Letters to the Editor

Aversiveness of carbon dioxide

We are grateful to Dr Ronald Wood for providing in his letter in the July issue [Wood 2005] his expert views and methodologies that could potentially be used in future to test the behavioural effects of airborne contaminants and irritants. Dr Wood seems to accept in his letter that carbon dioxide is aversive but he is unsure how aversive, and believes that it can be safely and humanely used in rodents. We, on the other hand, based on existing evidence, do not accept that concentrations that are capable of inducing unconsciousness can be humanely [i.e. to stop any animal suffering] used.

The main tenet of Dr Wood’s criticism of the work conducted by Leach et al. (2002) is that it is unable to differentiate between horizontal movements as a result of its depressant effect on the central nervous system (CNS) from those resulting from aversion to carbon dioxide. However, a CNS response of this type would probably take a minimum of 7-10s from inhalation of 80% carbon dioxide in air to produce such horizontal locomotory movement (Kohler et al. 1999), whereas we found that the withdrawal time [the time taken to leave a chamber containing carbon dioxide] occurred in less than 2s at low-to-moderate concentrations (26-36%) and less than 1s at high concentrations (>51%). This is further borne out by the dwelling times observed [time spent in the chamber over a 3-min period], with rats spending less than 2s at low and moderate concentrations and less than 1s at high concentrations [mice spent less than 6s at medium and high concentrations]. Therefore, with these types of response, there is insufficient time for carbon dioxide to stimulate a horizontal locomotory reaction due to CNS depression, rather an aversion.

Further, this notion that a CNS depressant gas, like carbon dioxide, induces animals to leave an atmosphere containing it, simply due to an increase in random horizontal movements by an animal, does not seem reasonable for a variety of reasons, including:

1) The aversive effect of carbon dioxide seems to be highly conserved across all species from an evolutionary perspective, and moreover makes good sense as it forms a potential threat to survival [van Luijtenaar & Coenen 1999, Raj 1999].
2) The aversive effect seems not one of tolerance that can be titrated as a simple dose-response curve, but is a threshold response physiologically driven.
3) Data from the groups in British Colombia have shown that even when rats are hungry, they avoid carbon dioxide, overcoming a basic physiological need to eat [Kirkden et al. 2005, Niel et al. 2005].

The ‘benchmark’ methodologies for the testing of inhalational irritants, including air pollutants and ammonia referred to by Dr Wood, may not be appropriate in the case of carbon dioxide. In this regard, many of the irritants tested in controlled laboratory studies using various protocols proposed by Dr Wood do not necessarily induce adverse physiological changes, such as stimulation of peripheral and central chemoreceptors, or induce breathlessness in a way that carbon dioxide does. Therefore, the various methodologies proposed by Dr Wood would have to be carried out with an understanding of these effects of carbon dioxide. Such effects have been shown in all other species of animals, including humans, this human experience giving us insight into why aversion to carbon dioxide is so widely conserved.

We feel that some of the criteria put forward by Dr Wood as being required for a conclusive demonstration are inappropriate for animal welfare studies, especially during euthanasia. For example, he states that the ‘duration of inhalant exposure tolerated must be inversely related to concentration’. This criterion assumes a linear relationship between the levels at which the gas concentration is irritant and the duration of tolerance, which may not be true with carbon dioxide. Although exposure to sub-lethal concentrations of carbon dioxide [e.g. 1-10% by volume] may show some relationship of this kind, the results of aversion testing suggest that rodents find concentrations of carbon dioxide [25% by volume or more] equally aversive. For example, the dwell time of rats in atmospheres containing 25%, 35% and 50% by volume of carbon dioxide was not significantly different [2, 1 and 0.7 s, respectively, Leach et al. 2003]. These results suggest two things: [1] the pungency effect of this gas forced the rats to demonstrate relatively shorter dwell times as concentrations increased, [2] the time to onset of breathlessness is quicker with the increasing concentrations. Note also that only concentrations of more than 40% can induce unconsciousness in a reasonable time period [i.e. less than 60s].

With reference to the experimental protocols put forward by Dr Wood, we would like to draw...
his and other readers’ attention to the work being conducted at the University of British Colombia. These studies have used the gradual fill methods proposed by the American Veterinary Medical Association [AVMA] in an attempt to further clarify aversion associated with carbon dioxide. We have contacted the authors of this work and understand that they will also be responding to Dr Wood’s letter. Therefore, we will leave the discussion of these proposed methods and their findings to them.

Finally, in the absence of conclusive evidence in Dr Wood’s letter, we are not convinced that he is correct in concluding [stated as his belief] that euthanasia of laboratory rodents with carbon dioxide is humane. We feel that this conclusion fails to take into account the wealth of literature supporting the aversiveness of carbon dioxide. We refer to the use of carbon dioxide as an ‘accepted’ pain stimulus in pain studies involving humans and other species (Thüräuf et al. 1991, Komai & Bryant 1993, Peppel & Anton 1993, Danneman et al. 1997). Therefore, when all of this information is taken into account, the balance of evidence does seem to indicate that carbon dioxide is not a humane method of euthanasia. We also feel that until conclusive experimental evidence can be provided that carbon dioxide can be used humanely, the only humane action is to stop using carbon dioxide to induce unconsciousness and death in animals in the currently accepted protocols.

Matthew Leach1, Mohan Raj1 and David Morton2
1Department of Clinical Veterinary Science, University of Bristol, Bristol BS40 5DU, UK. Email: m.c.leach@bris.ac.uk;
2Biomedical Services Unit, Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

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Wood RW (2005) Aversiveness of carbon dioxide (Letter) to the Editor. Laboratory Animals 39, 353-4

Aversion to carbon dioxide

We believe that Wood (2005) has raised some useful methodological points concerning the assessment of aversion. However, we do not believe that all of his criteria are necessary to demonstrate that a stimulus is aversive. Nor do we accept his assessment of carbon dioxide (CO2) euthanasia as humane.

We agree with Wood that it is important to rule out alternative explanations for the observed response. His criticism of the Leach et al. (2002) study, that the reduced dwelling time of rats and mice in a chamber containing CO2 might be attributed to CNS depression caused by the gas, rather than aversion to the gas, is plausible if the subjects in the Leach et al. study were simply exploring the apparatus. Detailed behavioural observations would seem to be required to fully evaluate this criticism, since it predicts that the animals’ behaviour at the time of leaving the gas chamber would have the appearance of undirected wandering, rather than a direct and swift movement toward the exit. In the absence of such observations, the coefficient of variation of dwelling time may be informative, as we predict this to be high in the case of undirected wandering.

In our laboratory, we have recently conducted several studies investigating the aversiveness of CO2 for rats. The findings are as yet published only in abstracts [Kirkden et al. 2005, Niel et al. 2005], but we believe they are worth mentioning due to their relevance to the debate. Like Leach et al. (2002), we found that our subjects always left the gas chamber before the CO2 rendered them unable to do so. An important difference between our tests of aversion and those of Leach et al. was that we offered our subjects an incentive to remain in the gas chamber. Specifically, we provided an attractive foodstuff at various levels of food deprivation. In several of our experiments we also employed a gradual fill procedure (a technique commonly used for euthanasia), in which the gas chamber was initially filled with air, after which the CO2 concentration was gradually increased. This design ensured that the rats were always eating before they decided to leave the chamber and made it easy to distinguish escape behaviour from general exploration. In fact, subjects showed highly directed escape behaviour