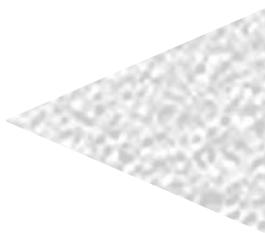


# GUIDELINES



## Use of CO<sub>2</sub> as a Euthanasia Technique

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Carbon dioxide is commonly used to euthanize laboratory animals (particularly rats and mice). However, its use is controversial. Amongst the community of scientists who have examined the use of CO<sub>2</sub> as a euthanasia technique, there is disagreement between those who believe that CO<sub>2</sub> cannot be used humanely under any circumstances and those who suspect CO<sub>2</sub> may be an acceptable method of euthanasia in some circumstances.

An international consensus meeting on carbon dioxide euthanasia of laboratory animals was held February 27-28, 2006 at the University of Newcastle-upon-Tyne, UK. The aim of the meeting was to place emphasis on the facts about the use of CO<sub>2</sub> on which there is agreement and which have direct implications for the way euthanasia is carried out. In addition, a secondary aim was to establish areas where further research is needed. Approximately 30 individuals were invited to participate in the meeting. These included investigators, representatives of the animal supply industry, animal caretakers, research regulators and animal welfare organizations. The intention was to ensure that the opinion of those directly involved with employing or regulating the use of euthanasia methods were represented and that the outcomes of the meeting would be widely disseminated. The meeting was funded by the UK National Cen-

tre for the Three Rs and Laboratory Animals Ltd, and organized by Huw Golledge, Matt Leach and Paul Flecknell, Comparative Biology Centre, University of Newcastle.

The experts at the meeting presented the results of experiments that had been carried out to try and understand the level of pain and/or distress experienced by the animals (principally rats) undergoing euthanasia by CO<sub>2</sub>. From studies carried out on human volunteers, involving taking a single breath of CO<sub>2</sub>, there appears to be little doubt that high concentrations of CO<sub>2</sub>, such as those experienced by rats in pre-filled chambers, are extremely painful. More difficult to measure and understand is the degree of distress experienced by rodents when subjected to slowly rising levels of CO<sub>2</sub>. Inhalation of 15 to 20% CO<sub>2</sub>, which is below the threshold for pain, has been found to cause severe air hunger, an unpleasant sensation of breathlessness, in humans. Interestingly, breathing CO<sub>2</sub> is used as a psychological test to determine anxiety states in humans, indicating that breathing concentrations of CO<sub>2</sub> below those shown to cause pain, are known to cause distress in humans.

Investigators based at the University of Newcastle-upon-Tyne had examined behavioural and physiological responses during pre-fill and gradual-fill CO<sub>2</sub> exposure, and found that pre-fill caused physiological signs of pain or nasal irritation before loss of consciousness while gradual-fill did not. Investigators based at the

Universities of Bristol and Lincoln in the UK and at the University of British Columbia in Canada, had all attempted to determine the extent of distress experienced by the animals by using preference tests to assess aversion to pre-fill and gradual-fill CO<sub>2</sub> exposure. In almost all cases, animals left the chamber when concentrations reached >16% CO<sub>2</sub>, even when food deprived and given access to highly desired treats. During forced exposure to a gradually increasing concentration of CO<sub>2</sub>, loss of consciousness does not occur before 28% CO<sub>2</sub>, which would suggest that rats do experience distress prior to loss of consciousness.

There was considerable debate at the meeting concerning whether euthanasia is a good death if it involves a short period of intense pain prior to loss of consciousness, versus a longer period of distress (i.e. how the time x intensity quotient should be interpreted). However, at least according to European legislation, the degree of pain must be minimized.

A report of the meeting has now been published and is available on the website of Laboratory Animals [www.lal.org.uk](http://www.lal.org.uk). Whatever method of euthanasia is employed, it must be reliable. It was agreed that ideally animals should be anaesthetized by overdose of barbiturates (recognizing that this involves handling stress and possible pain due to the barbiturate itself, unless administered with lidocaine) or by volatile anesthetic gases. However, in the cases where there is difficulty in accessing these drugs, CO<sub>2</sub> might be preferable to physical methods because of the need for a high level of competency to carry out physical euthanasia, in addition to operator burn-out, especially where large numbers of animals need to be euthanized.

CCAC is currently preparing *guidelines on: euthanasia* which include recommendations made by other jurisdictions. Recommendations emerging from this international meeting of experts will also be incorporated into the CCAC guidelines document. ●