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.

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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
when leaving the chamber. Rats would typically pause to sniff the air, collect food items in their mouths and then head directly for the chamber exit. They occasionally paused before ascending the exit tunnel, but never exhibited undirected locomotion in the chamber. In control trials, where air instead of gas flowed into the chamber, rats remained even after all food items had been consumed and were never observed to take food from the chamber.

In contrast to our results with rats, chickens have been reported to remain in an atmosphere containing up to 60% CO₂ until unconscious when offered food [Gerritzen et al. 2000] or social contact in the gas chamber [Webster & Fletcher 2004]. In these previous approach-avoidance studies, CO₂-induced activity increases did not cause the birds to leave the chamber prior to losing consciousness. These results suggest that the tendency of our rats to depart from an environment containing both CO₂ and food is not attributable to a general effect of the gas upon the neuronal control of behaviour.

Wood's other methodological suggestions are useful, but less important. The idea that subjects should be required to discriminate between two responses, only one of which terminates the aversive stimulus, in fact refers to one possible approach to demonstrating that the response is not a result of CNS depression [Wood 1978, 1979a]. This approach is particularly useful when the response is difficult to distinguish from general exploration, for example, the nose poke response used by Wood [1979b]. It is less relevant to studies such as ours and possibly that of Leach et al. [2002], in which the response was a clearly visible escape behaviour directed at a remote exit point. Wood's other points concern the establishment of a dose-response relationship. This provides additional information about a stimulus, for example, its aversive threshold [Wood 1982], but is not necessary to demonstrate that the stimulus is aversive. Nonetheless, we have tested rats with static CO₂ concentrations varying from 0% to 20%, and have demonstrated that residency times in the experimental chamber decline in a non-linear manner, starting at a concentration of about 10% [Niel et al. 2005].

We agree with Wood that both behavioural and physiological approaches have a place in the assessment of aversion to CO₂ in animals. However, unlike behavioural methods, physiological approaches do not yield direct evidence of aversion. Rather, they allow us to develop an argument by analogy [see Dawkins 1980]. In humans, noiceptive and respiratory responses can be correlated with psychophysical measures of pain and dyspnoea, allowing inferences to be drawn about the unpleasantness of CO₂ exposure in animals that exhibit similar anatomy and physiology. Thus, it has been found that the CO₂ threshold for the majority of nociceptors in the nasal mucosa is between 37% and 50% CO₂ [Peppel & Anton 1993]. Similarly, Théraul et al. [2002] reported a threshold of 42.6% CO₂ for the negative mucosal potential in the nasal mucosa of humans. This suggests that nociceptors in the nasal mucosa of rats and humans are responding to CO₂ in a similar manner. An average pain threshold of 50.4% has been reported for CO₂ in humans, and using the argument by analogy we can infer that CO₂ concentrations greater than 50% are also likely to be painful in rats. This suggests that pre-fill CO₂ exposure causes pain in rats, but that gradual-fill CO₂ euthanasia does not cause pain in rats as they typically lose consciousness at CO₂ concentrations below 40% [Smith & Harrap 1997]. However, pain is not the only unpleasant sensation associated with CO₂ exposure. In humans, CO₂ concentrations of 7–8% have been reported to be breathable but very unpleasant due to a sensation of air hunger or dyspnoea [Banzett et al. 1996, Masuda et al. 2001]. At a concentration of 35%, a single breath of CO₂ is highly unpleasant [Kaye et al. 2004]. If air hunger becomes severe, it can be a frightening experience that strongly motivates escape responses [Banzett & Moosavi 2001]. The physiological source of air hunger is not fully understood [Meek et al. 1998], so it is not possible to determine whether rats have the capacity for this sensation. However, it is likely that such a basic drive is common among mammals and this may explain why rats and mice appear to show aversion to concentrations of CO₂ that are not consistent with high pain.

In conclusion, we believe that, although CO₂ exposure is a practical method of euthanasia, there are significant welfare concerns arising from its use. Research is now required to compare alternative methods with CO₂ exposure, with the goal of discovering more humane methods for euthanising laboratory animals.

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