# Does Carbon Dioxide Euthanasia Cause Distress in Rats?

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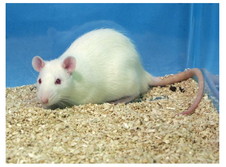
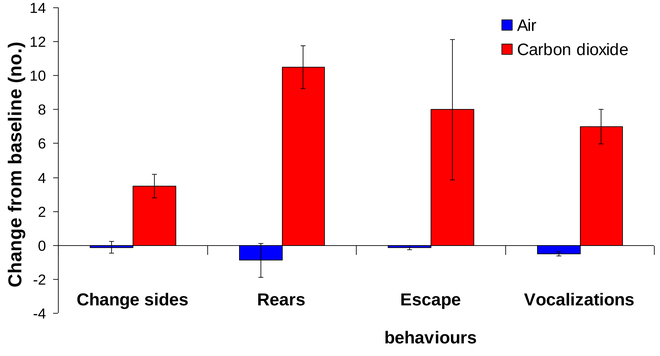


## *INTRODUCTION:*

Carbon dioxide ($CO_2$) is widely used for euthanizing laboratory rodents. Physiological data and human self-report data suggest that exposure to high concentrations of $CO_2$, such as those used during pre-fill euthanasia, is painful. During gradual-fill exposure rats generally lose consciousness at $CO_2$ concentrations of 30 to 40% and pain is unlikely. However, in humans even low concentrations of $CO_2$ (less than 8%) are associated with the unpleasant sensations of air hunger and increased respiratory effort, suggesting that there might also be welfare problems with gradual-fill exposure. These studies were conducted to determine whether gradual-fill $CO_2$ euthanasia causes distress in rats.

## *STUDY #1: Do rats show behavioural signs of distress during gradual-fill $CO_2$ euthanasia?*

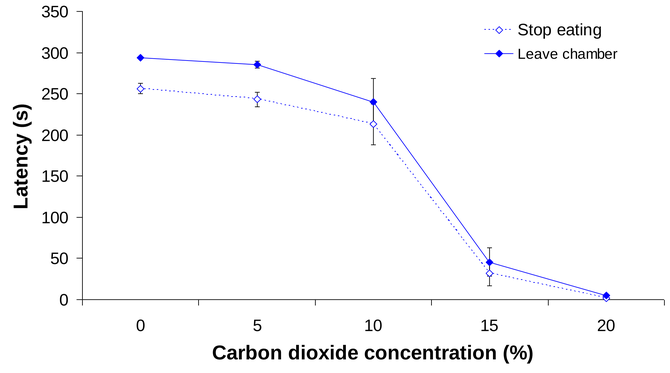
We measured the behavioural responses of 8 Sprague Dawley rats during exposure to either air or gradual-fill $CO_2$ at a rate of 17.25% per minute. Changes from baseline during air and $CO_2$ exposure were compared and data are presented as means ± standard errors.




During $CO_2$ exposure rats greatly increased their performance of side changes, rearing, escape behaviours and vocalizations. Similar changes were not observed during air exposure. These responses started increasing at a $CO_2$ concentration of about 5%, and on average the rats became recumbent after 106 s at a $CO_2$ concentration of 33%.

## *STUDY #2: Do rats avoid exposure to 5, 10, 15 and 20% $CO_2$ when it is paired with a food reward?*

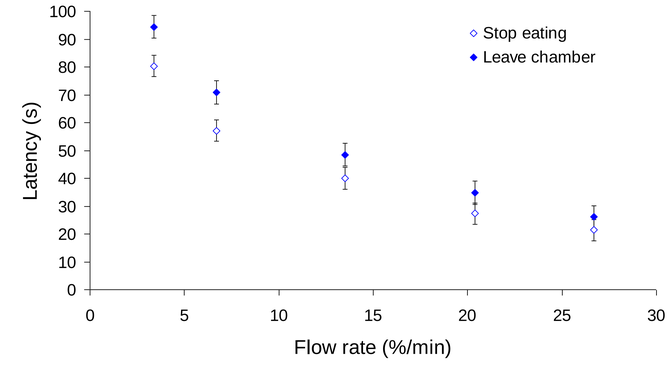
We trained 9 Wistar rats to enter a chamber for a food reward of 20 Honey-nut Cheerios. During testing sessions the chamber was filled with either air or $CO_2$ at concentrations of 5, 10, 15 or 20% and animals had 300 s to consume the reward. Data are presented as means ± standard errors.




The latency to stop eating and to leave the chamber was similar during test session with air and 5% $CO_2$, but these variables declined with 10, 15 and 20% $CO_2$. At 15% $CO_2$ one animal refused to eat, and at 20% $CO_2$ only two animals ate and each consumed only one or two Cheerios.

## *STUDY #3: Do rats avoid gradual-fill $CO_2$ exposure when it is paired with a food reward?*

We trained 8 Wistar rats to enter a chamber for a food reward of 20 Honey-nut Cheerios. During testing sessions $CO_2$ flow began when the rat started consuming the food reward, and we examined flow rates ranging from 3.4 to 26.7%/min. Data are presented as means ± standard errors.




The latency to stop eating and leave the test cage reduced with increasing $CO_2$ flow rate. This resulted in the rats leaving the test cage at similar concentrations of $CO_2$ for all flow rates. This concentration varied from a minimum of 13.0% at the 26.7%/min flow rate to maximum of 15.9% at 13.5%/min.

## *CONCLUSIONS:*

- During gradual-fill $CO_2$ euthanasia rats show behavioural signs of distress, and they will give up a food reward to avoid exposure to static and gradually increasing concentrations of $CO_2$
- Rats are averse to $CO_2$ exposure and we need to find better methods of euthanasia

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