RESEARCH ABSTRACT

ANIMAL WELFARE

Title: Nitrous Oxide for piglet euthanasia: validation by electroencephalography (EEG), **NPB #13-109** revised

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Scientific Abstract:

The identification and validation of humane methods to euthanize piglets is critical to address public concern that current methods are not optimal. The blunt force trauma procedure is humane when carried out correctly, but most people find it aesthetically objectionable. The use of carbon dioxide (CO_2) is often recommended, at the same time it is criticized as being aversive to swine. This research sought to: 1) evaluate the aversiveness of inhaling nitrous oxide and oxygen, using our previously validated approach which relies on the piglet's perspective, and 2) validate the effectiveness and humaneness of nitrous oxide to induce loss of consciousness by electroencephalogram (EEG) recording. Three experiments were conducted to determine the aversiveness and the effectiveness of different mixtures of gas to euthanize piglets. The gas mixtures tested were nitrous oxide ("laughing gas"; N_2O) and air (90%:10%; '90N'); nitrous oxide, oxygen and air (60%:30%:10%; '60N'); and carbon dioxide and air (90%:10%; '90C'). Experiment 1.a. allowed piglets to walk freely between one chamber filled with air and another chamber prefilled with either 60N or 90N. Experiment 1.b. performed the same test except the gas chamber held N_2O prefilled at 25%, 50%, or 75% concentration in air or CO_2 prefilled at 7%, 14%, or 21% concentration in air. *Experiment 1.a.* All eight piglets exposed to the 60N gas mixture finished the 10 min test. On the other hand, all eight of the piglets exposed to the 90N treatment had to be removed from the test (at 255.4 ± 65.5 s) before the allotted ten min because they started to flail due to the lack of oxygen. Piglets in 60N expressed more normal behavior (walking-investigating pen) compared to piglets in the 90N ($P<0.01$). The mean duration of disoriented behavior was much greater for the piglets in 60N ($P < 0.05$). There was no effect of treatment on heart rate ($P > 0.80$). *Experiment 1.b.* When gas concentrations were highest the test was shorter ($P < 0.001$) and had to be stopped when they succumbed to the test gas and flail or fall recumbent and non-responsive in the test chamber. The amount of time spent disoriented was greater ($P < 0.002$) for piglets when exposed to each gas in the middle concentration gradient (CO_2: 14%; N_2O: 50%). Flailing behavior tended to correlate ($r = .39$, $P < 06$) with increasing concentrations of CO_2; but not N_2O. Experiment 2 measured brain activity through EEG to assess loss of consciousness as they pigs were subjected to either 60N, 90N or 90C.

This experiment found that both 90N and 90C induced isoelectric EEG in 71 and 59 sec, respectively, but that 60N could never induce an isoelectric EEG, equivalent to brain death, over a 15 min period. Both 90N and 90C exposure resulted in a decrease in median frequency (F50), but only 90C resulted in a decrease in total EEG power (Ptot). The 60N treatment resulted in a slow decrease in F50 and a slow increase in Ptot over the 15 min period. The 95% spectral edge frequency (F95) was not affected by any treatment. The EEG results backed up the behavioral studies by demonstrating that there are differences between the 3 treatments in terms of effects on the brain. This means that the behavioral changes seen reflect differences in the animal's perceptive experience of the treatments rather than, for example, being alterations in motor function. In that sense, the EEG data strengthen the link between the behavioral results and the implications for animal welfare that nitrous oxide is less aversive than carbon dioxide. Furthermore, this project demonstrated that 90% nitrous oxide in air can kill piglets.