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Editorial
Carbon Dioxide for Euthanasia of Laboratory Animals

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Laboratory animals are normally euthanized during the course of, or at completion of, an experiment or research project. Investigators have moral and legal obligations to ensure that animals experience as little pain and distress as possible during a procedure. Thus, euthanasia should cause at most only minimal pain or distress. Euthanasia methods that involve injection require restraint of animals and have the potential for attendant distress. Inhalation methods, by contrast, can avoid this direct interaction. Euthanizing (or anesthetizing) animals in their home or a familiar cage using an inhalant agent to induce unconsciousness is an integral part of making the procedure less stressful, but is frequently overlooked or undervalued by scientists and others involved in decision-making (e.g., ethics committee members). We wish to emphasize these points pertaining to the use of carbon dioxide as an inhalant agent.

Most, if not all, inhalant agents of anesthesia and euthanasia are associated with some degree of aversion depending on type, concentration of the agent, and species (5-9). Thus Leach and coworkers found that as the concentration of fluorinated hydrocarbon anesthetics increased, so the aversion exhibited increased. Although the agents were equally effective at inducing and maintaining anesthesia, some agents were less aversive than others; for example, rats 'preferred' halothane and mice enflurane. Aversion can be measured by assessing an animal's response to exposure; that is, whether it voluntarily enters a gas mixture environment, repeatedly revisits the environment, or tries to escape as quickly as possible. Further, one can ask whether the degree of aversion varies between agents; e.g., can aversion be overcome by offering a counter-inducement such as food after a period of food restriction?

The results of experiments on several laboratory and farm animal species (2, 6) have shown that carbon dioxide is aversive to a far greater extent than other commonly used gaseous agents such as halothane, sevoflurane, desflurane and isoflurane (6) whether used alone or in combination with other gases. Moreover, aversion is observed to varying degrees regardless of whether carbon dioxide is presented in a pre-filled chamber, as a rising concentration, humidified, or combined with an inert gas or oxygen. There is also evidence to suggest that, in addition to aversion, animals unable to escape from an environment containing carbon dioxide are likely to experience considerable pain and distress before loss of consciousness (2, 3).

Additionally, trials involving human exposure to carbon dioxide indicate that it induces a sense of breathlessness prior to loss of consciousness, and 36 out of 40 persons reported adverse sensations at concentrations of 50% (1); a level similar to those used in animal anesthesia and exceeded when carbon dioxide is used for euthanasia. The sensation of breathlessness or dyspnea in humans is believed to originate from a direct activation of cerebral cortical sensory systems involved with respiration (i.e., conscious awareness of efferent motor command corollary discharge). It is also known that dyspnea during inhalation of carbon dioxide is due to activation of vascular chemoreceptors from increases in blood carbon dioxide levels (hypercapnea) and results in increased respiratory motor activity. It is worth noting that hypercapnea is a more potent respiratory stimulant than hypoxia or anoxia. It is reasonable to assume, based on current understanding of comparative respiratory anatomy and physiology, that laboratory animals also feel these effects experienced by humans.

One solution to the adverse effects of carbon dioxide is to induce unconsciousness with a less aversive gaseous agent such as halothane or isoflurane before using 100% carbon dioxide to complete euthanasia. Further, means should be sought to use non-stressful environments for euthanasia, such as the aforementioned familiar caging. As the research results are so clear-cut, carbon dioxide ought not be used as the sole agent for anesthesia or euthanasia, despite any inconvenience that may attend replacement or supplementation with anesthetic gases, unless and until there are other proven scientific reasons to do otherwise.

These concerns and recommendations are consistent with the important concept that animals, whose use helps advance biomedical research for the benefit of humankind, should also benefit from improved scientific knowledge.

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