

Assessing various carbon dioxide flow rates to minimize distress during laboratory mouse euthanasia

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Introduction

- Laboratory rodents are commonly euthanized by exposure to carbon dioxide (CO₂) gas using the gradual-fill method
- Current recommended flow rates (20-30% chamber vol/min) result in rodents experiencing dyspnea, a distressing sensation of breathlessness
- The aim of this study was to determine the flow rate that minimizes the duration of dyspnea**

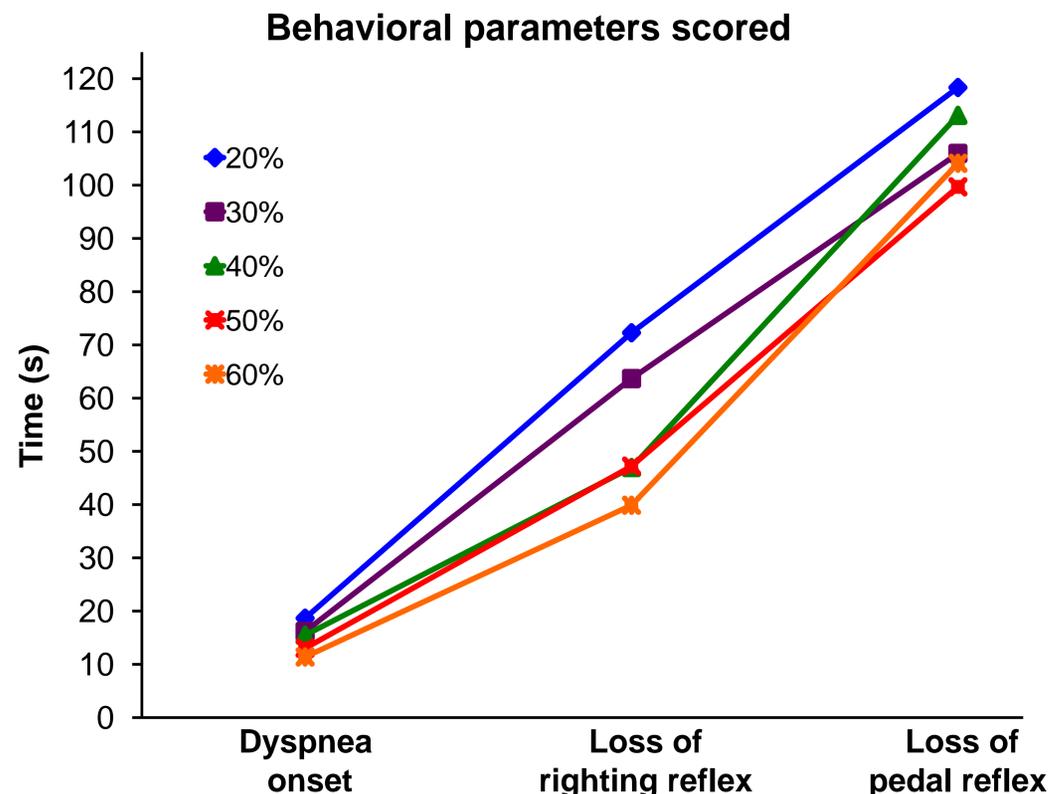
Methods

- Five flow rates were tested (20, 30, 40, 50, 60%) using female albino C57BL/6 mice
- Using an oxygen analyzer and a gas holding technique, CO₂ concentrations within the apparatus did not reach levels associated with pain (> 40%)
- A surgical glove was projected into the apparatus to allow one hand to be placed into the box during each trial
- Dyspnea onset was recorded when the mouse first showed labored breathing
- Upon the appearance of recumbency we tested loss of the righting reflex by placing the animal on its back
- Pedal withdrawal reflex was then tested every 10 seconds using a hemostat to pinch alternating hind paw inter-digital webbing, loss of reflex was recorded when the animal failed to respond to 3 consecutive pinches



Results

BEHAVIORAL PARAMETER	CO ₂ FLOW RATES					P value
	20%	30%	40%	50%	60%	
	LSM ± SE (s)					
Dyspnea onset	19 ± 2	16 ± 2	15 ± 2	13 ± 2	11 ± 2	0.0009
Loss of righting reflex	71 ± 3	64 ± 3	47 ± 3	47 ± 3	40 ± 3	<0.0001
Loss of pedal reflex	118 ± 9	106 ± 9	113 ± 9	100 ± 9	104 ± 9	0.2532
Dyspnea onset - loss of righting reflex	46 ± 9	42 ± 9	66 ± 9	52 ± 9	64 ± 9	0.1373
Dyspnea onset - loss of pedal reflex	100 ± 9	90 ± 9	98 ± 9	87 ± 9	93 ± 9	0.5703



Conclusions

- Dyspnea onset and loss of righting reflex occurred earlier with increasing flow rates, but loss of the pedal reflex did not vary with flow rate.
- Mice may suffer during the onset of dyspnea to loss of consciousness. Two different intervals assessed this potential - dyspnea onset to loss of righting reflex and dyspnea onset to loss of pedal reflex.
- The interval between dyspnea and loss of righting reflex was numerically lowest at the 30% flow rate, but neither interval showed a significant difference in relation to flow rate.