CO₂ Euthanasia

Performing CO₂ euthanasia is a critically important activity for those laboratories using rodents. Performing CO₂ euthanasia humanely is a critically important activity for the university and the animal care program. Performing CO₂ for certain ages of rodents – will not work. Failure to perform CO₂ properly requires a federal report to the NIH. These few facts are some of the most troublesome requirements of any animal care and use program. The guidelines below apply to mice, rats, hamsters, gerbils, guinea pigs, and rabbits:

A Summary of Guidelines for CO₂ Euthanasia

- **Gestational age 0 to gestational age 14:**
  - Euthanasia of the mother; or
  - Removal of the uterus/fetus

- **Gestational age 15 to birth:**
  - Skillful injection of chemical anesthetics; or
  - Decapitation with surgical scissors; or
  - Cervical dislocation

- **Birth to 14 days of age:**
  - Overdose of chemical anesthetics; or
  - Decapitation; or
  - Cervical dislocation.

- **15 days of age through weaning:**
  - CO₂ euthanasia (with extreme caution); or
  - Overdose of chemical anesthetics; or
  - Decapitation; or
  - Cervical Dislocation.

- **Post weaning through adulthood:**
  - CO₂ euthanasia (with extreme caution); or
  - Overdose of chemical anesthetics; or
  - Decapitation; or
  - Cervical Dislocation.

Caution: Animals younger than weaning may not respond to CO₂ as full grown adult do, some are resistant to CO₂ euthanasia for up to 30 days of age. ALWAYS USE A SECONDARY METHOD OF EUTHANASIA!

For more information on methods of euthanasia, consult:
Post- Approval Monitoring

Post-approval monitoring of Institutional Animal Care and Use (IACUC) protocols is performed to provide assurance to regulatory agencies and to the Duke community that animal experiments are performed in accordance with approved IACUC protocols. The Compliance Liaison serves as the eyes and ears of the IACUC and confirm consistent and accurate performance of the IACUC approved protocols, standard operating procedures (SOPs), and accepted veterinary practices.

Our goal is to monitor all active protocols on at least a yearly basis. In general, these monitoring sessions will be scheduled in advance, but unscheduled visits may also occur. Unscheduled visits may be performed due to convenience (in the right place at the right time) or ‘for cause.’

During the post-approval monitoring visit, the compliance liaison will compare the procedures conducted in the laboratory with those listed in the approved protocol. Any discrepancy will be noted and conveyed to the PI via email report.

The compliance liaison will refer to the DUIACUC post-approval monitoring guidelines during the visit. These guidelines include areas such as:

⇒ Protocol and personnel
⇒ Study procedures
⇒ Anesthesia
⇒ Surgery
⇒ Post-surgical care
⇒ Euthanasia
⇒ General record keeping
⇒ Laboratory

The compliance liaisons report all compliant and non-compliant issues to the IACUC on a monthly basis. A report is given indicating the number of protocols monitored within the past month, the number of protocols with no compliance issues (Attaboy/Attagirl), the number of protocols that were identified to contain non-compliant activities, and a listing of the non-compliant issues identified.

If you have questions regarding this policy, please refer to our website at http://vetmed.duhs.duke.edu/documents/iacuc/pdf/policy_on_compliance_monitoring.pdf

Test Your Knowledge

1. Toe clipping in mice must be performed prior to _____ days of age?
   A. 12
   B. 21
   C. 15
   D. 18

2. Anesthesia must be used performing tail clipping in mice over _____ days of age.
   A. 12
   B. 20
   C. 21
   D. 18

3. Institutional Animal Care and Use (IACUC) meetings are held
   A. As needed
   B. Monthly
   C. Weekly
   D. Every six months

4. Mice can be identified by using
   A. Ear tags
   B. Tattoos
   C. Micro chips
   D. All of the above

5. All individuals who have contact with or will be participating in the use of animals are required to complete Animal Handlers Parts I and II training prior to receiving approval on a protocol.
   True
   False

6. Euthanasia via CO2 must be ensured by a secondary method.
   True
   False

7. CO2 is an acceptable form of euthanasia for mice aged 0-14 days.
   True
   False

8. A protocol annual review will not be approved until all individuals listed on the protocol have completed CO2 training.
   True
   False

Answers: 1-A; 2-B; 3-B; 4-D; 5-T; 6-T; 7-F; 8-T
**QUESTION:** Do I need a protocol for experiments that only involve tissues?

**ANSWER:** This question can be a little tricky:

You **DO** need a protocol if:

1. You are collecting tissues from a live animal, or
2. An animal was euthanized so you could obtain tissue, or
3. You are administering any agent to the animal prior to euthanasia, or
4. You have a collaborator sending you tissues / fluids that will be injected into Duke animals.

You **DO NOT** need a protocol, if:

1. You are using tissues after the original investigator has euthanized the animal for the purposes of the original protocol, or
2. You are obtaining tissues from a meat processing plant or grocery store, or
3. A collaborating at another institution is sending you tissues to evaluate (which will not be injected into any animals).

If you **do not** need a protocol, you should still advise Duke OESO of your tissue / fluid use to assure safety issues are resolved. The OAWA requests an email of the non-protocol use of animals – in case we need to contact you.

**QUESTION:** Do I need a protocol for antibodies I am purchasing from a company?

**ANSWER:** This question also has specific caveats that must be considered:

You **DO NOT** need a protocol if you are purchasing an ‘off-the-shelf’ antibody commonly available from the vendor for any researchers.

You **DO** need a protocol if you are having a custom antibody produced by a third party which is not routinely available (e.g. you are providing a specific antigen for antibody production).

**QUESTION:** Do I need a protocol if DLAR is producing the antibody for me?

**ANSWER:** While DLAR has an antibody producing protocol, that document only has the process approved, and does not identify the antigen or the numbers of animals required. You will need a protocol to approve the antigen and the numbers of animals required BEFORE DLAR can produce the antibody you need.

**QUESTION:** I would like to transfer animals from the housing facility to my laboratory. Can I use my personal vehicle for this purpose?

**ANSWER:** Transportation in non-DLAR vehicles must have prior approval. This includes inclusion of the transportation activity in the protocol (or as an amendment to the protocol) and IACUC inspection. After initial inspection, the vehicle must be inspected every six months by two members of the IACUC. General criteria for an approved vehicle includes:

- Adequate heating and cooling to maintain general minimum comfort
- Protection from direct sun
- Protection from public view
- Method to contain waste (i.e. plastic sheet under the cage)
- Method to discourage allergens
- Method to secure the cage inside the vehicle (i.e. bungee cords)

To arrange an inspection of your personal vehicle for animal transportation purposes call the Office of Animal Welfare Assurance at 919-668-6720 or email iacuc@duke.edu.


Do you have a question relating to some aspect of the animal care program at Duke? Please send your questions for future columns to sonia.doss@duke.edu

Visit the Duke Animal Care and Use Program website at http://vetmed.duhs.duke.edu/