

PHS POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS CLARIFICATION REGARDING USE OF CARBON DIOXIDE FOR EUTHANASIA OF SMALL LABORATORY ANIMALS---Modified by UF IACUC

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NOTICE: NOT-OD-02-062

National Institutes of Health (NIH)-Policy Modified by UF

The Office of Laboratory Animal Welfare (OLAW) has determined a need to issue Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) guidance to Assured institutions clarifying current requirements regarding the use of carbon dioxide (CO₂) as a euthanasia agent for small laboratory animals. Guidance regarding prompt reporting of related serious noncompliance is included.

The Animal Care unit of the Animal and Plant Health Inspection Service, U.S. Department of Agriculture, has reviewed and concurs with this guidance as it relates to USDA-covered species.

BACKGROUND

PHS Policy requires Institutional Animal Care and Use Committees (IACUCs) to determine that methods of euthanasia utilized in research proposals are consistent with the Report of the American Veterinary Medical Association Panel on Euthanasia (AVMA Panel Report), unless a deviation is justified for scientific reasons in writing by the investigator. IACUC approval of such deviations must be project-specific and include critical review of assertions of scientific necessity. IACUCs may not otherwise disregard or issue blanket waivers of applicable AVMA Panel Report recommendations.

Applications and proposals for awards submitted to the PHS must contain, among other things, a description of procedures designed to assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of valuable research. Reliance on this overarching principle of minimization of pain and distress is especially useful in resolving apparent inconsistencies and gaps in the scientific literature and the specific guidance on CO₂ use.

CLARIFICATION CONCERNING USE OF CARBON DIOXIDE

Although CO₂ is generally considered an acceptable euthanasia agent for small laboratory animals when properly administered, its acceptability is predicated on the following:

- High concentrations of CO₂ may be distressful to some species. Accordingly, pre-filling the chamber should not be done. Chambers should be filled with CO₂ at a flow rate that results in rapid unconsciousness with minimum distress to the animals. Excessive noise or high velocity air movement from the introduction of CO₂ should be avoided.

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- Death must be verified by a physical method after euthanasia and prior to disposal, including neonates 15 days and older. Recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times or by other means. OLAW notes that thoracotomy after apparent death from CO₂ is one way to ensure the irreversibility of the procedure.
- Neonates 14 days and under in age should not be subjected to euthanasia by CO₂. However, neonates may be narcotized with CO₂ prior to decapitation. For acceptable methods of euthanasia for neonates 14 days and under, see IACUC Guidelines <http://iacuc.ufl.edu/Changes%20made%207-21-09/Modified%20GuidelinesforNeonatalRodentEuthanasia%207-21-09.pdf>
- Institutions must ensure that all individuals responsible for administering CO₂ euthanasia are appropriately qualified and monitored, and that they adhere to IACUC-approved protocols and institutional policies.
- Rodents to be euthanized should experience a minimum amount of stress prior to and during euthanasia. It is strongly recommended to euthanize rodents in their home cages (standard size: 7in x 11in x 5in h) without co-mingling animals from different cages. If rodents from different cages are combined, the following guidelines must be followed: A maximum of 10 mice is strongly recommended but an absolute maximum of 15 mice is permitted in the standard cage. Animals that have been co-mingled from different cages may only be kept together for a maximum of 30 minutes regardless of number. In this regard, it is also important to consider that mixing unfamiliar or incompatible animals in the same container may be distressful. Mixing unfamiliar males is unacceptable. Mice are to be under constant observation, and if signs of distress or incompatibility are observed, they are to be separated immediately. For rats, a maximum of 4 per cage (standard size: 10in x 19in x 8in h) is recommended for a maximum time of 30 minutes with constant observation. If stress is observed they are to be immediately separated.
- Compressed CO₂ in cylinders is the only AVMA Panel-recommended source of CO₂ for euthanasia purposes.

REPORTING OF NONCOMPLIANCE

Unintended recovery of animals after apparent death from CO₂ (e.g., in necropsy coolers) is a documented occurrence. Institutions are reminded that such incidents constitute serious noncompliance with the PHS Policy and serious deviation from the provisions of the *Guide for the Care and Use of Laboratory Animals*. As such, the IACUC, through the Institutional Official, must promptly provide OLAW with a full explanation of the circumstances and actions taken. Prompt reporting in accordance with PHS Policy requirements (PHS Policy IV.F.3) is an essential component of the formal relationship between OLAW and PHS-Assured institutions.

OLAW advises IACUCs to review their policies and practices regarding CO₂ euthanasia of small laboratory animals and take appropriate action as needed to conform to this guidance.

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