ALAT Training Manual Update

Chapter 14, Euthanasia, has been revised to incorporate the 2013 updates in the AVMA Guidelines for the Euthanasia of Animals. We are including the new version of this chapter to replace the one printed in the manual so you can study the most up-to-date resource on this topic.

If you have any questions about the ALAT Technician Certification exam, please email certification@aalah.org.

If you have any questions about this update or the contents of the manual, please email education@aalah.org

There are minor edits for other chapters. Please remember to check the Products Updates and Errata page for these updates and updates to other educational products: http://www.aalah.org/bookstore/errata.aspx

AALAS National Office
THE TERM “EUTHANASIA” IS DERIVED FROM Greek words eu, meaning “good,” and thanatos, meaning “death.” In the laboratory animal science field, euthanizing an animal means ending its life in a way that minimizes pain and distress. Another way to express the meaning of euthanasia is to give the animal a gentle death. Some common terms used to describe euthanasia are “put down,” “put to sleep,” or “sacrifice.” In the research setting, euthanasia of laboratory animals must meet both humane standards and the needs of the research study.

The Need for Euthanasia

In the research field, there are several reasons why animals may have to be euthanized. Many studies require that the animals’ organs and tissues be examined to obtain experimental data. Studies may make animals ill, so they are euthanized before they experience a significant amount of pain or distress. From an economic point of view, it is impossible to maintain large numbers of research animals once studies are concluded. In addition, laboratory animals may develop illnesses unrelated to the experimental treatment; and these may cause untreatable pain and suffering. These animals should be euthanized so that their suffering is not prolonged. It is the ethical responsibility of all personnel to ensure that the most humane standards are followed in performing euthanasia.

AVMA Guidelines on Euthanasia

The AVMA Guidelines for the Euthanasia of Animals, published on the AVMA’s website, is accepted by the research community as the standard for the euthanasia of animals.3 Animal care staff and researchers who perform, observe, or train others in euthanasia practices should familiarize themselves with the AVMA Guidelines for the Euthanasia of Animals and follow the recommendations carefully in order to treat the animals humanely. These guidelines take into consideration general animal welfare principles, the physiology and anatomy of each species, the characteristics of each method of euthanasia, and personnel safety. The euthanasia method chosen should produce a rapid loss of consciousness followed by a cessation of the heartbeat, respiration, and ultimately brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to the loss of consciousness. Most facilities have a policy to euthanize animals in an area apart from the colony in case sounds or smells generated by animals undergoing euthanasia cause distress to the other animals. Equipment used in euthanasia must be kept clean and be well maintained to ensure the animals are not subjected to unnecessary pain and distress. Regardless of the method of euthanasia, it is essential to confirm that the animal is dead before disposing of the carcass.

For each species covered, the AVMA Guidelines identify the euthanasia methods that are acceptable, acceptable with conditions, and unacceptable. These classifications are based on many factors, including whether the method consistently leads to a humane death for the animal and is safe for the individual performing the procedure.

Acceptable methods are preferred for use in animal research. Methods that are acceptable with conditions are appropriate for animal research provided certain conditions are met, such as weight limits on animals, specific personnel training. Adjunctive methods assure death after an animal has been made unconscious. Typically, these are physical methods such as exsanguination, decapitation, and pithing. All euthanasia methods must be specified in the animal use protocol and approved by the IACUC.
It is important to remember that the acceptability of a method is specific to the species and age of the animal. For example, some amphibians and reptiles can hold their breath for long periods, so inhaled anesthetics are usually not advisable for euthanasia of these species. Cervical dislocation is acceptable only for rodents weighing less than 200 g and other small mammals. Neonates of some species are resistant to carbon dioxide, so other methods are preferred for these animals.

All technicians should know what methods can be used with the species kept in their facility. In every situation, the method used must comply with the animal use protocol and facility SOPs, which are based on the AVMA guidelines. This document includes summary tables of information on the methods that are appropriate according to species.

**Methods of Euthanasia**

Euthanasia can be performed using chemical agents or physical methods. Responsible personnel must remain with the animals at the site where euthanasia is performed throughout the entire process. Having SOPs approved by the facility managers and veterinarians for each procedure ensures that proper and consistent techniques are used.

**Chemical Agents**

Chemical agents may be inhaled or injected. Aquatic species may be immersed in chemical baths.

**Gases for Inhalation**

Inhalant agents may be administered to animals individually or in groups, as in a euthanasia chamber. Euthanasia chambers must be an appropriate size for each species. Ideally, these chambers should be transparent so that the animal inside may be observed in case any problem arises in their reaction to the agent. Animals must not be overcrowded in the euthanasia chamber; facilities typically have SOPs specifying how many animals may be placed together. Chambers should be cleaned between groups of animals to minimize residual odors that may distress animals subsequently euthanized.

**Inhalant Anesthetics**

Inhalant agents are either compressed gases (typically carbon dioxide) or volatile anesthetics that are administered at a higher dose than is used to produce anesthesia. These liquids evaporate rapidly, generating a gas that induces anesthesia when inhaled. The animal breathes in the gas and loses consciousness, just as it would in preparation for a surgical procedure. The overdose of the anesthetic then causes the cessation of breathing and the heartbeat, and finally a cessation of brain activity. Halothane and isoflurane are examples of inhaled agents that produce rapid anesthesia and are highly effective for euthanasia of mammals.

Inhalant anesthetic may be used for euthanasia in the induction chamber (Figure 14.1) of an anesthesia machine. The animal is placed in the induction chamber, which is closed tightly, and the concentration of anesthetic is slowly increased past the level used for anesthesia. Your institution’s SOPs should specify the exact concentration and amount of time that the animals must remain exposed to the anesthetic. Waste gas is drawn into an approved waste system, where it can be absorbed or exhausted safely. This process is called waste gas scavenging.

Rodents can be euthanized by using an overdose of inhalant anesthetic in a bell jar, which is a clear glass or plastic chamber with a reservoir and wire mesh on the bottom. A cotton ball or other absorbent material is soaked with the anesthetic and placed below the grate, out of reach of the animals. Animals should be separated from the absorbent material because the anesthetic’s fast evaporation rate makes it cold to the touch, which would chill the animals’ paws and cause pain. This procedure must be conducted in a fume hood to protect the personnel from breathing the anesthetic vapor when the bell jar is opened to remove carcasses.

**Carbon Dioxide (CO₂)**

CO₂ is a gas that rapidly produces loss of consciousness when inhaled in a high concentration. It is one of the most commonly used chemical agents to euthanize rodents and other small mammals. CO₂ is an effective and inexpensive method of euthanasia for these animals, and it is reasonably safe for the operator. Animals inhale CO₂ and lose consciousness due to a reduced oxygen level in the brain and other tissues in the body (hypoxia). Eventually, they stop breathing and die. The flow rate should provide for a gradual displacement of 10% to 30% of the air inside the euthanasia chamber per minute. The CO₂ flow should be maintained for at least 1 minute after the animals cease to breathe, to assure death.

A compressed gas cylinder must be used as the source of the CO₂ gas. No other source of CO₂ is permitted. A low-pressure regulator must be used to control the flow of gas so that animals are not distressed by a turbulent inflow of gas. An example euthanasia apparatus is shown in Figure 14.1.

It is preferable for animals to be euthanized in their home cage or a dedicated chamber. The home cage can be placed within the euthanasia chamber, where the gas reaches the animals in their cage. Animals can also be placed directly into the chamber, although the chamber should not be overcrowded. The gas regulator is turned to a setting delivering gas at the rate of 10% to 30% of the chamber volume per
Euthanasia

minute. To fill a chamber of a 10-L capacity, for example, the flow rate should be 1–3 L per minute. After the animals become unconscious, the CO2 flow rate may be increased to hasten their death. The flow of CO2 should be maintained for at least 1 minute after the animals visibly stop breathing. When euthanizing more than 1 or several animals at a time, the euthanasia chamber should be cleared of CO2 after each animal or cage and kept clean to minimize odors that might cause distress in animals subsequently euthanized. Clearing out the CO2 between uses avoids having animals encounter a high level of CO2 when placed into the chamber.

Euthanizing mouse or rat neonates requires significantly longer exposure to CO2 than for adults. This is because neonates are resistant to the effects of hypoxia. Adequate time of CO2 exposure should be provided (for example, up to 50 minutes), or an adjunctive method (such as cervical dislocation or decapitation) should be performed.

Diethyl Ether

In the past, diethyl ether was used as both an inhalant anesthetic and euthanasia agent. It is now rarely found in animal facilities primarily due to safety issues associated with its storage and handling. Ether is unacceptable for use as a euthanasia agent.

Methoxyflurane

The FDA removed methoxyflurane (Penthrane®, Metofane®) from the US market due to problems with safety and effectiveness. This agent may cause irreversible toxicity to the liver and kidneys in humans and animals, which can be fatal. Methoxyflurane continues to be used in Australia, where it may be purchased by US laboratories, although it has been largely replaced in the US by other inhalants.

Chemicals for Injection or Immersion

Injectable euthanasia agents are generally administered intravenously and produce a rapid loss of consciousness followed by death. In rodents, these agents may be administered intraperitoneally. The use of injectable agents requires some skill on the part of the technician, especially for intravenous injection in the smaller species. These agents are typically solutions containing barbiturates, like pentobarbital, which have additional ingredients to help stop heart and brain function and provide local anesthetic effects. Most injectable euthanasia solutions are controlled substances because of the potential for human abuse. The federal government’s Drug Enforcement Administration (DEA) has strict requirements for handling, inventory control, and recordkeeping of these controlled substances.

Fish and aquatic amphibians can be euthanized by immersing them in a water bath containing an overdose of buffered MS-222 or other appropriate anesthetic. The anesthetic is absorbed through the gills of fish and the thin skin of the amphibians. An adjunctive method of euthanasia may be required, as is the case with Xenopus species.

The drugs in these euthanasia solutions remain chemically intact in the body after death and can kill scavenger animals that eat the carcass of a euthanized animal. Therefore, the remains of euthanized animals must be disposed of in ways that prevent their consumption by wild animals. Typically, carcasses are destroyed by incineration.

Physical Methods

Physical methods of euthanasia either destroy the brain or stop its functioning, thus killing the animal quickly. Physical methods may involve the use of equipment to apply a force or a cutting action with speed and precision, while safeguarding the operator.

One advantage of using a physical method of euthanasia is that it avoids contaminating the animal’s tissues with chemicals that could interfere with a study. Physical euthanasia methods are generally acceptable with conditions, and personnel using these methods must be trained and proficient in their use.

If properly done, physical methods are humane because the animal rapidly loses consciousness. However, as physical methods involve trauma, there are risks for both the animals and personnel. Animals may be distressed by the restraint necessary to position them in equipment, or they may be injured in a failed procedure. Personnel may be injured by a struggling animal or malfunctioning equipment. Even when carried out humanely, a physical method of euthanasia may cause emotional distress in onlookers. The proper functioning of the equipment is an important factor in determining
whether the method is humane for animals and safe for personnel. Knowledge and skill to restrain the animal and perform the procedure appropriately are essential to ensure that the animal rapidly loses consciousness and dies with minimal pain and distress.

Cervical dislocation is a physical method that involves the dislocation of the vertebrae in the neck, which is the cervical area of the spine. In cervical dislocation, the spinal cord is quickly separated from the skull, thus preventing transmission of nerve impulses in either direction between the brain and the body. When properly done, the animal quickly ceases to breathe and the heart stops beating. There may be muscle contractions continuing for some time, which causes motion of the limbs. However, since the body’s nervous system is disconnected from the brain, this motion is only a result of an automatic reflex and not the sensation of pain. Cervical dislocation is acceptable with conditions for mice, rats weighing less than 200 grams, rabbits, and some bird species.

Decapitation, another physical method, uses a special instrument called a guillotine to sever the head from the body. The person performing decapitation must be properly trained and comfortable with the technique. The guillotine must be cleaned between animals, and the blade must be kept sharpened. In neonatal rats and mice, a sharp blade, such as appropriately sized scissors or a scalpel, may be used.

Verification of Death

After euthanasia is performed, the death of the animals must be verified. For example, animals may stop breathing in a CO2 chamber, but they may not be dead. In these cases, the effect of the CO2 can wear off, allowing the animals to recover and wake up. Verification confirms death by making sure that the heart has completely stopped beating and respiration has ceased. For large animals, technicians may monitor the animal to verify the lack of a pulse, heartbeat, and breathing. In small animals, especially rodents, these vital signs are difficult to assess, making it necessary to use an adjunctive method to ensure that death has occurred. For example, cervical dislocation can be performed on rodents removed from a CO2 chamber to ensure that the animals are dead. Under the effects of prolonged high levels of CO2, a live animal would be unconscious and unable to feel any pain related to this second procedure. Another adjunctive method for rodents euthanized with CO2 is to open the chest cavity with scissors or a scalpel. This collapses the lungs and ensures death by preventing the resumption of breathing.

Learning to Perform Euthanasia

Because proper euthanasia technique is critical for animal welfare and occupational safety, individuals who perform euthanasia must be thoroughly trained in the methods used at their institution. This training includes the proper restraint and handling of the animals, the physiological processes involved with the euthanasia method, use and maintenance of equipment, handling and administration of euthanasia agents, verification of death in euthanized animals, and the method of carcass disposal. Practicing on animals that are either anesthetized or already dead helps technicians develop proficiency with any method.

Everyone must understand the emotional distress that can be experienced by the personnel involved in these procedures. Regardless of the reason, terminating the life of an animal can be a difficult task. Movements and vocalizations that are similar to those made by animals in distress may accompany even the most humane methods of ending an animal’s life. These involuntary reflexes of the unconscious animal may be uncomfortable for the technician to witness. It is normal to experience discomfort and anxiety when performing euthanasia procedures. A good way to deal with such feelings is to learn all you can about the procedures, train with those who have a lot of experience, and perform the techniques in the most humane way.

Taking the life of any animal can affect people in many different ways. Personnel should never minimize the emotional impact of performing euthanasia on the animals that had been under their or others’ care. Anyone may experience grief or bereavement over the loss of the animals. Sadness, depression, and disinterest in work can be signs of grief associated with the sense of loss. Suppressing or minimizing these feelings may lead to difficulty performing the task again, detachment toward the other animals in the facility, anxiety in the workplace, and conflict with coworkers. Sharing these feelings with coworkers, a supervisor, or a veterinarian can help technicians deal with these normal reactions. The AALAS publication “Cost of Caring: Recognizing Human Emotions in the Care of Laboratory Animals (Appendix C)” offers suggestions to staff and management on strategies for coping with grief over the death of animals in the workplace. A color brochure on this information can also be downloaded for free from the AALAS website.
Summary

The AVMA Guidelines for the Euthanasia of Animals describes the methods of euthanasia that are acceptable, acceptable with conditions, and unacceptable for each animal species. The method of euthanizing laboratory animals must comply with this document and be described in the approved IACUC protocol for the animal research. Acquiring the knowledge and skills to competently perform euthanasia is essential for safeguarding both animal welfare and personnel safety. Laboratory animal technicians should be aware that euthanizing animals they care for may cause them to grieve, which is a normal reaction shared by many individuals involved with animal research. Taking pride in having attained the technical competence to humanely care for laboratory animals, even at the time of their death, can help allay the grief felt at the loss of these animals. It is true that laboratory animals often have short lives because of their contributions to biomedical research. However, if quality of life can be valued more than length of life, then laboratory animal technicians who are caring and competent make great contributions to these animals throughout their lives.

Additional Reading