Letter to the Editor

Aversiveness of carbon dioxide

Carbon dioxide can produce aversive stimulation of the upper airways, and in their recent review Conlee et al. (2005) emphasized: ‘... Leach et al. (2002) concluded that CO₂, either alone or in combination with argon, cannot be used humanely at any concentration and is therefore unacceptable as a euthanasia agent for laboratory rodents, particularly when more humane methods exist. The findings of the studies discussed above demonstrate that CO₂ causes adverse effects that lead to stress, and perhaps distress, at various concentrations ranging from 25 to 100%...’

Typical ‘shuttle box’ or ‘maze’ experiments cannot be taken as an unambiguous demonstration of aversiveness. An agent that increases locomotor activity by the inhalation route (for example, almost any central nervous system [CNS] depressant) increases horizontal movement. Therefore, if the agent is inhaled in one chamber, activity increases and the likelihood of walking through an opening into an adjacent clean chamber increases. There is less agent-produced activity in the clean chamber, and a reduced likelihood of walking back into the exposure chamber. This may result in the outcome of less dwelling time in the exposure chamber without airway irritation playing any role whatsoever.

Such approaches neglect literature on the aversive properties of carbon dioxide in rodents, on the limitations of specific experimental methods, and on the experimental design considerations necessary for a conclusive demonstration of inhalant escape or avoidance, as well as for the accurate description of concentration–effect functions. Appropriate behavioural and physiological methods exist for the characterization of the irritancy of airborne contaminants, lacrimating agents, products of combustion, air pollutants, highly reactive chemicals, and ammonia, and are published in the inhalation toxicology literature.

I noted this literature in some detail on the COMPMED listserv (4 July 2002) and the issues are well summarized in a review [Wood 1979]: ‘... One way to characterize the aversiveness of a stimulus is to determine under what conditions an organism will respond to it off, i.e., escape from the stimulus. If the stimulus is intense enough so that its termination will support behaviour, then the stimulus is an effective negative reinforcer. Escape from aversive atmospheres has not yet been conclusively demonstrated. Escape from carbon dioxide has been reported in the mouse and the pigeon...and in the rat. Control procedures were not undertaken in these experiments to demonstrate that the reported performance did not result from a non-specific effect of carbon dioxide on response rate. Peterson and Andrews... reported a concentration-related reaction to ozone presented to one side of an annular plastic mouse chamber. Mice spent more time on the air side of the chamber. Position reversal controls were not performed. This experimental situation is different from electric shock escape or avoidance paradigms; in the ozone experiment the subject must present the stimulus to itself by walking into the exposure environment. Similar paradigms are used for the investigation of pheromones; the shortcomings of such designs have been discussed... A conclusive demonstration must meet the following criteria: (1) the duration of inhalant exposure tolerated must be inversely related to concentration; (2) the percentage of inhalant deliveries terminated by a response must be directly related to concentration; (3) the first two relationships must not be the result of a non-specific, rate-increasing property of the inhalant, and (4) there must be evidence that the subject discriminates between responses that terminate the delivery, and those that do not.’ Unambiguous precedents exist for other irritants (e.g. Wood 1979, 1981, Wood & Coleman 1995).

Carbonic-acid-induced stimulation of the trigeminal nerve is likely the principal aversive event resulting from carbon dioxide exposure [cf. Shusterman & Avila 2003]. Appropriate behavioural methods might be used to characterize this phenomenon, but the consistently associated trigeminal reflex could also be used for this purpose. Irritation of the upper airways results in a reduction in respiratory rate and bradycardia, endpoints readily measured and amenable to assessment with telemetry. A standardized test method has been promulgated (ASTM 1984) and widely used to evaluate sensory irritants, e.g. chlorine [Morris et al. 2005]. With computer control of carbon dioxide concentration in small experimental chambers with high airflow, it would be possible to define changes in sensitivity to clearly unpleasant concentrations as a function of the duration of exposure to nonaversive concentrations of carbon dioxide. The results of such studies could be used to determine exposure profiles (necessarily dependent on chamber architecture, volume, and flow rates) that could be used to ensure true euthanasia, i.e. death without aversive stimulation.

Figure 1 outlines a series of possible experiments to evaluate the aesthetic properties of carbon dioxide and its effects on the response to acute high-concentration exposures: (a) acute concentration–effect function using brief (30-60 s) exposures in a chamber where the ratio of flow to volume is at least 10:1, preferably 30:1; (b) evaluation of the effects of repeated brief exposure to 100% carbon dioxide; (c–e) evaluation of the effects of constant exposure to varying concentrations of carbon dioxide on the response to acute high-level pulsed exposure; (f) evaluation of the effects of 100% carbon dioxide pulsed exposures superimposed on exposure profiles bounded by the AVMA-recommended minimum ratio of flow to volume (0.2:1) and by those cited by the AVMA panel (2001) as being ‘without evident stress as measured by behavior and ACTH, glucose, and corticosterone concentrations in serum’, i.e. 0.476:1 [Hackbarth et al. 2005].
The ratio of flow (6 L/min) to volume was determined using a chamber volume of 12.6 L (cage information from Dr Hackbarth; personal communication). The illustrated concentration profiles were computed using established methods (Nelson 1971) and represent single-compartment exponential dilution kinetics under non-laminar (turbulent) flow conditions. Turbulent flow is likely to prevail when gases are introduced through narrow inlet tubes to a complex chamber stirred by animal movement. Note that an experiment of the type illustrated in panel ‘f’ could only be done under high-flow conditions that mimic the concentration profiles achieved under lower-flow conditions. If animals are prepared with telemetry devices, physiological responses to carbon dioxide exposure could then be evaluated in chambers typically used for euthanasia, thus validating extrapolation of the findings from a strictly controlled exposure environment to chambers typically used for euthanasia utilizing 100% carbon dioxide.

The practice of carbon dioxide euthanasia might be refined with appropriately conducted experimental work. As a behavioural toxicologist who has studied the behavioural effects of airborne contaminants extensively, including their properties as aversive airborne irritants, I do not think appropriate behavioural experiments are in hand to provide an adequate basis to alter regulation of euthanasia using carbon dioxide. Unlike other asphyxiants, carbon dioxide has good warning properties, and poses less intrinsic risk to personnel. It is my belief that carbon dioxide can be used safely and humanely in providing euthanasia to laboratory rodents.

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References
Leach MC, Bowell VA, Allan TF, Morton DB [2002] Aversion to gaseous euthanasia agents in rats and mice. Comparative Medicine 52, 249-57

Editor’s note
A reply from Leach and his colleagues is expected for the next issue.