

## ANIMAL WELFARE

**Title:** Evaluation of rate of administration of various gas mixtures using the Smart Box euthanasia device as a humane and effective method of piglet euthanasia  
– NPB #09-197

Revised

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### Scientific Abstract:

The objectives of this study were to compare the effectiveness and response of 100% CO<sub>2</sub> gas, relative to a 50:50 CO<sub>2</sub>:Argon (MIXED) gas mixture and different flow rates: slow (SL), medium (MD), fast (FT) and prefill (PF; 20, 35, 50, and prefill with 20%; chamber volume exchange per minute) as an effective tool for euthanizing when applied to piglets in two age categories: neonates (less than 3 days, n=160, BW 2.61 ± 0.81 kg) and weaned (16 to 24 days, n=180, BW 4.62 ± 0.76 kg). The slow flow rate 50:50 CO<sub>2</sub>:Argon gas mixture was not tested in neonates due to ethical concerns. A control treatment (CT) administered ambient air through the chamber followed by blunt force trauma. Male-female piglet pairs were placed in a chamber with lid and one side panel made of clear plastic to facilitate behavior observations. A Smartbox device (Euthanex Corp, Palmer, PA) was used to supply gas at controlled rates. Piglets were scored using direct observation and video for latency and duration of a variety of behavioral indicators of sensation, distress, insensibility, physiologic changes and efficacy. Behaviors were also scored as zero/one data to create percentages of piglets displaying for a variety of behaviors including loss of posture (LP), last movement (LM), gasping (GSP), open mouth breathing (OMB), defecation (DEF), urination (UR), vomiting (VM), licking and chewing (LC) and nasal discharge (ND). Piglet pair was the experimental unit. Analyses of zero/one and latency data were performed in R (v2.12.0, The R Foundation for Statistical Computing) as the univariate product-limit estimation of the survival curves, to determine significant differences. Significance was determined at  $P < 0.05$ . Analyses of durations were log transformed to equalize variance, when non-normal, and analyzed as a mixed model with fixed effects of sex and trt in SAS (v 9.2, SAS Institute Incorporated, Cary, NC). Significance was determined at  $P < 0.05$  using a Sidak correction. Raw means were calculated using Proc Means of SAS. Differences were observed for behavioral indicators of insensibility. Latency (seconds) to LM was shortest for PF & FT, followed by MD then SL (269, 274, 313, and 529 respectively). LP and GSP followed a similar pattern (means ranged over all 4 flow rates LP: 97- 200, GSP: 46-159). All other behaviors were observed with the exception of VM. A difference ( $P < 0.05$ ) was observed for OMB. No piglets displayed this behavior in the CT, while the gas treatments ranged from 80 – 100%; differences were not observed between the flow rates or gas type for OMB. No differences ( $P > 0.1$ ) were observed for any other measures: LC

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(5 – 70, %), ND (0 – 30, %), DEF (25 – 60, %), UR (5 – 35, %). Righting reflex occurred more frequently and for a longer duration ( $P < 0.05$ ) with the ST MIXED treatment relative to other flow rates. Differences were observed for the duration of behavioral indicators of sensation and distress. OMB was shortest ( $P < 0.001$ ) in duration for the PF treatment, followed by FT, MD and SL (19.6, 26.3, 33.7 and 44.8, seconds, respectively). OMB was also prolonged ( $P < 0.05$ ) with the addition of Argon gas for all flow rates. Ataxia was shortest ( $P < 0.05$ ) in the PF, FT, and MD flow rates relative to SL (13.5, 18.7, 20.7, and 38.6. seconds, respectively). The addition of Argon increased the duration of ataxia within all flow rates ( $P < 0.05$ ). Neonate piglets were euthanized as quickly as or faster than weaned piglets for all gases and flow rates (latency (sec): LP 99 vs. 142 ( $P=0.001$ ); LM 360 vs. 392 ( $P=0.045$ ); GSP 97 vs. 139 ( $P < 0.001$ ); for neonate and weaned piglets, respectively). Main effect of age was observed for the proportion of piglets displaying distress or discomfort for two of the four measured behaviors (% displaying: DEF 23 vs. 46 ( $P < 0.001$ ); ND 4 vs. 14% ( $P=0.017$ ); OMB: 97 vs. 94 (0.116); for neonate and weaned piglets, respectively). Gas by age interactions were observed. Differences were observed between the two age groups, with neonates succumbing to the gas effects faster than weaned piglets. In conclusion, MIXED and SL prolonged the duration to insensibility, as measured by LM, LP and GSP. When examined as percentage of animals displaying behaviors, OMB was the only behavioral measure to discern differences between treatments when examining percent of animals displaying, and this was for CT versus all other treatments. In general, more distress behaviors were observed for slower flow rates and with the use of MIXED gas. Thus, based on the parameters measured, 100% CO<sub>2</sub> and a faster flow rate decreases the duration of sensation and distress to the gas.