ºC). Homogenization of larger animal mass is carried out in several steps.

Between samples, each homogenizing blender must be decontaminated and disinfected to prevent contaminant carry-over from one sample to the next. To do this, blender parts should first be placed in a small bucket or container filled with a dilute (5 percent [%]) solution of hospital-grade Lysol brand disinfectant or hypochlorite bleach and left to soak under a fume hood for 10 minutes. The bucket can then be carried to a designated sink, the blender parts placed in the sink, and the disinfectant solution returned to the hood. Cleaning and chemical disinfection of blender parts can then be continued in the sink. When the soaking solution becomes dirty from blender debris it should be flushed down the drain with the addition of plenty of tap water and a fresh disinfectant solution should be prepared. After the blender parts have been disinfected, they should be decontaminated as specified in ERT SOP, *Sampling Equipment Decontamination* and the site-specific QAPP.

Tissue samples for residue analysis should be frozen and shipped using dry ice. Tissue samples used for histopathological analysis should be fixed in 10% neutral buffered formalin (37-40% formaldehyde), with the exception of male reproductive organs, which should be fixed with Bouin's fluid.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

The appropriate federal or state agency should be contacted to determine if threatened or endangered species have been recorded on or near the site. If so, only live trapping methods should be used to collect small mammals.

Small mammal populations can become depleted and community species composition can be altered if trapping is conducted for an extended time period. If populations become depleted, immigration into the trap area can occur and the resulting captures can include individuals not originally associated with the site. Thus, trapping should generally be limited to three or four consecutive nights.

Trapping methods may need to be modified based on regional or local factors, such as climate or interference by other animal species. For example, in some areas, ants may cause serious damage to bait or captured specimens. Predators, such as raccoons and foxes, can destroy trap lines and prey on captured animals. Extreme temperature conditions can affect survival of captured animals or alter tissue characteristics of both living and dead animals, biasing or preventing chemical and histopathological analyses. Under such conditions, trapping procedures may require special adjustments and the interval between trap checks should be shortened.

Statistical comparisons of body weight, organ weight, and other measures among areas of different contamination can be confounded by the age structure of the populations. It is important to ensure that comparisons are made within the same relative age and sex class. Some species show readily identifiable differences in fur coloration (pelage) that enable identification of age class in the field. For species in which age determination techniques are not described in the literature, eye lens weight and curves, body size and mass, tooth wear, and reproductive condition may be used to determine the relative age class (adult, sub-adult, or juvenile).

A single small mammal may not contain sufficient tissue mass for residue analysis. Individuals of the same species from locations within the same area of contamination may be composited for analysis. Multiple analyses of the same animal (e.g., metals and pesticides) may have to be prioritized if specimens do not provide sufficient tissue mass to conduct all of the required analyses. Percent moisture should always be included as an analytical parameter. If any contaminants of concern are lipophilic (e.g., polychlorinated biphenyls or dioxin), percent lipids should also be included in the analytical parameters.

5.0 EQUIPMENT/APPARATUS

5.1 Organizational and Safety Equipment

- Health and Safety Plan (HASP)
- UFP-QAPP
- Safety equipment (e.g., Tyvek, respirators with high-efficiency particulate air (HEPA) filters, surgical gloves, nitrile gloves, eye protection, first aid kit)
- Clipboard
- Maps & compass; GPS navigation/survey equipment
- Large container(s) or tubs for soaking traps

5.2 Trap Setting and Data Recording

- Digital Camera
- Data sheets
- Mylar Tape measure, 100-foot length
- Survey flags and flagging tape
- Waterproof markers
- Field log books
- Leather gloves
- Traps (live and/or kill)
- Bait (e.g., oats, peanut butter, mealworms, apple chunks, bacon grease)
- Cotton nestlets
- Animal field guides
- Plastic buckets, 5-gallon, with lids

5.3 Sample Preparation

- Surgical gloves
- Nitrile gloves
- Balance, top-loading (capable of weighing 0.01 grams)
- Wet ice
- Dry ice
- Ruler, 30-centimeter (cm)
- Small/large re-sealable plastic bags
- Dissecting kit, consisting of scalpels, forceps, probes, and needles
- Garbage bags
- Plastic sheeting to cover work surface
- Field-portable lights
- Aluminum foil
- Stainless steel trays
- Tables and chairs
- Duct tape

6.0 REAGENTS

The following is a list of reagents that may be required for small mammal sampling and processing depending on the scope of work outlined in the site-specific QAPP.

- Hypochlorite bleach or Lysol disinfecting solution
- 10% neutral buffered formalin (37% formaldehyde)
- Isotonic saline solution
- Bouin's fluid (fixative)

Decontamination solutions are specified in ERT SOP, *Sampling Equipment Decontamination* and the site-specific QAPP.

7.0 PROCEDURES

7.1 Office Preparation

A scientific collection permit may need to be obtained from the appropriate federal or state agency. This can often take as long as 45 days, so sufficient time must be allowed for permits to be obtained if needed prior to collecting samples. Most states have permit information available on the Internet. A natural heritage search for threatened or endangered species should also be requested from the state. In addition, permission from the landowner must be received prior to trapping at the site or reference area.

If the target species are known prior to the field investigation, information should be assembled on their life histories, appropriate aging techniques, and trapping methods. If the target species are not known, a literature review of distribution patterns, habitat requirements, and general abundance of species likely inhabiting the region of the site should be conducted. This information may be used in conjunction with site data to predict the species most likely to be encountered and trapped on the site.

Pertinent background information such as topographic maps, soil survey maps, previous site reports, and aerial photographs should be reviewed. Analytical requirements, including tissue mass requirements, sample holding times, report limits (RLs) and method detection limits (MDLs) for each analysis should be determined before the sampling plan is prepared and included in the UFP-QAPP. These should be discussed with the Work Assignment Manager (WAM), quality assurance (QA) personnel, subcontract laboratories, and other personnel involved with the project. If possible, a preliminary site visit should be conducted prior to initiation of the sampling. A statistically designed sampling plan should be developed, depending on the nature of the investigation, to ensure that the data collected are unbiased and that a sufficient number of samples are collected to meet site-specific objectives. Consultation with an environmental statistician is highly recommended.

All equipment must be cleaned and decontaminated prior to shipment to the site. Traps should be cleaned with tap water and scrub brushes, disinfected with bleach, and then allowed to thoroughly dry prior to packing. Detergents should not be used for cleaning traps. All traps should be inspected, the sensitivity of the trap mechanisms adjusted, and any necessary repairs made prior to shipment to the site. Traps should be handled minimally and using nitrile gloves to minimize scent.

Wooden snap traps, such as Museum Specials, rat traps, and mouse traps are prone to warping when they wick moisture from soil, absorb morning dew, or become wet from rain. When traps warp, they may trigger on their own, or they may not trigger at all. To prevent this, all wooden traps should be waterproofed with paraffin wax. Paraffin should be melted in a suitable container (e.g., an aluminum pan), using a suitable heat source (e.g., a hot plate), with sufficient ventilation (e.g., a fume hood). Since paraffin is flammable, an open flame must not be used, and the paraffin must never be left on the burner unattended. The paraffin will melt at 37 degrees centigrade (ºC). When the paraffin is completely melted, traps should be dipped briefly, and allowed to drip back into the paraffin. If the wax is not hot enough, the trap will get a thick coat of wax which will not penetrate the wood, and may flake off during use. When the paraffin is hot enough, the wood will be infused with wax, with very little wax coating the trap. The traps should be hung up to cool and dry overnight.

An approved site-specific health and safety plan (HASP) is required prior to fieldwork. The HASP should detail the appropriate precautions to prevent exposure to mammal or ectoparasite carried diseases (e.g., hantavirus, rabies, Lyme disease, and Rocky Mountain spotted fever).

Based on the results of a preliminary site visit (see below), the sampling design for the actual study should be developed, reviewed, and discussed in advance by the ERT WAM or their contractor's Task Leader (TL), project statistician, and analytical laboratory representative. This ensures that the number of specimens collected and location of trap grids are consistent with analytical requirements. This also ensures that the small mammal sampling is coordinated with other objectives of the study (e.g., soil or vegetation sampling).

7.2 Field Preparation and Preliminary Site Visit

Local suppliers of field supplies (e.g., wet and dry ice) and drop-off points for express courier services should be determined. Courier services should be contacted to confirm shipping requirements and potential restrictions for equipment and samples, since not all locations ship hazardous materials or provide overnight delivery service.

A general site survey should be conducted in accordance with HASP requirements. On-site sampling areas and a reference area should be identified. The habitat within the reference area must be similar to the site, yet outside of site influences or impacts. For example, if on-site trapping takes place in a red maple wetland, then a similar red maple wetland should ideally be selected as a reference area. If habitat can not be precisely matched between the on-site sampling areas and the reference area, a reference area with a different habitat type that contains the same target species as collected on-site should be used.

A preliminary site visit should be conducted before the actual fieldwork begins to obtain data on potential target species. Target species and sample design, including level of sampling effort, should be based on the results of the preliminary site visit. If possible, a variety of traps should be utilized during the initial site visit to determine the species present and most effective trapping technique. The area of the site, the diversity of habitat, and the trapping success should help to determine the number of trap nights to use during the actual sampling period. It is important to note that during the preliminary site visit, a trapping effort that is too extensive or performed too close in time to the actual study may potentially deplete small populations and affect the study. In areas of lesser habitat quality, sampling could deplete local populations. Therefore, live traps should be used whenever possible. As an alternative, traps can be set in areas outside the primary focus of the study, such as the site periphery, to minimize the level of disturbance to vegetation in the area. It is often, however, not practical or possible to set test traps in advance of the actual trapping effort.

7.3 Collection of Specimens

7.3.1 Determination of Trapping Method

The number and type of traps and the number of trap nights should be determined according to the study objectives. If those objectives include histopathological analysis, live trapping should be conducted. This is because tissue characteristics are less likely to change in a live animal than in a specimen that has been dead for several hours before collection. Alternative trapping methods such as snap trapping may be used for studies that do not require histopathological analysis or as a supplement to live trapping, especially if live trapping success is low.

The types of traps used should be appropriate for the target species. This can be determined by a literature review and previous experience. Several trapping techniques may be

employed if a variety of species are to be investigated, or if information on species diversity or community composition is required.

Once the trap types and target species are selected, the method of trap placement is determined. The habitat present, the selected target species, and the study objectives may affect the determination of the trapping method to be used. Typically either a grid, pace line, or sign method is used (DeBlase and Martin 1981) (Figure 1, Appendix B).

Grid Method

Grids consist of a series of parallel trap lines spaced at a set distance apart, with each line having the same number of traps (Figure 2, Appendix B). Traps are typically placed 10 meters (m) apart along the line but the distance between trap lines and traps may vary considerably (generally from three to 20-m between grid lines and traps) depending upon the species present, the habitat, and the type of study. Traps are placed in the best available spot (e.g., under a bush or next to a log) within about a 2 m distance of the grid node. Grids are best suited for mark and recapture studies (e.g., population studies) or where unbiased sampling is required.

Pace line Method

The pace line method places traps at set distances along a single trap line (Figure 1, Appendix B). The beginning and end of the trap line and every trap should be marked with flagging tape. This method is most useful for trapping along edge habitat or on sites with fragmented habitat where a grid cannot be established.

Sign Method

This method places traps at locations most likely to catch animals based on animal sign and microhabitat (Figure 1, Appendix B). It is biased towards trapping species that have conspicuous signs (e.g., readily observable burrows and runways). It therefore should be used when targeting specific species as opposed to taking an unbiased sample for determining community composition. The sign method typically provides the greatest trap success, but it is also the most time consuming to set. Since the traps are not placed at set distances apart, it is important to mark the location of each trap with a flag or tape. Depending on the habitat, additional notations in a field notebook or using a GPS to record trap locations may be necessary.

7.3.2 Sampling Effort

The sampling effort should be based on the size of the site and the number of animals required to meet the study objectives. For most small mammal investigations, three trap nights are sufficient to capture the required number of animals. However, the effort may be adjusted during the study as needed. When comparing areas (e.g., the on-site area compared to a reference area), an attempt should be made for equal trap success among areas to facilitate data analysis and interpretation. If the areas compared are of similar, relatively homogenous habitat, this may be achieved by expending equal trap effort per area. Additional trapping effort may be required in areas containing less than optimal habitats or otherwise low small mammal populations. Trap effort will need to be considered as a variable if community composition is being compared among areas.

7.3.3 Trap Placement and Marking

Upon arrival at the site, the traps are counted and placed in 5-gallon buckets, and the number of traps in each bucket are written on a piece of duct tape attached to the bucket handle. This is important for maintaining a trap inventory and ensuring that the correct number of traps are set and retrieved.

Trap areas are established in habitat suitable for the target species. Depending on the accuracy required, a measuring tape may be used, or points can be surveyed using surveying equipment or a GPS navigation and survey equipment. The start and end of each grid line or trap line is marked with a survey flag and/or length of conspicuous flagging tape tied to a branch at eye level. The flag or flagging should be labeled with the trap area, trap line, and trap number, using a thick waterproof marker. In heavily vegetated areas, individual trap locations may also be marked with a labeled survey flag. This simplifies trap relocation and reduces habitat destruction during subsequent trap checks. At locations where a survey flag is used, the flag is placed at the grid node. Traps are set at the most appropriate location within about 2 m of the grid node. Flags are placed so that they do not impede an animal's progress toward the trap.

At the beginning of each trap line, a labeled flag and a trap is dropped. Thereafter the person paces a distance of 10 m (or the distance necessary to meet the site objectives) in a straight line, drops the next trap, and places a survey flag (if required based on the habitat). This procedure is repeated until all the traps are dropped. Each individual who is trapping is responsible for ensuring that the distance between traps is accurate by measuring his/her pace in advance. Once the line of traps has been dropped, the person walks along the trap line in the opposite direction to bait and set each trap. In trapping areas (e.g., old fields) where the potential for habitat destruction is high compared to the potential for loss of orientation while dropping traps, traps may be dropped and set on the same pass without the need to return and set the dropped traps. By adhering to these techniques the amount of habitat disturbed is minimized.

Each trap area must have a unique name for identification (e.g., Area I, Area II, Reference Area). Each trap line is assigned its own unique number or letter. Trap lines are numbered or lettered sequentially. Trap lines that are part of a grid should be numbered according to their location within the grid. Each individual trap along the trap line is also assigned a number, based on its position along the line. For example, if the trap line contains 10 traps, they are numbered from one to ten. Traps are numbered so that low numbers are consistently located toward one end of the trap line. For example, trap location number Area III-D-2 denotes the second trap along trap line D in Area III (Figure 2, Appendix B).

The location and orientation of each trap grid should be sketched in field logbooks and on a single "master copy" of a map or aerial photo of the site. The simpler the sampling design, the easier it is to locate and document successful captures and to pick up traps at the end of the study. If the number of traps differs among grids, this is noted in logbooks and on the map.

7.3.4 Trap Types and Trap Setting

Figure 3 (Appendix B) contains a diagram of the trap types most commonly used for small

mammal trapping. No single trap type captures all species, sexes or age classes within a community with equal probability (Smith *et al.* 1975). Based on the objectives of the study, it is important to use the most appropriate trap type. For example, it is appropriate to use Longworth traps to capture voles and pitfall traps for trapping of soricid shrews (DeBlase and Martin 1981). If the target species is known before the initiation of the study, a trap type that would optimize trapping efficiency of the target species while satisfying the project objectives (e.g., live traps for histopathology) is selected. Trap size should be appropriate for the target species. If fossorial (burrowing) species are being trapped, the diameter of the trap should be approximately the same as the burrow size. If non-fossorial species are being trapped, the traps should allow enough space for animals to move around (Animal Care and Use Committee of the American Society of Mammalogists 1998). If the target species is not known prior to the initiation of the study or the project objectives dictate a small mammal community census, then multiple trap types and sizes should be used Several traps of different types can be placed at each grid node. If multiple trap types are used, the same proportion of each trap type and size should be used at on-site areas and reference areas.

The time of day traps are set depends upon the species being targeted. If only nocturnal species are being sought, traps are set in the late afternoon/early evening. Traps should then be kept closed during the day to avoid capturing diurnal species.

Traps are baited at the time they are set. Bait is carried in a re-sealable plastic bags and dispensed as needed. It is generally more efficient if each person carries his/her own bait bag. The bait used should be appropriate for the species being trapped and the type of trap used. For most species trapped in snap traps, bait should consist of a mixture of approximately 50:50 peanut butter and rolled oats. The relative proportions of each can be modified to suit field conditions (e.g., use less peanut butter in warmer weather). Bait can vary under conditions and target species. If shrews are among the target species, the traps should be baited with 50% bacon fat or melted suet and 50% peanut butter mixed with rolled oats. If shrews are required exclusively, then the traps may be baited with 100% bacon fat or suet. During summer months, paraffin may be added to the bacon fat to increase its melting point. Kill traps should be baited so that the bait does not fall off. Live traps are usually baited with a small amount (less than [<] 1 teaspoon) of rolled or crimped oats. A small piece of apple (1 x ½ inch) added to the trap often increases capture success and provides a source of moisture for trapped animals. Cotton nestlets may also be added as bedding material to increase survival during cool weather. If live shrews are required to meet the project objectives, an additional source of food (e.g., meal worms), as well as cotton bedding material, should be placed in the traps.

The technique of setting traps depends on the type of trap being set, although traps should always be set so that their release is not impeded by vegetation or other obstructions. Specific instructions for the most commonly used trap types are described next.

7.3.4.1 Museum Special Traps

Museum Special traps are snap traps designed to kill a small animal immediately. (Figure 3, Appendix B). A Museum Special trap that measures 5 ½ x 2 ¾ inches is generally used for mammals the size of mice and voles. It is set so that the pin is under the treadle toward the "fast" release end. This is generally located at the left side of the treadle.

Museum Special traps are set along trap lines, but individual traps are placed in areas most likely to be used by small mammals. Some species, such as voles, leave visible runways in grassy habitats. These runways typically are associated with high trap success. Runways or other animal paths should be inspected carefully for evidence of fresh cuttings, feces, or other signs of animal activity. Traps are placed accordingly to maximize trap success. Traps are seldom set in open areas, since small mammals usually avoid these areas due to the increased likelihood of predation. In some habitats, such as deserts or mine waste, this may be unavoidable. Nevertheless, success can still be increased by placing traps along fallen logs, large roots, or in brushy areas. However, traps should be placed so that the release is not impeded by vegetation. Care should also be taken to set individual traps within 2 m of the trap line to keep the trap line straight.

7.3.4.2 Mouse Traps

Mouse traps are similar to Museum Special traps (Figure 3, Appendix B), but are smaller in size (4 x 1 7/8 inches). Mouse traps are more suitable for smaller species (e.g., smaller mice or soricid shrews). Several species (e.g., meadow voles) that can be caught in a Museum Special may be too large to be captured consistently in a mousetrap. Unlike the Museum Special, the speed of the release mechanism is generally not adjustable by treadle placement. However, bending the trap pin slightly so that it releases from the treadle more easily can increase the sensitivity of the release. Mouse traps are placed in the same manner as Museum Specials.

7.3.4.3 Rat Traps

Rat traps are also similar to Museum Special traps (Figure 3, Appendix B), but they are larger in size (6 x 3 inches). Rat traps are more suitable for larger species of rodents (e.g., rats, chipmunks, and squirrels): smaller mammals (e.g., voles) can be trapped in rat traps but the trap success is reduced and the strength of the spring on the trap frequently destroys or damages the specimens. The speed of the release mechanism is generally not adjustable by treadle placement. However, bending the trap pin so that it releases from the treadle more easily can increase the sensitivity of the release. Rat traps are placed in the same manner as Museum Specials.

7.3.4.4 Sherman Traps

Sherman traps are lightweight aluminum box traps (Figure 3, Appendix B). They are available in several sizes and designed to capture animals alive. These traps are appropriate for capturing animals to be used for histopathological analysis, since postmortem autolysis of tissue is avoided. Sherman traps are also useful in preliminary studies designed to determine which species are present because animals may be released and local populations are not affected. Sherman traps are collapsible and easy to transport.

When setting Sherman traps, it is essential to check the effectiveness of the release mechanism by experimentally tripping the trap. The sensitivity of the release mechanism should be adjusted so that the trap releases easily if an animal

weighing approximately 10 grams (g) or more enters the trap. In practice, a light hand tap on the trap should trigger the release. To adjust the release mechanism, push down or back on the tab holding the "front panel" of the trap to the floor. Sherman traps should be cleaned regularly to ensure that no bait or other material becomes lodged under the panel or near the release mechanism, thereby inhibiting the ability of the trap to release.

Sherman traps are set so that the open end is facing the direction from which an animal is most likely to be traveling. For instance, if a trap is set near an opening within a tree stump, the open end of the trap should face the opening in the stump. Sherman traps are effective at catching a variety of species, including mice, voles, and chipmunks. Animals that burrow are especially prone to entering box traps if properly baited and set.

7.3.4.5 Longworth Traps

Longworth traps are best utilized for trapping soricid shrews (Figure 3, Appendix B). They are similar to Sherman traps, but consist of 2 parts, a tunnel through which an animal enters and a larger box where the animal is then confined. They are set by hooking the front (smaller) box into the larger box and securing the entrance door open. The sensitivity of the release can be modified slightly by bending the door pin.

7.3.4.6 Havahart traps

Havahart traps are live traps constructed of steel mesh (Figure 3, Appendix B). Like Sherman traps, they are available in a variety of sizes. Size 0 traps are generally used for mice and voles, while larger sizes (1, 2, 3) are used for rats and other mammals. Havahart traps are not collapsible, and are more difficult to set than Sherman traps or other box traps. However, they may be effective for live trapping some species that avoid entering Sherman traps if properly set. Havahart traps set in runways do not have to be baited. Care should be taken to ensure that the traps release effectively in the vegetation where they are set.

As with Sherman traps, the effectiveness of the release mechanism of Havahart traps should always be tested before the traps are set in the field. This is done after the traps are transported to the site, since in transport the sides of the trap may bend inward, resulting in only partial closure of the trap doors. The speed of the release can be adjusted by placing a rubber band along the upper end of the set pin, and extending it to the door latch. This may be done to both doors of the trap.

7.3.4.7 Tomahawk Traps

Tomahawk traps are also designed for live trapping of animals using bait. They are constructed of steel mesh, similar to Havahart traps, and are open on only one end. The release mechanism is not adjustable. When an animal trips the release, the door falls, capturing the animal. These traps are generally used for animals the size of chipmunks or rats. Spacing of Tomahawks is based on sampling requirements and the expected population densities of the target species.

7.3.4.8 Conibear Traps

Conibear traps come in a variety of sizes and are used for kill-trapping larger mammals (e.g., muskrat and mink) than the traps previously described (Figure 3, Appendix B). These traps are not usually baited but are placed along trails, runways, or other areas the targeted species frequent. When setting the traps, it is important to minimize human odor on the traps by wearing clean gloves. Scent bait may also be used to increase trap success. Unlike the traps discussed previously, Conibear traps are never set in grids but are set in trap lines following a stream or drainage. Conibear traps are illegal or restricted in several states, so state laws must be researched prior to use of these traps.

7.3.4.9 Pitfall Traps

If soricid shrews are included as a target species, or if the site objectives dictate an accurate estimate of the small mammal community composition, pitfall traps may be used. This trapping method requires extensive setup time and effort, and therefore may not be ideal for short-term investigations (one trapping period). It is ideally suited for long-term investigations and for studies where trapping is conducted over a number of trapping periods. Pitfalls should be used as kill-traps only when no other method will work (Animal Care and Use Committee of the American Society of Mammalogists 1998). If live trapping, the pits should contain food and nesting material. A small (pencil-width sized) hole should be drilled into the bottom to facilitate water drainage. During periods of actual or predicted heavy rainfall, pitfall traps should not be used for live trapping. Suitable for most shrew species, and the easiest to set, are small cans (e.g., coffee cans) set into holes made with a post-hole digger or shovel. Pitfall traps are often set in arrays interconnected with drift or silt fencing. The arrangement of traps and the optimum use of fencing may vary with the study objectives. Handley and Kalko (1993) present a review of the applications of different pitfall configurations.

7.3.5 Trap Checks

All personnel performing trap checks must wear appropriate personal protective equipment. This may include surgical gloves and an exterior pair of leather or thick rubber gloves (to prevent the interior gloves from getting torn on the sharp surfaces of the traps) and, if necessary, half face respirators fitted with HEPA filters. When checking traps in dry or dusty conditions, full-face respirators with HEPA filters (or half-face respirators with appropriate eye protection) will be worn, along with disposable coveralls (e.g., tyvek).

The species being trapped and weather conditions dictate the number of daily trap checks required to minimize stress to live animals and prevent damage to dead specimens from cold, heat, or scavengers. Generally, two checks per day are sufficient. Trap checks are ideally conducted as soon after dawn (less than two hours) as possible, and again in the late afternoon/early evening. If trapping for live shrews, traps should be checked every 4 to 6 hours and more frequently in cool or damp weather. For diurnal species in warm weather, traps should be checked approximately every two hours (Animal Care and Use Committee of the American Society for Mammalogists 1998).

Each two-person team arrives with a small cooler containing wet ice and a 5-gallon plastic bucket containing replacement traps. Each person carries marking pens, re-sealable plastic bags for specimens, and fresh bait for re-baiting traps. One person from each team should be responsible for field documentation of trap line records. That person notes the trap number and/or site of capture in a field log book. If a trap appears to have been visited but no small mammal is present (e.g., if bait has been eaten, urine or droppings are visible, or trap has been sprung), the trap is re-baited and reset.

Animals caught live are generally euthanized in the field by cervical dislocation. Animals may also be euthanized by asphyxiation with a chemical inhalant (e.g., CO_2). Cervical dislocation and chemical inhalants meet the criteria of the U.S.D.A. Animal and Plant Health Inspection Service for methods of euthanization for small mammals. Dead animals and animals not euthanized at the site of capture are transferred to individual sealable plastic bags labeled with the trap location number, genus, species, date, time of day, and collector's affiliation and initials. This information is recorded in a field logbook as well. Bags containing dead animals are placed on ice for transfer to the laboratory or processing area and the traps are re-baited and reset.

Upon completion of the study, traps are tallied as they are removed from the trap line. The number of traps in a bucket is again written on a piece of duct tape attached to the bucket handle before leaving the site. An attempt should be made to locate missing traps. Any damaged traps are marked so that repairs can be made prior to the next assignment. All traps used must be properly disinfected before being reused.

7.3.6 Trap Disinfection

To disinfect traps, at least one set of three 5-gallon buckets are set up in the designated small mammal processing area. One bucket is filled with dilute (5%) hospital-grade Lysol or hypochlorite bleach solution for disinfection and the other two are filled with tap water for rinsing. Traps are completely immersed in the disinfectant solution. Each Sherman trap should have a hinge pin removed to allow the trap to be opened flat, so that all surfaces come in contact with the disinfectant. Any visible dirt, fecal material, nesting material, or bait must be scrubbed off with a brush and the traps should be left to soak in the disinfectant for at least 10 minutes. After soaking, the traps are dipped in the first and then the second bucket of rinse water, and set out to dry. When the disinfectant solution or rinse water baths become dirty with debris from the traps, the liquid is disposed of properly, and new baths are prepared. All waste material from small mammal activities, including used paper towels, gloves, disposable coveralls, re-sealable plastic bags, table coverings, gauze, etc. are placed in a plastic bag. When processing is complete, bags should be tied or taped shut and disposed of properly and all work surfaces and equipment within the small mammal processing area are wiped down with a dilute 5% hospital-grade Lysol solution or a solution of 1% hypochlorite bleach.

7.3.7 Regional and Local Considerations

Factors such as climate and weather, habitat, and community composition need to be considered when trapping small mammals. Live animals may overheat, suffer hypothermia, or become otherwise stressed from capture, causing them to use fat reserves. Extreme temperature conditions can also alter tissue characteristics of both living and dead animals, making tissue unsuitable for analysis. Exposure of dead specimens to extreme

cold can freeze tissue, making histopathological analysis difficult. Exposure to extreme heat can result in rapid tissue decomposition and possibly impact tissue physiology, which could bias both chemical and histopathological analyses. Under such conditions, the interval between trap checks should be shortened.

Additional procedures may need to be followed to increase trapping success and/or survival rates of captured animals. For example, in hot, arid regions, a slice of apple can be added to traps and traps should be placed under cover to protect the animals from hot sunlight whenever possible. In cool or damp weather, cotton bedding material should be added; nestlets are particularly handy for field use (note, however, that cotton nesting material is not recommended for use during heavy rainfall). In the southeastern U.S., fire ants can rapidly consume a dead specimen. Live trapping may have to be used to reduce lost specimens, and oily bait such as peanut butter should be avoided when possible (this can sometimes also reduce damage to traps by other non-target species, such as raccoons). Alternatively, bait can be modified to prevent loss to ants by using cotton soaked with a mixture of peanut butter and water and rolled into balls (Atkinson 1997). Depending on the contaminants being investigated, an appropriate insecticide, may be sprayed in a circle about 0.5 m around the traps. This will decrease the loss of samples to fire ants without affecting trapping success (Mitchell *et al.* 1996). Insecticides must be used with caution and the applicator must take adequate protection to avoid exposure. Insecticides should not be used in sensitive habitats or where insecticides are a potential COC. Weather can also affect trap success; many small mammals stay in their burrows on moonlit nights to avoid exposure to predators, and heavy rain can affect small mammal foraging patterns. Under these conditions, additional trap nights may be needed to compensate for a decrease in animal activity and to obtain sufficient specimens for analysis.

7.3.8 Sample Processing

Processing should take place as soon after trap checks as possible to reduce potential degradation of the specimens. Live animals are euthanized, generally by cervical dislocation or asphyxiation with CO_2 or other inhalant for processing. Dead specimens are removed from the traps and transferred immediately to a re-sealable bag (one specimen per bag) labeled with the trap location number, genus, species, collector's initials, date, and time of day. Animals are removed from traps one at a time so that specimens are not mislabeled. The bags should be stored on wet ice in a small cooler for transport to the processing area or laboratory. Each animal is kept in its corresponding bag whenever possible to avoid mixing up sample information.

All personnel within the small mammal processing area should don safety gear as specified in the site-specific HASP. This may include disposable boot covers, disposable coveralls, and a full-face respirator equipped with a HEPA filter (or a half-faced respirator and eye protection). Only after all employees in the processing area are wearing the proper protective equipment are bags opened and animals taken out for identification and processing. After processing, all samples are placed in double containers (e.g., a sample jar inside a sealed ziplock bag or a sealed ziplock bag inside a second sealed ziplock bag). One person should be designated as “clean” and thus be able to assist the animal processors in packaging the animals for shipment by performing activities such as labeling clean bags or sample jars, holding bags or sample jars open while samples are placed inside, and placing packaged samples in the shipping coolers. This ensures that the outer bags and coolers are not contaminated when the samples arrive at their destination.

Tissue samples for residue analysis should be frozen using dry ice. Thick cotton or leather gloves should be worn when handling dry ice since it can cause serious skin burns. If tissue samples are to be shipped using dry ice, they should be thoroughly frozen prior to shipping and enough dry ice should be added to the shipping cooler to keep all samples frozen until they arrive at the laboratory. Dry ice is considered a dangerous good for air transport and requires special handling. Shippers are also required to have dangerous goods training. The design and construction of packaging used for dry ice shipments must prevent the buildup of pressure that could cause rupturing and packaging must comply with U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA) dangerous goods regulations. The quantity of the dry ice must be listed on the air bill.

All small mammal handling, processing, and homogenizing that take place in the laboratory must be done under a hood designed for protection against biological hazards. This is to prevent risk of exposure to hantavirus, a rare but potentially deadly disease, which may be transmitted from small mammal carriers. Two layers of chemical resistant surgical gloves or one layer of surgical gloves and one layer of thick nitrile gloves should be worn during processing. Each person should work with only one animal at a time. The bag in which an animal is contained should be placed within the hood prior to being opened. After processing, samples to be analyzed are placed in a container, which in turn is placed within an outer re-sealable plastic bag. Small mammal body parts or samples should not be removed from the hood prior to being placed in double sealed sample containers.

Tissue samples used for histopathological analysis should be fixed in 10% neutral buffered formalin (37% formaldehyde), with the exception of male reproductive organs, which should be fixed with Bouin's fluid. These solutions are carcinogenic and should be handled with caution as detailed on their respective safety data sheets (SDS). Tissue samples fixed in Bouin's fluid should be transferred to 10% neutral buffered formalin solution after 10 days.

8.0 CALCULATIONS

The calculations that can be made and conclusions that can be drawn from data acquired through small mammal sampling will depend upon the sampling plan. A statistical sampling plan should be designed according to the study objectives and in such a way as to ensure that the data collected are unbiased and that a sufficient number of samples are collected for appropriate and pre-determined statistical analyses. The actual number of samples needed to make statistically robust comparisons will be site and objective specific. Consultation with an experienced statistician is highly recommended.

Several calculations may be performed to examine the differences between the site and the reference area. Trap success is calculated by dividing the number of captures by the number of trap nights. Trap nights are calculated by multiplying the number of traps by the number of nights of trapping (e.g., 100 traps and 3 nights of trapping would equal 300 trap nights). Relative abundance should also be calculated for each species trapped during a given trapping period. This is done by dividing the number of each species trapped by the total number of animals caught during the trapping period. For example, if 12 masked shrews, 6 white-footed mice, and 6 short-tail shrews are caught during a study, the relative abundance of each species is 50%, 25%, and 25%, respectively.

Species diversity (e.g., Shannon Index of Diversity) may be calculated among trapping areas (Zar 1984). Trap effort should be equal among areas for comparisons to be valid. If similar habitats and habitat quality

exist between areas, the differences in species diversity and evenness at varying contaminant levels areas may indicate site impacts on terrestrial communities.

Statistical comparisons in species composition or population age structure, reproductive characteristics, or size may also be made between on-site areas and the reference area(s). Comparisons of body weight among areas and other similar comparisons require knowledge of the age of each specimen collection; thus, good age determination techniques are an essential prerequisite for such analyses.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

There are no specific QA activities which apply to the implementation of these procedures. However, the following general QA procedures apply:

1. All small mammal specimens must be documented in accordance with ERT SOP, *Sample Documentation*, and chain of custody records shall be completed according to ERT SOP, *Chain of Custody Procedures*.

2. A specimen data sheet (Appendix A) must be filled out for each specimen. Each specimen must be kept in its own re-sealable plastic bag, on which is written the trap location number, genus and species, date, and collector's affiliation and initials.

3. A bound field logbook must be maintained by field personnel to record daily activities. Separate entries should be made for each trap grid checked. Information recorded should include the total number of animals trapped, species trapped, weather conditions, and habitat. Field activities should be photo documented as well. The logbook must be maintained in accordance with ERT SOP, *Logbook Documentation*.

4. Records must be maintained, documenting the training of the operators that use instrumentation and equipment for the collection of environmental information.

10.0 DATA VALIDATION

Data verification (completeness checks) must be conducted to ensure that all data inputs are present for ensuring the availability of sufficient information. These data are essential to provide an accurate and complete final deliverable. The ERT contractor's TL is responsible for completing the UFP-QAPP verification checklist for each project.

11.0 HEALTH AND SAFETY

Based on Occupational Safety and Health Administration (OSHA) requirements, a site-specific HASP must be prepared for response operations under the Hazardous Waste Operations and Emergency Response (HAZWOPER) standard, 29 CFR 1910.120. Field personnel working for EPA's ERT should consult the Emergency Responder Health and Safety Manual for the development of the HASP, required personal protective equipment (PPE) and respiratory protection currently located at:
https://response.epa.gov/_HealthSafetyManual/manual-index.htm

According to the Centers for Disease Control and Prevention (CDCP), several species of small mammals have been found to carry and potentially transmit several related hantavirus to humans. Although relatively rare the disease can be deadly and is therefore of high importance. This disease is most common in the southwestern and western USA although it could potentially be found anywhere and it exists even outside

the USA. It generally spreads quicker at times when small mammal density is high. Details of the hantavirus may be found on the CDC website: https://www.cdc.gov/hantavirus/index.html. A Hantavirus Hotline has also been set up and may be reached toll free at (877) 232-3322. As of December 2018, there have been 754 cases of confirmed hantavirus. This virus can cause hantavirus pulmonary syndrome (HPS), which has been fatal to a high percentage (average of 36% as of December 2013) of exposed individuals including those whom were otherwise in peak health prior to exposure. Personnel who plan to trap, handle, process, or otherwise be involved in any activities related to small mammals should be educated about the inherent risks of such activities, as well as ways to minimize those risks.

During summer months, small mammals often carry external parasites such as ticks and fleas, which may potentially transmit diseases such as Lyme disease, Rocky Mountain Spotted Fever, or Plague. When residue analyses are being performed on the species being collected, insect repellant may not be used, if it will interfere with any of the site contaminants of concern. Personnel should carefully inspect their clothing and wear full body Tyvek when appropriate to avoid the possibility of infection by insect bites. In addition, all employees working with live animals should have a tetanus vaccination. If the potential exists for trapping animals that may be carriers of the rabies virus, the appropriate precautions should be taken, including vaccination against this virus. Because both hantavirus and rabies have the potential to be fatal to individuals exposed to them, the appropriate risk reduction/elimination measures should be included in the site HASP.

A limited number of people should be assigned to trap, handle and process small mammals. An area away from and downwind of human traffic, vehicles, equipment, and any domestic animals (including livestock) should be designated as a small mammal processing area. This area should only be entered by the personnel assigned to trap and handle small mammals. Food and drinking water should not be allowed in the small mammal processing area.

When setting and checking traps, disinfecting equipment and during processing of small mammals in the field personnel should wear the appropriate safety gear as specified in the site HASP. Gear may include chemical-resistant surgical gloves underneath an exterior pair of leather or thick rubber gloves to prevent the interior gloves from getting torn on the sharp surfaces of the traps and respiratory protection (e.g., half-face or full face respirators with HEPA filters).

Personal protective clothing and equipment should be doffed by first removing the outer layer of gloves, which should be discarded (if leather) or disinfected (if rubber) with a Lysol or hypochlorite solution. Coveralls should be removed next, followed by boot covers. The inner gloves should be washed in a disinfecting solution, washed with soap and water, and then removed and discarded. The respirator should be removed last. Personnel should then thoroughly wash their bare hands with disinfectant soap and water as soon as possible, and again before eating.

As previously stated, all small mammal handling, processing, and homogenization that takes place in the laboratory must be performed under a hood designed for protection against biological hazards. Personnel should wear two layers of chemical-resistant surgical gloves or one layer of surgical gloves and one layer of thick nitrile gloves. Each person should work with only one specimen at a time. The sealed bag in which an animal is contained should be placed within the hood prior to opening it. After processing, samples to be analyzed should be packaged in an inner container (e.g., sample jar) placed within an outer sealable plastic bag. Under no circumstances should a small mammal, any of its organs or parts, or a sample originating from a small mammal be removed from the hood prior to being placed in a double sealed sample container.

12.0 REFERENCES

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13.0 APPENDICES

A - Small Mammal Sampling and Processing Data Sheet
B - Figures
C - Hantavirus Information

APPENDIX A
Small Mammal Sampling and Processing Data Sheet
SOP: ERT-PROC-2029-21
January 2021

Small Mammal Sampling and Processing Data Sheet

Site Name:_____ Location No.: _____Sample No.:_____

Collector _____ Date Collected: _____
Processor _____ Date Processed: _____

Genus/Species _____ Trap Type: _____ Live Dead (circle one)
Total(mm): _____ Tail (mm): _____ Hind Foot (mm): _____ Ear (mm): _____
Weight(g): _____ Partial Whole (circle one)

Ectoparasites: Y N _____ Saved Discarded (circle one)
Endoparasites: Y N _____ Saved Discarded (circle one

MALE **FEMALE**

Testicle Wt (g): L _____ R _____ Ovary Weight (g): L _____ R_____

L Testicle (mm): L _____ W _____ Left Ovary (mm): L _____ W _____
R Testicle (mm): L _____ W _____ Right Ovary (mm): L _____ W _____

Seminal Vesicle Small Large (circle one) Placental Scars L _____ R _____
Epididymis Conv. Not Conv. (circle one) Embryos (no.) L _____ R _____
Mammaries Small Large Lactating (circle one)
Vagina: Inactive Cornified Turgid Plugged (circle one)
Repr. Stage: Nulli Semi Multi (circle one)

Uterus w/ Ovaries (g) _____ w/o Ovaries (g) _____

ORGAN	**WEIGHT (g)**	**COMMENTS**
Liver	_____	_____
Spleen	_____	_____
Adrenal	L_____ R_____	_____
Kidney	L_____ R_____	_____
Thymus	_____	_____
_____	_____	_____
_____	_____	_____

Dorsal Pelage Color _____ Ventral Pelage Color _____ Side Pelage Color _____

Age Based on Sex Organs: Juvenile Subadult Adult (circle one)
Age Based on Body Size: Juvenile Subadult Adult (circle one)
Age Based on Pelage: Juvenile Subadult Adult (circle one)

Comments:

APPENDIX B
Figures
SOP: ERT-PROC-2029-21
January 2021

FIGURE 1. Example Trap Placement and Trapping Methods for Different Trap Types and Habitats

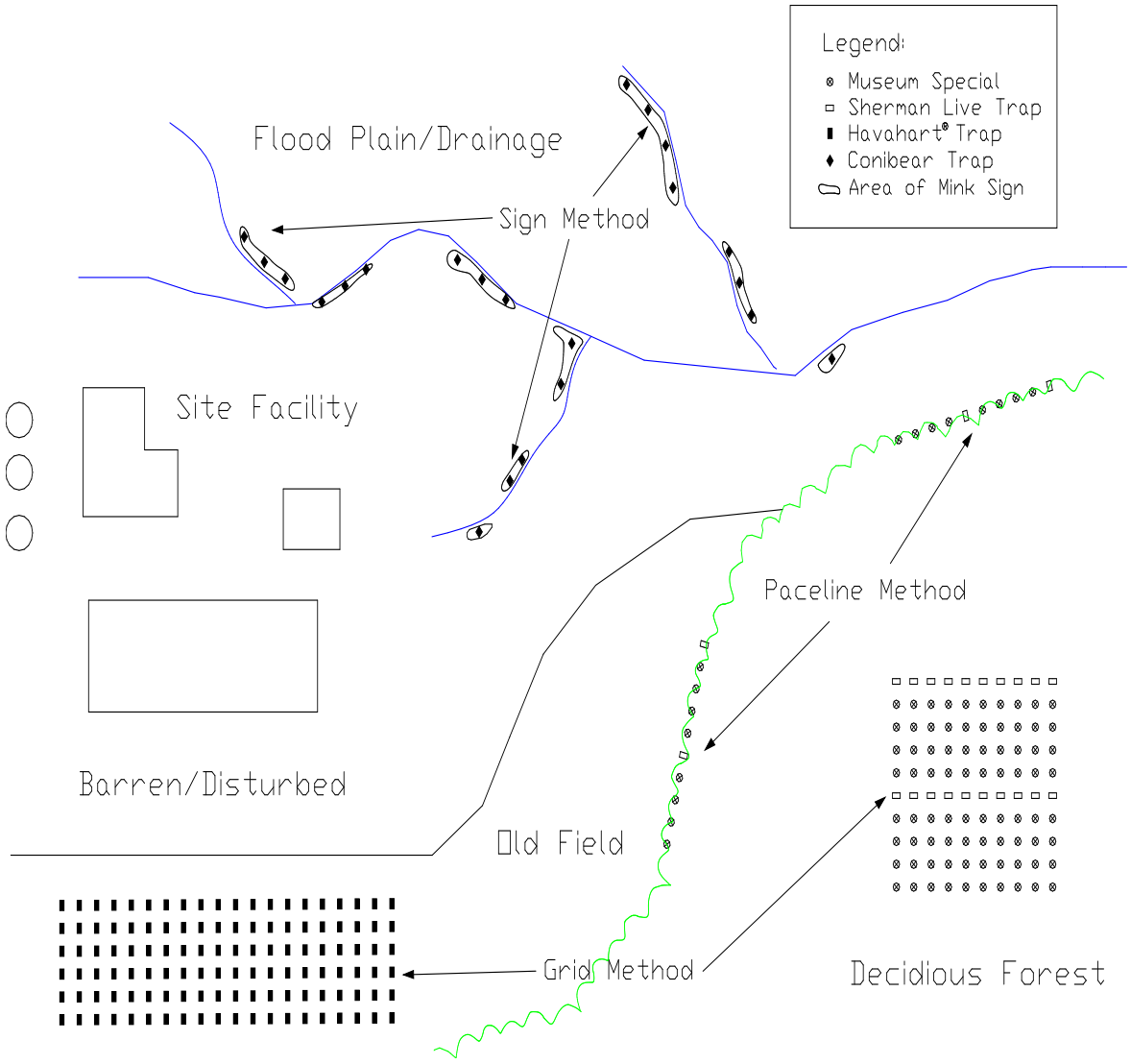


FIGURE 2. Typical Grid Layout and Identification of Trap Placement Using Grid Name, the Trap Line, and the Trap Number.

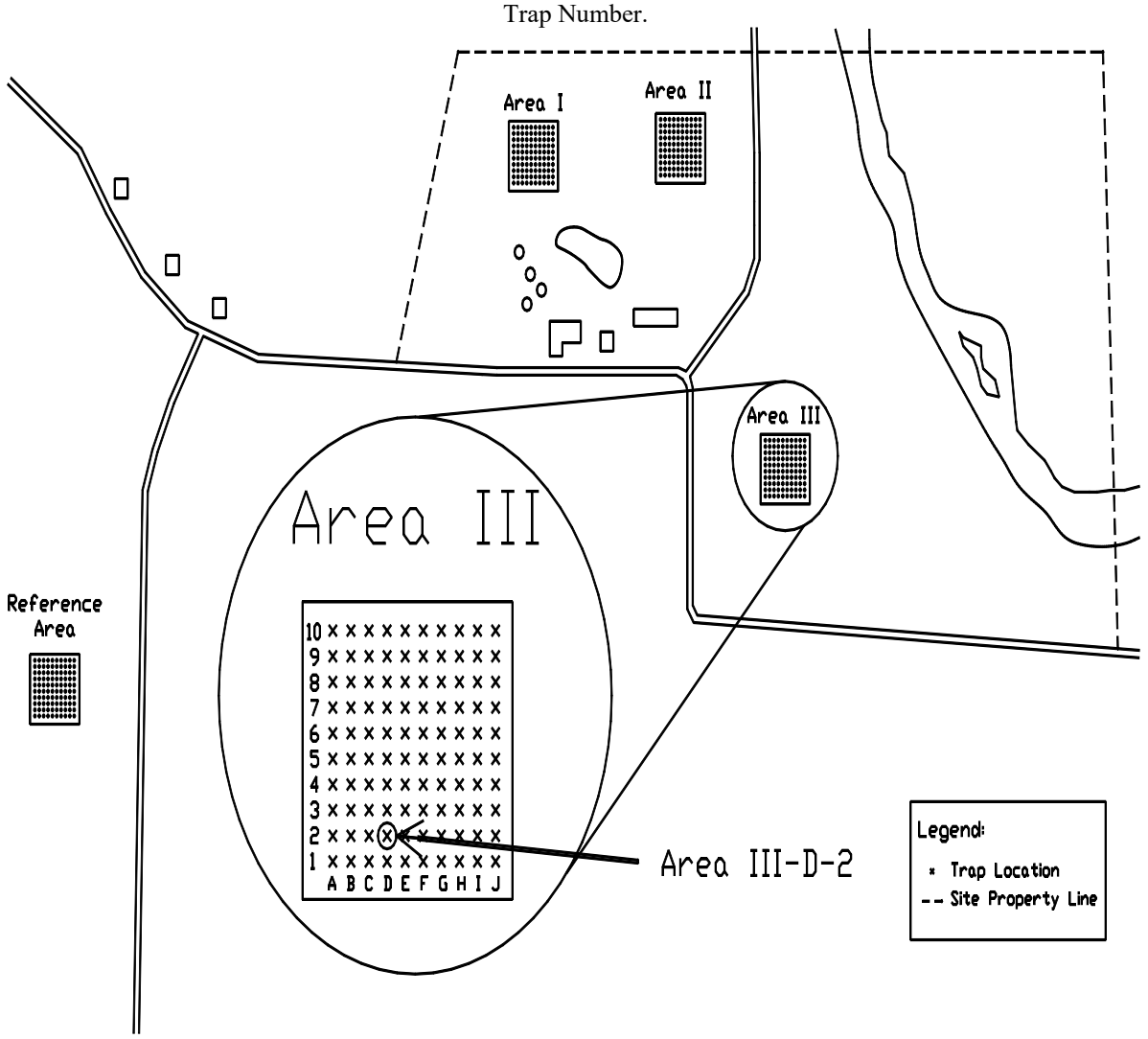
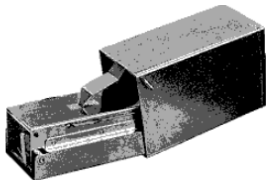


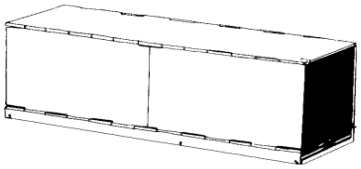
FIGURE 3. Comparison of Small Mammal Trap Types (DeBlase and Martin 1981)

Mousetrap



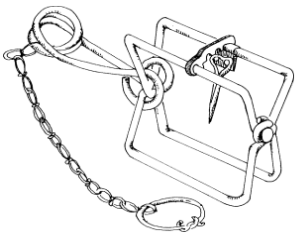
Longworth Trap

Museum Special

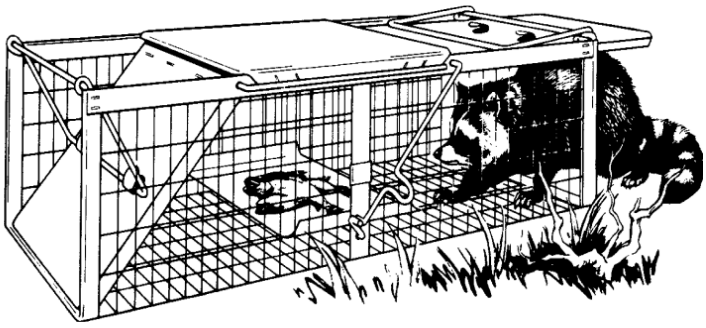


Sherman Live Trap

Rattrap



Conibear



Havahart Trap

APPENDIX C
Hantavirus Information
SOP: ERT-PROC-2029-21
January 2021

Hantavirus Information

Exposure Routes

Hantavirus can be transmitted through the urine, feces, saliva, and fresh organs of its rodent hosts. The primary exposure route for humans is via inhalation of aerosols or dusts contaminated with rodent urine, feces, saliva, or fresh tissue. However, the virus can also be introduced into the body via mucous membranes broken skin, and possibly by accidental ingestion with food or water. People may also be infected by being bitten by rodents (Mills *et al.* 1995).

Symptoms and Effects of Exposure

Although Hantavirus appears harmless to its rodent hosts, it can cause severe illness and often death in humans who have been infected by it. People who are infected develop initial symptoms of HPS within 1 to 6 weeks of initial exposure. Early symptoms often include a high fever (101 degrees Fahrenheit or above), muscle aches, headache, cough, abdominal pain, nausea, vomiting, and diarrhea. Symptoms do not include sore throat, runny nose, or watery eyes (Bradshaw 1994, Mills *et al.* 1995, Wlazelek 1998). Patients can also exhibit an increased heart rate and abnormal blood counts (Wlazelek 1998). Early symptoms are either accompanied or followed by shortness of breath, and, in approximately 50% of the cases, death ensues due to respiratory failure (Bradshaw 1994).

Respiratory failure is the result of leaking capillaries in the lungs causing the lungs to rapidly fill with blood. It can develop shortly after the onset of shortness of breath, sometimes in a matter of hours (Mandelbaum-Schmid 1993). Therefore, early detection of the disease, and thus early hospitalization, greatly increases the chances of survival. If a person who has been trapping, handling, dissecting, or otherwise coming in contact with small mammals' experiences symptoms within 45 days of potential exposure, they should seek medical attention immediately. The medical provider should be notified of the person's contact with small mammals and the possibility of hantavirus infection. Blood samples should be taken and sent through the state health department to the Center for Disease Control and Prevention (CDCP) to be tested for the Hantavirus antibody. If a person has difficulty breathing, he/she should be taken to the emergency room immediately and the hospital staff should be alerted of his/her potential exposure to hantavirus (CDCP 1996).