2013

Effects of flow rate, gas type and disease status on the welfare of sucking and weaned pigs during gas euthanasia

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by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Biomedical Sciences (Physiology)

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Iowa State University
Ames, Iowa
2013

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ABSTRACT

Gas euthanasia of swine on farms is increasingly common. However, there is controversy regarding pig welfare during gas euthanasia and research must be conducted to establish best practices ensuring minimal pain and distress. The objectives of these studies were to determine pig welfare and efficacy of processes with various gas euthanasia procedures: gas type (carbon dioxide, argon, carbon dioxide:argon mixture), flow rate (20%, 35%, 50%, prefill), age (neonate, weaned) and disease status of the pig (respiratory disease/depression vs. other reasons). Euthanasia with inhalant gases can produce confounding effects on physiologic responses, therefore behavior was chosen as the primary outcome of interest for welfare assessment. The results of these studies indicate a carbon dioxide:argon gas mixture and slower flow rates (20%) should be avoided when euthanizing weaned or neonate pigs. Neonate pigs succumb to the effects of gas euthanasia quicker than weaned pigs and display fewer signs of distress, however differences are not great enough to warrant procedures adapted for specific age groups. When comparing induction of anesthesia between 100% carbon dioxide and 100% argon, with implications for piglet processing, carbon dioxide was associated with superior pig welfare (lower distress calls, escape attempts, ataxia, righting response). However, infrastructure currently in place for on-farm gas euthanasia was not reliable for inducing depth and duration of anesthesia necessary for piglet processing. Depression score in suckling pigs and respiratory disease in nursery pigs did not affect responses

associated with efficacy or welfare when carbon dioxide was used. Conversely, with argon suckling pigs with high depression scores displayed longer latencies for loss of posture than pigs euthanized for other reasons and nursery pigs with respiratory disease lost posture faster than pigs euthanized for other reasons. Regardless of disease status, when assessed from behavioral indicators of distress carbon dioxide, relative to argon, was associated with superior pig welfare. Regardless of application method, including all methods tested with carbon dioxide, distress is still observed therefore, ingenuity and research are still needed to identify practical on-farm euthanasia methods that will further reduce pig distress.

CHAPTER 1. INTRODUCTION

Killing of animals has long been required due to mercy, when suffering is present, and as a routine practice in harvesting. In 1958 the U.S. highlighted the growing attention to animal pain and suffering, including during the killing process, with the passage of the Humane Methods of Slaughter Act (Welty, 2007). As described by the American Veterinary Medical Association (AVMA; 2013), “Euthanasia is usually used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress”. Additionally they note, “[We] recognize that complete absence of pain and distress cannot always be achieved”. Similarly, Merriam-Webster (Merrium-Webster, 2013) defines euthanasia as “the act or practice of killing or permitting the death of hopelessly sick or injured individuals in a relatively painless way for reasons of mercy”. Both of these definitions recognize pain and distress are inherent in the process. For the process to be humane, or termed as euthanasia, the goal must be to minimize these during the killing process. Gas euthanasia of swine on farms has been increasing in popularity. However, there is controversy regarding pig welfare during gas euthanasia and research must be conducted to establish best practices ensuring minimal pain and distress. This literature review will address research done in other ages of pigs as well as other species if not explored in the pig, in order to extrapolate and guide research in the young pig. Specifically, this introduction will address key factors involving pain and distress during the gas killing process that may contribute to pain and distress but have not yet been researched fully in the young pig including gas type, flow rate and age of the animal. Additionally, guidelines for application of gas to young pigs will be addressed. First, the U.S. swine industry will be examined and discussed in terms of prevalence and need of euthanasia, including the need for alternative options such as gas euthanasia, establishing the associated need for research in this area.

1.1 Euthanasia in U.S. swine industry

There are several factors to consider when selecting a euthanasia method; of principal importance is the humaneness of the process. The AVMA 2013 euthanasia guidelines cite 14 factors to consider when evaluating a euthanasia method: 1) ability to induce loss of consciousness and death with a minimum of pain and distress, 2) time required to induce loss of consciousness, 3) reliability, 4) safety of personnel, 5) irreversibility, 6) compatibility with intended animal use and purpose, 7) documented emotional effect on observers or operators, 8) compatibility with subsequent evaluation, examination, or use of tissue, 9) drug availability and human abuse potential, 10) compatibility with species, age, and health status, 11) ability to maintain equipment in proper working order, 12) safety for predators or scavengers should the animal's remains be consumed, 13) legal requirements, 14) environmental impacts. Additionally, they note, "… usually there are other mitigating factors that are relevant besides ones pertaining only to animal welfare or the animal's interest(s)". The research contained in this thesis is guided predominantly by the first listed principle, minimizing pain and distress during the euthanasia processes. However, consideration is also given to interests and needs of the swine industry, ensuring this research will be of practical use for the producer and subsequently have strong potential to be implemented by the industry to aid in the reduction of pain and distress experienced by pigs during the euthanasia process. Thus, it is critical we first understand the current state of euthanasia of young pigs in U.S swine industry.

Swine producers and veterinarians generally agree that euthanasia is the best choice for low viability pigs, especially when there is suffering due to injury or illness. The USDA estimates ~120 million piglets are born annually in the United States (Anon, 2010). There are various reasons for pre-weaning mortality, with crushing as the number one reason cited by producers (42%), followed by starvation (30%) and scours (13%) (USDA, 2009). These broad categories do not distinguish specific causes of death. Additionally, the recorded cause is often only the terminal factor. There may have been one or more predisposing influences leading up to death. Not all piglets born alive are viable, with some individuals suffering from physiologic ailments, deformities or underdevelopment that will predispose them to the more broadly defined categories of mortality. Neonate piglets (less than 3 days of age) represent a vulnerable subpopulation due to their small size, limited body reserves and poor immunological status, resulting in susceptibility to crushing and starvation (Alonso-Spilsbury et al., 2007). The average sow gives birth to ~12 live pigs per litter, but weans 10 (NAHMS, 2008). Thus, an estimated 20 million piglets do not survive to weaning per year in the U.S. More than half of these deaths occur in the first 3 days after birth (Alonso-Spilsbury et al., 2007). Pigs less than 3 days of age are classified as neonates, and physiologically differ from older pigs. The potential effects of age during the euthanasia process will be addressed later in this review. During the nursery phase, which is from weaning until around 32 kg in the U.S., there is an estimated 2.9% mortality rate (USDA, 2009). By a large margin, respiratory disease is identified by producers as the leading cause during this phase of production, accounting for nearly 54%. This is followed by starvation at 14%. Based on the 100 million pigs entering the nursery phase per year in the U.S., 2.9 million pigs die, with over 1.5 million attributed to respiratory disease. Thus in total, annually in the U.S. swine industry an estimated 23 million pigs born do not make it to the grow/finish phase. Even when estimated conservatively, if a relatively small proportion of mortalities are euthanized rather than dying naturally, the U.S. swine industry is euthanizing millions of suckling and nursery aged pigs annually. Thus, implications for research findings in this topic have the potential to affect millions of young pigs and the caretakers performing these procedures on an annual basis.

Research is limited regarding swine euthanasia techniques and as such, methods implemented may not be scientifically supported. Traditionally, blunt force trauma has been utilized (Daniels, 2010). Recently, many producers have been moving to gas euthanasia, often for reasons that are not related to animal welfare including recognizing some caretakers find blunt force trauma physically and psychologically difficult to perform (AVMA, 2007 p 4,5; Morrow et al., 2010), safety of personnel, availability of gas, safety for scavengers and environmental impact and legal requirements. Additionally, there has been growing pressure from interest groups not affiliated with industry, to discontinue the use of blunt force trauma (StarTribune, 2012; Huffington Post, 2012;

Smithfield Foods, 2011). When gas is utilized, carbon dioxide (CO_2) is the gas most often used (Daniels, 2010). CO_2 is the only gas listed for use in the *On-Farm Euthanasia of Swine* guide (National Pork Board, 2008). It is identified as an acceptable inhalant method of euthanasia for pigs because it is a rapid depressant with established analgesic and anesthetic properties (AVMA, 2007). High concentrations of CO_2 cause central nervous system depression leading to loss of consciousness and subsequent death (Martoft et al., 2002). Yet, several of the factors, identified in the AVMA 2013 guidelines, have not yet been examined in the young pig, including and perhaps the most importantly, what procedures should be implemented to reduce or eliminate distress and pain. Additionally, time required to induce loss of consciousness, reliability and effects of age and health statuses have not yet been explored for the young pig.

1.2 Guidelines

It is important that guidelines for the implementation of gas euthanasia be critically evaluated, recognizing these documents not only reflect the latest research, but also the general sentiment towards the procedures including public perception and industry interests. These guidelines not only recommend best practices for application of euthanasia technologies but also influence future research. In the United States, gas euthanasia of young pigs is guided predominately by two publications, the AVMA guidelines for euthanasia and the American Association of Swine Veterinarians (AASV)/National Pork Board (NPB) *On-Farm Euthanasia of Swine: Recommendations* *for the Producer*. The research contained in this thesis was conducted prior to the release of the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* and thus was designed with the guidance of the previous edition, which was released in 2007. There are several important differences between these two documents. First, the 2013 guidelines are more detailed than the 2007 version, providing recommendations for both technique and species. However, the 2013 version provides conflicting guidance, not only relative to 2007 guidelines, but also within the document. The 2007 guidelines Appendix 1 (*Agents and methods of euthanasia by species*), lists barbiturates, CO_2, potassium chloride in conjunction with general anesthesia and penetrating captive bolt as acceptable methods for swine. Whereas the 2013 guidelines lists injectable barbiturates as the only acceptable method. The use of CO_2 and argon are listed as acceptable with conditions relating to specific flow rates and concentrations, which will be discussed below.

Guidance for flow rate of CO_2 in these documents is conflicting and unclear. The AVMA 2007 guidelines state flow rate should be at least 20% chamber volume per minute or prefilled. Prefill would seem to be recommended as the 2007 guidelines notes on page 6, "in most cases, onset of loss of consciousness is more rapid, and euthanasia more humane, if the animal is rapidly exposed to a high concentration of the agent". Whereas the 2013 version, under section M1.6 Carbon Dioxide, states a displacement rate of 10-30% should be utilized and that prefill is "unacceptable". In appendix 2 (*Some acceptable agents and methods of euthanasia*), the AVMA 2013 document states only gradual fill methods may be used. In contrast to this, in the same document, under section S.3.3.2.2.1 of the 2013 guidelines, it notes “pigs may be exposed to CO_2 by gradually displacing ambient gases (introducing CO_2 into the container) or by introducing the animals into the prefilled environment”. Prefill is described as the box filled with CO_2, the pigs placed within and gas initiated again to create an 80-90% CO_2 concentration for a minimum of 5 minutes. In the 2007 version, guidance for prefill notes a concentration greater than 70% must be established and maintained for at least 1 minute after clinical death. The 2013 guidelines further clarify maintenance of CO_2 for at least 1 minute after respiratory arrest.

The recommendations for use of argon have seen a dramatic change between the 2007 and 2013 documents. The recommendations for application are similar; the 2007 guidelines recommend achieving an oxygen concentration less than 2% quickly and maintaining this level for greater than 7 minutes. The 2013 guidelines note prefill **must** (emphasis added) be utilized and O_2 levels maintained for more than 7 minutes. The major difference is observed with the 2007 guidelines noting the animal must be **heavily sedated or anesthetized** (emphasis added) for the use of argon, whereas the 2013 guidelines do not mention sedation or anesthesia for swine. The 2013 guidelines have two peculiar statements with relevance to the use of argon. The first comes under the direction for the use of CO_2 for pigs, which states, “if air exchange rates are not carefully controlled and monitored, animals may suffer substantial stress from suffocation prior to loss of consciousness and death” (page 60). As argon is a noble gas, and likely unreactive throughout the physiological systems, suffocation is its likely mode of action. The second statement comes on page 24, “[argon is] unacceptable for other mammals”.

To date, research does not support a unique quality to the pig's respiratory system to warrant isolation from all other mammalian species.

The AASV/NPB document is similar to the guidelines recommended in the 2007 version of the AVMA, recommending either gradual or prefill and creating a CO_2 concentration of 80-90% for at least 5 minutes. Both the AVMA and AASV/NPB guidelines provide additional guidance that is outside the scope of this thesis, addressing many of the other mitigating factors such as the practicality, relative cost of the procedure and safety of personnel.

The conflicting information within the 2013AVMA guidelines seems to illustrate, even among experts, there is ongoing debate about what methods should be utilized. Additional research conducted since the release of the 2007 guidelines is not cited to support the change so it is surprising the guidelines would change so dramatically. These discrepancies highlight the need for additional research in young pigs, as they are likely a result of the limited available research, thus requiring speculation and allowing room for bias by public perception and industry interests.

The 2013 guidelines highlight the need for additional research: "There is little published research on appropriate techniques for euthanizing young (neonatal and growing) pigs". In addition, both sets of guidelines mention specific areas of recommended research. The 2007 guidelines note, "Neonatal animals appear to be resistant to hypoxia and because all inhalant agents ultimately cause hypoxia, neonatal animals **likely** take longer to die than adults" (emphasis added). The 2013 guidelines note, "Carbon dioxide may be effective as a method of euthanasia for small groups of neonatal piglets; however, the parameters of the technique need to be optimized and published to ensure consistency and repeatability”. Additionally, the 2013 document notes, “in particular the needs of piglets with low tidal volume must be explored.” Under the Carbon Dioxide guidance, the 2013 guidelines also note, “small or incapacitated piglets have low tidal volumes and will not die as rapidly as larger, more viable pigs”. However, there is not research cited for this statement. The suggested targeted research areas in the AVMA guidelines for gas euthanasia of pigs are addressed throughout this thesis.

1.3 General physiologic control of carbon dioxide

Before deciding on measurement techniques to assess distress associated with the gases, it is important to understand the mammalian body’s natural response to them. This is especially interesting with CO_2, which is a natural byproduct of metabolism and highly regulated in the mammalian system. Respiration serves as the primary method for regulation of CO_2. Gas exchange occurs across the respiratory membrane of the alveoli; oxygen (O_2) and CO_2 are lipid-soluble and easily cross this membrane. Under normal atmospheric conditions, O_2 is pulled into the body and CO_2 is expelled. CO_2 is diffused approximately 20 times faster than O_2, allowing for quick removal of CO_2 from the respiratory system under normal atmospheric conditions (Martini et al., 2003). Similarly, in a modified atmosphere with increased CO_2, diffusion into the system would also be quick. The respiratory exchange surface receives blood from the pulmonary circuit, which then distributes the gases throughout the body. In a modified atmosphere with increased CO_2 concentrations, more CO_2 will be carried to the body. Upon entering the tissue capillaries, CO_2 almost instantaneously undergoes a series of physical and chemical reactions. Under normal conditions, 7% is transported as CO_2, 23% is transported with hemoglobin, but the majority is transported as bicarbonate (Martini et al., 2003).

There are 3 primary systems that regulate the H+ (CO_2) concentrations in the body fluids (Guyton and Hall, 2010, chap.40): 1) chemical acid-base buffer systems, which immediately combine with acid or base to prevent excessive changes in H+ concentrations 2) the respiratory center, which regulates removal of CO_2 (and hence bicarbonate) from the extracellular fluid and 3) the kidneys, which can excrete either acidic or alkaline urine, effectively altering extracellular fluid H+ concentrations. The buffering system responds in a fraction of a second to changes in acid or base balance, but is quickly overwhelmed when abnormal conditions are presented, such as during euthanasia with $CO_{2.}$ The respiratory system serves as the secondary line of defense and responds to increases in CO_2 within seconds to minutes. During euthanasia with gas CO_2, the body’s natural response (increased respiration and blood flow) to the CO_2 actually speeds up the intake of CO_2. Finally, the kidney requires hours or even days to produce a meaningful response and, as such, is not of consequence during a typical gas euthanasia process. Considering the physiology of these responses, the respiratory system is of primary concern for gas euthanasia techniques.

Respiration (rate and depth) is controlled by the respiratory centers, an integration of 3 pairs of loosely organized nuclei in the reticular formation of the pons and medulla (Guyton and Hall, 2010, chap.41). These centers can be further divided by their function; rate is controlled by the respiratory rhythmic center of the medulla oblongata. Under quiet respiration, only neurons from the inspiratory centers fire, with exhalation being passive. The respiratory center of the pons can adjust the respiratory rate and depth in response to sensory stimuli, emotional states or vocalization patterns. Reflex control of respiration is under the control of both mechanoreceptors and chemoreceptors. The inflation and deflation reflexes (Hering-Breuer reflexes) are under the control of mechanoreceptors and ensure the lungs do not over-expand or expire too much. Chemoreceptor reflexes respond to blood and cerebrospinal fluid. Chemoreceptor centers in the carotid and aortic bodies are sensitive to the pH, P_{CO2} and P_{O2}. Receptors in the medulla oblongata respond only to pH and P_{CO2}. During hypercapnia (decreased pH), these receptors stimulate the respiratory centers to both increase rate and depth of respiration. It is rare under natural conditions for the O_2 receptors to be activated; however, during hypoxia, these centers also stimulate the respiratory centers to increase rate and depth of respiration. The increased H+ concentration or O_2 deficiency quickly causes dilation of the cerebral vessels, almost doubling normal blood flow. During hypercapnia, the increased H+ concentration greatly depresses neuronal activity. During euthanasia with argon, O_2 would be quickly used up by metabolism, resulting in hypoxia. With inadequate oxygen levels, normal brain function ceases and eventually coma is induced. Exposure of animals to hypoxia induced with argon, nitrogen or other inert gases causes depolarization and intracellular metabolic crisis leading to death in neurons (Rosen and Morris, 1991; Hsu and Huang, 1997). Brain oxygen deprivation leads to accumulation of extra-cellular potassium and depletion of energy substrates and accumulation of lactic acid in the neurons. This generated brain damage is likely not a factor during a successful euthanasia effort, but would be of concern if the animal were allowed to recover.

In the cat model, it is observed that an abrupt change of CO_2 concentrations in inhaled air leads to a gradual change in $P_{CO2,}$ with ½ value being reached in approximately 1 minute and equilibrium in 5 to 10 minutes. Once normal atmospheric conditions are restored, recovery is complete in approximately 1 minute. In general, low doses of CO_2 have an excitatory effect throughout the body, whereas high doses result in depressive effects (Krnjevic et al., 1965). CO_2 is a known depressant of the cortex and brain stem, with both anesthetic and analgesic properties (Mischler et al., 1994; Mischler et al., 1996). Due in part to these effects, CO_2 has been commonly used for euthanasia of laboratory animals and poultry (Hackbarth et al., 2000; Raj and Gregory, 1995). Additionally, it has been used for over 60 years to stun market hogs prior to exsanguination (Rodríguez et al., 2008). This has led to the majority of research in this field being conducted on market weight pigs, with CO_2 being studied for its effectiveness as a stunning agent and its effects on meat quality (Dodman, 1976; Nowak et al., 2007). The specific effects of CO_2 in the young pig still need to be explored.

1.4 Welfare of pigs exposed to carbon dioxide

1.4.1 Terminology

A brief tangent will address terminology, allowing comparisons within published research. It is critical when comparing gas euthanasia techniques and subsequent pig welfare that, as within any science or discipline, a universal terminology is developed. Currently, when assessing the gas euthanasia process, it can be difficult to compare studies due to differences in terminology utilized between authors. For example, open mouth breathing is observed during the gas euthanasia process. This response is a physiological reaction and has been noted by many researchers, often using different terms (hyperventilating in Martoft et al., 2002; respiratory distress in Raj and Gregory, 1996; gasping in Rodríguez et al., 2008; breathlessness in Liotti et al., 2001). When describing behavioral responses, it is important to utilize terminology that describes the behavior, e.g. open mouth breathing, rather than potentially associated pain or distress, e.g. breathlessness or respiratory "distress". The term hyperventilation is used to describe a condition that is triggered by a lack of oxygen to the brain (Blood et al., 2007, p.920). This is not a result of a lack of oxygen throughout the body; rather it is due to sub-normal blood CO_2 levels, which cause constriction of the blood vessels to the brain, depriving it of oxygen and other required hormones and nutrients that maintain proper function of the nervous system. In humans, this condition may result from anxiety or if one exercises above VO_2 max (Guyton and Hall, 2010, chap.42). As low CO_2 levels are an important part of the hyperventilation process, this terminology is not applicable when CO_2 is utilized as the euthanizing agent and as such is not used appropriately by Martoft et al., 2002.

Following open mouth breathing, a second and distinct respiratory pattern is observed. In humans, the chemoreceptors responsible for the open mouth breathing response are located in the medulla (Guyton and Hall 2010 Ch 41). Once regular breathing fails, which is controlled by the ventral respiratory group and includes open mouth breathing, gasping is recruited (St John, 2009), which is an indicator of loss of consciousness (Miura et al., 1996; St John, 2009). When describing this behavior, emergency medical personal often refer to it as agonal breathing, but this term should be avoided as it correlates the concept of “agony” with the breathing response. Since the pig is unconscious during this behavior, it would be misleading to refer to it as such.

1.4.2 Assessment of welfare during euthanasia

Euthanasia is comprised of two stages: (1) induction of unconsciousness (insensibility) and (2) death. It is the induction phase that is critical to ensure the welfare of the pigs due to the potential for suffering. The entire process, including death, is important to ensure practical implementation. Additionally, irreversibility is important to the welfare of the pig that they are not allowed to regain sensibility. The degree of pain and distress created by CO_2 during induction is contested, along with its suitability as a euthanatizing agent. CO_2 is mildly acidic, which may cause irritation to the mucus membranes (Danneman et al., 1997). CO_2 has long been criticized and legislated, and is not allowed for stunning of market hogs in the Netherlands since 1980 (Hoenderken, 1983).

Pain and distress are affective states and can only be measured indirectly in humans and animals. No single parameter is able to definitively indicate if an experience is painful or distressing. Measuring the hypothalamic-pituitary-adrenal (HPA) axis activity is a common method to assess pain, distress and general welfare of pigs, as well as other mammals (Mormède et al., 2007; Chapados et al., 2009; Möstl and Palme, 2002). The hormones (cortisol, epinephrine, norepinephrine) produced by the HPA axis have been shown to be good indicators of acute stress, but may not be appropriate measures in chronic reactions. Large differences have been observed in responses between species. There are several reasons why measurements from the HPA axis in pigs may not be a viable option when assessing pain and distress, starting with the collection process. In the pig, collection of blood samples can be difficult and time consuming due to the pig's anatomy (Mormède et al., 2007). This can make it challenging to differentiate the distress of the acute incident from the pain and distress due to the collection method. Although this can be overcome with catheterization, this may not be practical in euthanasia studies due to 1) movement, during both conscious and unconscious phases, making maintenance of catheter patency difficult 2) recovery time is needed post-catheterization allowing the HPA-axis hormones to recover prior to initiating the euthanasia process, which may not be practical due to the health of the pig 3) catheterization may change the behavior of the pig, which is especially relevant if behavior is utilized in tandem as an assessment tool of welfare. Additionally, in newborn piglets, a non-responsive phase exists, in which pain and stress will produce no or minimal changes in the HPA hormones (Mormède et al., 2007). Finally, there is a lag time between the distressing or painful incident and measurable cortisol response. In an adult pig it takes 10 minutes to reach maximum cortisol level (Mormède et al., 2007). As such, when applied to euthanasia, which may have differing durations, results may be confounded.

Substances such as CO_2, which are produced by the body and are present under normal conditions, are especially difficult to assess, as it is difficult to ascertain what is a direct physiological response to the gas rather than a response to psychological distress of gas application. For example, increased respiration is often used in human studies as an indicator of pain (Weissman and Matson, 1999; Franck et al., 2000). However, when exposed to a modified atmosphere with increased concentrations of CO_2, the body responds by increasing the respiratory rate. Additionally, heart rate and heart rate variability are common measures when assessing distress (Ritter et al., 2009; von Borell et al., 2009; von Borell et al., 2007). Here again, CO_2 has a depressant effect on the heart rate in pigs (Martoft et al., 2002) and would confound any results. Thus, it is important that a variety of measures be taken and assessed to create a convincing body of evidence regarding welfare during gas euthanasia. This is not to say these measures are useless, rather it highlights the importance to not regard any single measurement as the sole assessment of welfare during euthanasia.

Behavioral response to pain and distress provide more sensitive measures of the animal’s experience than physiologic responses (Rault et al., 2011; Marchant-Forde et al., 2009), particularly since euthanasia with inhalant gases can produce confounding effects on physiologic responses (Burkholder et al., 2010). Behavioral responses, such as escape attempts, open mouth breathing, sneezing, coughing, head shaking and vocalization among others have been utilized to assess pain, distress and sensation to gas euthanasia (Dodman, 1976; Gregory et al., 1990; Raj and Gregory, 1996; Raj et al., 1997; Velarde et al., 2007; Rodríguez et al., 2008). Results and implications from studies utilizing physiological and behavioral response will be addressed later in this thesis.

As noted, the vocalization response of pigs has been utilized to assess pain and distress in pigs. This measurement has received unique attention in the pig and warrants further discussion. It has been shown pigs encode information about the callers condition when vocalizing, allowing assessment of the caller's functional state (Weary et al., 1998). Assessment and analysis of the vocal response can be conducted through a number of techniques, from simple measurements such as number of calls (Sutherland, 2011; Grandin, 1998) and subjective assessment using human perception to discriminate a distress calls from other vocalizations (Hartmann et al., 2010). Less refined methods such as these may result in errors and misinformation, since it is unclear if the vocalizations are truly related to distress. Objective measures of vocalization are also possible. Vocalization calls during known painful and distressing events (castration, restraint, back test) have been compared to vocalizations during benign events (in pen) or distressing but non-painful events (removed from dam or conspecifics). The vocalizations during these events have been characterized by frequency with clear indication of vocalizations made during painful and distressing events (Weary et al., 1998; Puppe et al., 2005). To further increase the objectivity of assessing pig vocalizations, a program, STREMODO, has been developed based on these established parameters and "training" of the program to distress calls. STREMODO automatically detects the presence and duration of calls made from a pig in distress. This program has been validated and proven reliable (Schön et al., 2004; Schon et al., 2001).

1.4.3 Central nervous system depression and corresponding behavioral reaction (establishing insensibility)

Although variations in behavior are observed during induction of insensibility, it is difficult to ascertain whether these are accurate indicators of distress since these behaviors may coincide with the induction process or be observed after the piglet is insensible. Establishing loss of consciousness (insensibility) has not been straightforward. Forslid (1987) was the first to describe and examine CO_2 induction of insensibility in detail, including several aspects related to welfare such as evaluating distress caused during induction. EEGs showed neocortical slow waves started to increase and slow wave activity became dominant a few seconds before muscle excitation began, indicating these movements were not voluntary. However, others have questioned this interpretation of the EEG pattern (Raj et al., 1997; Velarde et al., 2010). In addition, it has been demonstrated that EEGs are not reliable sole predictors of the depth of anesthesia (Raj et al., 1997). In an effort to better assess this issue, Raj and colleagues (1997) examined somatosensory evoked potentials in pigs and found that during euthanasia with CO_2, pigs experienced moderate to severe respiratory distress for a "considerable period of time" [4-15 seconds] prior to loss of consciousness. More recently, Martoft and colleagues (2002) used somatosensory evoked potentials matched with EEGs, and blood gas parameters for oxygen (P_{O2}) and carbon dioxide (P_{CO2}). They specifically aimed to assess muscular activity, which had previously been described as an indicator of extreme distress (Raj et al., 1997). Muscle excitation was observed 13 to 30 seconds after exposure to the gas, but they also observed suppression of the central nervous system and changes in the blood gas parameters almost immediately upon exposure into the gas, with a temporal relationship existing between depth of anesthesia, somatosensory evoked potentials and blood P_{CO2}. They concluded that capability to experience pain or distress from exposure to gas was diminished and responses were likely physiologic in nature rather than psychological. In a similar study using the same measures, Rodríguez and colleagues (2008) found a latency of 60 seconds for loss of auditory evoked potentials. Although they also found a temporal relationship with depression of the central nervous system and blood gas parameters, they concluded that muscle excitation was conscious movement. These conflicting results highlight the difficulty even “objective” measures have when assessing pain and distress and the importance of inclusion of a variety of measures when assessing affective states in pigs. While the exact point of loss of consciousness is debated, an important aspect can be extrapolated. When conducting studies utilizing unadulterated behavior (motor pattern of the free moving pig), it is reasonable to assess loss of consciousness utilizing loss of posture, since these two events roughly correspond (5 to 10 seconds). Other less technical measures can also be utilized, such as the brainstem reflexes including corneal, palpebral and pupillary light reflexes. Spinal reflexes can also be tested, including pedal reflex or nose prick. Other indicators of insensibility include the presence of gasping, lack of jaw tone, lack of muscle tension and tonic or clonic seizures, although the latter of these may be difficult to differentiate from conscious movement (Klide, 1996).

1.4.4 Assessment of stress hormones in pigs during CO_2 exposure

Forslid (1987) has observed increases in stress hormones during a simulated stunning process, including a three-fold increased plasma cortisol from handling (prior to exposure to CO_2), with no significant increase post-exposure. Epinephrine and norepinephrine increased 15 and 50 fold respectively during stunning. His research also demonstrated that a market pig with 15 seconds exposure to 90% CO_2 showed pronounced arterial hypoxia, hypercapnia and acidosis. Acidosis is a powerful stimulant of the HPA-axis, and thus increased epinephrine and norepinephrine do not provide direct evidence that exposure to CO_2 is a psychological stressor. Nowak and colleagues (2007) showed that pigs exposed to 80% CO_2 had lower pH values than pigs exposed to 90% CO_2. These decreased pH values corresponded to pig movement and heartbeats during the exsanguination process. Additionally, these pigs had final meat pH values below the level desired for meat quality. This lower tissue pH value may be due to the observed movements and consequent buildup of lactic acid. Ultimately, these authors concluded better pig welfare and meat quality were achieved with exposure of 90% CO_2 for 100 seconds relative to 80% CO_2 for 70 seconds, 80% CO_2 for 100 seconds or 90% CO_2 for 70 seconds. In the young pig during gas euthanasia with CO_2, Sutherland (2010) found a 200 fold increase in cortisol levels relative to baseline. Additionally, final cortisol levels did not differ between gradual fill and prefill gas flow rates. However, as noted previously, such a physiologic response would be expected during exposure to CO_2 regardless of psychological response. Likewise, and as expected, epinephrine levels were significantly increased.

1.4.5 Behavioral assessment of pigs during CO_2 exposure

Jongman and colleagues (2000) used aversion learning techniques to demonstrate that CO_2 concentrations of 90% and 60% were less aversive than a shock from an electric prod and not different than a control treatment, in which pigs were moved through the stunning apparatus without exposure to CO_2. Lagerweij and Utrecht, (1990) found pigs exposed to 30% or 70% CO_2 refused to eat and attempted escape, yet showed no conditioned avoidance response to the induction box one day post-exposure, suggesting the experience was not aversive. These two studies would seem to indicate pigs do not find CO_2 highly aversive, but there is concern amnesia may have been produced by the CO_2 exposure rendering subsequent measurements of aversiveness unreliable. Velarde and colleagues (2007) attempted to address this issue. After habituating pigs to a dip-lift and ambient air for 3 days, pigs were exposed to 66 ± 3% or 85 ± 3% CO_2 concentrations for 1 second, plus 30 (66%) or 20 (85%) seconds descent and ascent into and out of the pit for three consecutive days. This method ensured the pigs did not lose consciousness and subsequently may retain memories of the experience. By the last day of exposure to the CO_2, pigs exposed to 66% entered the crate faster, with fewer escape attempts and more often voluntarily relative to the 85%. However, the experiments were conducted on different days with different pigs, allowing day and pig effects to contribute to these observed differences, confounding the results. Independent of concentration, after exposure to CO_2, fewer pigs would voluntarily enter the crate, a greater number attempted to escape, and time to enter the crate increased, indicating that when pigs retain their memory, they find CO_2 aversive.

At this time, research indicates that the use of CO_2 at any level or flow rate results in some level of sensation and distress for all currently tested categories of pigs (Raj and Gregory, 1995; Sutherland, 2011). If CO_2 is to be used for on-farm euthanasia, further research is needed to identify the methods which will produce the least pain and distress.

1.5 An alternative gas to carbon dioxide?

Some individuals are adamantly opposed to the use of CO_2 to induce loss of consciousness, citing studies on humans and the conflicting literature regarding the welfare of market hogs during CO_2 exposure. At 10% CO_2 concentrations, the majority of human subjects report experiencing breathlessness, described as being unpleasant, and 50% CO_2 concentration is reported as being very pungent (Gregory et al., 1990). Additionally, results indicating perceived distress observed in market pigs during anesthetic induction (Raj and Gregory, 1995) have led to questions about the humaneness of CO_2 for pig anesthesia or euthanasia (Wright et al., 2009; Raj et al., 1997; Raj and Gregory, 1996; Rodríguez et al., 2008). Argon has been proposed as an alternative inhalant agent for euthanasia (Raj and Gregory, 1996). Argon is a noble gas, and as such is likely unreactive throughout the physiological systems (Mann et al., 1997). Loss of consciousness and death are produced through hypoxia, creating the physiological state hypocapnic anoxia (Raj, 1999).

During hypoxia, which is produced by argon during euthanasia, most tissues of the body can live without oxygen for several minutes and some for as long as 30 minutes. During this time, cells obtain their energy through anaerobic metabolism, and although this process requires a great deal of energy (glucose and glycogen), it keeps these tissues alive. The brain is only capable of very limited anaerobic metabolism and depends on second-by-second delivery of oxygen from the blood. Without this oxygen, unconsciousness occurs quickly (Yue et al., 1997).

The European Food Safety Authority recommends stunning with 30:60 CO_2:argon or 90:10 argon:air (EFSA, 2004). Market weight pigs do not display behavioral indicators of aversion and will repeatedly enter a chamber for a food reward, remaining in the chamber until ataxia causes them to fall out of the chamber and into atmospheric air (Raj and Gregory, 1995). Surprisingly, Raj and colleges (1997) concluded somatosensory evoked potentials indicated the passive effects (hypoxia) of 90% argon, or a 30:60 CO_2:argon gas mixture, resulted in decreased latency to loss of somatosensory evoked potentials relative to the active effects (hypercapnia) of 80% CO_2, requiring 15 and 17 seconds for argon and CO_2:argon, respectively vs. 21 seconds for CO_2 alone. In the young pig, Sutherland (2011) found increased cortisol and epinephrine levels relative to baseline when pigs were stunned with argon. Additionally, signs of distress including open mouth breathing, escape attempts and vocalizations were observed during the exposure to argon, bringing into question whether distress and pain are lessened by the use of argon relative to CO_2.

Argon is thought to be a promising alternative to CO_2, yet remains understudied. As with all euthanasia techniques, refinement of the process is necessary to ensure minimal distress. Additionally, while successful implementation of CO_2 has been demonstrated on-farm, the practicality of using argon on farms has not yet been explored.

1.6 Reliability and latency to death

In market hogs, it is critical to the welfare of the pig that consciousness is not regained prior to death via exsanguination. Likewise, return to sensibility during euthanasia on farm is likely to result in compromised welfare. When used on farms it is critical the euthanizing gas proves successful to not only cause loss of consciousness, but also death. Whereas the interpretation of EEGs does not lead to a clear conclusion regarding loss of consciousness, it certainly is the current gold standard for assessing death. Studies examining death rather than latency to recovery are very limited in the pig. In the market hog, different gas concentrations, exposure times and latency to recovery post-exposure have been explored. Nowak and colleagues (2007) found 11% of pigs displayed a corneal or palpebral reflex when hogs were checked 25 to 35 seconds after exposure to 90% CO_2 for 100 seconds. Raj (1999) found that the majority of market weight pigs exposed to 80 to 90 percent CO_2 died after 7 minutes of exposure. Sutherland (2010) found in pigs 1 to 6 weeks of age latency to loss of posture was shorter for prefill vs. gradual fill procedures (~50 vs. ~150 for prefill and gradual, respectively). However, in the neonate pig efficacy still needs to be explored including latency to death (respiratory and cardiac arrest). This is especially important as differences have been observed in the way neonates of other species respond to gas euthanasia. This will be discussed in detail later in this review.

1.7 Critical factors that may affect the euthanasia process, current research and why they may matter

1.7.1 Flow rate

Whereas gas concentration and type have been relatively well-studied and debated, it is somewhat surprising the flow rate or latency of exposure to full concentration has not received more attention. Two studies have examined flow rate in the rat model, one each with CO_2 and argon. Niel and colleagues (2008) examined flow rates from 3 to 27% chamber volume exchange rates per minute. During slow flow rates, rats would remain in the box for a food reward until concentrations reached ~16%, whereas during fast flow rates they would leave when concentrations reached 13%. Regardless of flow rate, no rats remained in the box until loss of consciousness, thus leading the authors to conclude that regardless of flow rate, rats find CO_2 aversive. In a similar study, effects of flow rates of 120% to 239% chamber volume exchange rate per minute were examined when using argon (Makowska et al., 2008). Makowska and colleagues found that regardless of flow rate, rats left the cage when O_2 levels decreased to around 7%, concluding that hypoxia produced by argon, regardless of flow rate, is aversive to rats. The Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals (Hawkins et al., 2006) examined the literature available for rodents and humans available up to 2006. They noted that humans reported concentrations above 50% to be painful whereas low concentrations were reported as only causing breathlessness. Primarily based off these human studies, they concluded exposure to high concentrations of CO_2 should be avoided and a 20% chamber volume exchange rate per minute was preferable for rodents. It is important to note that this recommendation was based from studies where humans were exposed to an instant concentration of 50%, rather than an increasing concentration of CO_2. The human studies are not sufficient to indicate that a 20% chamber volume exchange rate per minute is preferable to other flow rates, but may imply that slower flow rates would be preferred over prefill conditions. However, this conclusion is not supported in pigs. Sutherland (2010) examined gradual fill (20% chamber volume exchange rate) vs. prefill conditions in young pigs (age not provided) during on-farm application. Differences were not observed in measures of welfare (duration of escape behavior, plasma cortisol levels) while the process was prolonged (465 ± 23 seconds gradual vs. 313 ± 56 seconds) with the gradual fill. Since the duration of the process was prolonged with gradual and there were no observed benefits, prefill was recommended. Given that distress is observed with both CO_2 and argon, it is critical that flow rates beyond prefill or 20% chamber volume exchange rate per minute, be explored to identify procedures that produce the least distress, and correlate with highest welfare.

1.7.2 Age of pig

Since the use of gas had primarily been used for stunning of market hogs, little research has been conducted in the young pig. Yet the neonate pig may differ from the adult in several important aspects. The term neonate has a somewhat vague definition. In humans, this term generally refers to babies less than 1 month of age. In pigs, this term has been used to describe animals as old as 7 or even 28 days (Lecce and Morgan, 1962; Johansson and Karlsson, 1982; Matted and Carroll, 1997) , however more recently it has been used to describe piglets less than 72 hours of age (van der Lende and de Jager, 1991; Litmanovitz et al., 1994; Martin et al., 2005). Perhaps of critical importance for euthanasia is nervous system development and changes to blood composition, subsequently affecting affinity for oxygen and ability to transfer gases throughout the system. Some changes to the blood of the pig require several weeks before adult levels are reached. For example, 3 to 8 weeks are required for total lipids, cholesterol and low-density lipoprotein values to decrease to adult level. Conversely, high-density lipoprotein values remain high in 3 to 8 week old pig in comparison to the adult pig. The levels of total serum lipids, cholesterol, high and low density lipoproteins are low at birth in comparison to those of the adult pig, but increase to higher levels after the onset of colostrum and milk ingestion (Johansson and Karlsson, 1982). The serum of newborn pigs before suckling is characterized by a very low concentration of total proteins (approximately 25 mg mL^{-1}), low levels of albumin and transferrin and the lack of immunoglobulins. In contrast, α_1-acid glycoprotein and fetuin are present at high levels (approximately 12 and 5 mg mL^{-1} respectively). Martin and colleagues (2005) showed piglets undergo a very rapid metabolic maturation with regard to serum proteins, evolving from a characteristic ‘fetal’ pattern to ‘adult’ by 10 days of age. The evolution of serum levels of these proteins suggests that piglets must overcome a moderate acute-phase situation during the first week of life. This is particularly relevant for low viability pigs in the first 24 hours, since if they fail to nurse they are likely physiologically different.

Fetal and adult pig hemoglobin have similar affinity to oxygen but react differently to 2,3-diphosphoglycerate (2,3-DPG). This effectively increases O_2 affinity in fetabl hemoglobin. By 5 days of age, blood hemoglobin and 2,3-DPG levels are similar to the adult pig (Baumann et al., 1973). If differences are observed by age, they are likely to be in pigs less than 5 days of age. However, differences observed, in relation to efficacy of gas, are likely not solely due to differences in increased affinity for oxygen, since neonates of species in which no fetal hemoglobin is present, such as the mouse, still display resistance to the effects of CO_2 (Pritchett et al., 2005). Thus, resistance in neonates may be due to other factors, such as the decreased metabolic rate and general resistance of the brain to damage by hypoxia. Further research is needed to understand these differences.

Development of the lung structure and response to hypoxia may also affect the gas euthanasia process. Development occurs in the lungs of the young pig following birth, with lungs considered structurally similar to that of an adult at 12 weeks of age (Rendas et al., 1982). From birth until 9 to 12 weeks of age, within the lung, the relative volumes of respiratory bronchiolar and alveolar ducts increase. Circulatory system response to hypoxia is also altered relative to the adult pig until pigs are 9 to 12 weeks old. Rendas and colleagues (1992) showed an age affect to response of hypoxia (10% fractional inspired oxygen). Pigs 2 to 4 weeks old increase pulmonary arterial pressure 41% whereas pigs 9 to 12 weeks old increase 137% above baseline. Differences were also observed for total pulmonary resistance 66% vs. 139% above baseline for pigs 2 to 4 weeks old vs. 9 to 12 weeks old respectively. These differences likely result in decreased gas exchange in the young pig relative to the older pig.

Another important consideration is brain development and subsequently the ability to feel pain. The brain matures in an organized, predetermined pattern correlating with the functions the newborn performs at various stages of development. In the human neonate, the ability to perceive pain is questioned, since myelination of the white matter, which is responsible for transmission of neural impulses, has not reached full maturation. Though this is a debated topic, there is ample supporting research that a human neonate's capacity for pain is present at birth (Fabrizi et al., 2011) Though significant myelination development of the pig occurs through 5 weeks of age, as a precocial species it is likely capable of pain and has been shown to be able to respond to aversive conditions.

It has been demonstrated in several species that achieving successful euthanasia for neonates may take longer or require higher gas concentrations relative to the more mature animal (AVMA, 2007). In addition, anecdotal reports from stockpeople suggests neonates are more difficult to euthanize than older pigs. Sutherland (2010) examined pigs 1 to 6 weeks of age, and although statistical differences were observed for latency to death (~400 seconds for 6 weeks old vs. ~600 seconds for 3 weeks old), the variability within an age group was greater than 100 seconds and thus, from a practical stance, it is not necessary to develop procedures for each age group. The neonate (< 72 hours), which has showed differences in other species, has not yet been examined in the pig.

1.7.3 Disease status

In addition to limited research available on the young pig, researchers have generally examined stunning and euthanasia of healthy pigs. This is logical since healthy pigs are of interest for market and slaughter. Even for euthanasia studies, this is logical first step, since having a uniform population of pigs requires fewer replicates for statistical power. Additionally, sourcing or producing sick pigs for euthanasia studies can be logistically challenging. However, this leads to an information gap between the known research and the pigs on which euthanasia is most likely to be applied, the unthrifty or sick pig. Just as data from market weight pigs cannot always be extrapolated to the young pig, it is important not to assume the sick pig will respond to gases in a manner similar to the healthy pig. Specifically, pigs with compromised respiratory systems are of concern. Pigs with swine respiratory disease (SRD) will have reduced lung capacity and resulting efficiency due potentially to a variety of insults including hemorrhage, fibrous formation and edema (Straw et al., 1999). Inhalant euthanasia agents use the respiratory system to cause death; with the sick pig, and specifically those afflicted with illness affecting the respiratory system, there may be differences observed due to changes in the lungs. It is currently unknown how this system, when in a compromised state, will affect the efficacy and distress produced during gas euthanasia. Research regarding best euthanasia practices for SRD pigs has the potential to impact welfare for a significant number of animals requiring euthanasia every year.

1.8 Research objectives

Candidates for euthanasia are often in pain and suffering. A number of different factors must be considered to protect the pig welfare and to be consistent with the goal of a “good death”. The overall duration of the process is of utmost importance, specifically noting the intensity and duration of pain associated with inhalant euthanasia. Factors other than the inhalant may also contribute to pain and distress during the euthanasia process such as isolation, novel environment, restraint, safety and physical comfort for the animal. In addition, implementation of euthanasia techniques can vary greatly and without scientific justification to guide standard operating procedures, the welfare and efficiency of any method can be severely compromised. The research presented in this thesis will contribute to the body of knowledge regarding gas euthanasia and help ensure that best practices can be developed to protect pig welfare by minimizing pain and distress and increasing efficacy and speed of the euthanasia process.

1.9 References

Alonso-Spilsbury, M., R. Ramírez-Necoechea, M. González-Lozano, D. Mota-Rojas and M. Trujillo-Ortega. 2007. Piglet survival in early lactation: a review. Journal of Animal Veterinary Advances. 6, 76 – 86.

Anon. 2010. Quarterly Hogs and Pigs. USDA, National Agricultural Statistics Service. Available: http://usda01.library.cornell.edu/usda/nass/HogsPigs//2010s/2010/HogsPigs-12-27-2010.pdf.

AVMA. 2007. Guidelines on euthanasia, formerly report of the AVMA panel on euthanasia. American Veterinary Medical Association. Schaumber, IL, USA. Accessed January 2012

AVMA 2013 *AVMA Guidelines for Euthanasia of Animals: 2013 Edition*. American Veterinary Medical Association: Schaumber, IL, USA. http://www.avma.org/issues/animal_welfare/ euthanasia.pdf. Available: Accessed May 30, 2013.

Baumann, R., F. Teischel, R. Zoch and H. Bartels. 1973. Changes in red cell 2,3-diphosphoglycerate concentration as cause of the postnatal decrease of pig blood oxygen affinity. Respiration Physiology. 19, 153 – 161.

Blood, D.C., V.P. Studdert and C.C. Gay. 2007. Saunders Comprehensive Veterinary Dictionary. WB Saunders\ Elsevier Science. Philadelphia, USA von Borell, E., J. Baumgartner, M. Giersing, N. Jaeggin, A. Prunier, F. Tuyttens and S.A. Edwards. 2009. Animal welfare implications of surgical castration and its alternatives in pigs. Animal. 3, 1488 – 1496.

von Borell, E., J. Langbein, G. Després, S. Hansen, C. Leterrier, J. Marchant-Forde, R. Marchant-Forde, M. Minero, E. Mohr and A. Prunier. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals--a review. Physiology & behavior. 92, 293 – 316.

Burkholder, T.H., L. Niel, J.L. Weed, L.R. Brinster, J.D. Bacher and C.J. Foltz. 2010. Comparison of carbon dioxide and argon euthanasia: effects on behavior, heart rate, and respiratory lesions in rats. Journal of the American Association for Laboratory Animal Science: JAALAS. 49, 448.

Chapados, I., C.L. Chik and P.-Y. Cheung. 2009. Plasma cortisol response to ACTH challenge in hypoxic newborn piglets resuscitated with 21% and 100% oxygen. Shock. p.1.

Daniels C.S. 2010. Gas euthanasia methods in swine: process and physiology. In: 2010 AASV Annual Meeting: Implementing Knowledge; Swine Well-being: Euthanasia, Pigs & People. pp 447-450. American Association of Swine Practitioners, Perry, IA USA

Danneman, P.J., S. Stein and S.O. Walshaw. 1997. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Laboratory animal science. 47, 376 – 385.

Dodman, N.H. 1976. Observations on the use of the Wernberg dip-lift carbon dioxide apparatus for pre-slaughter anaesthesia of pigs. The British veterinary journal. 133, 71 – 80.

EFSA. 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal. 45, 1 – 29.

Forslid, A. 1987. Pre-slaughter Co2-Anaesthesia in Swine: Influence Upon Cerebral Electrical Activity, Acid/base Balance, Blood Oxygen Tension and Stress Hormones. Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Physiology.

Franck, L.S., C.S. Greenberg and B. Stevens. 2000. Pain assessment in infants and children. Pediatric Clinics of North America. 47, 487 – 512.

Grandin, T. 1998. Objective scoring of animal handling and stunning practices at slaughter plants. Journal of the American Veterinary Medical Association. 212:p.36–39.

Gregory, N., A. Mohan-Raj, A. Audsey and C. Daly. 1990. Effects of CO2 on man. The use of CO2 for stunning of slaughter pigs, pp 7 – 9.

Guyton and J.E. Hall. 2010. Guyton and Hall Textbook of Medical Physiology: Enhanced E-book. Elsevier Health Sciences. Philadelphia, USA

Hackbarth, H., N. Küppers and W. Bohnet. 2000. Euthanasia of rats with carbon dioxide-animal welfare aspects. Laboratory Animals. 34, 91 – 96.

Hartmann, H., G. Rindermann, C. Siegling-Vlitakis, G. Arndt, K. Wolf and R. Fries. 2010. Relationship between the response to the corneal reflex (depth of narcosis) and specific parameters in the slaughter blood of pigs narcotised with CO2. Animal Welfare. 19, 515 – 522.

Hawkins, P., L. Playle, H. Gooledge, M. Leach, R. Banzett, A. Coenen, J. Cooper, P. Danneman, P. Flecknell and R. Kirkden. 2006. Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals.

Hoenderken, R. 1983. Electrical and carbondioxide stunning of pigs for slaughter. Stunning of animals for slaughter. Boston: Martinus Nijhoff Publishers. pp 59 – 63.

Hsu, K.-S. and C.-C. Huang. 1997. Characterization of the anoxia-induced long-term synaptic potentiation in area CA1 of the rat hippocampus. British Journal of Pharmacology. 122, 671 – 681.

Huffington Post. 2012. Walmart, Christensen Farms Targeted In Mercy For Animals Investigation Focused On Gestation Crates. Huffington Post. Available: http://www.huffingtonpost.com/2012/07/18/walmart-christensen-mercy-for-animals-gestation-crates_n_1683811.html. Accessed April 25, 2013.

Johansson, M.B.N. and B.W. Karlsson. 1982. Lipoprotein and lipid profiles in the blood serum of the fetal, neonatal and adult pig. Neonatology. 42, 127 – 137.

John, W.M.S. 2009. Noeud vital for breathing in the brainstem: gasping—yes, eupnoea—doubtful. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2625 – 2633.

Jongman, E., J. Barnett and P. Hemsworth. 2000. The aversiveness of carbon dioxide stunning in pigs and a comparison of the CO2 stunner crate vs. the V-restrainer. Applied Animal Behaviour Science. 67, 67 – 76.

Klide, A.M. 1996. Lumb & Jones Veterinary Anesthesia. Anesthesia & Analgesia. 83. Available: http://journals.lww.com/anesthesia-analgesia/Fulltext/1996/12000/Lumb___Jones_Veterinary_Anesthesia.58.aspx.

Krnjevic, K., M. Randic and B.K. Siesjo. 1965. Cortical CO2 tension and neuronal excitability. Journal of Physiology. 176, 105 – 122.

Lagerweij, E. and Utrecht. 1990. CO2 Inhalation in the pig. Fleischwirtschaft. 1174. Available: http://agris.fao.org/agris-search/search/display.do?f=1990/DE/DE90072.xml;DE90M1451. Accessed February 6, 2013.

Lecce, J.G. and D.O. Morgan. 1962. Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in the neonatal pig and lamb. The Journal of Nutrition. 78, 263 – 268.

Van der Lende, T. and D. de Jager. 1991. Death risk and preweaning growth rate of piglets in relation to the within-litter weight distribution at birth. Livestock Production Science. 28, 73 – 84.

Liotti, M., S. Brannan, G. Egan, R. Shade, L. Madden, B. Abplanalp, R. Robillard, J. Lancaster, F.E. Zamarripa, P.T. Fox, et al. 2001. Brain responses associated with consciousness of breathlessness (air hunger). Proceedings of the National Academy of Sciences. 98, 2035 – 2040.

Litmanovitz, I., I. Dreshaj, M.J. Miller, M.A. Haxhiu and R.J. Martin. 1994. Central chemosensitivity affects respiratory muscle responses to laryngeal stimulation in the piglet. Journal of Applied Physiology. 76, 403 – 408.

Makowska, I.J., L. Niel, R.D. Kirkden and D.M. Weary. 2008. Rats show aversion to argon-induced hypoxia. Applied Animal Behaviour Science. 114, 572 – 581.

Mann, C., G. Boccara, V. Grevy, F. Navarro, J.M. Fabre and P. Colson. 1997. Argon pneumoperitoneum is more dangerous than CO2 pneumoperitoneum during venous gas embolism. Anesthesia & Analgesia. 85, 1367 – 1371.

Marchant-Forde, J.N., D.C. Lay, K.A. McMunn, H.W. Cheng, E.A. Pajor and R.M. Marchant-Forde. 2009. Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered separately. Journal of Animal Science. 87, 1479 – 1492.

Martin, M., M.A. Tesouro, N. González-Ramón, A. Piñeiro and F. Lampreave. 2005. Major plasma proteins in pig serum during postnatal development. Reproduction, Fertility and Development. 17, 439 – 445.

Martini, F., E.F. Bartholomew, W.C. Ober, C.W. Garrison, K. Welch and R. Hutchings. 2003. Essentials of anatomy & physiology. Prentice Hall New Jersey, USA.

Martoft, L., L. Lomholt, C. Kolthoff, B.E. Rodriguez, E.W. Jensen, P.F. Jørgensen, H.D. Pedersen and A. Forslid. 2002. Effects of CO2 anaesthesia on central nervous system activity in swine. Laboratory Animals. 36, 115 – 126.

Matted, R.L. and J.A. Carroll. 1997. Somatotroph function in the neonatal pig. Domestic Animal Endocrinology. 14, 241 – 249

Merrium-Webster. Euthanasia. Merriem-Webster. Available: http://www.merriam-webster.com/dictionary/euthanasia. Accessed May 29, 2013.

Mischler, S.A., M. Alexander, A.H. Battles, J.A. Raucci, J.W. Nalwalk and L.B. Hough. 1994. Prolonged antinociception following carbon dioxide anesthesia in the laboratory rat. Brain Research. 640, 322 – 327.

Mischler, S.A., L.B. Hough and A.H. Battles. 1996. Characteristics of carbon dioxide-induced antinociception. Pharmacology Biochemistry and Behavior. 53, 205 – 212.

Miura, M., J. Okada and K. Takayama. 1996. Parapyramidal rostroventromedial medulla as a respiratory rhythm modulator. Neuroscience Letters. 203, 41 – 44.

Mormède, P., S. Andanson, B. Aupérin, B. Beerda, D. Guémené, J. Malmkvist, X. Manteca, G. Manteuffel, P. Prunet, C.G. van Reenen, et al. 2007. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiology & Behavior. 92, 317 – 339.

Morrow, W.. M., R.E. Meyer and S.J. Matthis. 2010. In: 2010 AASV Annual Meeting: Implementing Knowledge; Swine Well-being: Euthanasia, Pigs & People. pp 447-450. American Association of Swine Practitioners, Perry, IA USA

Möstl, E. and R. Palme. 2002. Hormones as indicators of stress. Domestic Animal Endocrinology. 23, 67 – 74.

NAHMS. 2008. Highlights of Swine 2006 Part III: Reference of Swine Health, Productivity, and General Management in the United States, 2006. United State Department of Agriculture.

National Pork Board. 2008. On-farm euthanasia of swine: recommendations for the producer. National Pork Board. Available: http://www.aasv.org/aasv/documents/SwineEuthanasia.pdf. .

Niel, L., S.A. Stewart and D.M. Weary. 2008. Effect of flow rate on aversion to gradual-fill carbon dioxide exposure in rats. Applied Animal Behaviour Science. 109, 77 – 84.

Nowak, B., T.V. Mueffling and J. Hartung. 2007. Effect of different carbon dioxide concentrations and exposure times in stunning of slaughter pigs: Impact on animal welfare and meat quality. Meat Science. 75, 290 – 298.

Pritchett, K., D. Corrow, J. Stockwell and A. Smith. 2005. Euthanasia of Neonatal Mice with Carbon Dioxide. Comparative Medicine. 55, 275 – 281.

Puppe, B., P.C. Schön, A. Tuchscherer and G. Manteuffel. 2005. Castration-induced vocalisation in domestic piglets, Sus scrofa: Complex and specific alterations of the vocal quality. Applied Animal Behaviour Science. 95, 67 – 78.

Raj, A.B.M. 1999. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. Veterinary Record. 144, 165 – 168.

Raj, A.B.M. and N.G. Gregory. 1995. Welfare implications of the gas stunning of pigs 1. determination of aversion to the initial inhalation of carbon dioxide or argon. Animal Welfare. 4, 273 – 280.

Raj, A.B.M. and N.G. Gregory. 1996. Welfare implications of the gas stunning of pigs 2. stress of induction of anaesthesia. Animal Welfare. 5, 71 – 78.

Raj, A.B.M., S.P. Johnson, S.B. Wotton and J.L. McInstry. 1997. Welfare implications of gas stunning pigs: 3. the time to loss of somatosensory evoked potential and spontaneous electrocorticogram of pigs during exposure to gases. The Veterinary Journal. 153, 329 – 339.

Rault, J.-L., D.C. Lay Jr. and J.N. Marchant-Forde. 2011. Castration induced pain in pigs and other livestock. Applied Animal Behaviour Science. 135, 214 – 225.

Rendas, A., Branthwaite, M., Lennox, S., Reid, L., 1982. Response of the pulmonary circulation to acute hypoxia in the growing pig. J. Appl. Physiol. 52, 811–814.

Ritter, M.J., M. Ellis, D.B. Anderson, S.E. Curtis, K.K. Keffaber, J. Killefer, F.K. McKeith, C.M. Murphy and B.A. Peterson. 2009. Effects of multiple concurrent stressors on rectal temperature, blood acid-base status, and longissimus muscle glycolytic potential in market-weight pigs. Journal of Animal Science. 87, 351 – 362.

Rodríguez, P., A. Dalmau, J. Ruiz-de-la-Torre, X. Manteca, E. Jensen, B. Rodriguez, H. Litvan and A. Velarde. 2008. Assessment of unconsciousness during carbon dioxide stunning in pigs. Animal Welfare. 17, 341 – 349.

Rosen, A.S. and M.E. Morris. 1991. Depolarizing effects of anoxia on pyramidal cells of rat neocortex. Neuroscience Letters. 124, 169 – 173.

Schon, P.-C., B. Puppe and G. Manteuffel. 2001. Linear prediction coding analysis and self-organizing feature map as tools to classify stress calls of domestic pigs (Sus scrofa). The Journal of the Acoustical Society of America. 110, 1425 – 1431.

Schön, P.C., B. Puppe and G. Manteuffel. 2004. Automated recording of stress vocalisations as a tool to document impaired welfare in pigs. Animal Welfare. 13, 105 – 110.

Smithfield Foods. 2011. Smithfield Foods Response to 2010 HSUS Video - SFDFoodsToday_Content_PDF_FINAL.pdf. Available: http://files.shareholder.com/downloads/SFD/2124136574x0x635782/71c24a4b-0f87-4c32-b8c6-efd116036244/SFDFoodsToday_Content_PDF_FINAL.pdf. Accessed April 25, 2013.

Startribune, (Mercy for Animals). 2012. StarTribune - Print Page. Available: http://www.startribune.com/printarticle/?id=162790086. Accessed April 25, 2013.

Sutherland. 2010. Developing best management practices for on-farm euthanasia of young pigs using carbon dioxide gas. National Pork Board. Des Moines, USA.

Sutherland, M. 2011. The use of different gases and gas combinations to humanely euthanize young suckling pigs. National Pork Board.Des Moines, USA.

USDA. 2009. Nursery and Grower/Finisher Management in Swine 2000 and Swine 2006. USDA, APHIS.: www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2006/Swine2006_is_nursery.pdf. Accessed 10 May 2012

Velarde, A., J. Cruz, M. Gispert, D. Carrión, R. de la J.L. Torre, A. Diestre and X. Manteca. 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. Animal Welfare. 16, 513 – 522.

Velarde, A., X. Manteca and L. Weber. 2010. Response to Tolo, Christensen, Martoft and Forslid's letter "CO2-stunning in pigs."Animal Welfare. 19, 371 – 373.

Weary, D.M., L.A. Braithwaite and D. Fraser. 1998. Vocal response to pain in piglets. Applied Animal Behaviour Science. 56, 161 – 172.

Weissman, D.E. and S. Matson. 1999. Pain Assessment and Management in the Long-Term Care Setting. Theoretical Medicine and Bioethics. 20, 31 – 43.

Welty, J. 2007. Humane Slaughter Laws. Law and Contemporary Problems. 70, 175 – 206.

Wright, A.J., M. Whiting and A. Taylor. 2009. Letter to the Editor on the surgical castration of piglets. Animal: an international journal of animal bioscience. 3, 1474 – 1475.

Yue, X., H. Mehmet, J. Penrice, C. Cooper, E. Cady, J.S. Wyatt, E.O.R. Reynolds, A.D. Edwards and M.V. Squier. 1997. Apoptosis and necrosis in the newborn piglet brain following transient cerebral hypoxia–ischaemia. Neuropathology and Applied Neurobiology. 23, 16 – 25.

CHAPTER 2. EFFECTS OF FLOW RATE AND GAS MIXTURE ON THE WELFARE OF NEONATE AND WEANED PIGS DURING GAS EUTHANASIA[1]

A paper submitted to the Journal of Animal Science

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[1]This work was supported by National Pork Board (Project# 09-197) and VAST. The authors thank Stacey Therrien, Dimitar Spasov, Jay Lampe and the staff at the Iowa State University Swine Farms, Dr. Craig Rowles, Dan Sander and the staff of Elite Pork Partnership, and Daniel Able for technical assistance.

2.1 Abstract

The objectives of this study were to assess efficacy and welfare implications of gas euthanasia when applied to neonate and weaned pigs. Two age groups (neonate and weaned) were assessed in 9 gas treatments, arranged as a 2 x 4 factorial design with 2 gas types (CO_2 = 100% CO_2; MIXED = 50:50 CO_2:argon) and 4 flow rates (box volume exchange/min: slow = 20%, medium = 35%, fast = 50%, prefill = prefilled followed by 20%) and a control treatment in which ambient air was passed through the box. Ten pig pairs were enrolled per treatment. Pigs were placed in a modified Euthanex AgPro system (Euthanex Corp., Palmer, PA). Behavioral and physiological responses were observed directly and from video recordings for latency, duration, prevalence (percent of pigs affected) and frequency of occurrence. Data were analyzed as linear mixed models or with a Cox proportional hazard model as appropriate. Piglet pair was the experimental unit. For the weaned pig, welfare was superior with CO_2 relative to MIXED within one or more flow rates based on reduced duration of open mouth breathing, duration of ataxia, frequency of escape attempts and duration and frequency of righting response ($P < 0.05$). No measured parameters indicated superior welfare with the use of MIXED. Additionally, indicators of efficacy in terms of latencies to loss of posture and last movement favored CO_2 ($P < 0.05$). Faster flow rates were associated with reduced duration and frequency of behavioral indicators of distress relative to slow, in terms of OMB, ataxia and righting response ($P < 0.05$), as well as superior indicators of efficacy including latencies to loss of posture, gasping and last movement ($P < 0.05$). Weaned pigs were more likely to defecate ($P < 0.01$) and display nasal discharge than neonates, whereas neonates displayed shorter latency to loss of posture and last movement. Duration of ataxia was the only parameter associated with welfare for which neonates displayed superior welfare during euthanasia relative to the weaned pigs. As such, a 50:50 CO_2:argon gas mixture and slower flow rates should be avoided when euthanizing weaned or neonate pigs with gas methods. Neonate pigs succumb to the effects of gas euthanasia quicker than weaned pigs and display fewer signs of distress.

Key words: animal welfare, argon, carbon dioxide, gas euthanasia, piglet, swine

2.2 Introduction

Millions of suckling and weaned pigs are euthanized annually in the U.S. swine industry. Swine producers and veterinarians generally agree that euthanasia is appropriate when chances of survival are low and there is suffering due to injury or illness. Euthanasia is comprised of two stages: (1) induction of unconsciousness (insensibility) and (2) death. It is the induction phase that is critical to ensure pig welfare. The entire process, including death, is important to ensure practical and timely implementation. Euthanasia methodologies most commonly available to producers can be classified into two categories: mechanical and chemical (AVMA, 2007; National Pork Board, 2009). Blunt force trauma (BFT) is currently the most common euthanasia method for pigs less than 5.4 kg, but is recognized as being psychologically difficult for some caretakers to perform (Morrow et al., 2010) and has been receiving criticism (Daniels, 2010). These factors have prompted the U.S. swine industry to develop and refine alternative euthanasia methods for the pig, such as gas.

Carbon dioxide (CO_2) is the most commonly implemented gas for swine euthanasia (Daniels, 2010). CO_2 is mildly acidic, which may cause irritation to the mucus membranes (Danneman et al., 1997). This has led to questions about whether CO_2 is a humane option for pig anesthesia and euthanasia (Wright et al., 2009). Argon (Ar) or CO_2:Ar mixtures have been proposed as alternatives (Raj and Gregory, 1996). Ar is a noble gas, and as such is likely unreactive throughout the physiological systems

(Mann et al., 1997). There is little published research that addresses proper flow rates for gas euthanasia of neonates and weaned pigs. Therefore, the objectives of this research were to examine efficacy of CO_2 versus a CO_2:Ar gas mixture administered at 4 flow rates during euthanasia and effects on neonate and weaned pig welfare.

2.3 Materials and methods

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee.

2.3.1 Experimental design

A 2 x 9 experimental design was utilized. There were 2 age groups (neonate and weaned) and 9 gas treatments. These 9 treatments were arranged as a 2 x 4 factorial design with 2 gas types (CO_2 [100% CO_2] and MIXED [50:50 CO_2:Ar]) and 4 flow rates (box volume exchange/min [BVE/min]; slow 20%, medium 35%, fast 50%, prefilled followed by 20%). A control treatment was included, in which ambient (AMB) air was passed through the box. Ten pig pairs were enrolled per treatment. Pairs consisted of male-female matched littermates (neonate) or pen-mates (weaned). Familiar pairs were utilized to reduce isolation and social distress. One replication of all 9 treatments was conducted on a given day. Order of treatments was randomly assigned prior to the day of treatment. The first pig pair selected was assigned to the first treatment to be run, proceeding in this fashion sequentially until all treatments were filled.

2.3.2 Animals and housing

The experiment was conducted from May through September 2010. A total of 340 pigs were used. Neonates were classified as suckling pigs less than 3 d of age (80 females, 80 males). These pigs were housed and sourced from one of 2 locations, the Iowa State University Teaching Farm and a commercial swine farm located in western Iowa. Genetics were a composite of purebred genetics and crosses of those genetics including Duroc, Landrace, Yorkshire and Hampshire (Iowa State University Teaching farm) (9 females, 9 males) or a custom Landrace x Yorkshire cross x Duroc sire performance line (commercial farm) (72 females, 72 males). Pigs in this age category were 1.4 ± 0.1 d of age and weighed 2.61 ± 0.06 kg. Weaned pigs ranged from 16 to 24 d of age, weighing 4.64 ± 0.06 kg with PIC commercial line genetics sourced from the Iowa State University Swine Nutrition Farm (90 females, 90 males).

2.3.3 Euthanasia equipment

Gas was administered to the pigs via a Euthanex AgPro system. This gas delivery apparatus was designed by Euthanex Corporation (Palmer, PA), a manufacturer of gas delivery systems for rodents and small animals. The system allows for variable and precise administration of gas types, mixtures, flow rates and delivery time. To facilitate behavioral observation, the box was constructed of clear plastic on the top and front panels. The remaining 4 panels were constructed of opaque plastic. The inside dimensions of the box were 43 cm wide, x 60 cm long, x 30 cm high. The box had two 0.64 cm inlet valves located at 12.70 cm (CO_2) and 22.86 cm (Ar) from the side and 3.81 cm from the top. A 0.95 cm outlet valve was located on the opposite panel from the inlet valves, 30.48 cm from the side and 6.35 cm from top. The gas flowed through 3.25 m of 0.64 cm diameter hoses prior to entering the box. The floor was fitted with a rubber mat (Rubber floor mats, Kraco, Enterprises, LLC, Compton, CA) for traction. The CO_2 gas used was industrial grade (99% pure). The Ar had a guaranteed analysis of 99.99% pure. Constant and precise gas flows were provided using compressed gas cylinders equipped with compressed gas regulators and meters (Western Enterprises, Westlake, OH). Prior to each treatment, the box was cleaned out using pressurized air from an air compressor and disinfected with Roccal (Pfizer Animal Health, New York, NY).

2.3.4 Enrollment and euthanasia procedure

Pig pairs were identified and marked with an animal safe marker (LA-CO Ind.; Elk Grove, IL). Pigs were then removed from their home pens and carried to the testing room. The testing room provided isolation, minimizing noise and distractions. The room provided adequate ventilation ensuring escaped gases were not a concern to human safety. To habituate pigs to the euthanasia box, the pig pair was placed in the box for 10 min and then taken back to the home pen. A minimum of 1 h elapsed before the pair was placed back into the box. Upon placement, gas was immediately started and applied for 10 min. For gas treatments, pigs remained in the box until 10 min after last movement of both pigs was observed. The pigs were then removed and tested for insensibility and death. For the AMB treatment, pigs were removed from the box after 10 minutes and BFT applied.

For ethical reasons, pigs that displayed movement following 10 min of exposure to the gas were removed from the box and checked for insensibility. Pigs that displayed signs of sensibility were immediately euthanized using BFT. Pigs that were insensible were returned to the box and the euthanasia process, as described above, repeated. This modification was sufficient to induce cessation of movement (involuntary) and heartbeat in all pigs.

2.3.5 Confirmation of insensibility and death

Each pig was removed individually from the box and was immediately checked for signs of sensibility (Whelan and Flecknell, 1992; Kissin, 2000; National Pork Board, 2009; Grandin 2010). Three tests were conducted: (1) corneal reflex response, in which the eye was touched with the tip of a finger for absence of an eye blink or withdrawal response, (2) pupillary reflex, in which a light-beam (Mini MAGLite, Mag Instrument, Inc., Ontario, CA) was shone into the eye and pupil observed for absence of constriction and (3) nose prick, in which a 20 gauge needle was touched to the snout distal to the rostral bone for absence of a withdrawal response. After insensibility was confirmed, auscultation was used to confirm absence of heartbeat.

2.3.6 Modification of study design

At the individual pig level, 75% of the weaned pigs did not achieve last movement during the initial 10 min of gas application of the slow flow rate MIXED gas treatment. Of these, 47% of pigs were still sensible and BFT was immediately applied. Fifty-three percent were insensible, but maintained a heartbeat. These pigs were placed back in the box for up to an additional 10 min during which all achieved last movement. Due to ethical concerns regarding the high number of pigs requiring a secondary euthanasia step, the MIXED slow treatment was not examined in the neonates, creating an unbalanced study design for this age group.

2.3.7 Environmental conditions

A HOBO data logger (U23-001, Onset Computer Corporation, Cape Cod, MS) was placed within the box to record temperature (°C) and relative humidity (%), and was set to record every 10 s. Data were collected continuously throughout the treatment day and exported into Microsoft Office Excel (version 2007, Redmond, WA). For each pig pair, environmental data were extracted for three time periods: entry into the box, loss of posture and exit from the box.

Temperature within the box was relatively constant when gas was flowing regardless of treatment (Table 2.1 and Table 2.2), with the average temperature between treatments ranging less than 1 °C. Relative humidity showed slightly greater variation between treatments, with the average relative humidity between treatments ranging less than 8%. For weaned pig trials, mean starting temperature and relative humidity for all treatments was 26.24 °C and 68.35%, respectively. The neonate pig trials were conducted at a slightly lower temperature and relative humidity, 23.11 °C and 55.37%, respectively. Environmental differences likely resulted from procedures conducted on different farms and days. Temperature and relative humidity within the box changed little from when pigs were placed into the box until loss of posture. The average temperature change in the box, over all treatments, was only -0.16 °C, with the greatest average change within a single treatment of -0.35 °C in the MIXED prefill treatment. Relative humidity also showed little change during this time, increasing 3.91% over all treatments, with the greatest change occurring in the weaned pigs at the fast flow rate, 5.43%.

2.3.8 Behavioral observations

Behavioral data were collected directly and via video recording. For direct observation, one observer sat approximately 1.5 m from the box and recorded behavioral indicators of distress and insensibility (Table 2.3). Latency to last movement for the AMB treatment was determined from the time BFT was applied. Latency for all other behaviors in this study was determined from the point when each pig was placed into the box.

Video was captured utilizing a Noldus Portable Lab (Noldus Information Technology, Wageningen, NL). Two color Panasonic cameras (WV-CP484, Kadoma, Japan) were fed into a multiplexer, which allowed the image to be recorded onto a PC using HandiAvi (v4.3, Anderson's AZcendant Software, Tempe, AZ) at 30 frames/s. Behavioral data were collected by two trained observers, blinded to treatments, using Observer (v10.1.548, Noldus Information Technology, Wageningen, NL). Pigs were scored individually for behavioral and physiological indicators of distress and efficacy of the euthanasia process (Table 2.3). Prior to data collection, observers were trained to the ethogram. Scoring was not started until inter-observer reliability $k > 0.90$ was achieved. Inter-observer and intra-observer reliability were checked at the end of the observation period, and both were $k > 0.90$. Treatments were balanced between observers.

2.3.9 Statistical analysis

Scored behaviors were assessed as latency, duration, percentage of pigs (analyzed as number of pigs displaying) or frequency of occurrence as appropriate for the parameter (Table 2.3). Data were analyzed using linear mixed models fitted with the GLIMMIX procedure (SAS Inst. Inc., Cary, NC) or with a Cox proportional hazard model fitted with the PHREG procedure of SAS. Piglet pair served as the experimental unit. Least square means estimates for each treatment group and the corresponding standard errors (SE) are reported. The linear model included the fixed effect of gas type (CO_2/MIXED), flow rate (slow/medium/fast/prefill), age (weaned/neonate) and their 2- and 3-way interactions. A random blocking effect of litter or pen was included. The Kenward-Rogers method was utilized for determining the denominator degrees of freedom. Statistical significance was established at P-value ≤ 0.05 using a Sidak correction for multiple comparisons, unless otherwise noted. Sex of the pig was examined and found insignificant, and thus removed from all final models.

2.4 Results

2.4.1 Weaned pigs

For CO_2, pigs were heavier in slow versus fast and slow versus prefill (mean ± SE [0.17]; kg): slow = 4.93, prefill = 4.46, fast = 4.46; $P < 0.05$). Furthermore, there was a trend for a difference between CO_2 slow and MIXED prefill (4.51 ± 0.17; $P = 0.09$). Because of the unexpected difference between treatment groups, weight was included as a covariate in the model within the weaned age group for all parameters. However, weight was not significant ($P > 0.10$), and thus was removed from the final models. The possible causal factors of bias that created the unbalanced weight categories were unknown.

Duration of standing and locomotion (SL) did not differ between CO_2 and MIXED or between flow rates (Table 2.4). Duration of oral and nasal behaviors (ON) differed between gas treatments and flow rates, but only by a maximum of 11 s (Table 2.4). AMB was longer than gas treatments for both SL and ON ($P < 0.001$; Table 2.4). Duration of licking and chewing (LC) was shorter for CO_2 and MIXED prefill than other flow rates. Escape attempts were only observed in the MIXED treatment, and were performed by 10% of the pigs in the fast flow rate and 15% in all other flow rates (Table 2.5). There were no differences between gas types, flow rates and AMB in the percentages of pigs displaying defecation, urination, salivation or nasal discharge (Table 2.5). Ocular orbit discharge was displayed by two pigs, one in MIXED slow and one in CO_2 prefill. Blood was never visibly present in discharges, and vomiting was never observed.

Within CO_2, latency to OMB was shortest for prefill, did not differ between fast and medium and was significantly longer for slow (mean latency [s] ± 33 [SE]: prefill = 11, fast = 55. medium = 59, slow = 87; $P < 0.05$). For MIXED, latency to OMB was shortest for prefill (mean latency [s] ± 33 [SE]: prefill = 28, fast = 65, medium = 86, slow = 113; $P < 0.05$). MIXED slow and medium did not differ, but latency to OMB was significantly shorter for fast. Latency to OMB was significantly faster for CO_2 vs.

MIXED when applied at the slow and medium flow rates ($P < 0.05$). Percentages of pigs displaying OMB did not differ between gas type or flow rates, with 80 to 100% of pigs displaying this behavior (Table 2.4). In contrast, no pigs in the AMB displayed OMB ($P < 0.001$). Duration of OMB differed significantly between all flow rates within CO_2 (Table 2.4). A similar pattern was also observed within MIXED. When comparing MIXED vs. CO_2, duration of OMB was greater for MIXED at all flow rates.

Within CO_2, prefill was the quickest to induce LP (mean latency [s] ± 7 [SE]: prefill = 35, fast = 89, Medium = 102, Slow = 143; Figure 2.1). Fast and medium were similar for this parameter, whereas latency for LP was greatest for the slow flow rate. A similar pattern for latency to LP was observed within MIXED, with differences also observed between fast and medium (mean latency [s] ± 7 [SE]: prefill = 90, fast = 148, Medium = 174, Slow = 238; $P < 0.05$). LP occurred faster in CO_2 vs. MIXED for all flow rates (Figure 2.1). Prior to LP, 99% of pigs displayed ataxia. Within CO_2, the duration of ataxia differed at each flow rate ($P < 0.05$; Table 2.4). Within MIXED, differences were not observed between the prefill and fast, but were observed ($P < 0.05$) at all other flow rates. Comparison between gas types revealed a significantly longer duration of ataxia in MIXED relative to CO_2 ($P < 0.001$).

In some instances, a righting response was observed prior to loss of posture (10 to 60% of pigs; Table 2.4). The number of righting attempts by a single pig ranged from zero to 12 (maximum attempts CO_2: prefill = 4, fast = 1, medium = 5, slow = 6; MIXED: prefill = 12, fast = 5, medium = 4, slow = 10). When righting response did occur within CO_2, duration was shorter in prefill and fast relative to medium and slow ($P < 0.05$).

There was no discernible pattern within MIXED, but the shortest duration was observed in medium relative to prefill and slow ($P < 0.05$). The fast flow rate was shorter in duration than slow ($P < 0.05$).

Within CO_2, muscle excitation was observed less frequently in slow relative to all other flow rates (prevalence [% of pigs]: prefill = 65, fast = 60, medium = 60, slow = 40; $P < 0.05$). Within MIXED, the prevalence was lower in prefill and slow relative to fast and medium (prevalence [% of pigs]: prefill = 30, fast = 45, medium = 40, slow = 25). When comparing gas types, the prevalence of muscle excitation was lower for MIXED than CO_2 at all flow rates ($P < 0.05$). Within CO_2, mean duration of muscle excitation was less than 7 s vs. less than 4 s for the MIXED. All pigs displayed clonic movements, with the exception of one pig in the prefill CO_2 gas treatment. Within CO_2, the slow flow rate was associated with longer duration of clonic movements (mean duration [s] ± [SE]: prefill = 50 ± 7, fast = 55 ± 5, medium = 61 ± 5, slow = 84 ± 6). Similarly, within the MIXED, the slow flow rate was associated with longer duration of clonic movements relative to fast and medium, but did not differ from prefill. Prefill was similar to the fast and medium flow rates (mean latency [s] ± [SE]: prefill = 79 ± 6, fast = 65 ± 5, medium = 70 ± 5, slow = 93 ± 6). Between the two gas types, differences were only observed within the prefill flow rate.

GASP was performed by 90 to 100% of pigs in CO_2 and MIXED, and there were no differences between gas types or flow rates. None of the pigs in the AMB treatment displayed this behavior, and differences were observed between AMB and all other treatments. Within CO_2, duration of GASP was longest for the slow relative to other flow rates (mean duration [s] ± [SE]: prefill = 224 ± 12, fast = 174 ± 11, medium = 198 ± 12, slow = 346 ± 12). GASP was significantly longer in the prefill vs. fast, but did not differ from the medium flow rate. The same pattern was observed within MIXED, with the exception of differences observed between fast and medium (mean duration [s] ± [SE]: prefill = 371 ± 13, fast = 280 ± 12, medium = 344 ± 12, slow = 478 ± 12).

Latency to LM within CO_2 was shortest in the prefill and fast flow rates (Figure 2.2). Latency was greater for the medium flow rate, and greatest for the slow flow rate. For MIXED, the latency to LM was significantly longer than with CO_2 gas type at each flow rate. LM was quicker in the BFT treatment relative to MIXED at all flow rates and relative to slow and medium CO_2, but not prefill and fast CO_2 flow rates. Out of view and “Other” were scored for less than 0.1% of time for any individual pig.

2.4.2 Neonate pigs

Pigs differed in weight between AMB (3.02 ± 0.17) and CO_2 fast (2.38 ± 0.17; $P = 0.009$) and between AMB and MIXED fast (2.34 ± 0.17; $P= 0.006$). A trend was found between AMB and CO_2 slow (2.58 ± 0.17; $P = 0.07$). Pigs in the CO_2 prefill (1.8 ± 0.2 d) were older than in AMB (1.2 ± 0.2 d), CO_2 fast (1.2 ± 0.2) and MIXED medium (1.3 ± 0.2; $P<0.05$). Weight and age were analyzed as covariates in the models. However, weight and age were not significant and thus were removed from the final models.

Duration of SL did not differ between gas types or flow rates. Duration of SL was longer in AMB relative to all other gas types and flow rates (Table 2.6). Similarly, duration of ON was longer in AMB relative to all other gas types and flow rates. Duration of ON was significantly shorter in the prefill vs. other flow rates, but was a briefly observed behavior when pigs were exposed to the CO_2 and MIXED gas types. Duration of LC was less than 7 s and no differences were observed between gas types or flow rates (Table 2.6). Escape attempts were only observed in the prefill CO_2, prefill MIXED and AMB treatments at 5, 5 and 10% of pigs respectively (Table 2.7). There were no differences between gas types, flow rates or AMB for the percentage of pigs displaying defecation, urination, salivation and nasal discharge (Table 2.7). Blood was never visible in discharge. Ocular orbit discharge and vomiting were not observed.

Within CO_2, prefill elicited OMB fastest relative to other flow rates (mean latency [s] ± 33 [SE]: prefill = 7, fast = 45, medium = 54, slow = 67). Fast and medium did not differ from one another, but both rates elicited OMB faster than slow. Within MIXED, prefill elicited OMB faster than fast and medium (mean latency [s] ± 33 [SE]: prefill = 12, fast = 55, medium = 61). Differences were not observed for latency to OMB between the gas types. Percentages of pigs displaying OMB did not differ between gas types or flow rates, with 90 to 100% of the pigs displaying this behavior (Table 2.6). Conversely, only one pig displayed OMB in the AMB treatment ($P < 0.001$). Within CO_2, duration of OMB was shortest in prefill. Durations of OMB in fast and medium flow rates did not differ but were shorter in duration than the slow flow rate. Within MIXED, differences were observed for duration of OMB between all flow rates (Table 2.6). Comparison of CO_2 vs. MIXED revealed longer duration of OMB for MIXED at the fast and medium flow rates.

Within CO_2, prefill was quickest to induce LP (Figure 2.3). Fast and medium rates did not differ from one another for this parameter, whereas latency to LP was greatest for the slow relative to other flow rates. Similarly, within MIXED, prefill was quickest to induce LP (Figure 2.3). However, the fast flow rate induced LP significantly faster than the medium. LP was faster for CO_2 vs. MIXED for all flow rates. Prior to LP, 99% of the pigs displayed ataxia. CO_2 prefill produced the shortest duration of ataxia (Table 2.6). Fast and medium rates did not differ from one another, and slow produced the longest duration. Within MIXED, differences were observed at every flow rate (Table 2.6). Ataxia was significantly longer in duration in the MIXED treatments relative to the CO_2 treatments.

Prior to complete LP, 25 to 65% of pigs displayed a righting response (Table 2.6). Differences were not observed between any gas types or flow rates. The number of righting attempts within a response by a single pig ranged from zero to six (maximum attempts CO_2: prefill = 3, fast = 3, medium = 3, slow = 4; MIXED: prefill = 3, fast = 5, medium = 6). Differences were not observed in the prevalence of muscle excitation between any gas types or flow rates (prevalence [% of pigs]: CO_2: prefill = 10, fast = 5, medium = 0, slow = 0; MIXED prefill = 15, fast = 0, medium = 0).

GASP was performed by 100% of the pigs in all gas treatments. No pigs in the AMB treatment performed this behavior. Within CO_2, duration of GASP was longest for the slow relative to other flow rates (mean duration [s] ± [SE]: prefill = 210 ± 12, fast = 225 ± 11, medium = 247 ± 12, slow = 348 ± 12). GASP was significantly longer for the medium flow rate relative to prefill. Prefill and fast did not differ. Within MIXED, prefill and medium flow rates produced longer GASP durations than fast (mean duration

[s] ± [SE]: prefill = 374 ± 12, fast = 308 ± 13, medium = 346 ± 12). Longer duration of GASP was observed in MIXED vs. CO_2 at all flow rates.

Latency to LM within the CO_2 gas type was longest for the slow flow rate, did not differ between the medium and fast flow rates, and was shortest in the prefill flow rate (Figure 2.4). Within MIXED, latency to LM was longer for medium than fast, and prefill was similar to fast and medium (Figure 2.4). Latency to LM was longer for MIXED vs. CO_2 at all flow rates. Latency to LM was longer for BFT (313 ± 40 s) than CO_2 prefill, did not differ from CO_2 fast and medium, but was shorter than the CO_2 slow and all MIXED flow rates. Out of view and Other were scored for less than 0.1% of time for any individual pig.

2.4.3 Comparison between age groups

Weaned pigs displayed longer durations of licking and chewing relative to the neonate pigs in the CO_2 slow, CO_2 fast and the MIXED fast ($P < 0.05$; Tables 2.4 and 2.5). Weaned pigs were more likely to defecate relative to the neonate pigs ($P < 0.01$; Tables 2.6 and 2.7), and were more likely to display nasal discharge than neonates for the CO_2 slow and medium flow rates ($P < 0.05$; Tables 2.2 and 2.3).

Greater latency to OMB was observed in weaned pigs relative to neonates and was significant at all gas types and flow rates, except CO_2 fast and a trend for the MIXED medium flow rate ($P = 0.06$). Duration of OMB was also longer in the weaned pigs vs. neonate pigs for the CO_2 prefill and medium flow rates and the MIXED prefill and fast flow rates, with a trend in the MIXED medium flow rate ($P = 0.06$; Tables 2.4 and 2.5).

LP occurred later for the weaned pigs relative to the neonatal pigs in the CO_2 slow and medium flow rates and for all MIXED flow rates. The duration of ataxia was shorter for weaned relative to the neonate pigs, showing statistical differences for CO_2 fast and MIXED medium (Tables 2.4 and 2.5).

Numerically, longer latencies occurred for weaned relative to neonate pigs for gasping at every flow rate, and these were statistically different in CO_2 fast, medium and slow and MIXED prefill and medium flow rates.

Weaned pigs were more likely to display muscle excitation relative to neonates for all gas types and flow rates ($P < 0.01$) except MIXED prefill. Latency to LM was numerically longer for weaned pigs relative to the neonates for all gas types and flow rates except CO_2 fast and AMB, and was different in the CO_2 prefill and slow and the MIXED prefill ($P < 0.05$).

2.5 Discussion

Results from the current study indicate 100% CO_2 relative to a 50:50 CO_2:Ar gas mixture, and faster flow rates relative to slow, were advantageous for pig welfare and efficacy when euthanizing both neonate and weaned pigs. These conclusions are based on behavioral and physiological indicators of distress and efficacy. In this study, we separated the euthanasia process into two phases, conscious and unconscious. Behavioral indicators of distress along with normal behaviors were scored during the conscious phase and used to assess pig welfare. In our experiment, the transition from conscious to unconscious was determined by LP, which has been identified in previous research as an indicator for loss of consciousness (Forslid, 1987; Raj and Gregory, 1996; Velarde et al., 2007). However, there is a transition phase prior to LP during which a number of behaviors are typically observed, including OMB, ataxia, and righting response. The level of awareness, hence capacity of animals to suffer, during this transition is unclear, and we chose a conservative estimate to ensure pig welfare. While other and more precise methods for determining consciousness are available, such as EEG, it was important to allow the piglets to display a full and more natural repertoire of behaviors than can be achieved with these more invasive methods requiring restraint.

Behaviors chosen for welfare assessment included those associated with physiological distress, such as OMB (Forslid, 1987; Martoft et al., 2002; Mota-Rojas et al., 2012), or psychological distress, such as escape (Blackshaw et al., 1988; Velarde et al., 2007), righting response (Grandin, 1998; Kohler et al., 1999; AVMA, 2007; National Pork Board, 2009), defecation and urination. Once unconscious, which included the absence of audible vocalizations, the point of interest shifted from welfare to efficacy; it is vital that the process be practical for on-farm implementation. This experiment is the first to describe the duration of exposure at different flow rates required for reliable euthanasia of suckling and nursery age (weaned) pigs. These parameters are important in identifying when the process is not occurring within acceptable guidelines, indicating intervention is necessary. For the purpose of this study, LM was our best indicator of death since respiratory arrest (the cessation of gasping) was the last movement observed in gas treatments. For pig welfare and practical reasons on-farm, it is critical to reduce the number of animals that require a secondary euthanasia step.

2.5.1 Gas Type

Pig welfare was superior with CO_2 relative to MIXED based on a reduction in the duration of OMB, duration of ataxia, prevalence of escape attempts and righting response duration and intensity. None of the parameters we measured indicated superior welfare with the use of MIXED.

At 10% CO_2 concentrations, the majority of human subjects report experiencing breathlessness, described as being unpleasant, and 50% CO_2 concentration is reported as being very pungent (Gregory et al.,1990). Open mouth breathing is a physiological reaction associated with breathlessness, and has been identified as an indicator of compromised welfare in the pig (Velarde et al., 2007). It is important to note that several other researchers use different terms when describing this behavior (hyperventilating in Martoft et al., 2002; respiratory distress in Raj and Gregory, 1996; gasping in Rodríguez et al., 2008). While the onset of this behavior is noted by several researchers (Forslid, 1987; Raj and Gregory, 1996; Martoft et al., 2002), none reported duration of OMB. Using onset of OMB until onset of LP, duration of OMB can be calculated for some previous research, and values were similar to the current study for the CO_2 prefill treatment (12 s for 90% CO_2 in Raj and Gregory, 1996; 15 s for 90% CO_2 in Rodríguez et al., 2008). We argue OMB duration is an important measure of distress, and the MIXED treatment resulted in 60 to 90% longer duration of this behavior in weaned pigs.

Ataxia is likely an indicator of impaired function of the cerebellum, however it is unclear how this correlates to impaired cortical function. If ataxia indicates that the pig is aware of its surroundings, but is unable to react in a coordinated manner, this could be considered distressing to the pig. In this study, we defined ataxia as a potential stressor for the pig, and hence, a shorter duration of this behavior would correlate with improved welfare. Duration of ataxia was twice as long for weaned pigs with MIXED relative to CO_2, at prefill and fast flow rates. Latency to ataxia is discussed by Raj and Gregory (1995) and Troeger and Woltersdorf (1991), but duration of ataxia was not examined.

Escape attempts are noted by several researchers to be an indicator of compromised welfare and as such, the goal is to reduce its prevalence (Blackshaw et al., 1988; Raj and Gregory, 1995; Kirkden and Pajor, 2006; Velarde et al., 2007). Escape attempts in this study were rare relative to other studies (Raj and Gregory, 1996; Velarde et al., 2007). A maximum of 15% of weaned pigs per treatment displayed escape attempts, which only occurred in MIXED. Similarly, Raj and Gregory (1996) did not observe escape attempts when pigs were exposed to 80 or 90% CO_2, which they attributed to the pigs not having time to display the behavior. In our study, pigs were placed in ambient air before the atmosphere was modified for all but the prefill treatments. Hence, there was adequate time for pigs to display this behavior, as demonstrated in MIXED treatments. Velarde et al. (2007) observed higher prevalence of grower pigs displaying escape (33-93%). However, this is likely due to a more liberal definition that included pigs running across the dip-lift, which did not occur in our experiment due to the restrictive size of the box. Only neonate piglets attempted escape when exposed to ambient air. Escape was observed by Raj and Gregory (1996) when grower pigs were individually exposed to AMB, which they attributed to isolation and caging distress. Since pigs in the current study were placed in the box with a familiar pig, the novel environment and separation from the dam were more likely causes of this behavior in AMB in our study. Separation from the dam may also explain why escape attempts in AMB were only seen in the neonates and not the weaned pigs.

The lack of a righting response has been cited to be critical to ensure the pig has been successfully rendered unconscious prior to slaughter (Sandström, 2009; Grandin, 2010) and is cited as an indicator of unconsciousness (Anil, 1991; National Pork Board, 2009). However, to our knowledge, duration and frequency of righting responses have not been quantified within an individual pig as a measure of distress. Righting responses require coordinated brain activity, and are indicators of brain function. Since CO_2 and Ar are both heavier than air, it is possible that some of the righting responses observed reflect the animal's attempt to physically avoid the gas, rather than a reflex. Hence, duration and intensity (frequency) of righting responses are used as indicators of distress in this study. In the weaned pig, righting response duration was 9-fold greater and displayed by twice as many pigs with MIXED relative to CO_2 prefill.

Latency to LP was greater for MIXED at most flow rates. These results are in sharp contrast to Raj (1999), who found latency to LP was not affected by gas type when finisher pigs were exposed to 90% Ar, 80 to 90% CO_2, or 30:60 CO_2:Ar mixture. Additionally, latencies to LP (15, 17, 18 s respectively) were generally considerably shorter than observed in our study, perhaps due to differences in age and weight. It is important to note that Ar is a noble gas with no known effect on the body, and likely causes unconsciousness through hypoxia. Therefore, it is surprising that 90% Ar was capable of producing loss of posture in less than 20 s (Raj, 1999) vs. 45 s and 103 s (MIXED prefill) observed in neonate and weaned pigs respectively in this study. Another factor may be the method of gas application; when utilizing CO_2 to stun prior to slaughter, pigs are lowered into a pit where a constant modified atmosphere is present. In the current experiment, the prefilled box allowed some reintroduction of atmospheric air when the lid was opened to place the pigs inside, whereas gas flow was initiated after the pigs were placed in the box in other treatments. Both of these methods produced different exposure conditions when compared to slaughter conditions used by Raj and colleagues (1999) since we were simulating on-farm euthanasia procedures.

For both the weaned and neonate pigs, greater latency to death, as determined by LM, was observed for MIXED at all flow rates. In the weaned pig, latency to LM was 1.7 times greater for prefill MIXED versus CO_2. MIXED slow had an efficacy rate of 15% within the parameters of this experiment (10 min allowed for loss of consciousness and 10 min allowed for death post loss of consciousness), which we deemed unacceptable for both ethical and practical reasons. However, all other flow rates and gas type combinations were 100% successful. Dykshorn and Donovan (2010) found 100% CO_2 to be 83.9 to 97.7% effective, depending on the duration of exposure time. However, flow rate details were not provided in this paper making a direct comparison difficult.

2.5.2 Flow Rate

Faster flow rates were associated with lower duration and intensity of behavioral indicators of distress, as well as decreased latency of indicators of efficacy (LP, GASP and LM). Within CO_2, the slow flow rate more than doubled the duration of OMB, ataxia and righting response relative to prefill. Additionally, the slow flow rate resulted in a 5-fold increase in latency to loss of consciousness (LP) and 2-fold increase in latency to death (LM). These results brought us to the same conclusion as Sutherland (2010), who examined effects of prefill and slow flow rates with 90% CO_2 on latency to loss of brain activity and heart rate. Our findings conflict with recommendations for rodents reported from the Newcastle Census Meeting (Hawkins et al., 2006). While noting the optimal flow rate is uncertain, they concluded a 20% flow rate was preferred relative to prefill, based on many factors with heavy emphasis on the human experience, such as low CO_2 concentrations causing aversion due to dyspnea versus concentrations above 50% causing pain. Subsequent rodent research indicates that aversion occurs even at lower gas concentrations. In rats, Niel et al. (2008) examined 100% CO_2 with flow rates from 3 to 27% (chamber volume exchange rate per min) where rats were trained to enter the box for a food reward and allowed to exit at will. Minimal response to flow rates was observed, with rats leaving when CO_2 concentrations reached 11 to 16%. All rats left the chamber before loss of consciousness. In a similarly designed study, Makowska et al. (2008) examined 100% Ar with flow rates from 40 to 239% (chamber exchange rate per min). Again, minimal response to flow rate was observed, with rats leaving when O_2 concentrations reached 6 to 9%. All rats left the chamber prior to loss of consciousness. These results suggest that both hypercapnia and hypoxia are inherently aversive even at low levels, and call into question the prolonged gas exposure for euthanasia. Based on the parameters measured in our study and other studies involving swine and rats, slow flow rates prolong the duration of the process, and hence suffering, without providing benefits to animal welfare.

2.5.3 Age

It has been demonstrated in several species that achieving successful euthanasia for neonates may take longer or require a higher gas concentration relative to the more mature animal (AVMA, 2007). In addition, anecdotal reports from stockpersons indicated a belief that neonates are more difficult to euthanize than older pigs. This research indicated the opposite effect, since neonate pigs succumbed to the gases faster than weaned pigs for both the conscious (LP) and unconscious (LM) phases. Additionally, signs of distress were lower for neonates relative to weaned pigs as measured by defecation, nasal discharge and duration of OMB. Duration of ataxia was the single parameter for which neonate pigs displayed greater distress relative to weaned. Similarly, Sutherland (2010) observed small but significant differences for pigs aged 1, 2, 3, 4, 5 and 6 wk of age, and concluded the small differences did not merit development of different euthanasia methodologies for pigs of different ages.

2.6 Summary

When examining a euthanasia method, both animal welfare and efficacy are key components. Welfare is composed of both duration and intensity of distress. The results from this study indicate that pigs succumb faster when using 100% CO_2 vs. a 50:50 CO_2:Ar gas mixture. More importantly, it resulted in shorter durations of behavioral indicators of distress and physiological responses. Thus, proposed benefits of adding Ar were not observed. Likewise, the slow flow rate increased the durations of sensation and distress measures, while resulting in longer latencies to loss of posture and last movement. The current study is able to conclude that 50:50 CO_2:Ar gas mixtures and slower flow rates should be avoided when euthanizing weaned or neonate pigs with gas methods. Many farms are using a 2- or 3-min gas run time, followed by a 5-min dwell time, or a similarly timed procedure. It is important to note that if a procedure similar to slow flow in this trial had been followed on farm, most pigs would not have been successfully euthanized. It is critical that farms know the flow rate of their systems and avoid designing euthanasia procedures solely on timing.

2.7 References

Anil, M. H. 1991. Studies on the return of physical reflexes in pigs following electrical stunning. Meat Sci. 30:13–21.

AVMA. 2007. American Veterinary Medical Association Guidelines on euthanasia, formerly report of the AVMA panel on euthanasia. Accessed June 21, 2011. http://www.avma.org/issues/animal_welfare/ euthanasia.pdf.

Blackshaw, J. K., D. C. Fenwick, A. W. Beattie, and D. J. Allan. 1988. The behaviour of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. Lab. Anim. 22:67–75.

Blood, D. C., V. P. Studdert, and C. C. Gay. 2007. Saunders Comprehensive Veterinary Dictionary. No. Ed. 3. WB Saunders\ Elsevier Science. Harcourt Publishers Limited, London.

Daniels, C. S. 2010. Gas euthanasia methods in swine: Process and physiology. In: 2010 AASV Annual Meeting: Implementing Knowledge: Swine Well-Being: Euthanasia, Pigs & People: 447–450.

Danneman, P. J., S. Stein and S. O. Walshaw. 1997. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Lab. Anim. Sci. 47:376–385.

Dodman, N. 1977. Observations on the use of the Wernberg dip-lift carbon dioxide apparatus for pre-slaughter anaesthesia of pigs. Br. Vet. J. 133:71.

Dykshorn, D. and T. Donovan. 2010. Comparison of CO2, blunt force trauma, and non-penetrating captive bolt euthanasia methods in nursery pigs. In: 2010 AASV Annual Meeting: Implementing Knowledge: Swine Well-Being: Euthanasia, Pigs & People: 305–306

Forslid, A. 1987. Pre-slaughter Co2-Anaesthesia in Swine: Influence Upon Cerebral Electrical Activity, Acid/base Balance, Blood Oxygen Tension and Stress Hormones. PhD Dis. Swedish Univ. of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Physiology.

Grandin, T. 1998. Objective scoring of animal handling and stunning practices at slaughter plants. J. Am. Vet. Med. Assoc. 212:36–39.

Grandin, T. 2010. Recommended Animal Handling Guidelines & Audit Guide: a Systematic Approach to Animal Welfare. AMI Foundation. Accessed January 10, 2013. http://www.animalhandling.org/ht/a/GetDocumentAction/i/58425

Gregory, N., A. Mohan-Raj, A. Audsey, and C. Daly. 1990. Effects of CO2 on man. The use of CO2 for stunning of slaughter pigs; report of a meeting of experts (Lambooy E. Institut voor Veeteeltkundig Onderzoek `Schoonoord'; Zeist):7–9.

Hawkins, P., L. Playle, H. Gooledge, M. Leach, R. Banzett, A. Coenen, J. Cooper, P. Danneman, P. Flecknell, and R. Kirkden. 2006. Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals. February 26-7, 2006.

Hurnik, J. F., A. B. Webster, and P. B. Siegel. 1985. Dictionary of farm animal behaviour. University of Guelph.

Johnson, A. K., L. J. Sadler, L. M. Gesing, C. Feuerbach, H. Hill, M. Faga, R. Bailey, K. J. Stalder, and M. J. Ritter. 2010. Effects of Facility System Design on the Stress Responses and Market Losses of Market Weight Pigs During Loading and Unloading. The Professional Animal Scientist. 26:p.9–17.

Kirkden, R. D. and E. A. Pajor. 2006. Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. Appl. Anim. Behav. Sci. 100:29–47.

Kissin, I. 2000. Depth of Anesthesia and Bispectral Index Monitoring. Anesth. & Analg. 90:1114–1117.

Kohler, I., R. Meier, A. Busato, G. Neiger-Aeschbacher, and U. Schatzmann. 1999. Is carbon dioxide (CO2) a useful short acting anaesthetic for small laboratory animals? Lab. Anim. 33:155–161.

Makowska, I. J., L. Niel, R. D. Kirkden, and D.M. Weary. 2008. Rats show aversion to argon-induced hypoxia. Appl. Anim. Behav. Sci. 114:572–581.

Mann, C., G. Boccara, V. Grevy, F. Navarro, J. M. Fabre, and P. Colson. 1997. Argon pneumoperitoneum is more dangerous than CO2 pneumoperitoneum during venous gas embolism. Anesth. & Analg. 85:1367–1371.

Martoft, L., L. Lomholt, C. Kolthoff, B. E. Rodriguez, E. W. Jensen, P. F. Jørgensen, H. D. Pedersen, and A. Forslid. 2002. Effects of CO2 anaesthesia on central nervous system activity in swine. Lab. Anim. 36:115–126.

Meiszberg, A. M., A. K. Johnson, L. J. Sadler, J. A. Carroll, J. W. Dailey, and N. Krebs. 2009. Drinking behavior in nursery pigs: Determining the accuracy between an automatic water meter versus human observers. J Anim. Sci. 87:4173–4180.

Morrow, W. M., R. E. Meyer, and S. J. Matthis. 2010. Human factors influencing swine euthanasia. In: 2010 AASV Annual Meeting: Implementing Knowledge: Swine Well-Being: Euthanasia, Pigs & People:435–440.

Mota-Rojas, D., D. Bolanos-Lopez, M. Concepcion-Mendez, J. Ramirez-Telles, P. Roldan-Santiago, S. Flores-Peinado, and P. Mora-Medina. 2012. Stunning Swine with CO2 Gas: Controversies Related to Animal Welfare. Int. J. Pharmacol. 8:141–151.

National Pork Board. 2009. On-farm euthanasia of swine: recommendations for the producer. National Pork Board. Accessed June 12, 2012 http://www.aasv.org/aasv/documents/SwineEuthanasia.pdf.

Niel, L., S. A. Stewart, and D. M. Weary. 2008. Effect of flow rate on aversion to gradual-fill carbon dioxide exposure in rats. Appl. Anim. Behav. Sci. 109:77–84. Available: Accessed February 6, 2013.

Raj, A. B. M. 1999. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. Vet Rec. 144:165–168.

Raj, A. B. M. and N. G. Gregory. 1995. Welfare Implications of the Gas Stunning of Pigs 1. Determination of Aversion to the Initial Inhalation of Carbon Dioxide or Argon. Anim. Welf. 4:273–280.

Raj, A. B. M. and N. G. Gregory. 1996. Welfare Implications of the Gas Stunning of Pigs 2. Stress of Induction of Anaesthesia. Anim. Welf. 5:71–78.

Rodríguez, P., A. Dalmau, J. Ruiz-de-la-Torre, X. Manteca, E. Jensen, B. Rodriguez, H. Litvan, and A. Velarde. 2008. Assessment of unconsciousness during carbon dioxide stunning in pigs. Anim. Welf. 17:341–349.

Sandström, V. 2009. Development of a monitoring system for the assessment of cattle welfare in abattoirs. Sveriges lantbruksuniversitet.

Sutherland. 2010. Developing best management practices for on-farm euthanasia of young pigs using carbon dioxide gas. National Pork Board. Accessed June 12, 2012. http://www.pork.org/FileLibrary/ResearchDocuments/08-145-SUTHERLAND-TXTECH-revised-ABS.pdf

Troeger, K. and W. Woltersdorf. 1991. Gas anaesthesia of slaughter pigs 1. Stunning experiments under laboratory conditions with fat pigs of known halothane reaction type: meat quality, animal protection. Fleischwirtsch. 71:1063–1068.

Velarde, A., J. Cruz, M. Gispert, D. Carrión, R. de la J. L. Torre, A. Diestre, and X. Manteca. 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. Anim. Welf. 16:513–522.

Whelan, G. and P. A. Flecknell. 1992. The assessment of depth of anaesthesia in animals and man. Lab Anim. 26:153–162.

Wright, A. J., M. Whiting, and A. Taylor. 2009. Letter to the Editor on the surgical castration of piglets. Animal. 3:1474–1475.

Table 2.1 Temperature and relative humidity, weaned pigs

	Treatment									
Parameter	**CO_2 Prefill**	**SE**	**CO_2 Fast**	**SE**	**CO_2 Medium**	**SE**	**CO_2 Slow**	**SE**	**AMB**	**SE**
Starting air temperature[a], °C	26.43	0.76	26.34	0.76	25.81	0.76	26.10	0.76	26.30	0.76
Change in air temperature[b], °C	-0.10	0.07	-0.27	0.07	-0.15	0.07	-0.04	0.07	-0.27	0.07
Starting relative humidity[a], %	62.30	5.65	73.63	3.12	68.52	4.23	64.40	5.84	62.41	4.94
Change in relative humidity[b], %	2.05	2.78	5.43	2.78	4.68	2.78	3.89	2.78	2.03	2.78
	MIXED Prefill	**SE**	**MIXED Fast**	**SE**	**MIXED Medium**	**SE**	**MIXED Slow**	**SE**		
Starting air temperature[a], °C	26.30	0.76	26.22	0.76	26.21	0.76	26.51	0.76		
Change in air temperature[b], °C	-0.35	0.07	-0.17	0.07	-0.17	0.07	-0.10	0.07		
Starting relative humidity[a], %	70.61	5.41	72.32	5.21	71.61	5.03	63.42	5.30		
Change in relative humidity[b], %	2.48	2.78	3.71	2.78	4.53	2.78	3.77	2.78		

[a]recorded upon piglet placement, by gas type and flow rate

[b]change in temperature or relative humidity that occurred in the box from the time of placement until the piglets were removed, by gas type and flow rate

Chamber volume exchange rate; prefill followed by 20%, fast = 50%, medium = 35%, slow = 20%

Table 2.2 Temperature and relative humidity, neonate pigs

Parameter	Treatment									
	CO_2 Prefill	**SE**	**CO_2 Fast**	**SE**	**CO_2 Medium**	**SE**	**CO_2 Slow**	**SE**	**AMB**	**SE**
Starting air temperature[a], °C	22.64	0.76	23.55	0.76	23.47	0.76	23.30	0.76	23.08	0.76
Change in air temperature[b], °C	-0.02	0.07	-0.01	0.07	-0.07	0.07	-0.02	0.07	0.01	0.07
Starting relative humidity[a], %	55.41	4.76	46.00	3.23	57.36	5.35	61.16	4.38	79.22	4.36
Change in relative humidity[b], %	1.51	2.79	2.56	2.79	4.64	2.79	4.33	2.79	9.16	2.79
	MIXED Prefill	**SE**	**MIXED Fast**	**SE**	**MIXED Medium**	**SE**	**MIXED Slow[c]**	**SE**		
Starting air temperature[a], °C	22.70	0.76	22.77	0.76	23.32	0.76	X	X		
Change in air temperature[b], °C	-0.35	0.07	-0.17	0.07	-0.06	0.07	X	X		
Starting relative humidity[a], %	55.40	4.28	52.88	4.60	59.36	4.53	X	X		
Change in relative humidity[b], %	2.93	2.79	3.71	2.79	4.53	2.79	X	X		

[a]recorded upon piglet placement, by gas type and flow rate

[b]change in temperature or relative humidity that occurred in the box from the time of placement until the piglets were removed, by gas type and flow rate

[c]MIXED slow was not tested in the neonate age group

Chamber volume exchange rate; prefill followed by 20%, fast = 50%, medium = 35%, slow = 20%

Change in temperature and relative humidy was the change in temperature that occurred in the box from the time of placement until the piglets were removed, by gas type and flow rate

Table 2.3 Ethogram developed for investigating latency, duration, prevalence or frequency of behavioral indicators of indicators of distress and insensibility during euthanasia

Postures (state)	**Definition**	**Direct**	**Video**
Standing/ Locomotion	Maintaining an upright and stationary body position by supporting the body weight on the feet with the legs extended or movement derived from the repulsive force from the action of the legs [1]		X
Sitting	A body position in which the posterior of the body trunk is in contact with the ground, sides of the box or the other pig and supports most of the body weight [1]		X
Lying	Maintenance of a recumbent position [1]		X
Ataxic movement	Pig is moving in a seemingly uncoordinated fashion; lack of muscle coordination during voluntary movements [2]		X
Muscular excitation	Repeated muscular movement of the whole body, including head movements upwards; seemingly uncoordinated; categorizing posture is not possible due to rapid and frequent movements; severe excitation appear as major clonic convulsive seizures [3,4]		X
Righting response	Pig is making attempt to maintain either a standing or lying sternal posture but is not successful in maintaining the position, different than muscular excitation in that these are slower and seemingly coordinated movements. The event was defined as each time effort was made and the muscles relaxed		X
Out of view	Pig could not be seen clearly enough to identify the behavior or posture; or animal was removed from box		X
Other	Pig's posture was not defined in previous definitions		X

Table 2.3 (continued)

Behaviors (states)	**Definition**	**Direct**	**Video**
Oral Nasal Facial (ONF)	Rubbing, licking, biting, touching the mouth, snout or face to one of two modifiers: other pig or item (walls, flooring, cage) [5]		X
Licking and chewing	Pig is going through motions of licking and chewing, similar to oral nasal facial, but not interacting with and object or the other conspecific [5]	X[§]	X
Open mouth breathing	Pig's mouth is open, taking in quick breaths, with distinct thoracic movements; panting; upper and lower jaw being held open with the top lip pulled back, exposing gums or teeth and panting (pronounced inhalation and exhalation observed at the flanks) [6,7]	X[§]	X
Gasping	Rhythmic breaths characterized by very prominent and deep thoracic movements, with long latency between, may involve stretching of the neck; often occurs right before or after loss of posture [3,6]	X[§]	X
Out of view	Pig could not be seen clearly enough to identify the behavior or posture; or animal was removed from box		X
Other	Pig's behavior or posture did not fit in the above described behaviors or postures		X

Table 2.3 (continued)

Events	Definition	Direct	Video
Salivation	Fluid discharge coming from mouth, may be clear and fluid, viscous or blood. Type of discharge was noted	X	
Nasal Discharge	Discharge from the nasal cavity, may be clear and fluid, viscous or blood. Type of discharge was noted	X	
Eye orbit discharge	Discharge from the ocular orbit, may be clear and fluid, viscous or blood. Type of discharge was noted		
Defecation	Elimination of feces from the body [1]	X	
Urination	Discharge of urine from the body [1]	X	
Vomiting	Ejection of gastrointestinal contents through the mouth [1]	X	
Escape attempt, bout	Pig is raising their forelegs on the side of the wall of the box or pushing quickly and forcefully with their head or nose on the lid of the box; forceful coordinated movement against the exterior of the box; occurrences within in a 10 second period will be scored as a single bout [6,8]		X
Loss of posture	Pig is slumped down, making no attempt to right itself, may follow a period of attempts to maintain posture; considered the first indicator of loss of consciousness [6,8]	X	X
Last limb movement	No movement is observed by the pig's extremities for 1 minute		X
Last Movement	No movement, of any kind is observed by the pig.	X	

For video each pig was scored for 1 of 8 mutually exclusive postures and complementary for 1 of 6 mutually exclusive behaviors, along with event behaviors when occurred.

[§] All direct observations were scored as events

[1]Adapted from Hurnik et al., 1985, [2] Adapted from Blood et al., 2007, [3] Adapted from Dodman, 1977, [4] Adapted from Rodríguez et al., 2008, [5] Adapted from Meiszberg et al., 2009, [6] Adapted from Velarde et al., 2007, [7] Adapted from Johnson et al., 2010, [8] Adapted from Raj and Gregory, 1996

Table 2.4 Duration, when displayed (least square means ± standard errors; s), and percentage (%) of weaned pigs displaying behavioral indicators of distress by gas type and flow rate

	Flow rate of the gas							
Distress measures	**Prefill**	**%**	**Fast**	**%**	**Medium**	**%**	**Slow**	**%**
Standing and Locomotion (SL*)*								
CO_2	14.5 ± 29.7 [a]	100	57.6 ± 24.9 [a]	100	60.9 ± 14.5 [a]	100	78.7 ± 14.7 [a]	100
MIXED	23.0 ± 31.2 [a]	100	75.6 ± 26.0 [a]	100	85.6 ± 14.8 [a]	100	115.1 ± 15.1 [a]	100
AMB	240.7 ± 17.5 [b]	100	N/A		N/A		N/A	
Oral Nasal, all (ON)								
CO_2	0.0 ± 0.0 [a]	0	3.6 ± 1.3 [b]	40	3.6 ± 1.0 [b]	45	5.2 ± 1.3 [b]	65
MIXED	0.0 ± 0.0 [a]	0	5.1 ± 1.3 [b]	55	4.1 ± 1.5 [b]	40	11.1 ± 3.6 [b]	55
AMB	139.3 ± 5.0 [c]	90	N/A		N/A		N/A	
Licking and chewing (LC*)*								
CO_2	1.4 ± 0.4 [a]	5	20.7 ± 2.5 [b]	70	11.0 ± 1.7 [b]	60	18.4 ± 2.7 [b]	45
MIXED	4.0 ± 1.2 [a]	10	20.2 ± 2.9 [b]	50	13.1 ± 2.9 [b]	40	33.6 ± 7.5 [b]	55
AMB	27.3 ± 5.0 [b]	40	N/A		N/A		N/A	
Open mouth breathing (OMB)								
CO_2	19.6 ± 1.6 [a]	80	26.3 ± 1.9 [b]	100	33.7 ±2.3 [c]	100	44.8 ± 3.2 [d]	100
MIXED	35.4 ± 2.4 [c]	100	45.5 ± 3.0 [d]	90	63.9 ± 5.2 [e]	90	71.8 ± 4.9 [e]	100
AMB	0.0 [h]	0	N/A	N/A	N/A	N/A	N/A	N/A
Ataxia								
CO_2	13.5 ± 1.4 [a]	10	18.7 ± 1.9 [b]	25	20.7 ± 2.1 [c]	35	38.6 ± 3.9 [d]	20
MIXED	34.9 ± 5.0 [d]	60	39.5 ± 4.5 [d]	55	45.6 ± 4.6 [e]	60	52.2 ± 5.0 [f]	55
AMB	0	0	N/A		N/A		N/A	
Righting response								
CO_2	1.2 ± 0.7 [a]	20	0.3 ± 0.8 [a]	10	3.7 ± 1.7 [b]	25	4.2 ± 1.3 [b]	35
MIXED	11.2 ± 2.6 [c]	55	8.7 ± 2.2 [b,c]	60	4.7 ± 2.2 [b]	55	13.7 ± 3.2 [c]	60
AMB	N/A	0	N/A		N/A		N/A	

Table 2.4 (continued)

Superscripts indicate differences (P > 0.05) within a behavior, utilizing a post hoc Sidik correction for multiple comparisons. CO_2 was provided at 100% within the flow rates. MIXED constituted a mixture of 50% CO_2 and 50% Ar within the flow rates. Flow rates: slow = 20%, medium = 35%, fast = 50%, and prefilled = filled + 20%, chamber volume per minute. N/A= not applicable.

Table 2.5 Percentage (%) of weaned pigs displaying behavioral indicators of sensation and distress by gas type and gas flow rate

	Flow rate of the gas			
	Prefill	**Fast**	**Medium**	**Slow**
Defecation				
CO_2	25	45	45	50
MIXED	50	60	50	45
AMB	35	N/A	N/A	N/A
Urination				
CO_2	15	20	10	10
MIXED	30	35	35	5
AMB	10	N/A	N/A	N/A
Salivation				
CO_2	5	5	0	15
MIXED	10	10	30	50
AMB	15	N/A	N/A	N/A
Nasal discharge				
CO_2	0	10	20	25
MIXED	15	5	20	30
AMB	10	N/A	N/A	N/A
Escape attempts				
CO_2	0^{x}	0^{x}	0^{x}	0^{x}
MIXED	15^{y}	10^{y}	15^{y}	15^{y}
AMB	0^{x}	N/A	N/A	N/A

Superscripts indicate differences ($P > 0.05$) within a behavior (all flow rates and gas types), utilizing a post hoc Sidik correction for multiple comparisons. CO_2 was provided at 100% within the flow rates. MIXED constituted a 50% CO_2 and 50% Ar within the flow rates. Flow rates: slow = 20%, medium = 35%, fast = 50%, and prefilled = filled + 20%, chamber volume per minute. N/A= not applicable

Table 2.6 Duration, when displayed (least square means ± standard errors; s), and percentage (%) of neonate pigs displaying behavioral indicators of distress by gas type and flow rate

	Flow rate of the gas							
Distress measures	**Prefill**	**%**	**Fast**	**%**	**Medium**	**%**	**Slow**	**%**
Standing and Locomotion (SL)								
CO_2	3.6 ± 29.7 ^{a}	100	36.2 ± 14.5 ^{a}	100	36.6 ± 14.5 ^{a}	100	54.7 ± 14.8 ^{a}	100
MIXED	7.6 ± 31.2 ^{a}	100	48.3 ± 26.0 ^{a}	100	57.8 ± 14.8 ^{a}	100	X	
AMB	308.7 ± 17.5 ^{b}	100	N/A		N/A		N/A	
Oral Nasal, all (ON)								
CO_2	0.0 ± 0.0 ^{a}	0	1.4 ± 1.3 ^{b}	15	1.0 ± 1.0 ^{b}	25	0.7 ± 1.3 ^{b}	15
MIXED	0.0 ± 0.0 ^{a}	55	2.0 ± 1.3 ^{b}	45	3.4 ± 1.5 ^{b}	35	X	
AMB	53.3 ± 21.6 ^{c}	90	N/A		N/A		N/A	
Licking and chewing (LC)								
CO_2	0.0 ± 0.0	0	5.3 ± 2.5	30	4.0 ± 1.7	30	5.2 ± 2.7	20
MIXED	1.3 ± 2.0	25	2.9 ± 3.0	40	2.0 ± 3.0	30	X	X
AMB	6.9 ± 4.8	5	N/A		N/A		N/A	
Open mouth breathing (OMB)								
CO_2	12.1 ± 1.6 ^{a}	90	23.3 ± 1.9 ^{b}	90	23.6 ±2.3 ^{b}	100	39.1 ± 3.2 ^{c}	100
MIXED	13.5 ± 2.4 ^{a}	100	33.6 ± 3.0 ^{c}	100	49.3 ± 5.2 ^{d}	100	X	
AMB	0.0	5	N/A	N/A	N/A	N/A	N/A	N/A
Ataxia								
CO_2	14.3 ± 1.4 ^{a}	50	27.3 ± 1.9 ^{b}	60	25.9 ± 2.1 ^{b}	25	43.3 ± 3.9 ^{c}	50
MIXED	23.6 ± 5.0 ^{b}	45	47.1 ± 4.5 ^{c}	60	65.7 ± 4.6 ^{d}	40	X	
AMB	0	0	N/A		N/A		N/A	
Righting response								
CO_2	2.3 ± 0.7	50	3.6 ± 0.8	50	4.9 ± 1.7	60	1.9 ± 1.3	25
MIXED	3.7 ± 2.6	65	4.6 ± 2.2	45	8.0 ± 2.2	60	X	
AMB	N/A	0	N/A		N/A		N/A	

Table 2.6 (continued)

Superscripts indicate differences (P > 0.05) within a behavior, utilizing a post hoc Sidik correction for multiple comparisons. CO_2 was provided at 100% within the flow rates. MIXED constituted a mixture of 50% CO_2 and 50% Ar within the flow rates. Flow rates: slow = 20%, medium = 35%, fast = 50%, and prefilled = filled + 20%, chamber volume per minute. N/A= not applicable

Table 2.7 Percentage (%) of neonate pigs displaying behavioral indicators of sensation and distress by gas type and gas flow rate

		Flow rate of the gas		
	Prefill	**Fast**	**Medium**	**Slow**
Defecation				
CO_2	20	25	25	10
MIXED	20	30	30	X
AMB	30	N/A	N/A	N/A
Urination				
CO_2	20	35	25	20
MIXED	15	30	30	X
AMB	20	N/A	N/A	N/A
Salivation				
CO_2	5	10	5	10
MIXED	5	5	5	X
AMB	5	N/A	N/A	N/A
Nasal Discharge				
CO_2	0	0	5	5
MIXED	0	0	15	X
AMB	5	N/A	N/A	N/A
Escape attempts				
CO_2	5	0	0	0
MIXED	5	0	0	X
AMB	10	N/A	N/A	N/A

Significant differences were not observed between gas type or flow rate ($P > 0.05$), utilizing a post hoc Sidik correction for multiple comparisons. CO_2 was provided at 100% within the flow rates. MIXED constituted a 50% CO_2 and 50% Ar within the flow rates. Flow rates: slow = 20%, medium = 35%, fast = 50%, and prefilled = filled + 20%, chamber volume per minute. N/A= not applicable

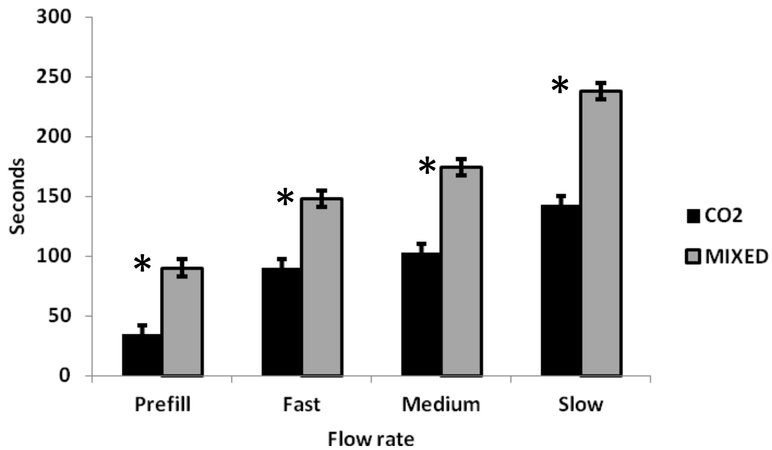

Figure 2.1 Least square means and SE for latency to loss of posture in the weaned piglets (n=180) by gas type (CO_2 = 100% CO_2, MIXED = 50:50 CO_2:Ar) within flow rate (prefilled = filled + 20%, fast = 50%, medium = 35%, slow = 20%, chamber volume exchange rate/min).

* = $P < 0.001$ between gas types

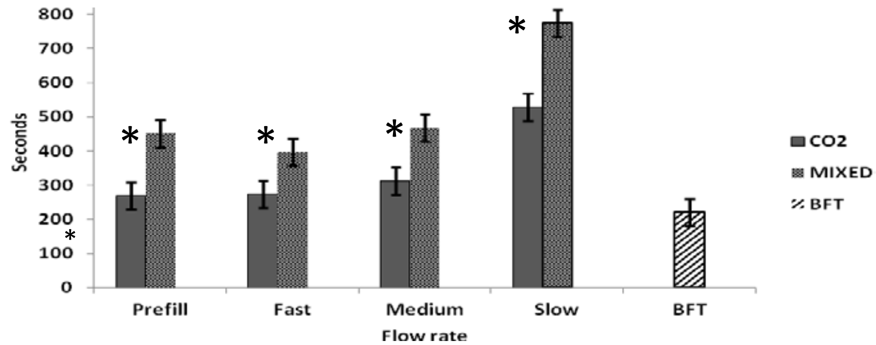

Figure 2.2 Least square means and SE for latency to last movement in the weaned piglets (n = 180) by flow rate (prefilled = filled + 20%, fast = 50%, medium = 35%, slow = 20%, chamber volume exchange rate/min) within euthanasia type (CO_2 = 100% CO2, MIXED = 50:50 CO_2:Ar, BFT = blunt force trauma). For gas methods, time was calculated from placement into the box until no movements were observed. For BFT, time was calculated from application of method until no movements were observed.

* = $P < 0.05$ between flow rates

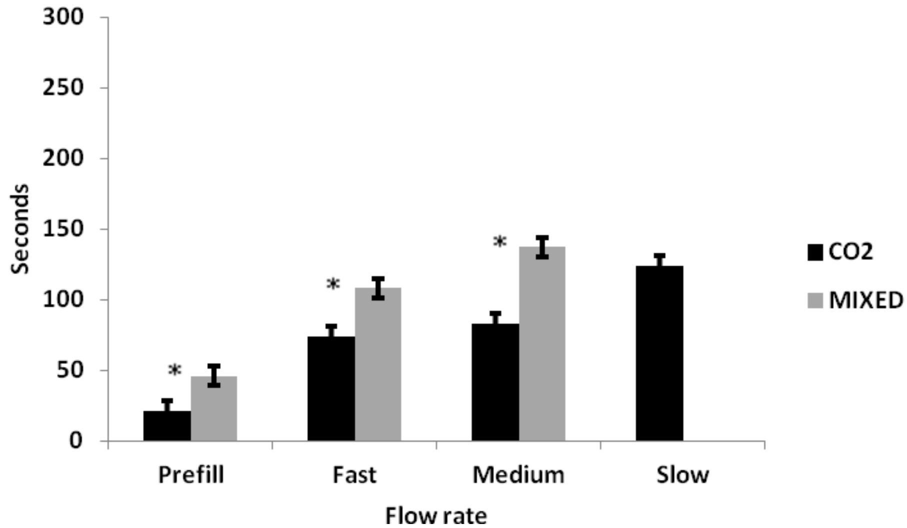

Figure 2.3 Least square means and SE for latency to loss of posture in the neonate piglets (n=180) by gas type (CO_2 = 100% CO_2, MIXED = 50:50 CO_2:Ar) within flow rate (prefilled = filled + 20%, fast = 50%, medium = 35%, slow = 20%, chamber volume exchange rate/min).

* = P < 0.001 between gas types

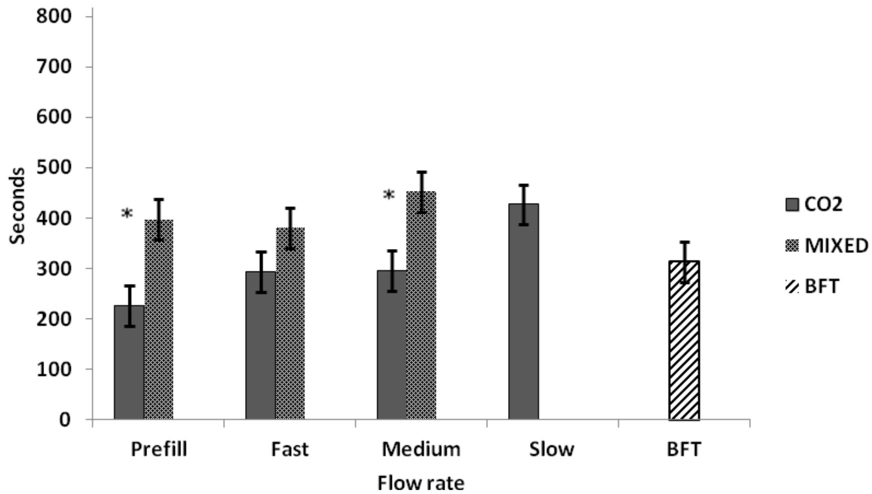

Figure 2.4 Least square means and SE for latency to last movement in the neonate piglets (n = 180) by flow rate (prefilled = filled + 20%, fast = 50%, medium = 35%, slow = 20%, chamber volume exchange rate/min) within euthanasia type (CO_2 = 100% CO_2, MIXED = 50:50 CO_2:Ar, BFT = blunt force trauma). For gas methods, time was calculated from placement into the box until no movements were observed. For BFT, time was calculated from application of method until no movements were observed.

* = $P < 0.05$ between flow rates

CHAPTER 3 DISTRESS ELICITED BY CARBON DIOXIDE OR ARGON GASES DURING INDUCTION OF ANAESTHESIA FOR SUCKLING PIGLETS

A paper to be submitted to the journal Applied Animal Behaviour Science

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3.1 Abstract

The objective of this experiment was to assess the distress elicited by induction of anaesthesia for piglet processing, produced from two gas types (100% carbon dioxide [CO_2], 100% argon [Ar]) relative to a control (100% air infused with a novel odour [ODOR]). Additionally, depth of anaesthesia, reliability and pig safety were assessed for the two gas treatments. Sixty-six, 3-d old, healthy male piglets were enrolled as piglet pairs. Piglets were habituated to the induction box over 4 days. The box was fitted with environmental enrichment (peat moss, Kong, jam, honey) to motivate the piglets to engage with the environment, allowing an assessment of distress relative to motivation to engage in rooting, play and investigation. On the 5th day, one of the three gasses was applied. For CO_2 and Ar, piglets remained in the box until 30 seconds after loss of posture (LP). ODOR piglets remained in the box for 14 min. Following removal, piglets were assessed for signs of sensibility, and were then placed in a pen for recovery and observation. Induction and recovery behaviours were collected live and with video. Audio recordings were captured during induction and analyzed for duration of distress vocalizations. Latency to LP and to regain posture (RP) were shorter in CO_2 relative to Ar (LP [$P < 0.001$] 100 ± 8 vs. 244 ± 8 s; RP [$P = 0.0461$] 74 ± 37 and 172 ± 37 s). No ODOR piglets displayed open mouth breathing (OMB), ataxia or a righting response (RR). All CO_2 and Ar piglets displayed OMB and ataxia (duration CO_2 vs. Ar: OMB [$P > 0.1$] 36 ± 7, 48 ± 7 s; ataxia [$P = 0.02$] 35 ± 5, 16 ± 5 s; RR [$P < 0.001$] 11 ± 4, 29 ± 4 s). Escape attempts were greater in Ar (64%) relative to ODOR (18%) and CO_2 (0%). Duration of distress calls from Ar (20 ± 1 s) treatments were longer ($P < 0.001$) relative to the CO_2 (2 ± 1 s) or ODOR (1 ± 1 s). Differences were observed between treatments for pupillary constriction, with CO_2 piglets less likely than Ar piglets to display this reaction ($P = 0.02$; 23% vs. 59%, respectively). In summary, both CO_2 and Ar elicited signs of distress during induction of anaesthesia in piglets, and Ar produced higher prevalence of escape, longer duration of distress calls, ataxia and RR while producing a lighter, more variable plane of anaesthesia. Results from this study preclude argon as an inhalant anaesthetic for piglet processing.

Keywords: swine, carbon dioxide, argon, anaesthesia, animal welfare

3.2 Introduction

Piglets in the U.S. swine industry are currently processed (castrated and tail docked) without anaesthesia or analgesia, in part due to the absence of drugs labelled for pain mitigation. These procedures are painful (Marchant-Forde et al., 2009), but in order for anaesthesia or analgesia to be widely implemented, interventions must be feasible in production settings (Rault and Lay, 2011; Sutherland et al., 2011). Varieties of anaesthetics are available for swine including xylazine, halothane, isoflurane, methoxyflurane and carbon dioxide [CO_2] (Klide, 1996). Of these, only CO_2 can be administered by non-veterinarians and thus is the only practical on-farm gas anaesthetic in the United States in the current regulatory environment. CO_2 is an unregulated gas with known anaesthetic and analgesic effects. In the piglet, CO_2:O_2 gas mixtures (70-80% CO_2, 20% O_2; 50:50 CO_2:O_2) have been demonstrated to produce general anesthetic effects for castration with analgesia observed 2 min post procedure (Gerritzen et al., 2008; Klide, 1996). Additionally, CO_2 has been shown to be quick to induce anaesthesia (< 30s; Klide, 1996) and is quickly reversible, which aids in practical application and reduces risks of hypothermia and crushing (Gerritzen et al., 2008; Mühlbauer et al., 2010; Sutherland et al., 2011). However, concerns about distress associated with CO_2 exposure and mortality risks have been raised.

Gas (CO_2) techniques for euthanasia are approved for swine by the American Veterinary Medical Association and are increasingly used for on-farm euthanasia of low viability piglets. Hence, infrastructure may exist to facilitate inhalant anaesthesia for piglet processing. A gas method of euthanasia involves a two-step process. First, induction of anaesthesia comprises all steps until the piglet is rendered unconscious. Second, cessation of respiratory and cardiac function results in death. The induction phase is critical for ensuring animal welfare during both euthanasia and anaesthesia. CO_2 is a mildly acidic gas and can irritate the mucus membranes (Danneman et al., 1997). At 10% carbon dioxide concentrations some human subjects report experiencing breathlessness, described as being unpleasant, and 50% CO_2 concentration is reported as being very pungent (Gregory et al., 1990). Prior to loss of consciousness, the piglet experiences severe laboured breathing (Liotti et al., 2001). This has led to questions about the humaneness of CO_2 induction (Raj & Gregory, 1996; Wright, Whiting, & Taylor, 2009). In contrast to euthanasia, anaesthesia during processing requires maintenance of respiratory and cardiac function for piglets to reliably return to consciousness. Deaths have occurred during piglet CO_2 anaesthesia, leading to questions regarding piglet safety.

Argon (Ar) has been proposed as an alternative to CO_2 for euthanasia. Argon renders a pig unconscious by creating a hypoxic state (Raj, 1999). Even though research in piglets is limited, Ar is listed as a comditionally accepted euthanasia method by the American Veterinary Medical Association (AVMA, 2013) and European Food Safety Authority (EFSA, 2004). Market pigs exposed to argon, in contrast to CO_2, will remain in a chamber for a food reward until loss of posture (Raj and Gregory, 1995). Conversely, in young pigs, a 50:50 CO_2:Ar gas blend increased distress relative to 100% CO_2 as measured by escape attempts, duration of open mouth breathing, ataxia, and righting response (Sadler et al., 2011). Sutherland (2011) utilized a unique welfare index, and found benefits with 100% Ar relative to CO_2, but Rault and colleagues (2013) dropped argon as a treatment after a preliminary trial due to welfare concerns. If Ar results in less distress during induction of unconsciousness, this gas has potential for both euthanasia and anaesthesia techniques. However, current research findings are conflicting; thus, further assessment is needed.

Several factors may contribute to piglet distress during induction of anaesthesia, one of which being the gas itself. Other potentially distressing factors include removal from the dam and conspecifics, mixing with unfamiliar piglets, the novel environment of the chamber, thermal stressors and physical comfort of the chamber. Hence assessing distress during induction of anaesthesia can be confounded by responses to these other stressors. Furthermore, these stressors can be viewed with the additive stressor model (McFarlane et al., 1989), in which the observed distress response is the summation of several factors. Pain and distress are affective states and can only be measured indirectly in humans and animals. Distress associated with CO_2 has been assessed in pigs using behavioural responses (Dodman 1977; Gregory et al., 1990; Raj & Gregory 1996; Velarde et al. 2007; Rodriguez et al 2008). Although variations in behaviour are observed during induction of insensibility, it is difficult to ascertain whether these are accurate indicators of distress since some of these behaviours also occur during involuntary neurophysiologic responses to the induction process, after the piglet is insensible. Also, changes in behaviour cannot be observed in piglets displaying a freezing response during acute fear. A motivational state model provides opportunity to circumvent these difficulties, allowing the distress from the gas to be teased apart from other sources of stress.

The objectives of this study were to isolate the distress caused by gas induction of unconsciousness using a competing motivational state model, and to compare distress and welfare implications associated with CO_2 and Ar gas anaesthesia during induction, recovery and post-recovery stages.

3.3 Materials and methods

3.3.1 Animals and enrolment procedures

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee.

Three gas types were explored: CO_2, Ar and air infused with a novel odour (ODOR). Sixty-six piglets were enrolled, with 11 piglet pairs placed in each treatment. Eleven litters were utilized with six healthy male piglets chosen from each litter. Within the litters, each of the three treatments was randomly assigned to one piglet pair. Piglets were 2 d of age on the day of enrolment. Piglet genetics were a custom maternal x performance line (Landrace x Yorkshire cross x Duroc sire line). Piglets were housed and maintained with the sow and other siblings, including those not enrolled in the trial. They were provided with customary care and husbandry, as standard to the farm. Piglets were housed indoors within farrowing stalls. Each stall had two rubber mats located on each side of the sow in the protected portion of the stall. A heat lamp was provided on one side. Piglets had access to a pelleted feed and a water nipple. All piglets were tail docked and castrated by 4 d of age.

At the time of enrolment, vital signs were collected and a behavioural assessment was conducted to identify healthy piglets. Parameters that defined a healthy piglet included rectal temperature < 39.7°C and respiratory breaths/min > 100 and < 300. Additionally, depression, diarrhoea and dehydration were scored for severity from 0 (normal) to 3 (moribund/severe). Piglets that qualified for enrolment were assigned a score of zero in all categories. All piglets within a litter, regardless of enrolment, were marked with an animal safe paint stick to facilitate behavioural observations and ear notched for identification throughout the trial. Paint was reapplied daily until gas testing was conducted.

3.3.2 Gas administration equipment and the habituation procedures

Gas was administered to the piglets via a modified Euthanex AgPro™ system (V-ast, Iowa, USA; Figure 3.1). To facilitate behavioural observations, the box was modified with clear plastic on the top and front panels. The remaining 4 panels were constructed of opaque plastic. The inside dimensions of the box were 60 cm long x 43 cm wide x 30 cm high. Two 0.64 cm diameter inlet valves were located on the side panel 12.70 cm (CO_2) and 22.86 cm (Ar) from the front panel and 3.81 cm from the top. A 0.95 cm diameter outlet valve was located on the opposite side panel from the inlet valves, 30.48 cm from the front panel and 6.35 cm from top. The gas flowed through 3.25 m of 0.64 cm diameter inlet hoses prior to entering the box. The CO_2 gas used was industrial grade (99% pure), whereas the Ar had a guaranteed analysis of 99.996% pure. The air was medical grade, with a guaranteed analysis of 99.995% pure. Constant and precise gas flow was provided by compressed gas cylinders outfitted with compressed gas regulators and flow meters (Western Enterprises, Westlake, OH, USA [CO_2 and Ar] and Praxair, Orangeburg, NY, USA [Air]). Gas was supplied at a 35% chamber volume exchange rate/min (21 air changes per hour [ACH], assuming complete mixing). For the novel odour treatment, air was passed through a cotton ball containing 2 mL of peppermint extract (Pure Peppermint Extract, McCormick, MD, USA). This produced a peppermint odour that was detectable in the exhaust air to a human.

In order to minimize distress due to novelty, several steps were taken to minimize outside distractions during testing and piglets were habituated to the process. The box was placed in a room that was isolated from all other animals, and testing was conducted after all farm staff had left for the day. A light screen (light was shown onto the testing box in a dark room) was created using a heat lamp bulb (125W, Infrared clear Heat Lamp, Havells, Inc., Atlanta, GA), which was also utilized in the home pen ensuring the lighting was not novel. The physical environment was designed to ensure comfort. The box was large enough to allow the piglets to move about freely, with adequate ceiling height to prevent contact with their heads. The box was constructed so that there were no pinch points or sharp protruding objects. Supplemental heat was provided with the heat lamp, which was placed approximately 0.4 m from the box. The floor was fitted with a rubber mat (Rubber floor mats, Kraco, Enterprises, LLC, Compton, CA, USA) to provide traction for the piglets. Peat moss was distributed at a depth of 2 cm throughout the entire floor (Premier Peat Moss, Premier Horticulture Inc., Quakertown, PA, USA) to provide additional traction and comfort. The peat moss also provided environmental enrichment. Additional enrichment items included three Kongs® (Classic- medium; Golden, CO, USA) filled with jam (Great Value Concord Grape Jam, Bentonville, AR, USA) and honey (Great Value Clover Honey, Bentonville, AR, USA). Piglets were only allowed access to these items when in the box. Enrichment items were chosen to elicit foraging/rooting, play and exploratory behaviour. Piglets exposed to the two gas treatments were compared relative to the baseline as established by the ODOR treatment, assessing motivation for rooting, play and exploration, relative to the distress of the gases. Although both CO_2 and Ar are odourless, a novel odour was included in the control treatment so that baseline included the effects of novel odours that could be produced from passing through equipment, which the piglets were not habituated to prior to testing.

Piglets were 3 d of age when they were first placed in the box. In this study, piglets were tested as familiar sibling pairs (n=11/treatment), always being placed with the same sibling in the box. Piglets were habituated for 4 consecutive days prior to testing, each time being placed in the box for 14 min. During these days, piglet pairs were collected from the home pen, placed in a basket and carried to the testing room. Following the habituation, they were again placed in the basket and carried back to the home pen. During this time, air was passed through the box at a 21 ACH flow rate, with gas flow started upon placement of the piglets into the box. This exposure provided an opportunity for piglets to habituate to the novel environment, including noise and airflow, as well as to explore the environmental enrichment before being returned to the home pen. The habitation process was started at the same time every day with piglet pairs placed in the box in the same order.

3.3.3 Testing procedures

Testing procedures were carried out on the 5th day post-enrolment and conducted similarly to the habituation process. Piglet pairs were tested in the same order, with treatment randomly assigned to each piglet pair. To avoid disruption of teat order and associated social conflict due to missed feedings, the entire litter was removed from the home pen on the day of testing and placed in a holding cart bedded with wood sawdust (TLC Premium Horse Bedding, Centerville, AR, USA). The entire litter was housed in the holding cart until all test piglets recovered from anaesthesia, after which they were returned to their home pen. Piglets enrolled in the study were carried in the basket to the treatment area as pairs and placed in the box, after which gas flow was initiated. The ODOR treatment piglets remained in the box for 14 min, after which they were returned to the cart. For the CO_2 and Ar treatments, piglets remained in the box until 30 s after loss of posture (LP). These piglets were then removed individually from the box and immediately checked for signs of sensibility (Whelan and Flecknell, 1992; Kissin, 2000; National Pork Board, 2009; Grandin 2010). Four tests were conducted: (1) corneal reflex response, in which the eye was touched with the tip of a finger for absence of an eye blink or withdrawal response; (2) pupillary reflex, in which a light-beam (Mini MAGLite, Mag Instrument, Inc., Ontario, CA) was shone into the eye and pupil observed for absence of constriction; (3) nose prick, in which a 20 gauge needle was touched to the snout distal to the rostral bone for absence of a withdrawal response; and (4) leg prick, in which a 20 gauge needle was sharply touched above the corneal band on the hind leg for absence of a withdrawal response. Following induction of anaesthesia and testing for signs of sensibility, piglets were placed in a recovery pen where they were monitored for return to sensibility and normal behaviour, defined as standing, vocalizing and exploring the novel environment without signs of ataxia. The recovery pen (Black E-Coat Exercise Pen, 550-24 61cm x 61cm panels, 7 panels used, Midwest Homes for Pets; Mancie, India) was novel to the piglets, and was arranged in a circular pattern ~1.3 m diameter. The floor in the recovery pen was fitted with rubber matting with a thin layer of peat moss. A heat lamp was fixed to the side of the pen to provide supplemental heat. Piglets were placed under the lamp during recovery. Prior to each treatment, peat moss was removed by vacuum (Shop Vac 10 Gallon Ultra Pro Vacuum, 185 CFM), a clean rubber mat was placed in the box and fresh peat moss was added. The vacuum was also utilized to remove gas traces, pulling air from the bottom of the box for a minimum of 4 min.

3.3.4 Collection of behaviour data

Induction and recovery behaviours were collected both live and through video. Live observations were conducted by two trained observers; each scoring one piglet from the pair. Video was captured, utilizing a Noldus portable lab (Noldus Information Technology, Wageningen, NL). Two colour Panasonic cameras (WV-CP484, Kadoma, Japan) were fed into a multiplexer, which then allowed the image to be recorded onto a PC using HandiAvi (v4.3, Anderson’s AZcendant Software, Tempe, AZ) at 30 frames per second. Video was not scored for one piglet pair in the Ar treatment due to technical difficulties. The collected video was scored by two trained observers, blinded to treatments, using Observer® (v10.1.548, Noldus Information Technology, Wageninger, NL). Piglets were scored individually during both the induction and recovery phase for normal behaviours as well as behaviours indicative of distress and sensation of the gases (Table 3.1). Prior to data collection, observers were trained to the ethogram, and scoring was not started until inter-observer reliability k > 0.90 was achieved. Inter-observer and intra-observer reliability were checked at the end of the observation period, and were k > 0.90 for both calculations. Treatments were balanced between observers.

3.3.5 Collection of vocalizations and distress calls

While in the box, digital audio recordings of the piglets were captured with a Marantz PMD 661 recorder (Marantz Corp., Kanagawa, Japan) and Crown PZM185 microphone (Crown Int., Elkhart, IN USA). Due to errors with the collection equipment, recordings from two piglet pairs were not captured, one from each ODOR and CO_2 treatments. The recorder digitized the audio into a wav file at 48 kHz. The created audio was analyzed with the STREMODO program (STREss Monitor and Documentation unit, Forschungsinstitutfu¨ r die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Germany) for duration of distress vocalizations. On a subset of the data (n=3/treatment), complete vocalizations, which included all grunts and squeals, were counted manually by a trained technician.

3.3.6 Production parameters collected

Individual weights were collected on the day of enrolment (age 2 d), the day of treatment (age 7 d), 1 day post-treatment (age 8 d) and prior to weaning (age 18 d). Morbidity and mortality were noted for all enrolled piglets until weaning.

3.3.7 Environmental parameters

Temperature and relative humidity were monitored within the box by a HOBO data logger (U23-001, Onset Computer Corporation, Cape Cod, MS) set to record every 10 seconds. Data was collected continuously throughout the treatment day. Oxygen levels were collected with an oxygen sensor (TR25OZ, CO2Meter.com, Ormond Beach, FL) attached to a HOBO data logger (U12, Onset Computer Corporation, Cape Cod, MS), which collected a reading every second. A CO_2 meter was placed in the box, but due to technical difficulties, these data were not collected.

3.3.8 Statistical analysis

Signs of sensibility were analysed using a mixed effects logistic regression model in SAS. Piglet pair was the experimental unit, blocked by litter, with fixed effect of gas type. Weights and behaviours were analysed using a linear mixed model in SAS with piglet pair as the experimental unit, blocked by litter, with fixed effect of gas type. The Kenward-Rogers method was utilized for determining the denominator degrees of freedom. Differences in weights between treatments, including at the beginning of the experiment, weight change one day post-treatment and ADG post-treatment were blocked by litter. Oral and nasal (ON) behaviours were analyzed individually (licking and chewing peat moss, other piglet, external Items and Kong) and summed. Statistical analysis for ON was assessed as a percent of time prior to LP. Least square means estimates for each treatment group and the corresponding standard errors (SE) are reported.

Audio data assessed by STREMODO were used to calculate duration of distress calls from the piglet pair during the treatment phase. This summarized data were analysed as a generalized linear mixed model, with the fixed effect of treatment and random effect of litter, utilizing the Kenward-Rodger method to compute the denominator degrees of freedom. For all analysis, a $P < 0.05$ was considered significant unless otherwise indicated. Least square means estimates for each treatment group and the corresponding standard errors (SE) are reported.

3.4 Results

3.4.1 Loss of posture and recovery

Piglets exposed to CO_2 took 100 ± 8 s to loss of posture (LP) with a range of 67 to 157 s. The Ar treatment piglets took 244 ± 8 s to LP, with a range of 222 to 292 s ($P < 0.001$; Figure 3.2). Once placed in the recovery pen, latency to regain posture (RP) was shorter for CO_2 relative to Ar ($P = 0.0461$; 74 ± 37 and 172 ± 37, respectively). The range for RP was 2 to 501 s for CO_2 and 0 to 680 s for Ar. Four piglets in the Ar treatment displayed RP prior to testing any signs of sensibility. This quick recovery was not observed in the CO_2 treatment. Latency to full recovery was shorter for CO_2 relative to Ar ($P = 0.03$; Figure 3.2) ranging from 117 to 501 s for CO_2 and within 240 to 1056 s for Ar.

3.4.2 Behaviours observed during induction

Eighteen percent of the piglets in the ODOR treatment attempted to escape (table 3.2). None of the piglets exposed to CO_2 attempted to escape, whereas escape attempts were observed in 64% of piglets exposed to Ar ($P = 0.9302$ Odor vs. CO_2; $P = 0.0582$ Odor vs. Ar; $P = 0.048$ CO_2 vs. Ar). No piglets in the ODOR treatment displayed open mouth breathing (OMB), ataxia or righting responses (RR), whereas all piglets exposed to CO_2 or Ar displayed OMB and ataxia. Righting response was displayed by 68% of the CO_2 and 90% of Ar pigs. Duration of OMB was not different between the two gas treatments ($P > 0.1$, 36 ± 7 s and 48 ± 7 s for CO_2 and Ar, respectively). The duration of ataxia experienced in Ar was more than twice as long (35 ± 5 s) as in CO_2 (16 ± 5 s; $P = 0.02$). When displayed, the duration of the RR was less for CO_2 (11 ± 4 s) relative to Ar (29 ± 4 s; $P < 0.001$). In addition, the number of righting attempts was lower in CO_2 relative to Ar, 4 ± 2 vs. 15 ± 2, respectively ($P < 0.001$).

When examining all ON behaviours combined (licking and chewing [LC], peat moss [PM], external item [EX], Kong, other piglet [OP]) as a percent of conscious time, ODOR pigs spent 17% vs. 3% engaged in this behaviour in CO_2 ($P < 0.001$), Ar pigs were intermediate at 6% ($P < 0.001$), but did not differ from CO_2 ($P = 0.12$). Overall, LC was most common behaviour observed of the ON behaviours, with ODOR displaying this 7% of the time, CO_2 3% and Ar < 1% ($P > 0.1$ [Odor vs. CO_2]; $P = 0.045$ [Odor vs.

Ar]); $P > 0.1$ [CO_2 vs. Ar]). ODOR spent 5% of the time engaged with the PM, differing from CO_2 piglets which displayed no interaction and Ar which interacted for 2% ($P < 0.001$); CO_2 and Ar did not differ. ODOR pigs engaged with an external item for 3%, whereas CO_2 and Ar engaged for 1% and < 1%, respectively ($P = 0.013$, Odor vs. CO_2; $P = 0.0556$, Odor vs. Ar; $P > 0.1$, CO_2 vs. Ar). Interaction with the Kong was infrequent with ODOR piglets, observed 2% of the time and < 1% for both CO_2 and Ar. Interaction with the other piglet was rarely observed, occurring < 0.03% of the time for all treatments.

Duration of piglets engaged in standing and locomotion prior to LP differed between all gas treatments with ODOR spending 467 s, followed by Ar at 152 s and CO_2 at 67 s. Time spent sitting was limited for all treatments with a difference observed between ODOR (21 s) and CO_2 (8 s; $P = 0.05$) and a trend between ODOR and Ar (9 s; $P = 0.082$).

Nasal discharge was observed in 14% of the ODOR pigs, and was less than that observed in CO_2 (27%) and Ar (23%; $P < 0.001$, ODOR vs. CO_2; $P < 0.001$, ODOR vs. Ar; $P > 0.1$, CO_2 vs. Ar). A trend ($P = 0.07$) was observed for oral discharge with 5% of both ODOR and Ar displaying this response, whereas 10% of CO_2 pigs exhibited this response.

3.4.3 Distress calls and vocalizations during induction

Duration of distress calls by the piglet pair during induction were longer for the Ar treatment relative to the CO_2 or ODOR treatment ($P < 0.001$; Figure 3.4). The Ar treatment resulted in 20 ± 1s of distress calling whereas CO_2 and ODOR produced virtually no recognizable distress calls (2 ± 1 and 1 ± 1 s, respectively). When comparing manual vocalization counts for piglet pairs (n=3/treatment), it was observed that the piglet pairs in the ODOR treatment were highly vocal, initiating 662 ± 144 vocalizations (includes all grunts, contact calls and distress calls) during the 14 min observation period. This is in contrast to CO_2, which only produced 40 ± 144 calls. Ar was intermediate, with 294 ± 162 calls. ODOR and CO_2 treatments differed (P = 0.03). These values equate to calls/min (prior to LP) of 44, 24 and 74 for ODOR, CO_2 and Ar respectively. In order to establish if the subpopulation was representative of the observed distress calls, distress calls from the subpopulation were statistically analysed. The subpopulation revealed numerical patterns similar to the whole data set, with a trend for a difference between CO_2 (0.2 s) and Ar (12.9 s; P = 0.10). While differences in this subset were not found between ODOR (4.1 s) and Ar, a similar numerical pattern was conserved within the full data set (P = 0.15).

3.4.4 Environmental conditions during induction

In the CO_2 treatment, LP occurred when oxygen (O_2) levels reached 14 ± 2%. In the Ar treatment, LP occurred at O_2 levels of 4 ± 1%. The ODOR group, which never lost posture, showed consistent O_2 levels at 21 ± 1%. On average, the Ar treatment piglets began OMB when O_2 levels were 7 ± 1%. In the CO_2 treatment, the onset of OMB occurred at an average O_2 level of 19 ± 1%. Ataxia in the Ar treatment, similar to OMB, also began around 7 ± 1%. CO_2 piglets displayed this behaviour at a slightly lower O_2 level than OMB at 17 ± 1%. Though data were not available for CO_2 levels, the monitored O_2 levels do indicate atmospheric air conditions were achieved prior to each application of treatment. Temperature in the box on treatment days averaged 25.6 °C, with a range of 23.3 to 27.2 °C. Relative humidity in the box during this time averaged 78% ranging from 74 to 93%.

3.4.5 Signs of sensibility

Differences were observed between gas treatments for pupillary constriction following induction, with the CO_2 piglets less likely than Ar to display this response ($P = 0.02$; 23% vs. 59%, respectively; Figure 3.3). Differences between gas treatments were not observed for the other three signs of sensibility. Independent of gas treatment, differences were observed between all signs of sensibility with the corneal reflex most likely to be observed (73%), followed by the nose prick (25%) and the leg prick (21%; $P < 0.001$).

3.4.6 Behaviours observed during recovery

Percent of piglets displaying OMB did not differ between the CO_2 and Ar treatments, nor were differences observed in the duration (86 ± 34 vs. 83 ± 34 s, CO_2 vs. Ar). Differences were not observed between the two gas treatments for piglets displaying ataxia. Differences ($P = 0.06$) were observed in the duration of the summed ON behaviours with CO_2 engaged for 32 ± 8 s compared to 6 ± 8 s for the Ar treatment. The majority of this difference came from interaction with external items (22 s). Only one CO_2 piglet made an escape attempt while in the recovery pen. Differences were not seen between treatments for the behaviours of SL or sitting. Duration of RR was 11 ± 4 s for

CO_2 and 29 ± 4 s for Ar ($P < 0.001$). The number of righting efforts was also different between the CO_2 and Ar treatments, 4 ± 2 vs. 15 ± 2 s, for CO_2 and Ar, respectively.

3.4.7 Post-treatment effects on performance

Two piglets died more than 7 d following treatment; one in the CO_2 treatment (starved out) and one in the Ar treatment (prolapse), as identified by stockpeople. Differences ($P > 0.1$) were not observed between treatments in the starting weight, weight change one day post-treatment or ADG post-treatment. At enrolment, piglets had a body weight of 1.9 ± 0.1 kg. Testing occurred when piglets were 7 days of age and weighed 3.27 ± 0.1 kg. At weaning, when piglets were 18 days of age, they weighed 6.86 ± 0.40 kg.

3.5 Discussion

The motivational model used in this experiment demonstrated that induction of anaesthesia from hypoxia and hypercapnia, produced with Ar and CO_2 gases respectively, was distressing to the piglets as indicated by presence of OMB, ataxia, righting response, increased nasal discharge and escape attempts. Carbon dioxide relative to Ar was associated with superior welfare, as indicated by lower prevalence of escape attempts, duration of distress calls, duration of ataxia and duration of righting response.

Piglet play and investigative behaviours have been described beginning 1 d after birth, directed towards objects and conspecifics (Blackshaw et al., 1997; Newberry et al., 1988). Several of the objects in this study were only available in the box and so it was expected that piglets would be more motivated to engage with these objects. Gas was started at a gradual rate after piglets were placed in the box, allowing the piglets to express normal behaviours before cognitive dysfunction. In this study, piglets exposed to the novel odour served as the control. Prevalence or duration of behaviours deviating from this treatment group was considered abnormal and interpreted as a result of the physiological stress or discomfort produced from hypoxia or hypercapnia. While engagement with the motivational objects was limited even within the ODOR treatment, differences were observed relative to the gas treatments. ODOR piglets engaged in ON behaviours approximately 10% of the time, whereas prior to loss of posture, CO_2 and Ar piglets, displayed these behaviours on a very limited basis (< 4 %). This would indicate the piglets in CO_2 and Ar were quickly aware and vigilant to the presence of the gas treatments. One possible explanation for discrepancies in ON behaviour may be latency to engage rather than alarm, in that latency to engage in these behaviours were censored by LP and sufficient time may not have been allowed for pigs in the gas treatments to begin interaction with the enrichment. This would indicate motivation to root and explore, with the given objects, was not strong enough to engage the piglets immediately upon placement into the box.

We expected LC to be activated by the creation of carbonic acid for the CO_2 gas on the mucus membranes, perhaps an indicator of mild pain (Raj, 1999). Humans do not generally find peppermint to be aversive and as such we assumed it would not cause pain or respiratory stimulation (Niel et al., 2008). Thus, it was surprising that LC was observed in ODOR treatment 7% of the time. This may indicate that a novel odour, rather than pain, was sufficient to elicit LC.

Ataxia is likely an indicator of impaired cerebellar function, however it is unclear how this correlates to impaired cortical function. If ataxia indicates that the piglet is aware of its surroundings, but is unable to react in a coordinated manner, this could be considered distressing to the piglet. Additionally the observed escape attempts and distress calls were often elicited during this phase indicating a distress is experienced. In this study, we defined ataxia as a potential stressor for the piglet, and hence, a shorter duration of this behaviour would correlate with improved welfare. The lack of RR has been cited as a critical indicator that a pig is successfully rendered unconscious prior to slaughter (Grandin, 2010; Sandström, 2009) and is cited as an indicator of unconsciousness (Anil, 1991; National Pork Board, 2009). Righting responses requires coordinated brain activity and are indicators of brain function. Since CO_2 and Ar are both heavier than air, it is possible that some of the RR observed reflect the animal's attempt to physically avoid the gas, as opposed to a reflexive behaviour. Hence, duration and intensity of RR are used as indicators of distress in this study. Duration of ataxia, RR duration and RR intensity (number of efforts/piglet) were twice as great in the Ar piglets relative to the CO_2 piglet, suggesting that Ar causes more distress in pigs than CO_2.

We hypothesized that the incidence of nasal and oral discharge would be increased in the CO_2 treatment due to irritation of mucus membranes. The trend observed with increased oral discharge was consistent with this hypothesis, but the increase in nasal discharge in Ar was unexpected. This may indicate that the increase in nasal discharge was due to a stress response and resultant increases in tidal volume rather than irritation of the mucus membranes by acidity of CO_2.

Latency to LP was more than doubled with Ar. This result is in sharp contrast to Raj (1999), who found latency to LP was not affected by gas type when finisher pigs were exposed to 90% Ar, 80 to 90% CO_2, or 30:60 CO_2:Ar mixture. It is important to note that Ar is a noble gas with no known effect on the body, and likely causes unconsciousness through hypoxia. Therefore, it is surprising that 90% Ar was capable of producing LP in less than 20 s, as observed by Raj (1999), even in a prefill environment. Examining a variety of O_2:CO_2 gas mixtures (CO_2 30-70%; O_2 10-30%), Gerritzen et al., (2008) found RP times similar to those observed in this study (6 to 67 s; depending on gas concentrations). Studies designed to investigate processing facility conditions also found results consistent with our study. Raj (1999) found that when pigs were exposed to 90% Ar or a 30:90 CO_2:Ar mixture for 3 min, many pigs would regain consciousness (signs of sensibility) after 45 s.

3.5.1 Anaesthesia depth and effects on performance

In this study, a majority of piglets displayed a corneal reflex. The corneal reflex may persist into deep planes of anaesthesia, with surgery performed when it is present (Klide, 1996). It is utilized commonly to assess unconsciousness following gas exposure and prior to exsanguination during processing, since it indicates a very deep plane of anaesthesia (Klide, 1996). Nearly 30% of the piglets in both treatments had no corneal reflex, indicating this may be a good method for euthanasia, but a safe level of anaesthesia would be difficult to produce using methods from this study. Pupil constriction/dilation can be a measure of plane of anaesthesia along with a corresponding reaction to light, but is dependent on the drug utilized (Klide, 1996). In general, a dilated pupil indicates a deeper anaesthesia (Klide, 1996). To our knowledge, pupil dilation/constriction has not yet been described in pigs under hypercapnic or hypoxic induced anaesthesia. The results from this study seem to indicate hypercapnic anaesthesia produces pupil dilation with no response to light. It is likely that hypoxia also produces a lack of response to light and is an indicator of depth of anaesthesia. With more than twice as many Ar piglets displaying a response to the light, this study would indicate piglets exposed to hypercapnic conditions for 30 s after LP are in a deeper depth of anaesthesia relative to piglets exposed to hypoxic conditions for 30 s after LP. Using responses to a leg or nose prick, more than 20% of piglets, regardless of treatment, were not in a sufficient anesthetic plane for processing.

The procedures utilized in this study appear safe for piglets, resulting in no deaths or changes to measured performance parameters. However, the sample size of this study was chosen to establish differences in behavioural observations and may not have been large enough to detect differences in performance.

3.5.2 Vocalizations

Piglets reliably produce stress vocalizations during painful events (Puppe et al., 2005; Sutherland et al., 2011; Weary et al., 1998; Xin et al., 1989). The STREMODO program was designed for and shown to reliably detect stress vocalizations from piglets (Schön et al., 2004; Schon et al., 2001), while not detecting regular vocalizations. This program allows for an objective determination of distress. In this study, distress calls were only detected in the Ar treatment. To verify this was not due to the CO_2 piglets being unable to vocalize due to pain or dyspnea, a subset of data was examined for total vocalizations. This subset of data showed all piglet pairs were vocalizing during the procedure at a high rate, and all had the ability to produce distress calls.

3.5.3 Conclusion

Results from this study do not support the use of on-farm gas euthanasia equipment for the purpose of inducing anaesthesia in piglets as a potential means to mitigate the distress caused by painful husbandry procedures. Gradually exposing piglets to CO_2 or Ar (21 ACH) in a specially designed chamber does not produce reliable anaesthesia. When gas is applied at a gradual fill rate, both CO_2 and Ar elicited a distress response in piglets during induction. Furthermore, Ar produced a greater level of distress and as such should not be utilized in piglets of this age for anaesthesia or euthanasia.

Acknowledgments and grant info

This project was supported by Agriculture and Food Research Initiative competitive grant no. 2012-67021-19363 from the USDA National Institute of Food and Agriculture.

The authors thank Stacie Gould, Alex Folkmann, Natalie White, Lauren Larsen, Kara Fiedler, Dr. Craig Rowles, Dan Sander and the staff of Elite Pork Partnership.

3.6 References

Anil, M.H., 1991. Studies on the return of physical reflexes in pigs following electrical stunning. Meat Science 30, 13–21.

AVMA, 2013. AVMA Guidelines for Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association: Schaumber, IL, USA. http://www.avma.org/issues/animal_welfare/ euthanasia.pdf. Accessed on 06 June 2013.

Blackshaw, J.K., Swain, A.J., Blackshaw, A.W., Thomas, F.J.M., Gillies, K.J., 1997. The development of playful behaviour in piglets from birth to weaning in three farrowing environments. Applied Animal Behaviour Science 55, 37–49.

Danneman, P.J., Stein, S., Walshaw, S.O., 1997. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Lab. Anim. Sci. 47, 376–385.

Dodman, N.H., 1976. Observations on the use of the Wernberg dip-lift carbon dioxide apparatus for pre-slaughter anaesthesia of pigs. Br. Vet. J. 133, 71–80.

EFSA, 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal 45, 1–29.

Gerritzen, M.A., Kluivers-Poodt, M., Reimert, H.G.M., Hindle, V., Lambooij, E., 2008. Castration of piglets under CO2-gas anaesthesia. animal 2, 1666–1673.

Grandin, T., 2010. Recommended Animal Handling Guidelines & Audit Guide: a Systematic Approach to Animal Welfare. AMI Foundation.

Gregory, N., Mohan-Raj, A., Audsey, A., Daly, C., 1990. Effects of CO2 on man. The use of CO2 for stunning of slaughter pigs 7–9.

Johnson, A.K., Sadler, L.J., Gesing, L.M., Feuerbach, C., Hill, H., Faga, M., Bailey, R., Stalder, K.J., Ritter, M.J., 2010. Effects of Facility System Design on the Stress Responses and Market Losses of Market Weight Pigs During Loading and Unloading. Prof. Anim. Sci. 26, 9–17.

Klide, A.M., 1996. Lumb & Jones Veterinary Anesthesia. Anesthesia & Analgesia 83.

Liotti, M., Brannan, S., Egan, G., Shade, R., Madden, L., Abplanalp, B., Robillard, R., Lancaster, J., Zamarripa, F.E., Fox, P.T., Denton, D., 2001. Brain responses associated with consciousness of breathlessness (air hunger). PNAS 98, 2035–2040.

Marchant-Forde, J.N., Lay, D.C., McMunn, K.A., Cheng, H.W., Pajor, E.A., Marchant-Forde, R.M., 2009. Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered separately. J ANIM SCI 87, 1479–1492.

McFarlane, J.M., Curtis, S.E., Shanks, R.D., Carmer, S.G., 1989. Multiple Concurrent Stressors in Chicks. 1. Effect on Weight Gain, Feed Intake, and Behavior. Poult Sci 68, 501–509.

Mühlbauer, I., Zöls, S., Otten, W., Palzer, A., Ritzmann, M., Heinritzi, K., 2010. Examination of CO2 gas anesthesia during piglet castration. Presented at the Proceedings of the 21st IPVS Congress.

National Pork Board, 2009. On-farm euthanasia of swine: recommendations for the producer (No. 04259-01/09). National Pork Board.

Newberry, R.C., Wood-Gush, D.G.M., Hall, J.W., 1988. Playful behaviour of piglets. Behavioural Processes 17, 205–216.

Niel, L., Kirkden, R.D., Weary, D.M., 2008. Effects of novelty on rats' responses to CO2 exposure. Applied Animal Behaviour Science 111, 183–194.

Puppe, B., Schön, P.C., Tuchscherer, A., Manteuffel, G., 2005. Castration-induced vocalisation in domestic piglets, Sus scrofa: Complex and specific alterations of the vocal quality. Applied Animal Behaviour Science 95, 67–78.

Raj, A.B.M., 1999. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. Veterinary Record 144, 165–168.

Raj, A.B.M., Gregory, N.G., 1995. Welfare Implications of the Gas Stunning of Pigs 1. Determination of Aversion to the Initial Inhalation of Carbon Dioxide or Argon. Animal Welfare 4, 273–280.

Raj, A.B.M., Gregory, N.G., 1996. Welfare Implications of the Gas Stunning of Pigs 2. Stress of Induction of Anaesthesia. Anim. Welf. 5, 71–78.

Rault, J.-L., Lay, D.C., 2011. Nitrous oxide by itself is insufficient to relieve pain due to castration in piglets. J ANIM SCI 89, 3318–3325.

Rault, J.-L., McMunn, K.A., Marchant-Forde, J.N., Lay, D.C., 2013. Gas alternatives to carbon dioxide for euthanasia: A piglet perspective. Journal of Animal Science 91, 1874–1883.

Rodríguez, P., Dalmau, A., Ruiz-de-la-Torre, J., Manteca, X., Jensen, E., Rodriguez, B., Litvan, H., Velarde, A., 2008. Assessment of unconsciousness during carbon dioxide stunning in pigs. Anim. Welf. 17, 341–349.

Sadler L., Hagen C., Wang C., Millman S., 2011. Comparison of CO_2 versus mixed CO_2:argon gas at different flow rates using the SmartBox euthanasia device as a humane and effective method of piglet euthanasia. J. Anim. Sci. 89(E2): 51

Sandström, V., 2009. Development of a monitoring system for the assessment of cattle welfare in abattoirs. Sveriges lantbruksuniversitet.

Schon, P.-C., Puppe, B., Manteuffel, G., 2001. Linear prediction coding analysis and self-organizing feature map as tools to classify stress calls of domestic pigs (Sus scrofa). The Journal of the Acoustical Society of America 110, 1425–1431.

Schön, P.C., Puppe, B., Manteuffel, G., 2004. Automated recording of stress vocalisations as a tool to document impaired welfare in pigs. Animal Welfare 13, 105–110.

Sutherland, M., 2011. The use of different gases and gas combinations to humanely euthanize young suckling pigs (Research Report No. NPB #09-199), Animal Welfare. National Pork Board.

Sutherland, M., Davis, B., McGlone, J., 2011. The effect of local or general anesthesia on the physiology and behavior of tail docked pigs. Animal 5, 1237.

Sutherland, M.A., Davis, B.L., McGlone, J.J., 2011. The effect of local or general anesthesia on the physiology and behavior of tail docked pigs. Animal 5, 1237–1246.

Velarde, A., Cruz, J., Gispert, M., Carrión, D., Torre, R. de la J.L., Diestre, A., Manteca, X., 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. Animal Welfare 16, 513–522.

Weary, D.M., Braithwaite, L.A., Fraser, D., 1998. Vocal response to pain in piglets. Applied Animal Behaviour Science 56, 161–172.

Xin, H., DeShazer, J.A., Leger, D.W., 1998. Pig vocalizations under selected husbandry practices. Transactions of the ASAE 32:6, 2181-2184.

Table 3.1 Ethogram used for behavioural observations during induction of and recovery from anaesthesia*

Postures (state)	**Definition** (expressed as latency, frequency and duration, as appropriate)
Standing/Locomotion (SL), duration	Maintaining an upright and stationary body position by supporting the body weight on the feet, with legs extended or movement derived from the repulsive force from the action of the legs [1]
Sitting, duration	A body position in which the posterior of the body trunk is in contact with the ground, sides of the chamber or the other piglet and supports most of the body weight [2]
Ataxic movement, latency and duration	Piglet is moving in a seemingly uncoordinated fashion; lack of muscle coordination during voluntary movements. This includes postures of standing, sitting and non-normal postures such as dropped down on one knee [3]
Righting response (RR), duration	Piglet is making an attempt to maintain either a standing or lying sternal posture from a standing, sitting or lying position but is not successful in maintaining the position. The event was defined as each time effort was made with subsequent muscle relaxation.

Table 3.1 (continued)

Behaviours (states)	Definition (expressed as latency, frequency and duration, as appropriate)
Oral Nasal (ON), duration	Rubbing, licking, biting, touching the mouth, snout or face to one of four modifiers: peat moss, item (walls or cage), Kong or other piglet[8]. For analysis, Licking and Chewing was combined as an additional modifier
Licking and chewing (LC), duration	Piglet is going through motions of licking and chewing, similar to ON, but not interacting with the other piglet or an item
Escape attempt (EA), number	Piglet is raising their forelegs on the side of the wall of the chamber or pushing quickly and forcefully with their head or nose on the lid or side of the box [4,5]
Open mouth breathing (OMB), duration	Panting; upper and lower jaw being held open with the top lip pulled back, exposing gums or teeth and pronounced inhalation and exhalation observed at the flanks [4,6]
Gasping (GASP), duration	Rhythmic breaths characterized by prominent and deep thoracic movements, with long latency between; may involve stretching of the neck; often occurs right after loss of posture [4,8]

Table 3.1 (continued)

Events	Definition
Loss of posture (LP)	Piglet is slumped down, making no attempt to right itself; may follow a period of attempts to maintain posture[3,4]
Righting response effort	See righting response under posture
Regain posture (RP)	Following induction of anaesthesia, piglet is standing on all four legs
Latency to recovery (LR)	Piglet is fully upright, possibly displaying exploring behaviour, with no inclination towards ataxic movement

*Behaviours of each piglet were scored continuously via video for latency, duration and number of occurrences. Each piglet was scored for a mutually exclusive posture and complementary mutually exclusive behaviour, along with event behaviour that occurred.

[1]Adapted from Hurnik et al., 1985
[2] Adapted from Johnson et al., 2010
[3] Adapted from Blood et al., 2007
[4] Adapted from Velarde et al., 2007
[5] Adapted from Raj & Gregory, 1996
[6] Adapted from Johnson et al. 2010[6]
[7]Adapted from N. H. Dodman. 1976
[8] Adapted from Rodriquez et al. 2008

Table 3.2 Latency (L; s), % of piglets displaying (%), duration (D, s), number of efforts (#), or % of time prior to loss of posture (%T) displayed by piglets exposed to two gas treatments until 30 seconds after loss of posture or a control exposed for 14 minutes

				Range	
	Means	**SE**	**%**	**High**	**Low**
Loss of posture (L)					
ODOR	-	-	-	-	-
CO_2	100	8	100	67	157
Argon	244	8	100	222	292
Regain posture (L)					
ODOR	-	-	-	-	-
CO_2	74	37	100	2	501
Argon	172	37	100	0	608
Full recovery (L)					
ODOR	-	-	-		
CO_2	401	62	100	117	501
Argon	597	64	100	240	1056
Escape attempts (%)					
ODOR	-	-	18	-	-
CO_2	-	-	0	-	-
Argon	-	-	64	-	-

Table 3.2 (continued)

	Means	SE	%	Range High	Range Low
Open mouth breathing (D)					
ODOR	-	-	0	-	-
CO_2	36	7	100	18	52
Argon	48	7	100	4	143
Ataxia (D)					
ODOR	-	-	0	-	-
CO_2	16	5	100	4	68
Argon	35	5	100	4	104
Righting response (D)					
ODOR	-	-	0	-	-
CO_2	11	4	68	0	53
Argon	29	4	90	0	63
Righting response (#)					
ODOR	-	-	0	-	-
CO_2	4	2	68	0	8
Argon	15	2	90	0	22

Table 3.2 (continued)

	Means
Oral/nasal- all (%T)	
ODOR	17
CO_2	3
Argon	6
Oral/nasal- licking and chewing (%T)	
ODOR	7
CO_2	3
Argon	< 1
Oral/nasal- peat moss (%T)	
ODOR	5
CO_2	0
Argon	2
Oral/Nasal- external item (%T)	
ODOR	3
CO_2	1
Argon	< 1
Oral/Nasal- Kong (%T)	
ODOR	2
CO_2	< 1
Argon	< 1

Table 3.2 (continued)	**Means**
Oral/Nasal-other piglet (%T)	
ODOR	< 1
CO_2	< 1
Argon	< 1
Nasal discharge (%)	
ODOR	14
CO_2	27
Argon	23
Oral discharge (%)	
ODOR	5
CO_2	10
Argon	5
Standing and locomotion (s)	
ODOR	467
CO_2	152
Argon	67
Sitting (s)	
ODOR	21
CO_2	8
Argon	9

Figure 3.1 Picture of piglets in euthanasia box during habitation

-

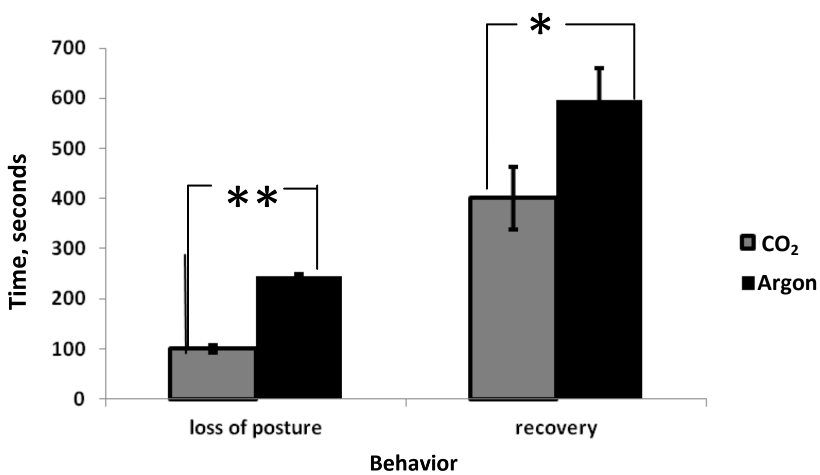

Figure 3.2 Least square means for latency to loss of posture and recovery during the induction and recovery phase, respectively, by gas type within phase (n=22 piglets/gas trt). * P = 0.03. ** P < 0.001

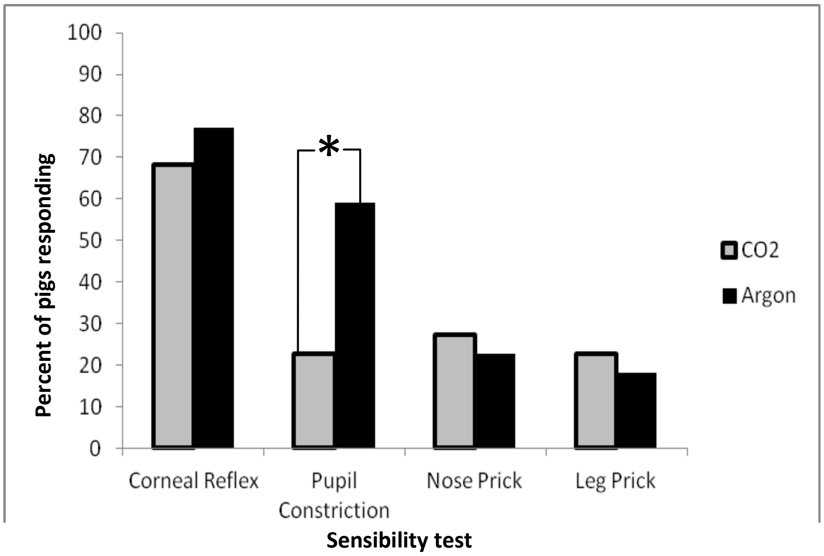

Figure 3.3 Percentage of piglets displaying a response to sensibility tests by gas type within test (n=22 piglets/gas trt) . Tests were performed 30 sec following loss of posture in the respective gas

* P = 0.02

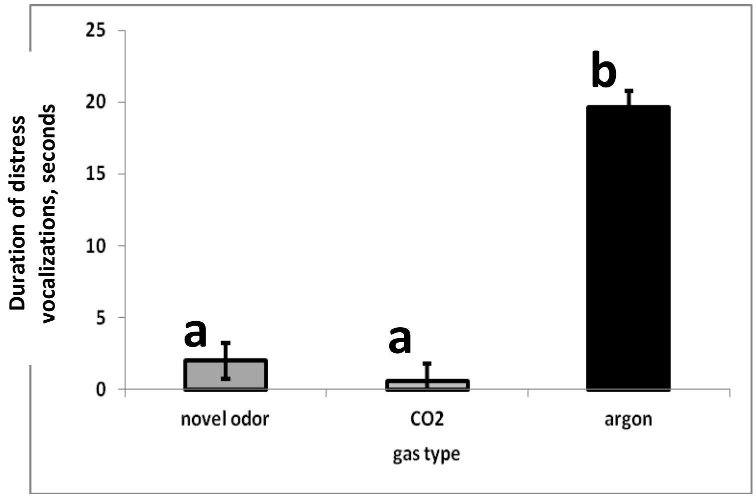

Figure 3.4 Least square means for distress calls made by piglets within gas type during exposure to the respective gas (n=11 piglet pairs/trt)

Superscripts indicate differences at P < 0.001

CHAPTER 4 ARE SEVERELY DEPRESSED SUCKLING PIGS RESISTANT TO GAS EUTHANASIA?

A paper submitted to the journal Animal Welfare

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4.1 Abstract

Severely depressed pigs exhibit differences in a number of important parameters that may affect gas euthanasia including decreased respiration rate and tidal volume. Hence the objectives of this study were to assess the efficacy and animal welfare implications of gas euthanasia of suckling pigs with varied disease severity (severely depressed [DP] vs. other [OT]). A 2 x 2 factorial design was utilized with two gas types (CO_2; argon [Ar]) and two flow rates (G=35% chamber volume exchange per min [CVE/min]; P=prefill + 20% CVE/min). Sixty-two pigs were enrolled and tested as DP/OT pairs in each gas treatment combination. Pigs identified for euthanasia were assigned a subjective depression score (0=normal to 3=severely depressed). Pigs scored 3 and ≤ 1 were categorized as DP and OT, respectively. Significantly lower respiration, rectal temperature, pulse and weight were observed for the DP pigs relative to OT. Pigs were assessed for behavioural indicators of efficacy and welfare. No differences were observed between DP and OT when using P-CO_2 or G-CO_2. However in P-Ar, DP relative to OT had greater latency to loss of consciousness (212±22 vs. 77±22, s), decreased latency to last limb movement (511±72 vs. 816±72, s), greater duration open mouth breathing (151±21 vs. 69±21, s), decreased duration ataxia (101±42 vs. 188 ±42, s) and righting response (27±11 vs. 63±11, s). The G-Ar treatment was dropped due to ethical concerns associated with prolonged induction. In conclusion, depression score did not affect pig responses to euthanasia with CO_2 gas, but did affect responses to Ar. Furthermore, Ar was associated with a prolonged euthanasia process, including frequencies and durations of distress behaviours.

Keywords: animal welfare, argon, carbon dioxide, euthanasia, moribund, swine

4.2 Introduction

Most swine producers and veterinarians agree that euthanasia is the best choice for low viability pigs, especially when there is suffering due to injury or illness. Low viability suckling pigs identified for euthanasia typically consist of two broad categories: unthrifty, ill and depressed pigs vs. injured or small but alert pigs. Carbon dioxide has been identified as an acceptable inhalant method for euthanasia of pigs because it is a rapid depressant with established analgesic and anaesthetic properties (AVMA 2013).

Carbon dioxide is commonly used for stunning market weight pigs at slaughter, and remains the most commonly implemented gas for on-farm euthanasia of suckling and nursery age pigs in the USA (Daniels 2010). The American Veterinary Medical Association Panel on Euthanasia notes, "… parameters of the technique need to be optimized and published to ensure consistency and repeatability. In particular, the needs of piglets with low tidal volume must be explored" (AVMA 2013 p61). Additionally, anecdotal reports from stockpeople suggest efficacy is decreased when euthanizing the moribund (severely depressed) pig relative to a more robust and alert pig, and this may account for failed euthanasia attempts in which additional exposure to the gas or a secondary euthanasia method is required.

Severely depressed pigs differ from robust pigs in several physiological parameters that may be important for gas euthanasia. Several causal factors could contribute to creating the depressed state, including disease, injury and underdevelopment. These pigs tend to have low respiration rates and tidal volumes. This would lead to decreased total volume exchange rates of gases into and out of the body (Guyton & Hall 2010 Ch 37). Pigs with low birth weights (< 0.8 kg) are often considered underdeveloped and more than 60% do not survive (Straw et al 1999). There are a number of factors following birth that may contribute to decreased survival, including greater latency to udder contact, greater latency to colostral intake and a greater than average decrease in temperature post-birth. These low birth weight pigs are often in a state of severe respiratory acidosis (Straw et al 1999). Furthermore, severely depressed pigs are likely hypoglycaemic, contributing to a variety of symptoms observed including low temperature, convulsions and comatose state (Straw et al 1999).

Carbon dioxide is mildly acidic, causing irritation to the mucus membranes in humans (Danneman et al 1997), leading to questions regarding the humaneness of this gas for pig euthanasia (Wright et al 2009). Argon has been proposed as an alternative inhalant in slaughter facilities for stunning and killing pigs to improve animal welfare (Raj 1999). Argon is a noble gas, and as such is likely unreactive throughout the physiological systems (Mann et al 1997). Hence, loss of consciousness and death are produced through hypoxia, creating the physiological state of hypocapnic anoxia (Raj 1999). According to the AVMA (2013), argon is considered conditionally acceptable as a euthanasia inhalant for swine. The European Food Safety Authority (EFSA 2004) states that although gas euthanasia requires sophisticated equipment, this technology has been identified as having high potential for humane stunning and killing of animals. Furthermore, EFSA recommends the use of noble gases such as argon that induce unconsciousness through hypoxia rather than hypercapnia. Controlled atmospheric killing with argon gas is used in some commercial broiler processing facilities, and since 2002, animal protection organizations such as People for the Ethical Treatment of Animals (PETA 2002) and the Humane Society of the United States (HSUS 2009) have encouraged retailers to source their chicken meat from companies using this technology. Both AVMA and EFSA acknowledge the need for further research to identify best management practices for preferred gas mixtures and methods of application. Since the physiologic effects of these gases differ, it is important that both carbon dioxide and argon be examined in relation to the severely depressed pig.

Euthanasia is comprised of two stages: (1) induction of unconsciousness (insensibility) and (2) death. It is the induction phase that is critical to the welfare of the pigs. Duration of the entire process, including death, is important to ensure practical implementation. Pain and distress are affective states, and hence can only be measured indirectly in humans and animals. Humans report feeling pain and distress when exposed to carbon dioxide (Gregory et al 1990). Distress associated with carbon dioxide has been assessed in pigs using behavioural responses, such as escape attempts, hyperventilation, sneezing, coughing, head shaking and vocalizations (Dodman 1977; Raj & Gregory 1996; Velarde et al 2007; Rodriguez et al 2008). Although differences in behaviour are observed during induction of insensibility, it is difficult to ascertain whether these are accurate indicators of distress since these behaviours may coincide with the induction process or when insensibility has begun. Raj and colleagues (1997) found loss of somatosensory evoked potentials (SEP), indicative of brain responsiveness, occurred within 21 sec of exposure to 90% carbon dioxide and hence, signs of moderate to severe respiratory distress (coughing, open mouth breathing, squealing) occurring during this period are likely associated with conscious awareness, in the grower pig (40 kg). Similarly, on grower pigs (25-35 kg) in an experiment using middle latency auditory evoked potentials, Rodriguez and colleagues (2008) concluded that loss of consciousness occurred on average 60 sec after exposure to 90% carbon dioxide and prior excitatory movements (lateral head movement, sneezing, vocalization) were conscious movements associated with aversion.

In contrast with these recommendations, our previous research suggests decreased welfare during induction to loss of consciousness when pigs are stunned with 100% argon relative to 100% carbon dioxide applied at 35% CVE/min since argon was associated with increased latency to loss of posture, increased duration of open mouth breathing and distress calls (Chapter 3). However, efficacy of 100% argon at this flow rate for euthanasia vs. stunning has not been examined. Rault and colleagues (2013) examined argon as the first step in two-phase gas euthanasia of suckling pigs, but efficacy of argon gas as a single gas method for pig euthanasia has not been examined.

The primary objective of this research was to examine the efficacy and welfare implications when severely depressed pigs are euthanized using gas techniques. The study design of this experiment also allows a secondary objective to compare 100% carbon dioxide and 100% argon in gradual and prefill conditions. Our data will provide knowledge about best management practices for carbon dioxide or argon gas euthanasia for this vulnerable population.

4.3 Materials and methods

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee and the Environmental Health and Safety Office.

4.3.1 Experimental design

Pigs identified for euthanasia were allocated to one of two disease status categories, DP=severely depressed and OT=other. Effects of each disease status were assessed in a 2 x 2 factorial design with two gas types (CO_2=100% carbon dioxide; Ar=100% argon) and two flow rates (G = gradual fill at 35% chamber volume exchange per min [CVE/min]; P=prefill + 20% CVE/min). The experiment was designed to utilize eleven DP/OT pairs for each gas treatment combination. This design would utilize 88 pigs (2 disease statuses x 2 gases x 2 flow rates x 11 reps/gas treatment). Gas treatments were run in a randomized sequence. Previous work in our lab has indicated reduced welfare and efficacy with the implementation of a 20% CVE/min or 50:50 CO_2:Ar gas mixture relative to faster flow rates and 100% CO_2 (unpublished). Consequently, gradual flow rate in this experiment utilized a 35% CVE/min. On farm, prefill is currently the most commonly implemented flow rate, and thus it was of high priority to examine its efficacy.

4.3.2 Study animals and enrolment criteria

Pigs were sourced and housed from a commercial sow farm, and genetics were a Landrace x Yorkshire cross x Duroc sire line. Pigs were eligible for enrolment if they were less than 21 d of age, and were identified by farm staff as low viability or injured and in need of euthanasia. These pigs were placed in a testing room by farm staff and contained in a cart with wood shavings and a heat lamp. Pigs were assigned a subjective depression score by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims (FDA [U.S Department of Human and Health Services Food and Drug Administration] 2007). The depression score ranged from zero to three (0 = Normal –Alert, active, normal appetite, well-hydrated, normal coat; 1 = Mild – moves slower than normal, slightly rough coat, may appear lethargic but upon stimulation appears normal; 2 = Moderate – inactive, may be recumbent but is able to stand, gaunt, may be dehydrated; 3 = Severe – down or reluctant to get up, gauntness evident, dehydrated). Based on this four-point scale, pigs were placed into a disease category (3 = DP; 0 or 1 = OT); pigs that were scored a 2 were excluded from this study. Individual pigs were then randomly placed into DP/OT pairs. Pig pairs were marked with an animal safe marker (LA-CO Ind.; Elk Grove, IL).

4.3.3 Euthanasia equipment

Gas was administered to the pigs via a modified Euthanex AgPro™ system. This gas delivery apparatus was designed by Euthanex Corporation (Palmer, PA, USA), a manufacturer of gas delivery systems for rodents and small animals. To facilitate behavioural observation, the box was constructed of clear plastic on the top and front panels. The top panel was hinged for placing pigs into the box, with an air-tight foam seal. The remaining four panels were constructed of opaque plastic. The inside dimensions of the box were 43 cm wide, x 60 cm long, x 30 cm high. The floor was fitted with a rubber mat (Rubber floor mats, Kraco, Enterprises, LLC, Compton, CA, USA) and a layer of wood sawdust (~ 2 cm in depth; TLC Premium Horse Bedding, Centerville, AR, USA) to aid in traction and comfort for the pigs.

The box had two 0.64 cm inlet valves located at 12.70 cm (CO_2) and 22.86 cm (Ar) from the side and 3.81 cm from the top. The gas flowed through rubber hoses that were 3.25 m length and 0.64 cm diameter, prior to entering the box. A 0.95 cm outlet valve was located on the opposite panel from the inlet valves, 30.48 cm from the side and 6.35 cm from top, and was vented outdoors for worker safety. Constant and precise gas flow was provided by compressed gas cylinders equipped with compressed gas regulators and meters (Western Enterprises, Westlake, OH, USA). The CO_2 gas was industrial grade (99% pure). The Ar had a guaranteed analysis of 99.99% pure. Prior to each treatment, sawdust was removed from the chamber by a vacuum (Shop Vac 10 Gallon Ultra Pro Vacuum, 185 CFM), a clean rubber mat was placed in the box and fresh sawdust was provided. The vacuum was also utilized to remove gas traces, pulling air from the bottom of box for a minimum of 3 min.

4.3.4 Environmental conditions

A HOBO data logger (U23-001, Onset Computer Corporation, Cape Cod, MS, USA) was used to record temperature (°C) and relative humidity (%) within the chamber. The data logger was set to record every 10 s. Oxygen levels were collected every second at pig level with an oxygen sensor (TR25OZ, CO2Meter.com, Ormond Beach, FL, USA) attached to a HOBO data logger (U12, Onset Computer Corporation, Cape Cod, MS, USA). Data was exported into Microsoft Office Excel (version 2007, Redmond, WA, USA). A CO_2 meter (CO2IR-WR 100%, CO2Meter.com, Ormond Beach, FL, USA) monitored levels every 1.25 s. However, due to technical difficulties these data are not included.

4.3.5 Euthanasia procedure, confirmation of insensibility and death

On the testing day, vital signs (respiration, rectal temperature, pulse and weight) were collected for all pigs prior to placement in the box. Pigs were euthanized within 4 h of being identified by farm staff for euthanasia. The testing room provided isolation, minimizing noise and distractions. Pig pairs (DP/OT) were placed into the box standing, and gas was immediately started/restarted (gradual/prefill) and continued to run until the pigs were confirmed dead. One of two observers, randomly assigned to a pig, performed all signs of sensibility test and observation. Two min following respiratory arrest, pigs were removed individually from the box and checked for signs of insensibility (Whelan & Flecknell 1992; Kissin 2000; National Pork Board 2009; Grandin 2010). Three insensibility tests were conducted: (1) corneal reflex response, in which the eye was touched with the tip of a finger for absence of an eye blink or withdrawal response, (2) pupillary reflex, in which a light-beam (Mini MAGLite, Mag Instrument, Inc., Ontario, CA) was shone into the eye for absence of pupil constriction and (3) nose prick, in which a 20 gauge needle was touched to the snout distal to the rostral bone for absence of a withdrawal response. After insensibility was confirmed, cardiac arrest was confirmed by auscultation. If the pig showed signs of sensibility or cardiac activity, it was placed back into the box for an additional min of gas exposure. This process was repeated until confirmation of cardiac arrest (CA) to establish duration of dwell time necessary for death.

For ethical and practical reasons, the protocol was terminated if pigs displayed signs of consciousness (retained posture, making righting attempts, vocalizations, or had not transitioned to gasping) after 10 min of gas exposure. Gasping, an indicator in disruption of the ventral respiratory group was defined as rhythmic breaths characterized by very prominent and deep thoracic movements. Additionally, a ceiling value of 15 min was used for death (cardiac arrest) after loss of consciousness. For pigs that did not achieve these measures in the designated time, manual blunt force trauma (National Pork Board 2009) was applied as a secondary euthanasia method.

4.3.5.1 Modification to original study design due to ethical concerns

In this study, 60% of the pigs in the Ar treatments (16 total pigs) required a secondary euthanasia method. Of these pigs, 73% displayed signs of sensibility after 10 min. Due to ethical concerns regarding this high proportion of pigs requiring a secondary euthanasia step, G-Ar was terminated after two repetitions (two pig pairs) and P-Ar was dropped after six repetitions (six pig pairs). Thus G-Ar (n=2) was dropped from the statistical analysis, and a total of 62 pigs were enrolled in the study. In the first run of P-Ar, the originally designed protocol was followed, using 20% CVE/min following pig placement in the box, however in an effort to increases success for all other subsequent

Ar runs, gas was applied at 50% CVE/min. This was done to ensure low oxygen levels were reestablished, after placement of the pigs in the box, as quickly as possible.

4.3.6 Behavioural observations

Behavioural data was collected directly and via video recording. For direct observation, each observer sat approximately 1.5 m from the box and recorded behavioural indicators of distress, physiological responses and insensibility (Table 4.1). Video recordings were recorded utilizing a Noldus Portable Lab (Noldus Information Technology, Wageningen, NL). Two colour Panasonic cameras (WV-CP484, Kadoma, Japan) were fed into a multiplexer, allowing the image to be recorded onto a PC using HandiAvi (v4.3, Anderson's AZcendant Software, Tempe, AZ, USA) at 30 frames/s. Behavioural data from video was collected by a single trained observer, blind to disease status and treatments, using Observer® software (v10.1.548, Noldus Information Technology, Wageningen, NL). Data were collected for each individual pig for behavioural and physiological indicators of distress and efficacy of the euthanasia process (Table 4.1). Latencies for all behaviours were determined from the point when each pig was placed into the box.

4.3.7 Assessment of lungs

Immediately upon confirmation of death, necropsy was performed. Lungs were removed and scored by a single technician, blinded to disease status, for total macroscopic lung lesions as described by Opriessnig and colleagues (2004). The scoring system is based on gross visible damage and the approximate volume each lung lobe contributes to the whole lung: the right cranial lobe, right middle lobe, cranial part of the left cranial lobe and caudal part of the left cranial lobe contribute 10% each to total lung volume, the accessory lobe contributes 5% and the right and left caudal lobes contribute 27.5% each. Each lobe was scored as follows: 0% = no gross damage, 50% = some damage, with <50% of the lobe grossly affected, 100% = >50% of the lobe grossly affected. These lobe scores were aggregated for a total lung score, ranging from 0-100% affected.

Samples of the lung tissue were collected, with diseased tissue sampled when grossly visible. If no gross lesions were visible, two samples were collected from each of the left and right middle lobes. Samples were collected and fixed in 10% buffered formalin until scored. Histological examination was performed by pathologists at the Iowa State University Veterinary Diagnostic Laboratory (VDL), who were blind to disease status and gas treatments. Sections of formalin-fixed lung were embedded in paraffin, processed per the VDL protocol and stained with hematoxylin and eosin stains. A pathologist examined lung sections for evidence of antemortem haemorrhage or atelectasis and also characterized the lesions of pneumonia as nonsuppurative interstitial pneumonia or suppurative bronchopneumonia. Pleuritis, when present, was also noted.

4.3.8 Statistical Analysis

Behaviours were quantified as latency, duration, percent of pigs displaying or number of occurrences as indicated for the parameter. Data were analyzed using linear mixed models fitted with the GLIMMIX procedure (duration and frequency; SAS Inst. Inc., Cary, NC) or with a Cox proportional hazard model fitted with the PHREG procedure (latency) of SAS. Individual pig was the measurement unit for depression score whereas pig pair served as the experimental unit for gas treatments. Least square means estimates for each treatment group and the corresponding standard errors (SE) are reported. The linear model included the fixed effect of disease status (DP/OT) and gas treatment (P-CO_2, G-CO_2, P-Ar) and all 2-way interactions. A random blocking effect of pig pair was included. The Kenward-Rogers method was utilized for determining the denominator degrees of freedom. Statistical significance was established at P-value $\leq$ 0.05.

4.4 Results

Latency to loss of consciousness [LC], last limb movement [LLM], respiratory arrest [RA] and cardiac arrest [CA] did not differ between DP and OT pigs in either P-CO_2 or G-CO_2 (Table 4.2). In P-Ar, latency to LC was almost three fold longer for DP relative to OT, but latency to LLM was longer for the OT pigs than DP. However, no differences were observed for RA or CA. Comparing gas treatments, independent of disease status latency to LC was shortest in P-CO_2 (P-CO_2 vs. G-CO_2, $P = 0.0219$; P-CO_2 vs. P-Ar, $P = 0.0015$), whereas G-CO_2 and P-Ar did not differ. Similarly, latency to LLM was shortest in P-CO_2 (P-CO_2 vs. G-CO_2, $P = 0.0052$; P-CO_2 vs. P-Ar, $P <$ 0.0001), and was twice as long in P-Ar relative to G-CO_2 ($P < 0.0001$). Latency to RA did not differ between P-CO_2 and G-CO_2, and both were shorter than P-Ar (P-CO_2 vs. P-

Ar, *P* = 0.0008; G-CO_2 vs. P-Ar, *P* = 0.0016). Latency to CA did not differ between treatments.

All pigs displayed open mouth breathing (OMB) and ataxia (Table 4.3). Duration of OMB did not differ between DP vs. OT pigs in either P-CO_2 or G-CO_2. In P-Ar, duration of OMB was twice as long for DP relative to OT. Similarly, duration of ataxia did not differ between DP vs. OT pigs in either P-CO_2 or G-CO_2, but in P-Ar, duration of ataxia was greater for the OT. Proportion of pigs displaying the righting response (RR) did not differ between DP vs. OT pigs (DP P-CO_2 = 55%, OT P-CO_2 = 27%; DP G-CO_2 = 64%, OT G-CO_2 = 64%; DP P-Ar = 83%, OT P-Ar = 100%). When it was observed, duration of RR did not differ between DP vs. OT pigs in either P-CO_2 or G-CO_2 (Table 4.3). In P-Ar, duration of RR was half as long in DP than OT. The number of efforts made during the righting response by a single pig ranged from zero to 19. Number of efforts did not differ between DP and OT pigs in P-CO_2 (mean number of events ± SE: DP = 3.4 ± 1.3, s; OT = 0.7 ± 1.3, s), or in G-CO_2 (DP = 3.4 ± 1.2, s; OT = 2.4 ± 1.2, s). In P-Ar, fewer RR efforts were observed for DP than OT (DP = 4.3 ± 1.7, s; OT = 11.8 ± 1.7, s; *P* = 0.0030).

Proportion of pigs displaying escape attempts did not differ between DP vs. OT pigs, and were rare. Escape was displayed by one OT pig in G-CO_2 and by three DP pigs and three OT pigs in P-Ar. All four pigs in G-Ar made escape attempts. Oral discharge was also a rare event, displayed by one OT pig in P-CO_2, one OT pig in G-CO_2 and two DP pigs in P-Ar. Ocular discharge was only displayed by two OT pigs in P-CO_2, and nasal discharge was displayed by one OT pig in P-CO_2 and one DP pig in P-Ar.

Vomiting and sneezing were not observed. Out of view was observed for less than 1% of the total observation for any individual pig.

Comparing gas treatments, differences were not observed between P-CO_2 and G-CO_2 for duration of ataxia, OMB or RR. Greater duration of ataxia was observed in P-Ar relative to P-CO_2 (P = 0.0436) but did not differ relative to G-CO_2 (P > 0.1). Similarly, greater duration of OMB was observed in P-Ar relative to both P-CO_2 (P = 0.0026) and G-CO_2 (P = 0.0129). P-Ar was also associated with greater proportion of pigs displaying RR, and greater duration (P = 0.0005 [P-CO_2 vs. P-Ar]; P = 0.0037 [G-CO_2 vs. P-Ar]) and number of RR efforts (P = 0.0002 [P-CO_2 vs. P-Ar]; P = 0.0009 [G-CO_2 vs. P-Ar]). Differences in RR were not observed between the CO_2 treatments.

At enrolment, pigs in the DP group had lower respiration rates, lower body temperatures and lower weights relative to OT (Table 4.4). Pulse, respiration and weight were examined as covariates for all measures of efficacy (LC, LLM, RA, CA) and found not to be different (P > 0.10). Light pigs (weighing < 0.8 kg) were examined relative to heavier pigs across all treatments, while controlling for disease status. Light pigs had shorter latencies to RA (-252 s, P = 0.0272) and CA (-306s, P = 0.0261). Differences were not observed by weight category for LC or LLM.

As assessed during necropsy, total lung damage did not differ between DP and OT pigs (Table 4.4). This gross assessment also indicated there was minimal lung damage in this population of pigs. Histological examination confirmed gross lesion scoring, indicating haemorrhages, atelectasis or lesions in all but 4 pigs identified as having gross lesions. Additionally, all pigs indentified grossly as having healthy lungs, lacked histological indicators of damage.

Over all days, the average temperature was 19.9 °C ranging from 16.4 to 22.8 °C. Relative humidity averaged 50.9% and ranged from 31.2 to 83.4%. Over all trials, initial O_2 levels were 2-8%, 21% and 5-7% for P-CO_2, G-CO_2 and P-Ar, respectively. The designed protocol required the lid to be opened for placement of pigs for P flow rates, and for removal of pigs to confirm insensibility. Both CO_2 and Ar are heavier than atmospheric air and it was expected modified gas concentrations would stay relatively constant. However, the process of checking for sensibility made maintaining continuous O_2 levels below 3% difficult. Although gas was flowing the entire time, opening the lid resulted in increased O_2 levels (< 7%) in both the CO_2 and Ar treatments. O_2 levels < 3% were regained in less than 45 s. For G-CO_2, pigs lost posture when O_2 levels were 8-16%. As P-CO_2 and P-Ar were prefilled by definition, pigs lost posture at < 7% O_2 levels.

4.5 Discussion

In the current study, pigs classified as DP or OT did not differ in behavioural and physiological responses associated with efficacy or distress when euthanized using P-CO_2 or G-CO_2. However, with a small sample size, euthanasia of DP pigs took longer and resulted in differences for distress indicators when utilizing P-Ar. Additionally, Ar resulted in behaviour and physiologic responses that raise concerns about efficacy and welfare for all pigs euthanized with Ar, regardless of flow rate or disease status.

The subjective categorization of pigs into DP and OT health categories, performed by behavioural scoring of depression, was validated since the subsequent vital parameters indicated the pigs classified as DP had a higher compromised health status relative to the OT pigs. Although lung lesions were not different, respiratory rates were lower in the DP pig, which could directly affect the exchange of gas through the respiratory system. Our objective for this study was to assess efficacy and distress of euthanasia procedures with the experiment designed to simulate on-farm conditions. Although more invasive methods to assess efficacy and distress, such as EEG or ECG monitoring, can provide robust data in the laboratory, they are not practical on farm and cannot be used in tandem with measurement of naturally occurring behaviours that are induced during gas euthanasia procedures. Behaviour was chosen as the primary outcome of interest for distress since behavioural observations provide more sensitive measures of the animal's experience than physiologic responses, particularly since euthanasia with inhalant gases can produce confounding effects on physiologic responses (Burkholder et al 2010).

4.5.1 Efficacy- disease status

We examined four different behavioural and physiological indicators of efficacy (LC, LLM, RA, CA). All four of these measures indicated disease status of the pig, as defined in this study, was not a predicting factor for determining efficiency in P-CO_2 or G-CO_2. The results of this study contradict the current AVMA euthanasia guidelines which note an incapacitated pig "...will not die as rapidly as larger more viable pigs" (AVMA 2013 p61).

The first assessed indicator of efficacy was LC. In our experiment, the transition from consciousness to unconsciousness was determined in part with LP, which has been identified in previous research as an indicator of loss of consciousness (Forslid 1987; Raj & Gregory 1996; Velarde et al 2007). When using P-Ar, the DP pigs took approximately two times longer than OT to reach LC, but were quicker to achieve LLM. The increased latency to LC may be explained by the different physiologic effects of CO_2 vs. Ar. The use of CO_2 creates a hypercapnic state and affects multiple body systems due to decrease in pH, including in the blood and interstitial fluid, which may create a similar euthanasia process for animals regardless of disease status. In part, this may be due to the possibility that the DP pig may be in an acidotic state at the time of euthanasia. In contrast, Ar creates a hypoxic state, and will make euthanasia more difficult for diseased pigs with compromised lung function. Further studies are necessary to completely understand the physiological mechanisms of this observation.

4.5.2 Efficacy- gas type

When examining gas treatments, G-CO_2 took 2.6 times longer to LC relative to P-CO_2 and P-Ar took 3.8 longer than P-CO_2. These results are in sharp contrast to Raj (1999), who found latency to LC was not affected by gas type when finisher pigs were exposed to 90% Ar or 80- 90% CO_2. Additionally, latencies to LC (15 and 18 s respectively) in Raj (1999) were considerably shorter than observed in our study. It is surprising that 90% Ar, with no known effect on the body, was capable of producing LC through hypoxia in less than 20 s. This time frame is almost four times less than that observed for OT pigs and 10 times less than that observed in DP pigs exposed to Ar in our study. The differences between studies may be due to age or weight of the pigs. Another factor may be the method of gas application; in the current experiment, opening the chamber lid to place pigs inside allowed some reintroduction of atmospheric air. When utilizing gas to stun prior to slaughter, pigs are lowered into a pit where maintaining a constant modified atmosphere is more feasible. The findings in the current study are similar to the pattern observed in rats; Ar prolonged the euthanasia process (Sharp et al 2006).

Identification of expected LLM during gas euthanasia is important for stockpeople to recognize when the process is not occurring within acceptable guidelines and intervention is necessary. It also serves as a general indicator of efficacy of the process. This study indicates that the use of G-CO_2 and P-Ar prolongs the euthanasia process by two and four times respectively relative to P-CO_2. Hence, this parameter provides further evidence that Ar decreases efficiency of gas euthanasia.

Once regular breathing controlled by the ventral respiratory group fails, which includes OMB (Guyton and Hall 2010 Ch 41), gasping is recruited (St John 2009). Respiratory arrest (cessation of gasping) represents the point at which gases can no longer be introduced into the pig's respiratory system. This point is critical to the euthanasia process because the pig will not recover without intervention. During gas euthanasia, gasping will become slower and shallow until breathing finally ceases. In this study, RA was the last movement by the pig that was observed, which is consistent with observations conducted using mink, perhaps indicating the death process during gas euthanasia is conserved across mammals (Hansen et al 1991), and warrants further study. Surprisingly, even though latencies to LC and LLM were longer in G-CO_2 relative to P-CO_2, differences were not observed in RA. As expected, latency was increased by the use of P-Ar relative to P-CO_2 and G-CO_2. These results are consistent with previous work in our lab, which indicated G-CO_2 and a 50:50 CO_2:Ar gas mixture were associated with increased latencies to RA relative to P-CO_2 by 70 and 170 s respectively (Sadler et al 2011a). The duration between breaths can be close to 1 min. Two DP pigs in the P-Ar treatment seemed to achieve RA for more than 1 min, displaying no signs of sensibility. However, these pigs recovered a regular gasping response when they were removed from the box and checked for signs of sensibility (in atmospheric air). Researchers in our lab have euthanized hundreds of pigs with CO_2 or CO_2:Ar gas mixtures under similar experimental conditions and this was the first incidence of this phenomenon in our experience. This anomaly highlights a potential difficulty and unpredictability with Ar and warrants further exploration. It is also possible that breathing would become so shallow that it may not be detectable through visible observation. As such, we would advise guidelines be structured around the latency to CA (in thus study: CO_2 ~ 15 min; Ar unknown due to some pigs reaching the censored value). CA was the last detectable point in our study and a clear indicator of death, representing an appropriate and safe point to stop monitoring the euthanasia process in practice. Differences were not observed between gas treatments for CA. This is surprising given that differences were observed between the gas treatments for all other measures of efficacy, though may be due in part to censoring the pigs.

4.5.3 Welfare implications- disease status

In this study, we separated the euthanasia process into two phases, conscious and unconscious. There is a transition phase prior to LC during which a number of behaviours are typically observed, including OMB, ataxia and RR. The level of awareness, hence capacity of animals to suffer, during this transition is unclear, and we chose a conservative estimate by including all measures up to the point of LC to ensure appropriate pig welfare. Behaviours chosen for welfare assessment included those associated with physiological distress, such as OMB (Forslid 1987; Martoft et al 2002; Mota-Rojas et al 2012), or psychological distress, such as escape attempts (Blackshaw et al., 1988; Velarde et al., 2007) and RR (AVMA 2013; Grandin 1998; Kohler et al 1999; National Pork Board 2009). When CO_2 was utilized at either flow rate, disease status did not affect any welfare parameters measured. However, in P-Ar, differences were observed in duration of OMB, duration of ataxia and righting duration and intensity (number of efforts/pig).

Open mouth breathing is a physiological reaction associated with dyspnea, and has been identified as an indicator of compromised welfare in the pig (Burki & Lee 2010; Velarde et al 2007). In P-Ar, duration of OMB was approximately three times greater for DP relative to OT. Durations of OMB in P-CO_2 and G-CO_2 were similar to those reported previously in our lab (12 ± 2, s and 24 ± 2, s for P and G, respectively; Sadler et al 2011b). Ataxia and RR duration and intensity (number of efforts/pig) were greater in the OT relative to the DP pigs, with duration of ataxia approximately five times greater in OT. The duration of RR was more than doubled in the OT pigs relative to the DP pigs. Ataxia is likely an indicator of impaired function of the cerebellum; however it is unclear how this correlates to impaired cortical function. If ataxia indicates that the pig is aware of its surroundings, but is unable to react in a coordinated manner, this could be considered distressing to the pig. In this study, we defined ataxia as a potential stressor for the pig, and hence, a shorter duration of this behaviour would correlate with improved welfare. The lack of a RR has been cited as a critical indicator that a pig is successfully rendered unconscious prior to slaughter (Grandin 2010; Sandström 2009) and is cited as an indicator of unconsciousness (Anil 1991; National Pork Board 2009). RR requires coordinated brain activity, and is an indicator of brain function. Since CO_2 and Ar are both heavier than air, it is possible that some of the RR observed reflect the animal's attempt to physically avoid the gas, as opposed to a reflexive behaviour. Hence, duration and intensity (frequency) of RR are used as indicators of distress in this study.

4.5.4 Animal welfare- gas type

Comparing gas treatments, differences were not observed in measured parameters of welfare between P-CO_2 and G-CO_2. P-Ar pigs had decreased welfare relative to P-CO_2 and G-CO_2, as measured by increased duration of OMB. However, ataxia and intensity and prevalence of RR were decreased. The decreased welfare with the use of argon was surprising and conflicts with conclusions of researchers when Ar was applied to market weight pigs (Raj & Gregory, 1996) and with recommendations from EFSA

(2004). Our results would suggest that peripheral chemoreceptors are activated prior to loss of consciousness. This is expected, since these peripheral chemoreceptors detect low O_2 and stimulate increased respiration in an effort to prevent loss of consciousness. Guyton and Hall (2010) report using the human as a model, a 5-fold increase in respiration with the activation of the peripheral chemoreceptor while still conscious.

The results in the current study are consistent with results of two previous studies from our laboratory using a similar protocol and age of pig (unpublished); relative to CO_2, Ar produced greater behavioural and physiological responses associated with reduced pig welfare during induction. Sutherland (2011) also found, using a similar protocol to ours and with the same age of pig, that exposing pigs to 100% Ar resulted in an increase in the number and durations of vocalizations, onset to loss of consciousness and cardiac arrest compared with pigs exposed to 100% CO_2. However, the increase in the number and duration of escape attempts performed by pigs exposed to 100% CO_2 compared with Ar may conversely suggest that pigs found CO_2 more aversive, which is consistent with the fact that CO_2 is mildly acidic and can irritate the mucus membranes in humans (Danneman et al 1997). At this stage in our understanding of animal perception it is not possible to conclude whether the incidence of increased escape attempts signifies a greater distress response compared to increased vocalizations or vice versa. None the less, Sutherland (2011) established that transitional EEG occurred at 33 and 61 s after exposure to 100% CO_2 and Ar, respectively, which is considered incompatible with consciousness (Blackmore & Delany 1988), and isotonic EEG (undisputed loss of awareness) occurred at 46 and 69 s respectively. Therefore, exposure to 100% Ar appears to double the latency to unconscious in young pigs as compared to 100% CO_2, and as the behavioural response to CO_2 and Ar are similar if not exaggerated in pigs exposed to Ar, it may suggest that Ar should not be recommended as an alternative to CO_2 as a method of euthanasia for young pigs.

Our findings about the aversiveness of Ar euthanasia are similar to those found for rats by Sharp et al (2006). When CO_2 (10% CVE/min) vs. Ar (50% CVE/min) was applied to modify the atmosphere to a level that would produce biologic effects in rats, convulsions and gasping were more frequently observed in Ar, whereas rats exposed to CO_2 showed no adverse reactions (Sharp et al 2006). Though in studies which have examined only CO_2, aversion is observed with this gas (Hawkins et al 2006; Niel et al 2008). Rats were not taken to loss of posture by Sharp and colleagues (2006). In humans, exposure to CO_2 has been associated with pain and coughing (Guyton & Hall 2010 Ch 37). In our study, sneezing nor coughing were not observed in any of the gas treatments, which may indicate irritant receptors in the airways are not activated in pigs of this age, or this effect is not conserved among mammalian species.

4.5.5 Efficacy- low weight

In general weight did not have an effect on measures of efficacy, yet pigs weighing < 0.8 kg showed decreased latencies to measures of efficacy (RA and CA). This would support previous findings for pigs weighing < 0.8 kg as reported in Straw et al (1999), and indicates physiological differences that render them more susceptible to euthanasia. However, it is important to note differences described by Straw (1999) were in relation to birth weights, which is unknown in our pigs; the low weight here could represent pigs that had become severely emaciated.

4.6 Animal welfare implications

When utilizing prefill CO_2 or gradual CO_2 as a euthanizing agent, depression status of the pig does not need to be considered. Conversely, depressed pigs responded differently to Ar than pigs euthanized for other reasons. When utilized as a euthanizing agent for suckling pigs, Ar reduced efficacy and welfare compared to CO_2 and should not be considered for use in gas euthanasia for this age of pig. These concerns are especially relevant in pigs with highly compromised health.

4.7 References

Anil MH 1991 Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Science 30*: 13–21

AVMA 2013 *AVMA Guidelines for Euthanasia of Animals: 2013 Edition*. American Veterinary Medical Association: Schaumber, IL, USA http://www.avma.org/issues/animal_welfare/ euthanasia.pdf

Blackmore DK and Delany MW 1988 Slaughter of stock: a practical review and guide publication number 118. In: *Foundation for Continuing Education of the New Zealand Veterinary Association* Palmerston North, New Zealand

Blackshaw JK, Fenwick DC, Beattie AW and Allan DJ 1988 The behaviour of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. *Laboratory Animals 22*: 67–75

Blood DC, Studdert VP and Gay CC 2007 *Saunders Comprehensive Veterinary Dictionary*. WB Saunders: Philadelphia, USA

Burkholder TH, Niel L, Weed JL, Brinster LR, Bacher JD and Foltz CJ 2010 Comparison of carbon dioxide and argon euthanasia: effects on behaviour, heart rate, and respiratory lesions in rats. *Journal of the American Association for Laboratory Animal Science 49*: 448

Burki NK and Lee LY 2010 Mechanisms of Dyspnea. *Chest 138*: 1196–1201

Daniels CS 2010 Gas euthanasia methods in swine: process and physiology. *In: 2010 AASV Annual Meeting: Implementing Knowledge; Swine Well-being: Euthanasia, Pigs & People*.pp 447-450 American Association of Swine Practitioners, Perry, IA USA

Danneman PJ, Stein S and Walshaw SO 1997 Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Laboratory Animal Science 47*: 376–385

Dodman NH 1977 Observations on the use of the Wernberg dip-lift carbon dioxide apparatus for pre-slaughter anaesthesia of pigs. *The British Veterinary Jounral 133*: 71

EFSA (European Food Safety Authority) 2004 Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. *The EFSA Journal 45*: 1–29

FDA (U.S. Department of Human and Health Services Food and Drug Administration) 2007 Guidance for industry: recommended study design and evaluation of effectiveness studies for swine respiratory disease claims. *Guidance for industry* U.S. Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine Rockville MD, USA

Forslid A 1987 Pre-slaughter CO_2- anaesthesia in swine: influence upon cerebral electrical activity, acid/base balance, blood oxygen tension and stress hormones.

Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Physiology Uppsala, Sweeden

Grandin T 1998 Objective scoring of animal handling and stunning practices at slaughter plants. *Journal of American Veterinary Medical Association 212*: 36–39

Grandin T 2010 *Recommended Animal Handling Guidelines & Audit Guide: a Systematic Approach to Animal Welfare*. AMI (American Meat Institute) Foundation: Washington, D.C., USA

Gregory NG, Mohan-Raj AB, Audsey ARS and Daly CC 1990 Effects of CO_2 on man. *Fleischwirtschart 70*: 1173-1174

Guyton AC and Hall JE 2010 *Guyton and Hall Textbook of Medical Physiology, 11th edition*. Elsevier Saunders: Philadelphia, USA

Hansen NE, Creutzberg A and Simonsen HB 1991 Euthanasia of mink (Mustela vison) by means of carbon dioxide (CO2), carbon monoxide (CO) and nitrogen (N2). *British Veterinary Journal 147*: 140–146

Hawkins P, Playle L, Gooledge H, Leach M, Banzett R, Coenen A, Cooper J, Danneman P Flecknell P and Kirkden R 2006 Newcastle consensus meeting on carbon dioxide euthanasia of laboratory animals. *Animal Technology and Welfare 5*: 125-134

HSUS (The Humane Society of the United States) 2009 The HSUS urges Tyson Foods to reduce suffering of animals at its slaughter plants. http://www.humanesociety.org/news/press_releases/2009/09/tyson_resolution_090109.html

Hurnik JF, Webster AB and Siegel PB 1985 *Dictionary of farm animal behaviour.* University of Guelph Canada

Johnson AK, Sadler LJ, Gesing LM, Feuerbach C, Hill H, Faga M, Bailey R, Stalder KJ and Ritter MJ 2010 Effects of facility system design on the stress responses and market losses of market weight pigs during loading and unloading. *Professional Animal Scientist 26*: 9–17

Kissin I 2000 Depth of anesthesia and bispectral index monitoring. *Anesthesia & Analgesia 90*: 1114–1117

Kohler I, Meier R, Busato A, Neiger-Aeschbacher G and Schatzmann U 1999 Is carbon dioxide (CO2) a useful short acting anaesthetic for small laboratory animals? *Laboratory Animals 33*: 155–161

Mann C, Boccara G, Grevy V, Navarro F, Fabre JM and Colson P 1997 Argon pneumoperitoneum is more dangerous than CO_2 pneumoperitoneum during venous gas embolism. *Anesthesia & Analgesia 85*: 1367–1371

Martoft L, Lomholt L, Kolthoff C, Rodriguez BE, Jensen EW, Jørgensen PF, Pedersen HD and Forslid A 2002 Effects of CO_2 anaesthesia on central nervous system activity in swine. *Laboratory Animals 36*: 115–126

Mota-Rojas D, Bolanos-Lopez D, Concepcion-Mendez M ,Ramirez-Telles J, Roldan-Santiago P, Flores-Peinado S and Mora-Medina P 2012 Stunning swine with CO_2 gas: controversies related to animal welfare. *International Journal of Pharmacology 8*: 141–151

National Pork Board 2009 *On-farm euthanasia of swine: recommendations for the producer*. National Pork Board. Des Moines IA, USA. http://www.aasv.org/aasv/documents/SwineEuthanasia.pdf

Niel L, Stewart SA and Weary DM 2008 Effect of flow rate on aversion to gradual-fill carbon dioxide exposure in rats. *Applied Animal Behaviour Science 109*: 77–84

Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ and Halbur PG 2004 Experimental reproduction of Postweaning Multisystemic Wasting Syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and Porcine Circovirus Type 2. *Veterinary Pathology 41*: 624–640

PETA (People for the Ethical Treatment of Animals) 2002 Hundreds of thousands of hens are given a more humane death. http://www.peta.org/about/victories/Hundreds-of-Thousands-of-Hens-Are-Given-a-More-Humane-Death.aspx

Raj ABM 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. *Veterinary Record 144*: 165–168

Raj ABM and Gregory NG 1996 Welfare implications of the gas stunning of pigs 2: stress of induction of anaesthesia. *Animal Welfare 5*: 71–78

Raj ABM, Johnson SP, Wotton SB and McInstry JL 1997 Welfare implications of gas stunning pigs 3: the time to loss of somatosensory evoked potential and spontaneous electrocorticogram of pigs during exposure to gases. *The Veterinary Journal 153*: 329–339

Rault JL, McMunn KA, Marchant-Forde and Lay DC Jr 2013 Gas alternatives to carbon dioxide for euthanasia: a piglet perspective. *Journal of Animal Science 91*: 1874-1883

Rodriguez P, Dalmau A, Ruiz-de-la-Torre JL, Mantexa X, Jensen EW, Rodriguez B, Livan H and Velarde A 2008 Assessment of unconsciousness during carbon dioxide stunning in pigs. *Animal Welfare 17*: 341-349

Sadler L, Hagen C, Wang C and Millman S. 2011a Comparison of CO_2 versus mixed CO_2:argon gas at different flow rates using the SmartBox euthanasia device as a humane and effective method of piglet euthanasia. *Journal of Animal Science* 89(E2): 51

Sadler, L, Hagen C, Wang C and Millman S 2011b Effect of age on piglet distress associated with euthanasia by carbon dioxide or by a carbon dioxide:argon mixture. *Proceedings of the 45th Congress of the International Society for Applied Ethology (ISAE): Scientific evaluation of behavior, welfare and enrichment* Indianapolis, IN, USA p 8

Sandström V 2009 *Development of a monitoring system for the assessment of cattle welfare in abattoirs. Sveriges lantbruksuniversitet*. Swedish University of Agricultural Sciences Skara Sweden. http://ex-epsilon.slu.se:8080/archive/00003176/01/pdf_VS_Epsilon.pdf

Sharp J, Azar T and Lawson D 2006 Comparison of carbon dioxide, argon, and nitrogen for inducing unconsciousness or euthanasia of rats. *Journal of the American Association for Laboratory Animal Science 45*: 21–25

St John WMS 2009 Noeud vital for breathing in the brainstem: gasping—yes, eupnoea—doubtful. *Philosophical Transactions of the Royal Society B 364*: 2625–2633

Straw BE, D'Allaire S, Mengeling WL and Taylor DJ 1999 *Diseases of Swine 8th edition* Iowa State University Press: Ames, IA, USA

Sutherland 2011 The use of different gases and gas combinations to humanely euthanize young suckling pigs. *National Pork Board Research Report Animal Welfare* NPB #09-199 Des Moines, IA, USA

Velarde A, Cruz J, Gispert M, Carrión D, Torre R, de la JL, Diestre A and Manteca X 2007 Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. *Animal Welfare 16*: 513–522

Whelan G and Flecknell PA 1992 The assessment of depth of anaesthesia in animals and man. *Laboratory Animal Science 26*: 153–162

Wright AJ, Whiting M and Taylor A 2009 Letter to the Editor on the surgical castration of piglets. *Animal 3*: 1474–1475

Table 4.1 Ethogram developed for investigating latency (L), duration (D), prevalence (P) or frequency (F) of behavioural indicators of distress or sensation during euthanasia. [abbreviation used in text]

Behaviours (states)	**Definition**
Open mouth breathing (D,P); [OMB]	Upper and lower jaw being held open with the top lip pulled back, exposing gums or teeth and panting (pronounced inhalation and exhalation observed at the flanks) [1,2]
Ataxia (D,P)	Pig is moving in a seemingly uncoordinated fashion; lack of muscle coordination during voluntary movements [3]
Righting response (D,P,F); [RR]	Pig is making attempt to maintain either a standing or lying sternal posture but is not successful in maintaining the position, seemingly coordinated movements. The event was defined as each time effort was made and the muscles relaxed
Out of view (D)	Pig could not be seen clearly enough to identify the behaviour or posture; or animal was removed from box

Table 4.1 (continued)

Behaviours (events)	**Definition**
Oral discharge (P)	Fluid discharge coming from mouth, may be, clear and fluid, viscous or blood. Type of discharge was noted
Nasal Discharge (P)	Discharge from the nasal cavity, may be clear and fluid, viscous or blood. Type of discharge was noted
Ocular orbit discharge (P)	Discharge from the ocular orbit, may be clear and fluid, viscous or blood. Type of discharge was noted
Vomiting	Ejection of gastrointestinal contents through the mouth [4]
Escape attempt, bout (P)	Pig is raising their forelegs on the side of the wall of the box or pushing quickly and forcefully with their head or nose on the lid of the box; forceful coordinated movement against the exterior of the box; occurrences within in a 10 second period will be scored as a single bout [5]
Loss of consciousness (L); [LC]	Pig had loss posture: pig is slumped down, making no attempt to right itself, may follow a period of attempts to maintain posture, loss of attitude of position of the body; [1,5]; no vocalizations; pig is gasping: rhythmic breaths characterized by very prominent and deep thoracic movements, with long latency between, may involve stretching of the neck
Last limb movement (L); [LLM]	No movement is observed in the pig's limbs
Respiratory arrest (L); [RA]	No thoracic movement visible verified for 1 min duration
Cardiac arrest (L); [CA]	No cardiac activity confirmed by auscultation, verified for 30 s duration
[1] Adapted from Velarde et al 2007, [2] Adapted from Johnson et al 2010, [3] Adapted from Blood et al 2007 p 150, [4]Adapted from Hurnik et al 1985, [5]Adapted from Raj and Gregory 1996	

Table 4.2 Mean latencies (±SE) (seconds) for parameters of efficacy of gas euthanasia comparing disease status of suckling pigs within gas treatments. Means are based on non-zero values.

	Prefill CO_2[2]			Gradual CO_2[3]			Prefill Ar[4]		
Parameter	Depressed[1] (n=11)	Other[1] (n=11)	*P*-value	Depressed [1] (n=11)	Other[1] (n=11)	*P*-value	Depressed[1] (n=6)	Other[1] (n=6)	*P*-value
Loss of consciousness	37 ± 22	40 ± 22	> 0.1	99 ± 21	97 ± 21	> 0.1	212 ± 32	77 ± 29	0.0010
Last limb movement	142 ± 53	167 ± 53	> 0.1	289 ± 51	322 ± 51	> 0.1	511 ± 72	816 ± 72	0.0040
Respiration arrest	377 ± 80	400 ± 80	> 0.1	503 ± 55	388 ± 55	> 0.1	741 ± 223	1233 ± 223	> 0.1
Cardiac arrest	780 ± 93	828 ± 93	> 0.1	748 ± 89	736 ± 89	> 0.1	907 ± 125	1329 ± 125	> 0.1

[1]Pigs were assigned a subjective depression score by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims (FDA, 2007). Based on this four-point scale, the pigs were placed into a disease category (3 = DP; 0 or 1 = OT). Pigs scored 2 were not enrolled.

[2]Chamber was filled with carbon dioxide, pigs placed within and then gas supplied at 20% chamber volume per min

[3]Pigs placed within and then carbon dioxide supplied at 35% chamber volume per min

[4]Chamber was filled with argon, pigs placed within and then gas supplied at 50% chamber volume per min

Table 4.3 Mean durations (±SE) (seconds) of behavioural and physiological measures of distress for suckling pigs of different disease status within gas treatment. Means are based on non-zero values.

	Prefill CO_2[2]			**Gradual CO_2**[3]			**Prefill Ar**[4]		
Parameter	Depressed[1] (n=11)	Other[1] (n=11)	*P*-value	Depressed[1] (n=11)	Other[1] (n=11)	*P*-value	Depressed[1] (n=6)	Other[1] (n=6)	*P*-value
Open mouth breathing	14 ± 15	21 ± 15	> 0.1	35 ± 15	29 ± 15	> 0.1	151 ± 21	69 ± 21	0.0035
Ataxia	12 ± 31	16 ± 31	> 0.1	35 ± 29	35 ± 29	> 0.1	101 ± 42	188 ± 42	0.0370
Righting response	13 ± 8	3 ± 8	> 0.1	20 ± 8	10 ± 8	> 0.1	27 ± 11	63 ± 11	0.0030

[1]Pigs were then assigned a subjective depression score by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims (FDA, 2007). Based on this four-point scale, the pigs were placed into a disease category (3 = DP; 0 or 1 = OT). Pigs that scored 2 were not enrolled.

[2]Chamber was filled with carbon dioxide, pigs placed within and then gas supplied at 20% chamber volume per min

[3]Pigs placed within and then carbon dioxide supplied at 35% chamber volume per min

[4]Chamber was filled with argon, pigs placed within and then gas supplied at 50% chamber volume per min

Table 4.4 Means (±SE) of descriptive parameters prior to euthanasia for cull suckling pigs classified as severely depressed or other.

Parameter	**Depressed [1]** (n=31)[2]	**SE**	**Other[1]** (n=31)[2]	**SE**	***P* - value**
Respiration rate, #/10 s	8	2	11	2	0.0430
Pulse rate, #/10 s	24	3	32	3	< 0.0001
Temperature < 31.7 °C*, number of pigs	22	N/E	3	N/E	N/E
Temperature if >31.7 °C[2]	35.9	0.3	38.3	0.3	0.0236
Weight, kg	1.0	0.3	1.6	0.3	0 .0125
> 0.8 kg, number of pigs	15	N/E	8	N/E	N/E
Female, number of pigs	15	N/E	16	N/E	N/E
Male, number of pigs	16	N/E	15	N/E	N/E
Total lung damage, %	20	6	10	6	0.2498
* Thermometer utilized was not capable or recording temperatures below 89° F					

N/E = value not estimated

[1]Pigs were assigned a subjective depression score by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims (FDA, 2007). Based on this four-point scale, the pigs were placed into a disease category (3 = DP; 0 or 1 = OT). Pigs that scored 2 were not enrolled.

[2]For estimates of temperature if > 89°

CHAPTER 5 SWINE RESPIRATORY DISEASE MINIMALLY AFFECTS RESPONSES OF NURSERY PIGS TO GAS EUTHANASIA

A paper submitted to the Journal of Swine Health and Production

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5.1 Summary

Objective: 1) To assess effects of swine respiratory disease on nursery pig responses during gas euthanasia in terms of efficacy and welfare 2) To compare nursery pig responses to carbon dioxide (CO_2) or argon (Ar) gas euthanasia in terms of efficacy and welfare

Materials and methods: Fifty-four low viability pigs were identified for euthanasia due to swine respiratory disease (SRD, n=27) or other reasons (OT, n=27), and were enrolled in 1 of 3 gas treatments: prefill CO_2 (P-CO_2, n=18), gradual fill CO_2 (G-CO_2, n=18) or prefill Ar (P-Ar, n=18) in a 2x3 factorial design. Behavioral and

physiological indicators of efficacy and welfare were scored directly and from video. CO_2 and O_2 levels were collected throughout the process.

Results: Respiratory disease status did not affect behavioral or physiological responses associated with efficacy or welfare with P-CO_2 or G-CO_2. Conversely, SRD pigs lost posture faster than OT with P-Ar (130 vs. 270 ± 34 seconds, $P < .01$), performed shorter duration of open mouth breathing (15 vs. 63 ± 18 seconds, $P < .05$), but increased duration of ataxia (118 vs. 31 ± 33 seconds, $P = .0569$). Regardless of disease status, P-CO_2 was associated with superior animal welfare based on shorter latency to loss of posture than P-Ar, decreased duration of ataxia and decreased duration and intensity of righting responses.

Implications: Standard operating procedures for gas euthanasia utilizing CO_2 or Ar do not require adjustment for nursery pigs with respiratory disease. Based on our results, a minimum exposure of 10 minutes at > 70% CO_2 concentration is required to reliably produce respiratory arrest in nursery pigs. Duration of exposure to Ar required to reliably produce respiratory arrest (near 100% success rate), was not established for the nursery pig, though is > 10 minutes. The AVMA recommends exposure to < 2% O_2 for > 7 min when euthanatizing with Ar, but 10 min in a prefilled environment was not successful in reliably producing loss of posture. Further refinement of the box or methods might support the AVMA recommendations but this was not achievable with a box designed to simulate top on-farm conditions.

Keywords: swine, respiratory disease, gas euthanasia, carbon dioxide, argon

Swine producers and veterinarians generally agree that euthanasia is appropriate for low viability pigs, especially when there is suffering due to injury or illness. The National Animal Health Monitoring System reports respiratory disease is the primary producer-identified cause of mortality in nursery pigs (44.2%).[1] However, there is little empirical evidence for evaluating euthanasia techniques for pigs in this compromised state. Carbon dioxide (CO_2) is the most commonly implemented gas for swine euthanasia in the US [2] and the American Veterinary Medical Association notes, "… parameters of the technique need to be optimized and published to ensure consistency and repeatability. In particular, the needs of pigs with low tidal volume must be explored".[3] A pig suffering from swine respiratory disease differs from a healthy pig in several physiological parameters that may be important when utilizing gas as a euthanizing agent. Perhaps most importantly, the damaged lung would likely decrease gas exchange rates.

With CO_2, loss of consciousness and death result from hypercapnia when pigs are gradually exposed to the gas (such as gradual fill at 20% chamber volume exchange per minute) or from a combination of hypercapnia and hypoxia when pigs are placed in a prefilled chamber at 80% concentration.[4] Carbon dioxide is mildly acidic, which may cause irritation to the mucus membranes.[5] At 10% carbon dioxide concentrations human subjects report experiencing breathlessness, described as being unpleasant, and the majority of subjects report 50% carbon dioxide concentration as being very pungent and painful.[6] This has led to questions about whether carbon dioxide is appropriate for pig euthanasia.[7] Argon has been proposed as an alternative gas euthanasia method.[8] The European Food Safety Authority recommends stunning pigs with 30:60 carbon dioxide:argon or 90:10 argon:air.[9] Argon is a noble gas, and as such is likely unreactive throughout the physiological systems.[10] Loss of consciousness and death are produced through hypoxia, creating the physiological state of hypocapnic anoxia.[11] As the mechanism of carbon dioxide and argon are different, it is important that both be examined in the compromised pig.

Euthanasia is comprised of two stages: (1) induction of unconsciousness (insensibility) and (2) death. It is the induction phase that is critical to ensure the welfare of the pigs. The entire process, including death, is important to ensure practical implementation. The primary objective of this research was to examine the welfare implications of carbon dioxide and argon for euthanasia of nursery pigs suffering from swine respiratory disease. A secondary objective was to compare welfare implications of carbon dioxide and argon for euthanasia of nursery pigs, regardless of disease status.

5.2 Materials and methods

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee.

5.2.1 Experimental design

Pigs identified for euthanasia were allocated to 1 of 2 disease status categories (swine respiratory disease [SRD] vs. other [OT]). Pigs of each disease status were enrolled in 3 gas treatments: P-CO_2 = 100% CO_2 prefilled box followed by 20% chamber volume exchange rate per minute (CVR/min); G-CO_2 = 100% CO_2 at 20% CVR/min; P-Ar = 100% argon (Ar) prefilled box followed by 50% CVR/min. Eleven SRD/OT pig pairs were enrolled in each gas CO_2 treatment, and 5 SRD/OT pig pairs were enrolled in the Ar treatment for a total of 54 pigs (2 disease statuses x 3 gas treatments x 11 reps/ CO_2 gas treatment + 6 reps/ Ar gas treatment). Pigs from both the SRD and OT categories were randomly selected and paired. Gas treatments were run in a randomized order.

5.2.2 Study animals and enrollment criteria

Pigs were housed and sourced from a commercial nursery farm located in north central Missouri. Genetics were a custom Landrace x Yorkshire cross x Duroc sire performance line. Pigs were eligible for enrollment if they were weaned and 3 to 10 weeks of age. Enrolled pigs were chosen from a pool of pigs identified by farm staff as candidates for euthanasia. These pigs were then assigned a disease status, SRD or OT, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims.[12] Pigs were enrolled as SRD if: rectal temperature was ≥ 40.00 °C, respiratory score was ≥ 2 (2 = moderate – increased respiratory rate, some abdominal breathing; 3 = severe – increased respiratory rate with abnormal effort - open mouth breathing, grunting, dog sitting) and depression score was ≥ 2 (2 = moderate – inactive, may be recumbent but is able to stand, gaunt, may be dehydrated; 3 = severe – down or reluctant to get up, gauntness evident, dehydrated). Pigs were enrolled as OT if: rectal temperature was < 39.72 °C, respiratory score was 0 (rate and pattern normal, no abnormal nasal discharge) and depression score was ≤ 1 (0 = normal – alert, active, normal appetite, well-hydrated, coat normal; 1 = mild – moves slower than normal, slightly rough coat, may appear lethargic but upon stimulation appears normal). Pigs with respiration score 1 were not enrolled.

5.2.3 Euthanasia equipment

Gas was administered to the pigs via a modified Euthanex AgPro™ system (V-AST, Mason City, IA). This gas delivery apparatus was designed by Euthanex Corporation (Palmer, PA), a manufacturer of gas delivery systems for rodents and small animals. The system allows for variable administration of gas types, mixtures, flow rates and delivery time, and once set ensures precise and controlled administration of gases to the box. To facilitate behavioral observations, the box was constructed of clear plastic on the top and front panels. The top panel was hinged for placing pigs into the box. A foam gasket created an airtight seal. The remaining 4 panels were constructed of opaque plastic. The inside dimensions of the box were 53 cm wide, x 91 cm long, x 56 cm high.

On a side panel, the box had a 0.64 cm diameter inlet valve located 7.6 cm from the side, relative to the opaque panel, and 7.6 cm from the top. The exhaust valve was located on the same panel, 3.8 cm from the top and 44 cm from the opaque panel. The gas flowed through 3.25 m of 0.64 cm diameter rubber hoses prior to entering the box. The floor was fitted with a custom foam mat (1.3 cm thick) overlaid with a thin rubber mat (0.16 cm thick) and a layer of wood sawdust (~ 1 cm in depth; TLC Premium Horse Bedding, Centerville, AR) to aid in traction and comfort for the pigs. Constant and precise gas flow was provided by compressed gas cylinders equipped with compressed gas regulators and meters. The CO_2 gas was industrial grade (99% pure), and the Ar gas had a guaranteed analysis of 99.99% pure. Prior to each treatment, sawdust was removed from the chamber by a vacuum (Shop Vac 10 Gallon Ultra Pro Vacuum, 185 CFM), the rubber mat and box were cleaned and disinfected (Roccal, Pfizer Animal Health, New York, NY; Windex, S.C. Johnson, Racine, WI) and fresh sawdust provided. The vacuum was also utilized to remove gas traces, pulling air from the bottom of box for a minimum of 3 min.

5.2.4 Environmental conditions

A HOBO data logger (U23-001, Onset Computer Corporation, Cape Cod, MS) was used to record temperature (°C) and relative humidity (%) within the chamber. The data logger was set to record every 10 s. Oxygen levels were collected with an oxygen sensor (TR25OZ, CO2Meter.com, Ormond Beach, FL) attached to a HOBO data logger (U12, Onset Computer Corporation, Cape Cod, MS), which collected the oxygen level every second. Data were collected continuously throughout the treatment day and exported into Microsoft Office Excel (version 2007, Redmond, WA). A CO_2 meter (CO2IR-WR 100%, CO2Meter.com, Ormond Beach, FL) monitored levels every 1.25 seconds. All sensors were placed at a standing pig's level. Over all days, the average temperature in the chamber was 32.0 °C ranging from 25.7 to 38.5 °C. Relative humidity averaged 41.7% and ranged from 12.9 to 73.3%.

5.2.5 Euthanasia procedure, confirmation of insensibility and death

The experiment was conducted over 4 days in July 2012. For identification during behavior observations, pigs were marked with an animal safe marker (LA-CO Ind.; Elk Grove, IL). The testing area provided isolation, minimizing noise and distractions. Respiration rate, pulse rate, temperature and body weight were recorded for each pig prior to placement in the box. Upon SRD/OT piglet pair placement into the box, gas was immediately started/restarted (gradual/prefill) and continued to run until the pigs were confirmed dead. Two minutes following last movement (respiratory arrest), pigs were removed individually from the box and checked for signs of insensibility.[13–16] Three insensibility tests were conducted: (1) corneal reflex response, in which the eye was touched with the tip of a finger for absence of an eye blink or withdrawal response, (2) pupillary reflex, in which a light-beam (Mini MAGLite, Mag Instrument, Inc., Ontario, CA) was shone into the eye for absence of pupil constriction and (3) nose prick, in which a 20 gauge needle was touched to the snout distal to the rostral bone for absence of a withdrawal response. After insensibility was confirmed, cardiac arrest was confirmed by auscultation. If the pig showed signs of sensibility or cardiac activity, it was placed back into the box for an additional minute of gas exposure. This process was repeated until confirmation of cardiac arrest, allowing us to establish duration of dwell time necessary for death.

For ethical and practical reasons, the protocol was terminated if pigs displayed signs of consciousness (regained posture, made righting attempts, vocalizations or had not transitioned to gasping) after 10 minutes of gas exposure. Additionally, a ceiling value of 10 minutes was used for death (cardiac arrest) after loss of consciousness. For pigs that did not achieve these outcomes within the designated time, captive bolt was utilized as a secondary euthanasia method.

5.2.6 Assessment of lungs

Immediately upon confirmation of death, necropsy was performed. Lungs were removed and a single technician, who was blinded to disease status, scored the lungs for total macroscopic lesions as described by Opriessnig.[17] This scoring system was based on gross visible damage and the approximate volume each lung lobe contributes to the whole lung: the right cranial lobe, right middle lobe, cranial part of the left cranial lobe and caudal part of the left cranial lobe contribute 10% each to total lung volume, the accessory lobe contributes 5% and the right and left caudal lobes contribute 27.5% each. Each lobe was scored as follows: 0% = no gross damage, 50% = minimal damage, to < 50% of the lobe grossly affected, 100% = more than 50% grossly affected. These lobe scores were aggregated for a total lung damage score, ranging from 0-100%. Samples of the lung tissue were collected, with diseased tissue sampled when grossly visible. If no gross lesions were visible, 2 samples were collected from each the left and right middle lobes. Samples were fixed in 10% buffered formalin until scored. Histological examination was performed by pathologists at the Iowa State University Veterinary Diagnostic Laboratory who were blind to disease status and gas treatments. Sections of formalin-fixed lung were embedded in paraffin, processed routinely and stained with hematoxylin and eosin stains. To confirm gross observations as lesions, a pathologist examined lung sections for evidence of antemortem hemorrhage or atelectasis and also characterized the lesions of pneumonia as nonsuppurative interstitial pneumonia or suppurative bronchopneumonia. Pleuritis, when present, was also noted.

5.2.7 Behavioral observations

Behavioral data were collected by direct observation and via video recording. For direct observation, 1 observer per pig stood approximately 1.5 meters from the box and recorded behavioral indicators of welfare, physiological responses (Table 5.1) and tested insensibility. Videos were created utilizing a Noldus Portable Lab (Noldus Information Technology, Wageningen, NL). Two color Panasonic cameras (WV-CP484, Kadoma, Japan) were fed into a multiplexer, allowing the image to be recorded onto a PC using HandiAvi (v4.3, Anderson's AZcendant Software, Tempe, AZ) at 30 frames per second. Behavioral data was collected from video recordings by a single trained observer, blind to disease status and gas treatments, using Observer® software (v10.1.548, Noldus Information Technology, Wageningen, NL). Data were collected for the individual pig for behavioral and physiological indicators of efficacy and welfare of the euthanasia process (Table 5.1). Latencies for all behaviors were determined from the point when each pig was placed into the box.

5.2.8 Statistical Analysis

Behaviors were quantified as latency, duration, frequency of occurrence or percent of pigs displaying the behavior as indicated for the parameter. Data were analyzed using linear mixed models fitted with the GLIMMIX procedure (duration, number, prevalence; SAS Inst. Inc., Cary, NC) or with a Cox proportional hazard model (latency) fitted with the PHREG procedure of SAS. Individual pig was the measurement unit for SRD vs. OT pigs, while pig pair served as the experimental unit for gas type. Least square means estimates for each treatment group and the corresponding standard errors (SE) are reported. The linear model included the fixed effect of disease status (SRD/OT) and gas treatment (P-CO_2, G-CO_2, P-Ar) and all 2-way interactions. A random blocking effect of pig pair was included. The Kenward-Rogers method was utilized for determining the denominator degrees of freedom. Statistical significance was established at P-value < .05 unless otherwise noted. The GLIMMIX procedure of SAS was utilized to establish correlations between latency to behaviors and total lung damage, with the fixed effect of gas treatment and a random blocking effect of pig pair.

5.3 Results

Rectal temperature, respiration rate and weight were higher in SRD pigs relative to OT pigs (Table 5.2; Rectal temperature $P < .001$, respiration rate $P = .0494$, weight $P < .01$). Pulse rate did not differ by disease status ($P > .1$). Lung damage was higher in

SRD pigs relative to OT pigs ($P < .001$). Grossly scored lung damage was confirmed by histological examination with 100% agreement between gross and histological damage scores. Total lung damage was a predictor for loss of posture ($P < .05$), associated with ~0.5 second shorter latency for every 10% of identified damage.

Within a gas treatment, O_2 and CO_2 levels were similar for both SRD and OT pigs. O_2 concentrations (%) at loss of consciousness (LC) were at 5 ± 5, 17 ± 1 and 3 ± 3 for P-CO_2, G-CO_2 and P-Ar, respectively. CO_2 concentrations (%) at LC were 63 ± 4, 46 ± 2 and 0 ± 0 for P-CO_2, G-CO_2 and P-Ar, respectively.

In P-Ar, latency to LC was longer for SRD relative to OT ($P < .01$), but did not differ in P-CO_2 or G-CO_2 ($P > .1$; Table 5.3). Comparing gas treatments independent of disease status, latency to LC was shortest in P-CO_2 (P-CO_2 vs. G-CO_2, $P < .001$; P-CO_2 vs. P-Ar, $P < .001$), whereas G-CO_2 and P-Ar were not different ($P > .1$). Latency to last limb movement (LLM) and respiratory arrest (RA) did not differ between SRD vs. OT pigs in any gas treatments. Comparing gas treatments independent of disease status, latency to LLM was shorter in P-CO_2 vs. G-CO_2 ($P = .0003$). There was a trend for LLM to be shorter with P-CO_2 than P-Ar (P = .0678), whereas a difference was not observed between G-CO_2 and P-Ar. RA did not differ between gas treatments regardless of disease status. In P-CO_2, latency to cardiac arrest (CA) was shorter for SRD vs. OT pigs (Table 5.3; $P = .0497$). However, differences were not observed by disease status for G-CO_2 or P-Ar. Comparing gas treatments independent of disease status, latency to CA was shortest in P-CO_2 (P-CO_2 vs. G-CO_2, $P < .05$; P-CO_2 vs. P-Ar, $P < .05$), but did not differ ($P > .05$) between G-CO_2 and P-Ar. Two OT pigs in P-Ar required secondary euthanasia procedures; one each did not achieve LC or CA in the allotted time.

All pigs displayed open mouth breathing (OMB) and ataxia (AX). Duration of OMB did not differ between SRD vs. OT pigs in P-CO_2 or G-CO_2. However, in P-Ar, duration was greater for OT pigs vs. SRD pigs (Table 5.4). Independent of disease status, duration of OMB was lower in P-CO_2 relative to G-CO_2 ($P < .01$), but was not different than P-Ar. G-CO_2 and P-Ar were not different for duration of OMB. Duration of AX was not different in SRD vs. OT in P-CO_2 or G-CO_2 ($P > .1$). In P-Ar, there was a trend for increased duration of AX in SRD vs. OT pigs ($P = .0569$). Independent of disease status, duration of AX was lower in the use of P-CO_2 relative to both G-CO_2 and P-Ar (P-CO_2 vs. G-CO_2, $P < .05$; P-CO_2 vs. P-Ar, $P < .05$), but there was no difference between G-CO_2 and P-Ar. Righting response (RR) was displayed by 46% of both SRD and OT pigs in P-CO_2. In G-CO_2, 82% of the SRD pigs and 64% of the OT pigs displayed a righting response. All pigs in P-Ar displayed a righting response. When examining intensity of the RR (number of efforts per pig), differences were not observed between SRD and OT pigs within any gas treatment (SRD P-CO_2 = 1, OT P-CO_2 = 1, SRD G-CO_2 = 2, OT G-CO_2 = 1, SRD P-Ar = 3, OT P-Ar = 4). Independent of disease status, duration of righting response was lower in P-CO_2 and G-CO_2, relative to P-Ar (P-CO_2 vs. P-Ar, $P < .01$; G-CO_2 vs. P-Ar, $P < .05$). Duration did not differ between P-CO_2 and G-CO_2. When examining intensity of RR, P-Ar showed greater intensity than P-CO_2 or G-CO_2 (P-CO_2 vs. P-Ar, $P < .001$; G-CO_2 vs. P-Ar, $P < .01$), whereas P-CO_2 and G-CO_2 were not different.

Prevalence of escape attempts did not differ for disease status or gas type (% of pigs: SRD P-CO_2 = 45, OT P-CO_2 = 36, SRD G-CO_2 = 55, OT G-CO_2 = 9, SRD P-Ar = 20, OT P-Ar = 40), nor did the range of number of attempts per individual pig (0 to 3). Oral discharge was a rare event observed in 6 pigs, 1 each in SRD P-CO_2, OT P-CO_2, SRD G-CO_2 and 3 in OT G-CO_2; of these, 3 were prior to gas treatment application. Ocular and nasal discharges were each displayed by 1 pig, both in G-CO_2. Blood was never visible in the discharges. Sneezing, coughing, oral discharge and vomiting were not observed in this study.

Prefill conditions required the box to be filled with the designated gas and then the lid opened to allow placement of the pigs. This allows atmospheric air to enter, quickly changing conditions within the box. Over all trials, initial O_2 levels were 5-8%, 20-21% and 5-7% for P-CO_2, G-CO_2 and P-Ar, respectively. The designed protocol required the lid to be opened for confirmation of death, making it difficult to maintain continuous O_2 and CO_2 levels throughout each run. Opening the lid resulted in increased O_2 levels (Ar and CO_2 treatments; <7%) and decreased CO_2 levels (CO_2 treatments; >55%). Gas levels were regained (< 60 seconds) as gas flow was maintained throughout the procedure.

5.4 Discussion

The objectives of this study were to examine and assess the efficacy and welfare of nursery pigs suffering from swine respiratory disease during gas euthanasia with either CO_2 or Ar, and to compare efficacy and welfare, regardless of disease status, of gas euthanasia with either CO_2 or Ar. It was hypothesized that SRD pigs would have decreased respiratory membrane available for gas exchange, resulting in greater latency to measures of efficacy and reduced welfare during gas euthanasia. Contrary to our hypothesis, disease status did not affect behavioral or physiological responses associated with efficacy or welfare when euthanizing with P-CO_2 or G-CO_2. Minimal differences were observed between disease statuses with the use of Ar, increasing time spent conscious for the OT pigs vs. SRD pigs. Also in Ar, minimal differences were observed in measures of welfare between SRD and OT pigs, with SRD pigs displaying decreased OMB, but increased AX. When comparing prefilled conditions, CO_2 relative to Ar, resulted in improved welfare by shorter latency to LC, shorter duration of AX and shorter duration and lower intensity of RR, whereas differences were not observed in the other measures of welfare that were collected. Differences between disease statuses were small enough to not warrant changes to gas euthanasia procedures.

Weights of the SRD pigs were higher than OT pigs. This is likely due to variability in disease processes in these 2 groups. SRD pigs gradually develop disease symptoms, often being identified for euthanasia late in the nursery phase. Conversely, OT pigs were identified for euthanasia for multiple reasons (injury, exudative epidermitis, hernias, structural deformities and other reasons). It is unlikely that differences in weight account for differences in responses by SRD and OT pigs.

In this study, the euthanasia process was evaluated in 2 phases: conscious and unconscious. There is a transition phase prior to LC during which a number of behaviors are typically observed, including OMB, AX and RR. The level of awareness, hence capacity of animals to suffer, during this transition is unclear, and we chose a conservative estimate by including all measures up to the point of LC to ensure appropriate pig welfare. Behaviors chosen for welfare assessment included physiological distress, such as OMB, or psychological distress, such as escape attempts and RR.[15,18–25] Although more invasive methods to assess efficacy and welfare, such as EEG or ECG monitoring, can provide robust data in the laboratory, they are not practical on farm and cannot be used in tandem with measurement of naturally occurring behaviors that are induced during gas euthanasia procedures. Behavior was chosen as the primary outcome of interest for welfare since behavioral observations provide more sensitive measures of the animal's experience than physiologic responses, particularly since euthanasia with inhalant gases can produce confounding effects on physiologic responses.[26]

When CO_2 was utilized at either flow rate, disease status did not affect any welfare parameters measured. OMB is a physiological reaction associated with breathlessness, and has been identified as an indicator of compromised welfare in the pig.[27] When exposed to CO_2, durations of OMB were similar to those previously observed in nursery pigs for both prefill and gradual conditions (12 ± 2 s; 34 ± 2 seconds).[28] In P-Ar, duration of OMB was approximately 4 times greater for OT pigs relative to SRD pigs. Duration of OMB in P-Ar has not yet been reported in nursery pigs, though observed values in this trial are about 3 times less than that reported in suckling pigs (110 ± 21 seconds).[29]

Ataxia is likely an indicator of impaired function of the cerebellum, however it is unclear how this correlates to impaired cortical function. If ataxia indicates that the pig is aware of its surroundings, but is unable to react in a coordinated manner, this could be distressing to the pig. In this study, we defined ataxia as a potential stressor for the pig, and hence, a shorter duration of this behavior would correlate with improved welfare. In Ar, duration of AX was almost 4 times greater in SRD pigs relative to OT pigs. This longer display of AX may be attributed to the general health status of these pigs.[30,31] With a higher depression score, they may be more likely to display AX even without application of gas. As such, the increase may be explained by the longer latency to LC, rather than an adverse effect of the gas. Regardless of disease status, reduced welfare was observed with the use of Ar and the gradual flow rate relative to P-CO_2. The lack of RR has been cited as a critical indicator that a pig is successfully rendered unconscious prior to slaughter.[13,23] Hence, duration and intensity (number of efforts) were used as indicators of welfare in this study. Righting response was not affected by disease status in any gas treatment. In the prefilled gas treatments, decreased welfare was observed with the use of Ar, as indicated by a 6-fold increase in duration and 4-fold increase in number of attempts vs. CO_2. The reduced welfare observed in the gradual flow rate was not surprising, since it is consistent with previous research in our laboratory in which welfare was superior with the use of prefill or a faster flow rate (50% CVR/min).[28] The original protocol called for G-CO_2 to be run at 35% CVR/min and P-CO_2 followed by 50% CVR/min. However, due to technical difficulties during the trial, only a 20% CVR/min was achieved in the system. Other flow rates not examined in this study may be advantageous to the pig. Given that disease status did not affect pig responses in the two extreme flow rates tested with CO_2, it is likely SRD disease status would not be a factor at any rate in-between.

In addition to minimizing the potential distress caused by the gases, an important goal for euthanasia of these compromised pigs includes minimizing latency to LC to ensure the most humane process is achieved. In Ar, pigs in the OT category took more than twice as long to lose consciousness, being conscious for nearly 4.5 minutes. Latency to LC was increased with Ar and the gradual flow rate relative to P-CO_2. This is similar to what was observed in suckling pigs.[29] During the gas euthanasia process, once regular breathing (including OMB) controlled by the ventral respiratory group fails, gasping is recruited.[32,33] Respiratory arrest (cessation of gasping) represents the point at which gases can no longer be introduced into the pig's respiratory system. This point is critical to the euthanasia process because the pig will not recover without intervention. During gas euthanasia, gasping will become slower and shallower until breathing finally ceases. In this study, RA was the last movement by the pig that was observed, and is consistent with that found in suckling pigs undergoing gas euthanasia.[29] Current recommendations for CO_2 state exposure should be for > 5 min.[3,15] In CO_2, the longest observed latency to RA was 585 seconds, suggesting that a minimum of 10 minutes exposure to high CO_2 concentrations is indicated in gas euthanasia. Current recommendations for Ar state exposure should be for > 7 min.[3] In Ar, 1 pig was still conscious after 10 minutes of exposure and thus a longer unknown duration would need to be implemented when using this gas. Surprisingly, despite the difference in diseased lung tissue between SRD and OT pigs, the only observed difference occurred in latency to CA, when utilizing CO_2 as a euthanatizing agent. Since CA occurs post LC and RA, it is likely this difference is not of consequence to either welfare or practical implementation because the pig is insensible and gases can no longer be introduced into the pig's system.

Pigs which had been clinically identified as SRD were confirmed to have severely diseased lungs, almost 3 times more damage than the OT pigs. The visible assessment of the lungs was confirmed through histology, with 100% agreement on identification of gross lesions. During respiratory disease, the pulmonary membrane becomes inflamed and highly porous, allowing fluid to leak into the alveoli, effectively decreasing functional respiratory membrane. Additionally, respiratory disease causes inflammation and decreased diameter or blockage of infected airways. This obstruction makes expiration difficult, trapping air which may be reabsorbed, leading to collapse of the affected lung sections. The consequences of decreased functional respiratory membrane include hypoxemia and hypercapnia.[32] To compensate for the hypoxic and hypercapnic state, the SRD pig displayed tachypnea. Pigs were assessed for a respiratory score as part of the selection process. These scores were collected under both normal and stressed conditions. First, a respiratory score was assigned while the pigs were minimally disturbed in the sick pen; second, assessment was conducted while the pig was restrained by a technician and was presumably in a stressed state. It is interesting to note that the physiological and compensatory effects of lung damage were observed in both normal and stressed conditions. Assessment of respiratory rate under stressed conditions is the likely cause of this value being higher for both SRD and OT pigs relative to expected values (25-40 breaths/minute in a normal nursery pig vs. SRD 96 and OT 78 breaths/minute).[34] Although total lung damage significantly affected LP, the effects were minor (5 seconds difference between zero and 100% lung damage) and not substantial enough to merit modifications of standard operating protocols for euthanasia.

5.5 Implications

- With respect to efficacy or pig welfare, a successful gas euthanasia protocol that utilizes CO_2 does not need to be adjusted for pigs with respiratory disease
- P-CO_2, relative to G-CO_2 or P-Ar, provided superior welfare when euthanizing nursery pigs, on the basis of reduced latency to LC and reduced duration of OMB, AX and RR
- Producing O_2 levels necessary for the euthanasia with Ar is difficult with current on-farm equipment, nor were welfare benefits observed with its use; as such Ar is not recommended as a euthanatizing agent on nursery swine farms

5.6 References

1. NAHMS (National Animal Health Monitoring System). *Highlights of swine 2006 part III: reference of swine health, productivity, and general management in the United States, 2006*. United State Department of Agriculture. 2008.

2. Daniels CS. Gas euthanasia methods in swine: process and physiology. *Proc AASV*. Omaha Nebraska.2010;447–450.

3. AVMA (American Veterinary Medical Association*). Guidelines for the euthanasia of animals: 2013 edition*. American Veterinary Medical Association, Schaumber, IL; 2013.

4. Raj ABM, Johnson SP, Wotton SB, McInstry JL. Welfare implications of gas stunning pigs: 3. the time toloss of somatosensory evoked potential and spontaneous electrocorticogram of pigs during exposure to gases. *Vet J.* 1997;153:329–339.

5. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Lab Anim Sci*. 1997: 47: 376–385.

6. Gregory N, Mohan-Raj A, Audsey A, Daly C. Effects of CO_2 on man. *Fleischwirtschart.*1990;70:7–9.

7. Wright AJ, Whiting M, Taylor A. Letter to the Editor on the surgical castration of piglets. *Anim Int J Anim Biosci*.2009:3:1474–1475.

8. Raj ABM, Gregory NG. Welfare implications of the gas stunning of pigs 2. stress of induction of anaesthesia. *Anim Welf.* 1996;5:71–78.

9. EFSA (European Food Safety Authority). Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. *EFSA J*.2004;45:1–29.

10. Mann C, Boccara G, Grevy V, Navarro F, Fabre JM, Colson P. Argon pneumoperitoneum is more dangerous than CO_2 pneumoperitoneum during venous gas embolism. *Anesth Analg.* 1997;85:1367–1371.

11. Raj ABM. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. *Vet Rec.* 1999;144:165–168.

12. FDA (U.S. Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine). Guidance for industry: recommended study design and evaluation of effectiveness studies for swine respiratory disease claims. U.S. Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine. 2007.

13. Grandin T. *Recommended Animal Handling Guidelines & Audit Guide: a Systematic Approach to Animal Welfare*. AMI (American Meat Institute) Foundation. 2010.

14. Kissin I. Depth of anesthesia and bispectral index monitoring. *Anesth Analg*. 2000;90:1114–1117.

15. NPB (National Pork Board)/AASV (American Association of Swine Veterinarians). *On-farm euthanasia of swine: recommendations for the producer.* National Pork Board, Des Moines, IA. 2009.Available at: http://www.aasv.org/aasv/documents/SwineEuthanasia.pdf. Accessed 11 November 2012.

16. Whelan G, Flecknell PA. The assessment of depth of anaesthesia in animals and man. *Lab Anim*. 1992;26:153–162.

17. Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng X-J, Halbur PG. Experimental reproduction of Postweaning Multisystemic Wasting Syndrome in pigs by dual infection with mycoplasma hyopneumoniae and Porcine Circovirus Type 2. *Vet Pathol*. 2004;41:624–640.

18. Forslid A. *Pre-slaughter CO_2-anaesthesia in swine: influence upon cerebral electrical activity, acid/base balance, blood oxygen tension and stress hormones*. Swdish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Physiology Uppsala, Sweeden.1987.

19. Martoft L, Kolthoff C, Rodriguez BE, Jensen EW, Jorgensen PF, Pedersen HD, Forslid A. Effects of CO_2 anaesthesia on central nervous system activity in swine. *Lab Anim*. 2002;36:115–126.

20. Mota-Rojas D, Bolanos-Lopez D, Concepcion-Mendez M, Ramirez-Telles J, Roldan0Santiago P, Flores-Peinado S, Mora-Medina P. Stunning swine with CO_2 gas: controversies related to animal welfare. *Int J Pharmacol*. 2012;8:141–151.

21. Kohler I, Meier R, Busato A, Neiger-Aeschbacher G, Schatzmann U. Is carbon dioxide (CO_2) a useful short acting anaesthetic for small laboratory animals? *Lab Anim*. 1999;33:155–161.

22. Grandin T. Objective scoring of animal handling and stunning practices at slaughter plants. *J Am Vet Med Assoc*. 1998;212:36–39.

23. Sandström V. Development of a monitoring system for the assessment of cattle welfare in abattoirs. *Sveriges lantbruksuniversitet*. Swedish University of Agricultural Sciences Skara Sweden. 2009. Available at:http://ex-epsilon.slu.se:8080/archive/00003176/01/pdf_VS_Epsilon.pdf. Accessed 15 December 2012.

24. Anil MH. Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Sc*i. 1991;30:13–21.

25. AVMA (American Veterinary Medical Association). *Guidelines on euthanasia, formerly report of the AVMA panel on euthanasia*. American Veterinary Medical Association, Schaumber, IL, USA. 2007.

26. Burkholder TH, Niel L, Weed JL, Brinster LR, Bacher JD, Foltz CJ. Comparison of carbon dioxide and argon euthanasia: effects on behavior, heart rate, and respiratory lesions in rats. *J Am Assoc Lab Anim Sci.* 2010;49:448.

27. Velarde A, Cruz J, Gispert M, Carrion D, Torre R, Diestre A, Manteca X. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. *Anim Welf*. 2007;16:513–522.

28. Sadler L, Hagen C, Wang C and Millman S. Comparison of CO_2 versus mixed CO_2:Argon gas at different flow rates using the Smart Box euthanasia device as a humane and effective method of piglet euthanasia. *J Anim Sci.* 2011;89(E2): 51.

29. Sadler, L, Hagen C, Wang C and Millman S. Effects of depression score on welfare implications of CO2 and Argon gas euthanasia of suckling piglets. *Proceedings of the 47th Congress of the International Society for Applied Ethology (ISAE) Florianoplis*, Brazil. 2013;p44.

30. The Pig Site. *Hypoglycaemia - Disease of Pigs*. Available at http://www.thepigsite.com/diseaseinfo/53/hypoglycaemia. Accessed 11 November 2012.

31. Fernandez NJ, Barton J, Spotswood T. Hypoglycemia in a dog. *Can Vet J*. 2009;50:423–426.

32. Guyton, Hall, JE. *Guyton and Hall Textbook of Medical Physiology, 11th edition*. Elsevieer Saunders: Philadelphia USA. 2010.

33. John W MS. Noeud vital for breathing in the brainstem: gasping—yes, eupnoea—doubtful. *Philos Trans R Soc B Biol Sci*. 2009;364:2625–2633.

34. Straw BE, D'Allaire S, Mengeling WL, Taylor DJ. Diseases of Swine. Iowa State University Press, Ames, IA. 1999:841-845

35. Johnson AK, Sadler LJ, Gesing LM, Feuerbach C, Hill H, Faga M, Bailey R, Stalder KJ, Ritter MJ. Effects of facility system design on the stress responses and market losses of market weight pigs during loading and unloading. *Prof Anim Sci*. 2010;26:9–17.

36. Blood DC, Studdert VP, Gay CC. *Saunders Comprehensive Veterinary Dictionary* p150 WB Saunders: Philadelphis USA. 2007.

37. Hurnik JF, Webster AB, Siegel PB. *Dictionary of farm animal behaviour*. University of Guelph. 1985.

Table 5.1 Ethogram developed for investigating latency (L), duration (D), prevalence (P) or frequency (F) of behavioral indicators of welfare or sensation during euthanasia.

Behaviors (states)	**Definition**
Open mouth breathing (D,P)	Upper and lower jaw being held open with the top lip pulled back, exposing gums or teeth and panting (pronounced inhalation and exhalation observed at the flanks) [27,35]
Ataxic (D,P)	Lack of muscle coordination during voluntary movements [36]
Righting response (D,P,F)	Pig is making an attempt to maintain either a standing or lying sternal posture but is not successful in maintaining the position. The event was defined as each time effort was made and the muscles relaxed
Sham licking and chewing, (D,P)	Pig is going through motions of licking and chewing but is not making contact with any substrate or object
Out of view (D)	Pig could not be seen clearly enough to identify the behavior or posture; or animal was removed from box

Table 5.1 (continued)

Behaviors (events)	**Definition**
Oral discharge (P)	Discharge coming from the mouth, may be clear and fluid, viscous or blood. Type of discharge was noted
Nasal Discharge (P)	Discharge from the nasal cavity, may be clear and fluid, viscous or blood. Type of discharge was noted
Ocular orbit discharge (P)	Discharge from the ocular orbit, may be clear and fluid, viscous or blood. Type of discharge was noted
Sneezing or coughing (P)	To expel air forcibly from the mouth and nose in an explosive, spasmodic involuntary action
Vomiting	Ejection of gastrointestinal contents through the mouth [37]
Escape attempt, bout (P)	Pig is raising their forelegs on the side of the wall of the box or pushing quickly and forcefully with their head or nose on the side or lid of the box; forceful coordinated movement against the exterior of the box; occurrences within a 10 s period were scored as a single bout [8]
Loss of consciousness (L)	Pig has lost posture: pig is slumped down, making no attempt to right itself, may follow a period of attempts to maintain posture,[27,8]; no vocalizations; pig is gasping: rhythmic breaths characterized by very prominent and deep thoracic movements, with long latency between, may involve stretching of the neck
Last limb movement (L)	No movement is observed of the pig's extremities
Respiratory arrest (L); [RA]	No thoracic movement visible verified for a 2 minute duration
Cardiac arrest (L); [CA]	No cardiac activity confirmed by auscultation, verified for a 30 second duration
[27] Adapted from Velarde et al., 2007, [35] Adapted from Johnson et al., 2010, [36] Adapted from Blood et al., 2007, [37]Adapted from Hurnik et al., 1985, [8] Adapted from Raj and Gregory, 1996	

Table 5.2 Means (±SE) of descriptive parameters of enrolled pigs, taken prior to placement in the box.

Parameter	**SRD**[1] (n=27)	**SE**	**OT**[1] (n=27)	**SE**	***P*** **-value**
Female	16	--	18	--	--
Male	11	--	9	--	--
Pulse rate, #/10 s	28	1	30	1	> .1
Respiration rate, #/10 s	16	1	13	1	.0494
Temperature, °C	40.4	0.2	39.2	0.2	< .001
Weight, kg	15.4	1.4	10.0	1.4	< .01
Total lung damage, %	64	7	24	7	< .001

SRD = nursery pigs identified for euthanasia suffering from swine respiratory disease

OT = nursery pigs identified for euthanasia not suffering from SRD

[1]Pigs were assigned into a disease status category by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims.[12]

Table 5.3 Mean latencies (±SE) in seconds for parameters of gas euthanasia efficacy comparing disease status of nursery pigs within gas treatments. Means are for non-zero values.

	Prefill CO_2[2]			**Gradual CO_2**[3]			**Prefill Ar**[4]		
Parameter	SRD[1] (n=11)	OT[1] (n=11)	*P*-value	SRD[1] (n=11)	OT[1] (n=11)	*P*-value	SRD[1] (n=5)	OT[1] (n=5)	*P*-value
Loss of consciousness	35 ± 16	36 ± 16	> .1	149 ± 13	158 ± 13	> .1	130 ± 34	270 ± 34	< .01
Last limb movement	145 ± 40	157 ± 40	> .1	367 ± 33	329 ± 33	> .1	274 ± 53	255 ± 53	> .1
Respiration arrest	426 ± 81	314 ± 81	> .1	434 ± 68	433 ± 68	> .1	317 ± 110	408 ± 121	> .1
Cardiac arrest	485 ± 39	574 ± 39	.0497	623 ± 32	647 ± 32	> .1	619 ± 52	700 ± 58	> .1

SRD = nursery pigs identified for euthanasia suffering from swine respiratory disease

OT = pigs identified for euthanasia not suffering from SRD

[1]Pigs were assigned into a disease status category by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims. [12]

[2]Chamber was filled with carbon dioxide, pigs placed within and then gas supplied at 20% chamber volume per minute

[3]Pigs placed within and then carbon dioxide supplied at 20% chamber volume per minute

[4]Chamber was filled with argon, pigs placed within and then gas supplied at 50% chamber volume per minute

Table 5.4 Mean durations (±SE) in seconds for welfare behavioral measures of gas euthanasia comparing disease status within gas treatments.

	Prefill CO_2[2]			**Gradual CO_2[3]**			**Prefill Ar[4]**		
Parameter	SRD[1] (n=11)	OT[1] (n=11)	*P*-value	SRD[1] (n=11)	OT[1] (n=11)	*P*-value	SRD[1] (n=5)	OT[1] (n=5)	*P*-value
Open mouth breathing	16 ± 13	14 ± 13	> .1	47 ± 11	58 ± 11	> .1	15 ± 18	62 ± 18	.0491
Ataxia	12 ± 22	15 ± 22	> .1	48 ± 20	62 ± 20	> .1	118 ± 30	31 ± 33	.0569
Righting response	5 ± 5	2 ± 5	> .1	11 ± 4	8 ± 4	> .1	16 ± 6	28 ± 6	> .1

SRD = nursery pigs identified for euthanasia suffering from swine respiratory disease

OT = pigs identified for euthanasia not suffering from SRD

[1]Pigs were assigned into a disease status category by a single technician, based on the Guidance for Industry Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims. [12]

[2]Chamber was filled with carbon dioxide, pigs placed within and then gas supplied at 20% chamber volume per minute

[3]Pigs placed within and then carbon dioxide supplied at 20% chamber volume per minute

[4]Chamber was filled with argon, pigs placed within and then gas supplied at 50% chamber volume per minute

CHAPTER 6 GENERAL DISCUSSION

6.1 Conclusions

Killing of animals has long been recommended due to mercy, when suffering is present. Gas euthanasia on swine farms has been increasing in popularity. However, there is controversy regarding pig welfare during gas euthanasia and research is needed to establish best practices, ensuring minimal pain and distress. The entire process, including death, is important to ensure practical implementation. Additionally, it is important to the pigs' welfare that they are not allowed to regain consciousness. Pain and distress are affective states and can only be measured indirectly in humans and animals. No single parameter is able to definitively indicate if an experience is painful or distressing. Euthanasia with inhalant gases can produce confounding effects on physiologic response, therefore behavioral responses to pain and distress were chosen as primary outcomes to add to the current body of literature for gas euthanasia of young pigs.

The research presented in this thesis on young pigs utilized methods which simulate well controlled on farm gas euthanasia conditions, ensuring results can be applied to the millions of pigs which are currently euthanatized annually within the U.S. swine industry. When examining a euthanasia method, both animal welfare and efficacy are key components. This thesis answers questions about the gas euthanasia process related to gas type, flow rate and age of pig.

In Chapter 2, research is presented that addresses gas type, flow rate and age of pig. Two age groups (neonate and nursery) were assessed in 9 gas treatments, arranged as a 2 x 4 factorial design with 2 gas types (carbon dioxide (CO_2); 50:50 CO_2:argon) and 4 flow rates (chamber volume exchange rate per minute [CVR/min]: 20%, 35%, 50%, prefill) and a control treatment in which ambient air was passed through the box. Based on behavioral and physiological assessments, it was demonstrated that pigs succumb faster when using 100% CO_2 vs. a 50:50 CO_2:argon gas mixture. More importantly, 100% CO_2 resulted in shorter durations of behavioral indicators of distress and physiological responses. Thus, proposed benefits of adding argon were not observed. Likewise, the 20% CRV/min increased the durations of sensation and distress measures, while resulting in longer latencies to loss of posture and last movement. When utilizing gas as a euthanizing agent for young pigs (neonate or nursery) faster flow rates ($\geq$ 35% are preferable to slow (20% CRV/min). In general, neonates succumb to the effects of the gas faster and with lower duration and intensity of distress outcomes relative to the nursery aged pigs.

Results from Chapter 2 demonstrated 50:50 CO_2:argon gas mixtures and slower flow rates should be avoided when euthanizing nursery or neonate pigs with gas methods. Additionally, differences between the two examined age groups were not great enough to warrant development of separate gas euthanasia protocols. Many farms within the U.S. swine industry utilize 2- or 3-min gas run time, followed by a 5-min dwell time, or similarly timed procedures. It is important to note that if a procedure similar to slow flow in this trial had been followed on farm, most pigs would not have been successfully euthanized. It is critical that producers know the flow rate of their systems and avoid designing euthanasia procedures solely on timing.

Following this study, key questions emerged. In young pigs, would distress be reduced with 100% argon relative to 100% CO_2, as had been previously reported for market weight pigs? What is the appropriate duration of gas flow and dwell time to ensure a high-level of efficacy (near 100%) and welfare? Are distress and efficacy, associated with gas euthanasia, altered in the young pig by health status?

In Chapter 3, research utilizing a motivational state model is presented assessing induction of anesthesia with 100% CO_2 and 100% argon. The motivational model utilized allowed for highly refined assessment of the distress produced during induction from the gases. Piglets in the U.S. swine industry are currently processed (castrated and tail docked) without anesthesia or analgesia. These procedures are painful, but in order for anesthesia or analgesia to be widely implemented, interventions must be feasible in production settings. Gas techniques for are commonly used for on-farm euthanasia, and hence infrastructure may exist to facilitate inhalant anesthesia for piglet processing. A gas method of euthanasia involves a two-step process. First, induction of anesthesia comprises all steps until the piglet is rendered unconscious. Second, cessations of respiratory and cardiac functions result in death. If the processes could be successfully controlled by removing the pig after the first step but prior to the second, the resultant procedure may serve as a method to induce general anesthesia for piglet processing. In addition to depth and control of anesthesia produced with equipment utilized on-farm, the distress produced and the reliability of depth and recovery from the anesthesia method were assessed when utilizing CO_2 or argon.

In this study, suckling pigs were habituated to factors, other than the gasses, that may elicit a distress response, and hence distress produced from the gases was better assessed. Although variations in behavior are observed during induction of insensibility, it is difficult to ascertain whether these are accurate indicators of distress resulting from exposure to the inhalant agents since these behaviors may be involuntary neurophysiologic response to the induction process or may be observed after the piglet is insensible. A motivational state model was chosen as one experimental technique to circumvent these difficulties, allowing the distress from the gas to be teased apart from distress associated with novelty. Results from this study do not support the use of on-farm gas euthanasia equipment for anesthesia. Placing piglets in gradual fill CO_2 or argon does not produce reliable anesthesia. When gas is applied at a gradual fill rate, both CO_2 and argon produced distress during induction. Furthermore, argon produced a greater level of distress and is not recommended for pigs less than 7 days of age for anesthesia or euthanasia.

In Chapter 4, research is presented that explored the effects of CO_2 or argon gas euthanasia in suckling pigs that are physiologically depressed. Severely depressed pigs exhibit differences in a number of important parameters that may affect gas euthanasia including decreased respiration rate and tidal volume. When utilizing prefill CO_2 or gradual CO_2 as a euthanizing agent, depression status of the pig did not affect the gas euthanasia process. Conversely, depressed pigs responded differently to argon than pigs euthanized for other reasons, taking longer to lose consciousness and increasing the duration of open mouth breathing. Independent of disease status, argon relative to CO_2 was associated with a prolonged euthanasia process, including frequencies and durations of distress behaviors. This research demonstrated current guidelines (AVMA, 2013; National Pork Board, 2008) directing euthanasia recommending 80% CO_2 concentrations for at least 5 minutes creates a risk of suckling pigs recovering after an attempted euthanasia. Based on my findings, I recommend exposure time to be increased to 15 minutes. Depression score did not affect pig responses to euthanasia with CO_2 gas, and thus does not need to be considered when establishing euthanasia protocols. When utilized as a euthanizing agent for suckling pigs, argon reduced efficacy and welfare compared to CO_2 and based on my findings, I recommend argon not be used for gas euthanasia of suckling pigs.

In Chapter 5, research was presented that showed nursery pigs clinically identified as suffering from Swine Respiratory Disease (SRD) and confirmed to have severely diseased lungs (almost 3 times more damage than the other [OT] pigs) did not differ in regards to efficacy or welfare when euthanized with CO_2. Conversely, SRD suffering pigs lost posture faster than OT with argon, performed shorter duration of open mouth breathing, but increased duration of ataxia. Regardless of disease status, prefilled CO_2, was associated with superior animal welfare, relative to prefilled argon, based on shorter latency to loss of posture, decreased duration of ataxia and decreased duration and intensity of righting responses. This research demonstrated current guidelines (AVMA, 2013; National Pork Board, 2008) recommending 80% CO_2 concentrations for 5 minutes create risks for nursery pigs by recovering after attempted euthanasia. Therefore, CO_2 gas exposure time should be increased to 15 minutes. When creating protocols for euthanasia, this research demonstrated SRD status does not need to be taken into account.

6.2 Challenges and future research

In the presented research, distress is observed during gas euthanasia of young pigs, regardless of flow rate tested (20% to prefill). However, flow rates less than 20% were not examined. Rates slower than this may provide an alternative that allows the pig to succumb to the affects of CO_2 while minimizing distress. Alternatively, final gas concentrations that are lower than 80% should be examined, as this lower concentration may render a young pig unconscious, followed by death, with potential benefits to pig welfare.

Furthermore, the research did not establish the maximum gas concentration young pigs will tolerate before aversive behaviors are displayed. Investigation into gas concentrations that pigs find aversive could be completed with motivational studies. Motivational studies involving gas anesthesia have proven difficult due to potential amnesia and loss of mobility. Slower flow rates might allow for completion of motivational studies and identification of the gas concentration that pigs find unpleasant. In the presented research, in addition to the use of relatively fast flow rates, the environmental sensors (CO_2 and O_2) in this research were not operational until the final study. These challenges made it difficult to associate behavioral responses with gas concentrations. Future research establishing these associations could be beneficial to further refine gas euthanasia guidelines. Blood PCO_2 and PO_2 values could help differentiate and classify the behavioral response with the physiological state of the pig. Open mouth breathing is a behavioral response that is observed in pigs with a wide range of variation from subtle to maximized tidal volume and respiration rate. Potentially all variants of this process are not painful/distressing. If the pig is not in an anesthetic state when maximal tidal volume and rate are achieved, the likelihood of pain increases. It would be useful to continue investigating the young pig and establish the time spent conscious at these maximal values. If this data is obtained, guidelines that are more complete could be provided to minimize exposure to distressing gas concentrations. Additionally, minimal gas concentrations could be established, allowing the producer to minimize input cost. For example, if a 60% vs. 80% final CO_2 concentration could be used to produce loss of consciousness and death, the producer could use less gas and thus conserve resources.

The technology employed in this study would allow for practical on-farm two-step gas euthanasia in which the pig is first anesthetized with one flow rate/gas type and killed with a second flow rate/gas type. A two-step process has potential to utilize methods that are currently not practical nor explored. A two-step process could be applied in a number of different manners, including the use of two gases or two flow rates. Currently, there is little research on gases which are cost prohibitive or have proven difficult to produce death such as isoflurane, nitrous oxide or CO_2:O_2 gas mixtures. The two-step process could utilize these gases to anesthetize the pig followed by CO_2 to produce death, potentially making them viable alternatives. Alternatively, the two-step process could involve two flow rates. For example, a slow flow rate that does not induce distress could be continued until distress is incurred (at a concentration still to be established), then flow rate would be rapidly increased ensuring rapid loss of consciousness and minimal time spent in distress.

In the current research, all studies were done with piglet pairs, in a uniform box. Dynamics of other aspects of the euthanasia process such as stocking density, which would alter effective box volume, stimulation from conspecifics, and creation of microclimates within the box have yet to be considered. Additional factors that may contribute to distress of the gas euthanasia process should also be explored. Some examples of these factors include flooring type, lighting levels, thermal comfort, space requirements and general design of the box. Alterations to these factors may prove beneficial in alleviating distress during the euthanasia process.

Although current guidelines indicate argon is preferable to CO_2, this was not observed in young pigs (AVMA, 2013; EFSA 2004). Because of this, further research of argon is warranted in the older/larger pig to establish best practices within these age/wieght groups. Finally, it is important to recognize that regardless of pig classification (age or disease status) or method tested (0% to prefill; 100% carbon dioxide, 100% argon and gas mixture) distress is observed during gas euthanasia of pigs. Thus, research into alternative, non-gas methods is warranted.

References

AVMA (American Veterinary Medical Association), 2013. AVMA guidelines for the euthanasia of animals: 2013 edition. Accessed 15th of June 2013 at: https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

EFSA. 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal. 45:p.1–29.

NPB (National Pork Board), 2008. On-farm euthanasia of swine: recommendations for the producer (No. 04259-01/09). National Pork Board. Des Moines, USA