Guidelines for Euthanasia of Rodents Using Carbon Dioxide (CO₂)

CO₂ inhalation is one of the more common methods of euthanasia used for rodents. More than one Purdue investigator has found it necessary to contact the LAP / PACUC office regarding the use of carbon dioxide for the euthanasia of rodents. In a few instances this has been the result of questions posed by a granting agency based on the description of the CO₂ euthanasia procedure provided by the investigator. In addition, the use of carbon dioxide was discussed in a number of facilities during the recent AAALAC site visit with a variety of procedures being described. As such, the following from the PHS Office of Laboratory Animal Welfare (OLAW) and the NIH Animal Research Advisory Committee is offered regarding the use of carbon dioxide:

1. Rodents must be euthanized by personnel that have been appropriately trained. Euthanasia should not be performed in the animal room.

2. The CO₂ euthanasia chamber should allow ready visibility of the animals. The chamber should not be overcrowded (all animals in the chamber must be able to make normal postural adjustments). It is important to also consider that mixing unfamiliar or incompatible animals in the same container may be distressful and is to be avoided.

3. Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled. Without pre-charging the chamber*, place the animal(s) in the chamber and introduce 100% carbon dioxide at the rate of 10-20% of the chamber volume per minute so as to optimize reduction in distress. (For a
10-liter volume chamber, use a flow rate of approximately 1-2 liter(s) per minute.) After the animals become unconscious, the flow rate can be increased to minimize the time to death.

*Sudden exposure of conscious animals to high concentrations of CO₂ (e.g., 70% or greater) has been shown to be distressful to some species. Therefore it is recommended that euthanasia chambers be pre-filled with CO₂ only if it is known not to cause distress in the species being euthanized.

4. **DEATH MUST BE VERIFIED AFTER EUTHANASIA AND PRIOR TO DISPOSAL.** Animals should be left in the container until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and exposure times followed by thoracotomy (opening of the thorax) or by other means (e.g., ascertaining cardiac and respiratory arrest, noting an animal’s fixed and dilated pupils) will assure the irreversibility of the procedure. This is very important, because unintended recovery of animals after apparent death from CO₂ (e.g., in dead animal coolers) constitutes a serious noncompliance with the PHS Policy and must be reported promptly to OLAW with a full explanation of the circumstances and actions taken to correct the noncompliance.

If an animal is not dead following CO₂ exposure, another approved method of euthanasia (e.g. decapitation or cervical dislocation) must be added while the animal is under CO₂ narcosis to assure death.

5. Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended. Carbon dioxide may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Keeping neonates warm during CO₂ exposure may decrease the time to death.


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**New and Triennial Protocol Applications**

The PACUC office has been receiving a significant number of protocol applications (new and triennial re-writes) recently that do not have the signatures of the principal investigator and department head on the last page.

Please remember to obtain these signatures prior to mailing the application to the PACUC office. We cannot process
the application until such time that the signature page is complete.

Thank you for your attention to this.

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Reminders on Recently Revised PACUC Policies

“Policy (revised) on Wire-Bottom Caging for Rodents”

This policy was revised to include the following statement effective March 1, 2005. “Rodents housed in wire bottom cages should be provided environmental enrichment such as, but not restricted to, PVC tubes, large marbles, or small nyla-bones. Environmental enrichment objects should only be added after review by, and discussion with, impacted research groups. Request for exceptions to this policy will require review and approval by the PACUC.” Any requests should be made via an amendment form (PACUC Form 1A).

To date, the PACUC office has only received one request for exemption to this policy. Please be advised that while performing spring animal facility inspections, if PACUC finds rodents housed in wire bottom caging with no environmental enrichment provided and no request for exemption was received, it will be listed as an item of non-compliance in your facility.

A complete copy of the Policy is attached to this newsletter for your convenience.

“Policy on Availability of Approved Protocol(s) At or Near the Animal Facility”

PACUC has approved a policy that a copy of approved protocols and amendments be available at or near the site where the animals are housed. This will ensure that inspection teams (PACUC, USDA, AAALAC-I) and laboratory animal veterinarians/veterinary technicians have easy and ready access should a question arise while they are visiting the facility where animals are being housed. This will also ensure that all animal care staff has sound knowledge of the procedures that are being performed on the animals so they may anticipate what health problems might occur as part of the study/course. This will allow them to distinguish such problems from naturally-occurring ones and to also alert investigator’s to any such problems when they occur.

It is the responsibility of the Principal Investigator to supply a copy of the approved protocol(s) and amendment(s) to the Animal Facility Supervisor.

If a Principal Investigator does not wish for a copy of their protocols and amendments to be available in the animal housing area, a request for exemption to this policy must be made to Lisa Snider, PACUC Administrator, in writing. The exemption request will be forwarded to the full membership of PACUC for review.

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