

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. CO₂ 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

increase distress for the animals. There is no conclusive evidence that adding pure oxygen to carbon dioxide makes this procedure less stressful to animals.^{13,20,29,39} 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

B. Biological Effects of Euthanasia Techniques

Table 1. Biologic effects of decapitation^{3,5,16,49,56,60,66}

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 ^{5,53,61} _{a,b}	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

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.