

Welfare evaluation of euthanasia of rodents using inert gases – searching for humane methods

Thomas C. Gent¹, Alexei L. Vyssotski², Daniel S.J. Pang³, Sarah Isler⁴, Martin Wehrle⁴, Regula Bettschart-Wolfensberger¹. ¹Vetsuisse Faculty, University of Zürich, CH-8006 Zürich. ²Neuroinformatik, University of Zürich, CH-8006 ³Faculty of Veterinary Medicine, University of Calgary, Canada. ⁴Natur & Tierpark Goldau, CH-6410 Goldau.

Key words

Mouse, Euthanasia, Inert Gas, Laboratory Animal Welfare

Aim of the study

Mice are the most frequently used laboratory animal and often euthanased using carbon dioxide. The use of carbon dioxide has several welfare concerns and this study aimed to compare behavioural stress correlates during euthanasia with other inert gases.

Material and methods

C57/Bl6 mice were implanted with electrodes to record EEG/EMG. After 7 days they were randomised to undergo euthanasia with one of the following: carbon dioxide, nitrous oxide, nitrogen, helium, argon, or xenon with a minimum of 6 animals per group. Following a 5 minute baseline of 21% oxygen, gases were pumped into a chamber at 30% of the chamber volume per minute and for carbon dioxide an additional experiment at 80%. In addition to electrophysiological data, gas concentrations at loss of consciousness were measured as well as locomotor activity analysed by video tracking. Nociception was determined using the mouse grimace scale.

Results and significance

Carbon dioxide, xenon and nitrous oxide resulted in animals losing consciousness at significantly higher oxygen concentrations (14.0 ± 0.6 , 9.6 ± 0.6 and 7.6 ± 0.6 respectively) than other gases. There was no difference for fast versus gradual fill carbon dioxide. Argon ($4.5 \pm 0.2\%$) and helium ($3.9 \pm 0.3\%$) were not significantly different from nitrogen ($4.5 \pm 0.3\%$) which caused loss of consciousness due to hypoxia. This suggests a narcotic effect for carbon dioxide, xenon and nitrous oxide. Carbon dioxide was aversive resulting in a median of three jumps per animal to escape the chamber compared to a median of 0 jumps for all other gases. Fast flow of carbon dioxide resulted in a median of 2 jumps per animal. Carbon dioxide also resulted in behavioural excitation causing a mean increase in locomotor activity of $15.8(\pm 8.6)\%$ for the first 30 seconds of exposure. Nitrogen, helium nitrous oxide and argon did not change initial locomotor activity whereas xenon ($-25.6 \pm 10.0\%$) resulted in a significant reduction. All gases except carbon dioxide resulted in an increase in delta power (marker of sedation) during the first 30 seconds of exposure. Xenon and nitrous oxide produced the largest increases ($139.9 \pm 35.1\%$ and $120.4 \pm 20.0\%$ respectively) whereas carbon dioxide resulted in a $20.0(\pm 17.8)\%$ decrease. Muscle fasciculations were noted with all gases except carbon dioxide and xenon, however these occurred after the onset of an isoelectric EEG. Carbon dioxide exposure at sub-narcotic concentrations had a nociceptive effect causing a median grimace score of 4 within the first 30 seconds of exposure. No other gases caused a significant increase in grimace score over baseline.

Carbon dioxide used alone as a euthanasing substance in mice results in electrophysiological and behavioural endpoints indicating stressful excitation and nociception. Alternative methods need to be established as a matter of urgency on animal welfare grounds. From the gases investigated in this study, xenon and nitrous oxide were not aversive and a degree of sedation was noted. Nitrogen alone did not produce any sedation however death resulted from hypoxia only. These three gases are feasible alternatives to carbon dioxide euthanasia.

Publications, posters and presentations

Experimentation and data analysis has only just concluded. Submission for publication will be forthcoming within the next six months.

Project 2.13.04

Project duration March 2014 – January 2015