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*Public Statement*

# Report of the ACLAM Task Force on Rodent Euthanasia

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The ACLAM Task Force on Rodent Euthanasia was appointed by President Lynn Anderson in 2002 in response to growing concerns and controversy regarding techniques that were commonly used for rodent euthanasia. Three issues were targeted as the focus of the report: euthanasia of fetal and neonatal rodents, the use of carbon dioxide for rodent euthanasia, and the impact of euthanasia techniques on data. The charge to the Task Force was to create a document that summarized in a scholarly and comprehensive manner all available data-based literature relevant to these topics, to assess the scientific merit of the design and conclusions of those studies, and to compile valid information into a concise and cohesive document that could be disseminated to veterinarians, IACUC members, regulatory bodies, and research scientists.

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### C. Euthanasia Chambers

1. Euthanasia chambers should be kept clean and free of debris and excreta.
2. The euthanasia chamber should be large enough to permit each animal to stand on the floor of the chamber with all 4 feet and have sufficient space to turn around and perform normal postural adjustments.

### D. CO<sub>2</sub> Gas Delivery Systems

1. Sufficient carbon dioxide must be introduced into the chamber to totally displace the residual air by both mixing and dilution. Ideally, the inlet for delivery of CO<sub>2</sub> and any diffusion devices in the euthanasia chamber should provide a predictable and controllable elevation in CO<sub>2</sub> concentration.
2. Excess gas must be allowed to escape from the chamber in a way that allows a gradual increase in the concentration of CO<sub>2</sub> at the floor of the container that holds the animal. Escape of the gas mixture through a

increase distress for the animals. There is no conclusive evidence that adding pure oxygen to carbon dioxide makes this procedure less stressful to animals.<sup>13,20,29,39</sup> A fill rate of 20% of the chamber volume per minute with carbon dioxide, added to existing room air in the chamber should be appropriate to achieve a balanced gas mixture to fulfill the objective of rapid unconsciousness with minimal distress to the animals.

### F. Cautionary Information

1. Animal carcasses should not be exposed to room air until death has ensued with high certainty, as the anesthetic effects of CO<sub>2</sub> can be quickly reversed in the presence of oxygen.
2. Individual rodents may become apneic at certain concentrations of CO<sub>2</sub>, giving the false impression that death has occurred.<sup>9</sup>
3. Confirmation of death should be based not on a single sign, such as cessation of breathing.

**B. Biological Effects of Euthanasia Techniques**

**Table 1. Biologic effects of decapitation<sup>3,5,16,49,56,60,66</sup>**

Effect	Mechanism
Increase in plasma sodium	
Increase in plasma potassium	
Increase in GABA concentrations (brain)	
Increase in Alanine (brain)	
Increase in plasma ascorbic acid (30-40% > resting state)	Hemolysis
Increase in blood catecholamine levels	Continued postmortem neurochemical alterations
Increased plasma calcium, magnesium	
No change in vasoactive intestinal peptides (brain)	
No change in neuropeptide Y (brain)	
Alteration in rat heart mitochondria function	
Increase in serum corticosterone	Stress stimulus → mobilization from tissues to blood; generalized metabolic response secondary to sympathoadrenal response some handling related stimulation.
	Possible handling stress

**Table 2. Effects of physical and pharmacological euthanasia methods**



Table 4. Biologic effects of euthanasia induced by pharmacologic and/or physical methods

Method of euthanasia	Effect	Mechanism
Injectable Pentobarbital <sup>5,53,61</sup> <sub>a,b</sub>	Decreased muscular contractility in isolated muscle preps Decreased GI smooth muscle contractility when given orally or intravenously; not seen in intraperitoneal route	Decreased calcium transport

Table 5. Anesthetics – ketamine hydrochloride, pentobarbital, chloral hydrate, chloralose and halothane in combination

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15. Committee on Guidelines for the Use of Animals in Research

62. Sharp J, Zammit T, Azar T, Lawson D. 2003. Stress-like responses to common procedures in individually and group-housed female

69. Walter G. 2000. Effects of carbon dioxide inhalation on hematology, coagulation, and serum clinical chemistry.