Evaluation of Methods for On-Farm Euthanasia of Commercial Meat Rabbits

by

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ABSTRACT

EVALUATION OF METHODS FOR ON-FARM EUTHANASIA OF COMMERCIAL MEAT RABBITS

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Dr. Patricia V. Turner

There are no validated euthanasia methods for meat rabbits. The goal of this research was to evaluate blunt force trauma (BFT), assisted manual cervical dislocation (AMCD), a non-penetrating captive bolt (NPCB) device and two fill rates of carbon dioxide (CO₂) across several age groups of rabbits as methods for on-farm euthanasia. Physical methods were evaluated for their ability to cause immediate sustained insensibility until death occurred. Chamber fill rates for CO₂ euthanasia gassing were compared based on signs of distress prior to loss of sensibility, time to insensibility and time to death. A 58% CO₂ displacement rate resulted in a significantly shorter time to insensibility and death compared to a 28% displacement rate, with neither rate inducing significant distress behaviours. Our findings indicate that AMCD, a specific NPCB device, and CO₂ inhalation at the rates studied are suitable methods for on-farm rabbit euthanasia, while blunt force trauma is ineffective.
Acknowledgements

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Declaration of Work Performed

All the work presented in this thesis was performed by me except for the following:

1. Aaron Percival assisted with data collection and data entry for the physical euthanasia trials (Chapter 2).
2. Survey radiographs were conducted at the OVC Health Sciences Centre (Chapter 2).
3. Brains from rabbits euthanized by physical euthanasia methods were trimmed by Aaron Percival, made into microscope slides by the Animal Health Laboratory, University of Guelph, and then assessed microscopically by a veterinary pathologist, Patricia V. Turner (Chapter 2).
4. Brianne Mercer assisted with data collection and data entry for the gassing trials (Chapter 3).
5. John Van de Vegte (OMAFRA) assisted with design and in vitro testing of equipment for gassing trials (Chapter 3).
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### Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMCD</td>
<td>Assisted-manual cervical dislocation</td>
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<tr>
<td>AVMA</td>
<td>American Veterinary Medical Association</td>
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<td>BFT</td>
<td>Blunt force trauma</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CD</td>
<td>Cervical dislocation</td>
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<tr>
<td>CO$_2$</td>
<td>Carbon dioxide</td>
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<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>d</td>
<td>day</td>
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<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
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<tr>
<td>GH</td>
<td>Gross hemorrhage</td>
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<tr>
<td>h</td>
<td>Hour</td>
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<tr>
<td>L</td>
<td>Litres</td>
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<tr>
<td>MH</td>
<td>Microscopic hemorrhage</td>
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<td>min</td>
<td>minutes</td>
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<tr>
<td>mo</td>
<td>month</td>
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<tr>
<td>N</td>
<td>Sample size</td>
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<tr>
<td>No.</td>
<td>Number</td>
</tr>
<tr>
<td>NPCB</td>
<td>Non-penetrating captive bolt</td>
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<tr>
<td>O$_2$</td>
<td>Oxygen</td>
</tr>
<tr>
<td>P</td>
<td>Probability value</td>
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<tr>
<td>PCB</td>
<td>Penetrating captive bolt</td>
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<tr>
<td>s</td>
<td>seconds</td>
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Chapter 1: Literature Review

1.1 Introduction

Commercial meat rabbit industry

Globally, the commercial meat rabbit industry produces 1.2 billion rabbits for meat each year (FAOSTAT, 2012). The industry is highly region-specific and is based on demand, with China producing the most rabbit meat in overall production. In Europe, commercial meat rabbits are the second largest livestock industry by number next to poultry, with France, Spain, and Italy dominating most of this market (FAOSTAT, 2012). In Canada, approximately 500,000 rabbits are raised for meat each year and Ontario is the largest producing province in Canada (AAFC, 2014). Rabbits that are raised for meat are termed growers. Juvenile rabbits are termed kits and are weaned from their dams, the doe, at approximately 5 weeks of age, at which time they are regrouped and housed with other growers until they reach market weight at 2.5kg around 11-12 weeks of age (Ontario Rabbit, 2013). A buck is a mature male rabbit that is kept for breeding purposes. The purpose of this literature review is to evaluate the published information on different methods of euthanasia to determine their effectiveness for on-farm euthanasia across different age groups of commercial meat rabbits.

Euthanasia

The term euthanasia describes a method of humanely killing an animal that minimizes or eliminates pain and distress (AVMA, 2013). Preferred killing methods are ones that affect the brain first, rapidly rendering the animal insensible (unconscious), followed by death (AVMA, 2013; CVMA, 2014). Herd veterinarians are encouraged to guide their clients through end of life decisions, but are not always present when such decisions are being made (Turner and Doonan, 2010). In such cases, euthanasia becomes the responsibility of the individual producer. Euthanasia is conducted on-farm for a variety of reasons including rabbits that are sick or injured and that do not respond to treatment, unthrifty or runt rabbits that will not survive to market.
weight, and removal or culling of rabbits that are no longer needed for breeding and reproduction and are not profitable or fit to send to slaughter. Ontario commercial meat rabbit mortality rates sit around 33% for breeding does, 15% for pre-weaned kits, and 7% for growers from weaning to slaughter (Kylie et al., 2016). To minimize suffering, sick and injured animals must be identified and euthanized in a timely manner. When choosing a euthanasia method, consideration must be given to ensure that the method is reliable, effective, practical, safe, easy to use, and as esthetically pleasing as possible. Although esthetics should not be the primary consideration when choosing a method, as it has no direct effect on the animal being euthanized, it is an important consideration for personnel. Euthanasia is a challenging task and in some situations it can be hard to rationalize, leading to detrimental mental effects on the operator and any observer (Whiting and Marion, 2011). A producer might also be resistant to euthanizing a sick animal if the method is esthetically displeasing (Walsh et al., 2016).

The method selected for euthanasia needs to minimize animal distress, something best accomplished by rendering the animal rapidly insensible. Producers need effective, humane methods to be validated for use. They also need to ensure that the method is applied correctly such that the animal is rendered insensible and does not recover. To date there is no on-farm euthanasia research available specific to meat rabbits so this literature review will borrow research from other species and from other uses of rabbits.

1.2 Assessing Insensibility, Degree of Brain Injury, and Time to Death for Euthanasia Methods

The brain and consciousness

Consciousness or sensibility describes a state of wakefulness or awareness in which sensations, sights, sounds, and feelings register within an animal (Turner and Knapp, 1995; Verhoeven et al., 2015a). Sensibility relies on a system of sensory inputs and outputs from specific areas in the brain: the cerebral cortex, thalamus, and brainstem (Verhoeven et al., 2015a). Within the brainstem is a large neural network, the reticular formation, feeds information to the cerebral cortex. The reticular formation occupies most of the central and dorsal part of the
brainstem, extending from the lower medulla to the upper midbrain (Terlouw et al., 2016). It is an essential component of sensory input and is a targeted brain structure for causing insensibility. The brainstem also controls vital functions, such as breathing, heartbeat, and homeostasis (Terlouw et al., 2016).

Insensibility refers to the inability to perceive sensory inputs. Pain is a conscious experience and the intention of using a validated euthanasia method is for any associated pain and distress to be minimized or eliminated (Antognini et al., 2005; AVMA, 2013; CVMA, 2014). This outcome can best be achieved by a rapid onset of insensibility, as activity in nervous system pathways sent by harmful or noxious stimuli is not pain until it is interpreted by the brain and becomes a psychological state (Antognini et al., 2005). Insensibility can be achieved by damage to the reticular formation, ascending reticular activating system, the thalamus, or the cerebral hemisphere on a large scale (Terlouw et al., 2016). Neurological events involved in insensibility include lack of oxygen to the brain (anoxia), inadequate blood supply (ischemia), depolarization, acidification and destruction of neurons (Terlouw et al., 2016).

There are multiple indicators that can be used to assess insensibility. The following section will describe these in further detail. The focus of this research is to find validated indicators of insensibility in rabbits that can be used on-farm by producers to confirm the success of their euthanasia methods.

**Electroencephalogram**

An electroencephalogram (EEG) is a tool used to measure electrical brain waves to determine neurophysiologic activity. Electrical activity can be evaluated non-invasively by placing metal electrodes on the scalp surface or more invasively by placing electrodes directly on the cortical surface of the brain (Teplan, 2002). Neurophysiologic activity is recorded from electrical signals and categorized based on wave amplitude and frequency (Teplan, 2002; Gibson et al., 2009). Providing a direct measure of brain activity makes an EEG one of the most accurate methods for determining onset of insensibility. An EEG is a research tool, but an impractical method for routine use by producers to check for insensibility after application of a method on-
It is also not practical to use an EEG to assess methods causing physical damage to the brain due to the risk of damaging expensive EEG equipment. One study on chickens and turkeys did use an EEG to examine the effect of blunt force trauma (BFT) on insensibility (Cors et al., 2015). An animal can be considered insensible if conscious responses to auditory-evoked potentials and visual-evoked potentials are no longer recorded by the EEG (Cors et al., 2015). In the poultry study brain activity was recorded for one minute before application, but was removed during application to protect the equipment, and reapplied seconds afterwards (Cors et al., 2015). The removal and reapplication of equipment and the electrical noise created from convulsions makes it difficult to interpret these results. Thus, it is preferable to use an EEG to validate other indicators of insensibility, such as brainstem and spinal cord reflexes, which do not require specialised equipment (Sandercock et al., 2014; Verhoeven et al., 2015b). In rabbits, this EEG validation approach has been used to validate loss of posture, when rabbits were exposed to CO$_2$ (Dalmau et al., 2016).

**Brainstem and spinal cord reflexes**

Cranial nerve reflexes stem from specific nervous system signalling pathways within the brainstem, so called brainstem reflexes. Spinal cord reflexes are motor responses to a sensory evoked stimulus, such as pain, and loss of these reflexes is used as an indicator of insensibility. The presence of an expected reflex response indicates that the pathway is intact, the brainstem is functional, and the animal remains sensible. Research on rabbits examining the time to insensibility has used brainstem reflexes such as the corneal, pupillary, nose prick, and palpebral reflexes (Dennis et al., 1988; Schutt-Abraham et al., 1992; Hellebrekers et al., 1997; Anil et al., 1997; Anil et al., 2000; Maria et al., 2001; Nodari et al., 2008; Li et al., 2012; Dalmau et al., 2016). The corneal reflex evaluates the response to touching the surface of the eye. There is varying success when using this reflex as a sole indicator of insensibility in rabbits. Maria et al. (2001) used brainstem reflexes in their studies of electrical stunning and determined that the corneal reflex did not reliably differentiate between sensibility and insensibility, concluding that it should not be used independently in rabbits. In contrast, Nodari et al. (2008) concluded that the corneal reflex was the best indicator of recovery of sensibility in rabbits. These researchers
evaluated effectiveness of electrical stunning in a Spanish abattoir, and observed that rabbits that fully recovered to sensibility had an earlier positive response to the corneal reflex. However, caution should be used when validating methods from electrical stunning trials. Other studies have suggested that brainstem reflexes are present long after electrocution and in animals confirmed insensible via EEG, perhaps due to damage to areas other than the brainstem (Vogel et al., 2011; Verhoeven et al. 2015a). The conclusion from these studies is that the corneal reflex disappears at a deep stage of anesthesia and depends on damage to the brainstem. The presence of a corneal reflex does not necessarily indicate sensibility, but its absence is strongly correlated with insensitivity (Cors et al., 2015). Use of the corneal reflex is appropriate for testing for sensibility, but if it is present after a euthanasia attempt, another method should be used to confirm sensibility.

Another brainstem reflex is the pupillary reflex, which involves shining a light into the eye and evaluating pupillary constriction or avoidance in response to a bright light. The pupillary reflex disappears earlier than the corneal reflex (Li et al., 2012). Li et al. (2012) evaluated the strength of force applied during application of blunt force trauma to rabbits. They found that when insufficient force was applied, the pupillary response was absent, but the corneal response and other withdrawal responses were weak, but still present. The pupillary reflex could be used in combination with the corneal reflex to ensure insensibility but is insufficient on its own for monitoring insensitivity.

Nose prick is a cranial nerve reflex that has been used for rabbits by pricking the inside of the nose with a sharp object, and examining head withdrawal in response. Anil et al. (1997) discovered that a response to the nose prick stimulus did not occur until after the corneal reflex and rhythmic breathing had returned, and only seconds before the animal regained posture. Maria et al. (2001) also determined that response to nose prick was not a reliable indicator of sensibility as most rabbits regained posture before a response occurred. It is thought that perhaps this area is not sensitive enough in rabbits (Maria et al., 2001). Based on this information, withdrawal response to nose prick returns too late to be a useful tool to assess insensibility in rabbits undergoing euthanasia.

The palpebral reflex evaluates the response to tapping around the eye near the eyelid with blinking being the expected conscious response. This reflex has not been used previously in
studies assessing for insensibility of rabbits after applying a stunning or euthanasia method, but has been used in veterinary medical research examining depth of anesthesia and for neurologic exams. The corneal reflex and palpebral reflex have not proven useful as indicators of depth of anesthesia because of the variability in their rate of disappearance (Hellebrekers et al., 1996). Checking for insensibility during surgery differs from evaluating irreversible insensibility after application of a euthanasia method. The limited usefulness of these reflexes for examining anesthetic depth does not infer that they cannot be used to check for insensibility after a euthanasia method is applied, but that they should be validated first. Response to ear and toe pinch have been effective measures when evaluating depth of anesthesia in rabbits, and are suggested to be good reflexes to use to judge insensibility (Hellebrekers et al., 1996; Hedenqvist et al., 2002). These reflexes could be used in combination as a tool for producers to assess insensibility.

**Physiologic measures**

Possible physiologic measures of insensibility for commercial meat rabbits include rhythmic breathing, pupil diameter change, loss of posture, and convulsions. Rhythmic breathing, which refers to the normal pattern and frequency of breathing, has been observed to be the first sign of return to sensibility for rabbits by researchers evaluating electric stunning (Anil et al., 1997; Maria et al., 2001) and CO₂ stunning (Dalmau et al., 2016). Nodari et al. (2008) found rhythmic breathing to be an impractical indicator following electric stunning because bleeding from the nose or mouth made observations of the nostrils difficult. Dalmau et al. (2016) observed it to be the first sign of regained sensibility, with rhythmic breathing returning before the basal EEG signal. It is predicted to return before an EEG signal because breathing is controlled by the brainstem while depending on electrode placement an EEG typically measures cortical activity. If external bleeding from the nose is minimal, the normal nostril movements that are seen during rhythmic breathing could be used as a reliable tool to assess onset of insensibility on-farm.

Pupillary diameter has been evaluated as an indicator of insensibility in rabbits without success. Changes in pupillary diameter following BFT are inconsistent during the time between
insensibility and death (Li et al., 2012). Pupillary dilatation is thought to occur in response to reduced and then absent blood flow, and can be used as a surrogate marker of cardiac arrest (Larson and May, 2002). Pupil dilation could be used to confirm death but not as a measure of insensibility. Involuntary whole body movements, referred to as clonic and tonic convulsions, can occur after loss of sensibility. In broiler chickens, a correlation was found between the end of convulsions and time of brain death, as indicated by a flat line EEG (Dawson et al., 2009). In a study by Dalmau et al. (2016) EEG recordings of insensibility after rabbit CO₂ stunning, all convulsions occurred after loss of sensibility. The earliest observed indicator of insensibility in rabbits following CO₂ gassing for stunning is loss of posture (recumbency) (Llonch et al., 2012; Dalmau et al., 2016). Dalmau et al. (2016) also found that loss of posture coincided with an EEG reading of loss of sensibility. Chisholm and Pang’s (2016) work comparing different measures of loss of sensibility in rats following multiple common euthanasia practices determined that loss of posture is an inaccurate measure of insensibility occurring before true loss of sensibility. Loss of righting reflex was a more reliable estimation of loss of sensibility in rats and in a majority of their trials loss of posture occurred before loss of righting reflex. Loss of posture is a validated indicator that can be used in CO₂ stunning trials on rabbits to predict onset of insensibility but loss of righting reflex might be a more sensitive measure.

**Behaviour**

In situations where it is not possible to directly interact with the animal to test reflexes, behavioural indicators of sensibility must be used. In a study evaluating rabbit exposure to CO₂, response to a loud noise (auditory-evoked sensation) was used to assess sensibility (Hayward and Lisson, 1978). The behavioural indicator used in response to the noise was not specified by Hayward and Lisson (1978) and it was not used in combination with another measure to confirm insensibility, limiting its validity. Vocalization is another possible behavioural indicator, as rabbits only vocalize purposely when conscious and experiencing marked pain or distress (Mayer, 2007). Response to noise, vocalization, and other indicators of pain can be used to verify that the animal is sensible. Behavioural indicators are not as reliable as other brainstem and
spinal cord reflexes, and physiologic measures for evaluating insensibility in rabbits as they may be challenging to interpret.

**Scoring brain damage**

Scoring visual damage to the regions of the brain responsible for sensibility and vital functions can be used to confirm method efficacy and cause of death. Regions of the brain responsible for sensibility include the cerebral cortex, thalamus, and brainstem (Verhoeven et al., 2015a). Hemorrhage in the brain is indicative of ruptured blood vessels, internal brain injury, and inadequate delivery of blood containing nutrients and oxygen to the brain (Erasmus et al., 2010b). Insensibility is associated with multifocal brain damage, specifically diffuse axonal injury (Finnie, 2001). In human medicine, the most common predictors of a coma and thus insensibility are intracerebral hematoma, subarachnoid hemorrhage, and subdural hemorrhage (Fabbri et al., 2008). Hemorrhage that results from a physical euthanasia method is commonly scored on a macroscopic and microscopic level as total damage to specific areas of the brain (for example, Finnie et al., 2000; Finnie et al., 2003; Erasmus et al., 2010b; Casey-Trott et al., 2013; Casey-Trott et al., 2014). Hemorrhage is generally scored grossly based on different injury categories using the percentage of area that the hemorrhage covers (Veltri and Klem, 2005). This scoring system was originally used to compare fatal bird injuries from collisions with towers and windows (Veltri and Klem, 2005), but has been adapted and slightly modified to score injuries following application of physical euthanasia methods, such as blunt force trauma, cervical dislocation, and non-penetrating captive bolt devices (Erasmus et al., 2010b; Casey-Trott et al., 2013).

Degree of brain damage has also been found to be correlated with the effectiveness of the euthanasia method. Li et al. (2012) found a correlation between the extent of parenchymal injury and mortality in rabbits after BFT. Erasmus et al. (2010b) proposed that macroscopic subdural hemorrhage, microscopic subarachnoid and parenchymal hemorrhage, can be used as follow up evidence for rapid insensibility in turkeys. In a study validating a non-penetrating captive bolt (NPCB) device, piglets were rendered immediately insensible with successful progression to death and had moderate to marked microscopic subdural and parenchymal hemorrhage scores in
at least one section (cerebral cortex, midbrain including the thalamus, and the brainstem) (Casey-Trott et al., 2013). In comparison, all piglets survived application of the NPCB device in a study by Finnie et al. (2003). These piglets had only mild and localized microscopic brain hemorrhage (Finnie et al., 2003). This research suggests that conclusions about sensibility, technique irreversibility, and brain death can be augmented by scoring gross and microscopic hemorrhage. Assessment of hemorrhage is a useful tool and can also provide information on whether localized or widespread damage occurred. Contusions are localized damage in the form of ruptured blood capillaries and appear as micro hemorrhages beneath the impact site (coup) or opposite to the site of impact (contrecoup). Widespread hemorrhaging and ventral brainstem hemorrhage illustrate that adequate concussive force was applied to physically jolt the brain within the cranium, and these findings are generally, although not always, associated with loss of sensibility (Gaetz, 2004).

Caution should be used when making conclusions about subcutaneous hemorrhage. In experiments by Erasmus et al. (2010b), cervical dislocation (CD) (stretching of the spinal cord and associated vertebral blood vessels) did not result in immediate insensitivity and there was no direct damage to the brain, but there was significant subcutaneous and subdural hemorrhage. It was suggested that when a method ruptures the carotid arteries, blood flowed under the scalp as well as pooled around the brain (Erasmus et al., 2010b). For methods that cause injury to areas other than just the brain, scoring subcutaneous hemorrhage may not be a useful parameter.

Another method used to directly assess damage caused by physical euthanasia methods is examination for skull fractures. Skull fractures have been used to score damage caused by BFT and the NPCB device (Erasmus et al., 2010b; Casey-Trott et al., 2013). Scoring hemorrhage on a macroscopic and microscopic scale and examining for skull fractures are all direct measure of damage to the brain and correlates to the success of the method. These methods are only useful for physical methods of euthanasia. Insensibility can occur via a different physiologic route, such is the case for inhaled gasses or injectable substances like pentobarbital.

Based on available research on indicators of insensitivity, it would seem that various indicators are best used in combination. Brainstem reflexes, spinal cord reflexes, physiologic measures, and behavioural responses can all be used by the operator to confirm successful application of a method. In rabbits, the corneal and pupillary reflexes can be used together to
assess brainstem reflexes. Spinal cord reflexes could be used in a similar way, but none have been validated for use on rabbits (Chisholm and Pang, 2016; Dalmau et al., 2016). Cessation of convulsions is correlated only with brain death in poultry. Rhythmic breathing is a sign of sensibility or that sensibility might return shortly. Loss of posture during CO$_2$ exposure is a validated indicator of loss of sensibility in rabbits but loss of righting reflex might be a more sensitive method. These indicators can be used in rabbits, but should not be used on their own. Behavioural responses are harder to interpret but are more direct indicators of the conscious experience of the animal. Electroencephalograms and histopathology scoring for brain damage are limited to experimental studies evaluating the effectiveness and humaneness of killing methods used on-farm, and can be used to validate other measures.

1.3 Euthanasia Methods and Modes of Action

1.31 Physical Methods

Physical methods of euthanasia cause direct destruction to the brain and spinal cord. The physical methods that will be discussed for rabbits are blunt force trauma (BFT), cervical dislocation (CD), decapitation, electrocution, and non-penetrating and penetrating captive bolt devices (NPCB, PCB). When conducted correctly by a well-trained and competent operator, physical methods have the ability to induce immediate insensibility (AVMA, 2013). The operator’s knowledge, experience, physical strength, and level of fatigue are factors that play a major role in the humane application of physical euthanasia methods.

Blunt Force Trauma

According to the American Veterinary Medical Association (AVMA), the correct method for BFT is a sharp blow to the central skull with sufficient force to cause immediate depression of central nervous system function and destruction of brain tissue. Assessment of the anatomical features of the species is important to ensure that impact is targeted to the correct location on the
head and that the skull can be breached with the applied force. For this reason BFT is only recommended for neonates with a thin cranium. Neonatal calves do not meet this requirement, and BFT is deemed an inappropriate method for this species (AVMA, 2013). Blunt force trauma is not listed as an AVMA-approved method for any age group of rabbits. This is not because it is an unapproved method for rabbits, rather because the AVMA Guidelines on Euthanasia have never covered commercial meat rabbits and there are more appropriate methods available for laboratory and companion rabbits, such as intravenous barbiturate overdose. It is also important to note that the Ontario Ministry of Agricultural Food and Rural Affairs banned the use of BFT for stunning meat rabbits in Ontario abattoirs in 2005, as it was thought to be an inconsistent method and thus was deemed inhumane (OMAFRA, 2016).

No formal research to date has been conducted to evaluate the use of BFT for any age group of rabbits. Research has been conducted using rabbits as a BFT brain injury model for humans, because of anatomic similarities. This work provided some useful information, such as the amount of applied force needed to induce skull damage, and the resulting pathology. In an experiment by Li et al. (2012), a machine operated iron bar (diameter=14mm, length=80mm, and weight=85g) was applied on the sagittal axis of the rabbit’s head aligned with the top of the brain. Blunt force trauma was applied using this device in 10 adult (2.2 ± 0.2kg) rabbits in the mild injury group at a force of 65psi and in 10 adult rabbits in the marked injury group at 87psi. The data on BFT from this experiment is particularly useful because a machine controlled for correct anatomic location and force, aspects that are difficult to ensure when the technique is conducted manually by an operator on a live animal. At an applied force of 65psi rabbits were not immediately rendered insensible, but at an applied force of 87psi, they were. Rabbits in the mild injury group were also less likely to die from their brain injuries with only 10% mortality compared to 60% mortality in the marked injury group (Li et al., 2012).

Differences in brain damage was assessed as present or absent, allowing correlations to be made between applied force, onset of insensitivity, and mortality. When BFT was applied at a force insufficient to cause immediate insensitivity and unlikely to cause death, subdural and subarachnoid hemorrhage were still present, thus subdural and subarachnoid hemorrhage may not be indicative of effective BFT application. In this study, hemorrhage was only assessed as present or absent, and not scored by a pathologist thus lacking in specificity. Skull fractures
might be a good indicator of effective application of BFT as these were present in all rabbits in the marked injury group with minimal skull fractures found in the mild injury group. High mortality in the marked injury group was positively correlated with extensive parenchymal injury (Li et al., 2012).

Respiratory recovery time, survival time, and degree of brain damage were significantly different between rabbits in the two groups. In the mild injury group, rabbits returned to breathing faster than those in the marked injury group (Li et al., 2012). Return to rhythmic breathing is significant as it indicates that none of the rabbits in this group were irreversibly insensible. The experimenters intervened after 1 min to give assisted respiration to those rabbits that were not spontaneously breathing. This intervention might have affected the probability of recovery to sensibility as well as survival time; however the purpose of this research was to develop an animal model. The paper did not mention the precise number of rabbits that returned to breathing or those that returned to breathing but still died. This would have been interesting information to draw conclusions about the effectiveness of BFT at a force of 87psi, and correlations between reversible insensibility and brain damage.

Despite blunt force trauma being widely used on livestock, the practicality and effectiveness of its use has only been formally evaluated in turkeys and piglets, and never meat rabbits (Erasmus et al., 2010a; Whiting et al., 2011). Erasmus et al. (2010a) examined the effectiveness of BFT on-farm for turkey euthanasia. They compared two devices; a metal pipe and metal bat, and found no difference in effectiveness. They did find a difference across the age groups, in that BFT needed to be reapplied 22% of the time when applied to turkey broilers (3.9kg) and only 3% of the time when applied to turkey toms (13.1kg) (Erasmus et al., 2010a). Possible explanations are a smaller head and therefore smaller targets for broilers (Erasmus et al., 2010a) or because of differences in skull thickness and development. Whiting et al. (2011) evaluated the use of BFT on weaned piglets and concluded that the method was difficult to apply in practice, had a high method failure rate (24% failure rate, n=50) and was esthetically displeasing to personnel. Blunt force trauma has the potential to induce immediate insensibility, if correct force and application are further examined on a per species basis. However, a key condition, regardless of species, is excellent restraint of the animal to ensure that the target is struck accurately.
Blunt force trauma efficacy is limited by technical aspects of force and location, as well as operator aspects of skill, experience, fatigue and esthetics. Uncertainty of success often results in repeated application and can result in delay in using the technique (Whiting et al., 2011; Walsh et al., 2016). For these reasons, the AVMA recommends that personnel using this method seek alternative options. This method is practical for on-farm euthanasia as it does not require specialized equipment or drugs. If force is sufficient and makes contact with the targeted location in the first attempt, this method could be used for on-farm euthanasia of commercial meat rabbits.
### Cervical Dislocation

Cervical dislocation involves stretching the neck, to separate the vertebral column from the cranium, damaging the spinal cord, and rupturing cervical and cranial blood vessels (Sparrey et al., 2014). Cervical dislocation can cause insensibility through direct damage to the brainstem or lack of blood flow to the brain (Terlouw et al., 2016). Cervical dislocation is listed as acceptable with conditions by the AVMA for use on laboratory rabbits. The condition is that the rabbit must weigh <1kg, as older, heavier rabbits have a large neck muscle mass making manual stretching physically difficult for the operator (AVMA, 2013). Similarly the Canadian Council on Animal Care does not allow CD for rabbits greater than 2 kg (CCAC, 2010). Cervical dislocation is a physical method in which operator fatigue can cause errors in application. For this reason the Council of the European Union has set a limit for CD on poultry at 70 birds per person per day (Sparrey et al., 2014).

A device can be used to assist operators with CD, reducing the strength needed to create a clean dislocation. In mice, a closed hemostatic clamp or pair of forceps can be placed at the base of the skull for assistance. Carbone et al. (2012) compared different CD techniques commonly used for mice and found that 21% of the time overall when conducted by experienced operators CD was unsuccessful. When conducting manual CD (thumb at the base of the skull) or hemostat-assisted CD in mice, lesions occurred in locations other than just the cervical area. Only when using the technique of pushing the head away and twisting did damage result only in the desired location (Carbone et al., 2012). For mice it appears that devices do not assist with correct technique.

In poultry, tools such as a heavy stick or broomstick, is often used to assist with CD. The tool is placed over the back of the bird’s neck while resting on a flat surface and held in place by the operator standing on it on either side of the animal’s neck. The device allows for extra applied force from the legs and back muscles as the operator pulls the bird straight up to dislocate the neck. The disadvantage of this method is there is a period of time in which the bird is choking prior to CD (Sparrey et al., 2014). Commercial meat rabbit producers reported using similar tools, such as a broomstick, to assist with CD (Walsh et al., 2016).
Another device that has been used to assist with poultry CD is the burdizzo (crushing device used for permanently damaging the spermatic cord in bull calves in lieu of surgical castration). The concern with such a device is that crushing the vertebrae often causes a fracture versus stretching the neck to cause a dislocation. This is also a concern for the previously mentioned technique where standing on a tool could result in inhumane vertebral crushing prior to death or loss of sensibility. Bader et al. (2014) compared manual CD on chickens and non-penetrating forceps (similar in concept to the burdizzo) on turkeys, both stunned first with BFT. In all animals, a dislocation occurred. The incidence of fractures and the location of fractures were similar between groups as was the chance of the dislocation occurring between C1 and C2 vertebrae versus the base of the skull and C1, where it is supposed to occur (Bader et al., 2014). Although the study compared different species of poultry, it demonstrated that manual CD and non-penetrating forceps had the same probability of incorrect placement and risk of fractures. There is no available research addressing the effectiveness of using a device to assist with CD of rabbits. Research from other species suggests using survey radiographs as a follow up to ensure that vertebral crushing or fracture is not occurring (Erasmus et al., 2010b; Carbone et al., 2012).

The CCAC emphasises the importance of manual palpation after performing CD in any species, to ensure spinal cord damage and vertebral separation have occurred (CCAC, 2010). In a study by Cartner et al. (2007) operators palpated the neck after application of CD on anesthetized mice to judge whether they had caused a clean dislocation, which was then confirmed with a radiograph. Ten percent of the time there was no dislocation at the base of the skull even though operators reported to have felt it. The vertebral separation distance after dislocation is quite significant in some species, making it easier to palpate. For example, in chickens this distance is approximately 3cm (Bader et al., 2014). If the vertebral separation distance after dislocation is similarly broad in rabbits, palpation could be used to evaluate technique effectiveness.

One concern with CD is it does not consistently cause immediate insensibility. In broiler turkeys, the nictitating membrane (brainstem reflex) did not disappear before 43s (Erasmus et al., 2010a). Current slaughter practice for guinea pigs in the Andean region is CD in combination with exsanguination. Limon et al. (2016) evaluated insensibility after such practice and observed that 97% of guinea pigs retained at least one behavioural or cranial nerve/spinal reflex response immediately after application, concluding that CD should not be recommended as a slaughter
method for guinea pigs. The use of exsanguination with CD limits the conclusions that can be drawn about CD alone. No studies have been conducted in rabbits to indicate how long sensibility persists after CD. Limited cerebral ‘concussion’ is thought to be caused by the acceleration or deceleration of the head during CD causing nervous tissue compression and fragmentation (Shaw, 2002; Li et al., 2012). Concussive force is hypothesized to underlie immediate insensibility with CD and depends on technique (Sparrey et al., 2014).

It is not possible to extrapolate whether CD is an effective method for on-farm euthanasia of rabbits. The AVMA suggests that manual CD can be used in juvenile rabbits. For adult rabbits with a larger muscle mass device-assisted CD might be a better option because it allows more force to be applied. Applied correctly, the device should cause a clean dislocation of the skull from the vertebral column. In mice, CD is difficult to assess accurately via palpation and it is unknown whether this is also the case for rabbits and whether technique varies with age and weight. For CD to meet the requirements of euthanasia, it needs to induce rapid insensibility. Research in other species suggests that this does not always occur (Erasmus et al., 2010; Martin et al., 2016; Limon et al., 2016). To date, there is no research on CD in rabbits of any age and the research available on other species does not suggest it would be an appropriate method for on-farm euthanasia.

Decapitation

Decapitation involves using a sharp instrument or automated device to completely sever the head from the body. The blade must be maintained in good working order to ensure a clean, rapid cut. Ability to properly restrain the animal and apply this method safely is a concern (CCAC, 2010; AVMA, 2013). Another concern is the potential length of time that brain activity persists after the head has been separated from the body. In rats, sensibility is rapidly lost after decapitation but EEG activity consistent with consciousness is observed for up to 50s, making it unclear what the animal experiences after application (Van Rijn et al., 2011). There is no research available on decapitation as a method of euthanasia for rabbits. Decapitation is approved by the AVMA for small and neonatal rabbits (AVMA, 2013). Concerns with using this
as an on-farm method of euthanasia are esthetics, safety, as well as biosecurity and sanitation concerns with blood loss.

**Electrocution**

Electrocution can be used to render an animal insensible by passing an electrical current through the brain, resulting in massive depolarization of neurons in both cerebral hemispheres (Terlouw et al., 2016). Electrocution can kill an animal if the current is also sent through the heart inducing cardiac fibrillation (Denicourt et al., 2010). For euthanasia purposes both need to occur so that the animal remains insensible until the heart stops. This can be achieved with one-step-head-to-body-electrocution, in which the current simultaneously passes through the head and heart (Denicourt et al., 2010).

In Europe, electrocution is the most common pre-slaughter stunning method used for rabbits in abattoirs (Lafuente and Lopez, 2014). The device used is a “V” shaped metal electrode with jagged edges for better contact with the head (Maria et al., 2001). The two arms of the device make contact with the rabbit’s frontal sinus, between the eyes and ears. Research has focused on the appropriate voltage, current, and duration of application to stun rabbits, but has not examined electrocution as a euthanasia method for rabbits. Anil et al. (1997) determined that an appropriate current is more critical than voltage, recommending a minimum of 140mAmp. A well-designed device will not operate unless it is at the minimum current to limit unsuccessful applications (Nodari et al., 2008). Nodari et al. (2008) observed 1020 rabbits being stunned with this method at a Spanish slaughterhouse, and noted incorrect application in 110 rabbits (10.8%). Failure was often due to incorrect placement such that the current was applied too far down the head, missing the brain and passing through the sinuses, or when the animal resisted and a forelimb got in the way. Although electrocution is a commonly used stunning method in Europe, it has not been perfected and potential safety concerns have limited industry adoption in Canadian abattoirs.

To date there has been no research on one-step-head-to-body-electrocution use for euthanasia of rabbits. A purpose-built device would need to be designed and validated.
Appropriate restraint and operator safety are concerns. Electrocution might be limited in effectiveness on dehydrated animals (Grandin et al., 2010), reducing its usefulness as an on-farm euthanasia technique. Due to logistical and safety reasons this is not a method being considered for on-farm euthanasia of rabbits in this project.

**Captive Bolt**

*Penetrating Captive Bolt*

The penetrating captive bolt is designed to apply kinetic force to the skull resulting in mechanical destruction of the brain. Concussion of the skull causes shock waves to travel through the brain leading to detrimental tears and lesions, and increased intracranial pressure from hemorrhage. Passage of the bolt into the brain is intended to cause damage and reach the reticular formation or ascending reticular activating system leading to insensitivity (Terlouw et al., 2016). The AVMA (2013) lists the PCB device as acceptable with conditions for laboratory rabbits. Conditions include correct positioning and operator safety. At least two PCB devices are marketed for use on rabbits. The devices are commonly cartridge-, spring- or elastic-powered. The Dick KTBG (Friedr. Dick GmbH and Co., Deizisau, Germany) is a spring-powered model that has been evaluated for use on rabbits (Schutt-Abramah et al., 1992) and kangaroo joeys (Sharp et al., 2014). This is the only PCB device designed for use on small mammals that has been studied for its ability to cause insensitivity and death. The device produces 4.88J of kinetic energy with a bolt velocity range of 8.4-9.4mm/s and a mean depth of penetration into a kangaroo joey’s skull of 28.3mm (Sharp et al., 2014). The bolt is 4.7mm in diameter and 30mm long.

When considering if this device could be used for on-farm euthanasia there are a number of considerations. The length of the bolt, skull thickness, and correct placement are factors that limit the bolt’s ability to cause damage to the parts of the brain responsible for sensibility and death. A PCB device is commonly used in slaughter facilities to stun cattle. A modified device for cattle euthanasia shoots 15psi of air through the end of the bolt to cause more extensive brain
damage (Derscheid et al., 2016). Assessing these potential limiting factors will determine if the PCB is suitable to euthanize commercial meat rabbits.

Skull thickness has a direct impact on the penetration of the bolt, and therefore degree of fatal physical destruction to the brain. The rabbit skull is thickest along the midline at 3 mm, but slightly paramedian thickness is only 1.8 mm (Schutt-Abraham et al., 1992). Skull thickness impacted Schutt-Abraham et al. (1992) findings such that the best stunning results were obtained when applying the PCB device into the parietal bone near the sagittal line without hitting the bone sutures. This led these researchers to suggest that the target location on a rabbit when using a PCB device is slightly paramedian and as close to the ears as possible (Schutt-Abraham et al., 1992). Assessment of joey cadavers determined that the skull is thinnest (1 mm) at the highest point on the head midline, leading the researchers to conclude that this was the most appropriate target location (Sharp et al., 2014).

Precise placement of the bolt on the head of an animal is of critical importance to ensure the bolt penetrates into the brain, causes a large degree of damage and hits the targeted location of the reticular formation. When testing the PCB device as a euthanasia method on sheep, marksmanship was a significant factor in method efficacy (Gibson et al., 2012). Placement too far caudally on any animal results in the bolt penetrating the nasal cavity instead of the brain. Sheep that were not immediately insensible had been shot in the incorrect location due to poor marksmanship. In extreme cases the bolt penetrated only the thick pad of tissue above the sheep’s skull, barely grazing the skull and leaving the brain untouched.

Operation manuals for use of the PCB device on rabbits vary in their suggested application location. Some vaguely describe the site of application to be high on the forehead, others suggest the targeted area to be a finger width above the eyes and some suggest that the PCB device should be applied between the ears (Holtzmann and Loeffler, 1991). Schutt-Abraham et al. (1992) compared application in two targeted areas; between the ears (applied from behind the rabbit angled forward) and at a cross section between the eyes and the ears. A more successful stun rate was achieved when the PCB was applied between the ears into the parietal bone (Schutt-Abraham et al., 1992). Dennis et al. (1988) applied a cartridge-powered PCB device at the crossing point midway between the eyes and ears on five 2.5-5kg rabbits. This resulted in immediate loss of the corneal reflex and cessation of breathing in 4 of 5 rabbits.
(Dennis et al., 1988). Low study numbers and lack of comparison between locations make it challenging to compare this study to that of Schutt-Abraham et al. (1992). There is a lack of information to suggest an optimal location bolt placement on a rabbit to ensure death. Based on these results it is suggested that for the best stunning results the correct placement of the PCB device is on the parietal bone as close to the ears as possible and slightly paramedian.

Placement of the PCB device should occur where the skull is the thinnest, but at a point where the bolt can travel the full length of the brain into the reticular formation. These discrepancies and requirement for location judgement create a high risk of error leading to an ineffective application of this method (Sharp et al., 2014). Schutt-Abraham et al. (1992) emphasized that for correct application, proper restraint was needed including firm restraint of the rabbit’s head. Restraining the head of a rabbit, of any size, when applying a captive bolt leads to a concern for operator safety due to misfire or the bolt penetrating entirely through the head and out the other side.

Whether this device can cause enough damage to be used for euthanasia was evaluated by Sharp et al. (2014). Their analysis of the Dick KTBG on kangaroo joeys might translate to rabbits, as they are approximately of the same weight range (0.5-3kg), but differ in anatomy, development, and skull thickness. Testing this device on joey cadavers resulted in a wound almost the full length of the brain, from the cerebellum into the brainstem. Even though the bolt was relatively thin, it caused a large entrance cavity twice its diameter. When testing the device in the field on live joeys, Sharp et al. (2014) found it rendered them immediately insensible 62% of the time. Of the animals that were immediately insensible, 31% were reported to return to sensibility. The researchers did not specify whether these animals died on their own or were euthanized with another method. This information does not provide strong evidence that the PCB device can be used to euthanize animals alone without a secondary method. The results of this study demonstrate that although the device has the potential to cause damage to a large area of the brain, it may be unreliable for induction of insensibility and death. This study concluded that spring-powered PCB bolt devices should not be used as a euthanasia method because they are ineffective at rendering kangaroo joeys insensible.

Although other PCB devices are advertised for sale online for euthanasia of rabbits, research has only evaluated the Dick KTBG’s ability to stun rabbits before slaughter. Schutt-
Abraham et al. (1992) evaluated corneal reflex and respiration after this device was used on 78 mixed breed rabbits weighing 3kg. The device caused an effective stun 72% of the time, leaving 23% of rabbits only temporary stunned, regaining respiration in 30-120s. The other 5% of rabbits were ineffectively stunned and described to have weak tonic convulsions, but maintained respiration and response to corneal reflex (Schutt-Abraham et al., 1992). Although this research showed that the device could render some animals irreversibly insensible, it was only being tested for its ability to stun the rabbit, with exsanguination occurring as a secondary step. Research for the purposes of stunning means only market weight animals were examined, use for on-farm euthanasia would need a method validated for all age groups of rabbits with different brain sizes. There is no research examining the ability of the device to induce irreversible insensibility. This research concluded that the spring-powered PCB device could be used to stun rabbits, but that exsanguination should occur as soon as possible after application to prevent animals returning to sensibility. The researchers do not recommend it being used for euthanasia and predicted that failure rate would be high if attempted. While a PCB device has the potential to be a humane euthanasia method, there are many constraints that must be satisfied for effective operation and the relative levels of success are poor.

**Non-Penetrating Captive Bolt Device**

The non-penetrating captive bolt is a pneumatic-powered device designed to cause indirect destruction to the brain and skull without skin penetration. The barrel is placed directly onto the head of an animal and the bolt extends a small distance from the end of the barrel, differing from the PCB. The force applied to the skull causes jarring movement of the brain and fractures the skull, with these fragments becoming lodged into the brain also directly damaging brain tissue. The marked brain hemorrhage caused by the NPCB device leads to increased intracranial pressure, and lack of oxygen and nutrients reaching the brain. This mechanical force causes widespread damage including coup-countrecoup contusions and diffuse injury to the central nervous system (Finnie et al., 2000; Casey-Trott et al., 2013). It is not only the force the device applies that is important, but also the shape and length that the bolt protrudes from the barrel. For a piglet study, Casey-Trott et al. (2014) used a redesigned NPCB device used in a
pilot study on piglets by Widowski et al. (2008). The bolt was redesigned to be mushroom-shaped and to extend further from the barrel than a similar device. Even though the force applied remained the same, this modification eliminated method failure. The NPCB device has been referred to as a controlled form of BFT, as the goals for damage and effect are identical. The major advantage of the NPCB device is that it can be used to repeatedly deliver the exact applied force needed every time, allowing for more consistent damage. Placement of the barrel directly on the head allows for more accurate alignment. Some NPCB devices need to be attached to an air compressor to operate, slightly limiting the portability of the device and adding a potentially frightening noise for the animals.

Similar to the PCB device, skull thickness is important in determining whether the NPCB device can inflict sufficient injury to cause insensibility and death. Finnie et al. (2003) compared efficacy of a cartridge-powered NPCB device (model MKL, Karl Schermer & Co, Karlsruhe, Germany) between 4-5wk old lambs and 7-8wk old pigs. The device caused less damage to pigs than lambs, and the authors concluded that pig skulls were too thick to sustain an appropriate degree of injury with this device (Finnie et al., 2000; Finnie et al., 2003). Experiments with a different NPCB device contradicted these results. Casey-Trott et al. (2013) examined the use of a modified pneumatic nail gun (Zephyr-E prototype, Bock Industries, PA) to euthanize piglets. For piglets less than 3 days-old (~1kg) the device resulted in death of 94% of piglets (Casey-Trott et al., 2013). When tested on piglets from 3-9kg (<7wk old) the device resulted in the death of 99% of piglets (Casey-Trott et al., 2014). Hemorrhage throughout the brain was milder in heavier, animals whereas skull fracture displacement was greater in this group. This was thought to occur because older piglets have thicker skulls with more calcification making them more likely to fracture (Casey-Trott et al., 2013). Skull thickness and development likely altered the efficacy of the NPCB device. This is an important consideration when trying to find an on-farm euthanasia method that will work across all age groups of commercial meat rabbits.

There is conflicting information on the use of NPCB devices with different designs for euthanasia. For both lambs and piglets (7-8wk old) Finnie et al. (2000; 2003) determined that the device did not cause death. Results from this study lead the AVMA to classify the NPCB device as a stunning tool that requires a follow-up secondary method to ensure death. Studies from
Casey-Trott et al. (2013) indicate that the NPCB device does cause euthanasia when used on piglets <3 days old (~1kg) and on piglets weighing 3-9kg (<7wks old). Predicted reasons for this difference include different models of the NPCB device tested, differences in applied force, and different target locations. The device Casey-Trott et al. (2013) used was air-powered, allowing it to be applied at a force of 120psi, whereas the device used in Finnie et al. (2000) study was cartridge-powered operating at an undetermined applied force. Finnie et al. (2003) concluded that the applied force emitted by the device was insufficient to cause the required damage to properly stun a pig. Both devices were tested on piglets of similar age, but were applied at different locations and different number of applications; once at the temporal fossa (Finnie et al., 2003) compared to twice to the frontal bone and once behind one ear (Casey-Trott et al., 2013). Application of the pneumatic NPCB device twice in rapid succession at the same location is recommended because it increases the probability of immediate insensibility (Erasmus et al., 2010a). Thus, it appears that the NPCB device can be used to euthanize piglets <7wks old when applied twice at a pressure of 120psi to the frontal bone and once behind the ear. The results from Casey-Trott’s et al. (2014) work suggests that their device could replace BFT as a more controlled, accurate method.

No research has been conducted evaluating the use of the NPCB device for euthanasia of commercial meat rabbits. An informal study by OMAFRA investigated the use of the NPCB device to replace BFT stunning at Ontario abattoirs. Use of the device to stun market weight rabbits was most effective when applied at the base of the ears versus behind the ears at the back of the head (Rau, personal communication). This informal study provided some direction for formal study design for evaluating the NPCB device use on rabbits. Limitations of the NPCB device for on-farm euthanasia include portability, and the need to determine the appropriate pressure based on skull thickness and age for commercial meat rabbits.

The information available on physical methods of euthanasia provides guidance as to which methods may be appropriate for on-farm euthanasia of rabbits. Blunt force trauma can be an effective method if applied correctly. It is theoretically an ideal method for on-farm euthanasia because it requires minimal equipment and financial investment, although this technique is banned for stunning of rabbits in Ontario abattoirs (OMAFRA, 2016). Manual
cervical dislocation is only appropriate for small (<1kg) rabbits because of older rabbits’ neck muscle strength. Assisted-manual cervical dislocation could be an effective method to assist with the force needed to dislocate the vertebrae of larger rabbits. Decapitation is only recommended for use on undefined small and neonatal rabbits, but no research is available specifically for rabbits. One-step-head-to-body-electrocution can be used to euthanize, but due to potential human safety issues, restrictions of size, and concerns for dehydrated animals, it is not an appropriate on-farm method. Utility of both the PCB and NPCB devices may be limited by skull thickness and it is unknown whether they cause enough damage to the brain to cause irreversible insensibility. The penetrating captive bolt is a concern because correct safe restraint is needed to allow for application. Due to operator safety concern, the PCB device is not being considered further for this project. More research is needed with all these methods before any can be recommended for on-farm euthanasia of rabbits.

1.32 Inhaled Gasses

Inhaled gasses can cause direct brain damage by changing circulating oxygen concentrations and through direct physiologic effects. The gasses discussed for potential use for euthanasia of rabbits are carbon dioxide (CO₂) and carbon monoxide (CO). The advantages of gasses over physical methods include reduced animal handling and the ability to kill multiple animals at once. A major disadvantage is that insensibility is not immediate and is highly dependent on flow rate. Animals lose consciousness faster if they are exposed to higher concentrations; however, this may result in a more distressing death, depending on the agent used (AVMA, 2013). A challenge with assessing gassing methods is human safety, making it difficult to directly interact with the animal to test for insensibility and death. Research usually relies on behavioural observations as surrogate indicators of pain or distress.

Rabbits will breath-hold when exposed to an unpleasant or unfamiliar odour possibly delaying onset of insensibility (AVMA, 2013). This can make use of inhaled gasses challenging, particularly if they have an odour. Observed signs of distress in rabbits include rapid blinking, immobility, vocalizations, and standing in a hunched position (Gigliotti et al., 2009). Behavioural indicators of respiratory irritation in rabbits include rapid nose twitching, tearing of
the eyes, and audible obstruction of respiration by secretions (Oliver and Blackshaw, 1979; Gigliotti et al., 2009). These behavioural indicators of distress are important to assess and consider when evaluating the effect of gasses.

**Carbon Dioxide**

Carbon dioxide (CO\(_2\)) inhalation causes hypoxia leading to brain damage and subsequent death. Insensibility is caused by a depression of brain activity from oxygen deprivation, and rapid acidification of the cerebrospinal fluid and brain cells (Terlouw et al., 2016). Carbon dioxide is a gas detected directly by central and peripheral chemoreceptors leading to a change in respiration rate (McKeegan et al., 2007). Air hunger, observed as deep breathing, gasping and open mouth breathing, is likely distressful. Exposure to CO\(_2\) also results in a rapid decrease in intracellular pH, and the formation of carbonic acid when in contact with water, causing irritation of the mucus membranes (Danneman et al., 1997). The benefits of CO\(_2\) are that it is inexpensive, readily available, and relatively safe for the operator. A significant disadvantage of CO\(_2\) is that it has potential aversive properties. Distress from these aversive properties can be limited by reducing the duration of exposure with a rapid onset to insensibility, or by using a lower CO\(_2\) concentration during the induction phase. The AVMA lists CO\(_2\) as conditionally acceptable for use in laboratory rabbits and suggests that rabbits be sedated or anesthetized prior to exposure, although this recommendation is not well verified by specific research.

Use of CO\(_2\) inhalation as a euthanasia method requires the appropriate flow rate and exposure time. In a study by Hayward and Lisson (1978), rabbits (1.5kg) were exposed to 30%, 40% and 50% CO\(_2\) at a steady flow rate (2-3L/min). Time to insensibility was determined by an undefined startle response to a sudden loud noise (auditory-evoked). Time to death was defined as respiratory arrest as measured by an electrocardiogram. Heart rate was also monitored and cardiac arrest occurred soon after to respiratory arrest. At 30% CO\(_2\), 4 of 5 rabbits were reported to be alive and semiconscious at the 180min cut-off. At 40% and 50% CO\(_2\), the rabbits became insensible in 4-10mins. The researchers concluded that at a concentration between 40-50%, CO\(_2\) effectively caused insensibility and death. A concern with making recommendations based on this research is that nociceptors in humans are activated in the range of 40-50% CO\(_2\) (Anton et
al., 1992). Hayward and Lisson (1978) suggested that rabbits exposed to CO$_2$ concentrations >40% could be in a state of distress. Unfortunately, behaviour was not observed in this study, limiting further interpretation of the data. This study does provide a basis for time to insensibility and death at a set flow rate and concentration of gas.

Nitrogen/CO$_2$ mixtures have been evaluated as a method to reduce aversion to CO$_2$. The only nitrogen/CO$_2$ research done specifically in rabbits evaluated their use for stunning before slaughter. Llonch et al. (2012) observed the behavioural and physiologic responses of rabbits when lowered into a prefilled gassing pit of 90% CO$_2$ compared with a mixture of 80% nitrogen/20% CO$_2$, for a total exposure time of 1min. Rabbits in this study were used as their own control group and exposed to air while in the gassing pit the day before exposure to the test gas. A limitation of this study is that the control group trial occurred on a different day, and was always the first time in the gassing pit. Rabbits were less active before the onset of insensibility, when exposed to 90% CO$_2$ compared to their control, but had no change in activity when exposed to the nitrogen/CO$_2$ mixture. Activity level is not a validated measure of distress in rabbits and predicted reasons for activity level change varied from immobile fear response, exploratory behaviour or escape behaviour, preventing clear conclusions. Almost all rabbits exposed to 90% CO$_2$ (97%) demonstrated signs of respiratory distress compared to less than half of animals (40%) exposed to the nitrogen/CO$_2$ mixture. Loss of posture and muscle twitching were used as signs of insensibility and occurred sooner for the nitrogen/CO$_2$ mixture. Llonch et al. (2012) suggest that 80% nitrogen/20% CO$_2$ causes less respiratory distress and induces insensibility sooner than 90% CO$_2$. Exposure time was less than 1min and 90% CO$_2$ is a much higher concentration than would be used for euthanasia purposes.

Data on rabbits’ responses to CO$_2$ was analyzed by Dalmau et al. (2016). Although this research evaluated rabbits’ response to being lowered into a CO$_2$ gassing pit prior to slaughter, use of an EEG and evaluation of duration of insensibility provide useful data. Loss of posture was confirmed to correlate with a change in EEG power, and occurred more quickly as CO$_2$ concentrations increased from 70% to 80%, 90% and 98%. Validation of loss of posture confirms the findings of Llonch et al. (2012) for loss of sensibility. At 90% CO$_2$, sensibility was lost at relatively the same time in both studies, at approximately 30s. Dalmau et al. (2016) investigated how effective CO$_2$ stunning is for creating sustained insensibility for 5min once
rabbits were removed from the gassing pit. The four concentrations (70%, 80%, 90%, and 98%) were examined at different exposure times. Maximum exposure time of 360s was ineffective in sustaining insensibility for all animals. Thus, exposure times were not long enough at any of the examined concentrations to be useful for euthanasia.

Distress was evaluated in both CO\textsubscript{2} rabbit stunning studies. Both Llonch et al. (2012) and Dalmau et al. (2016) attempted to use activity level as a sign of distress, but were unable to make solid conclusions from the results. Llonch et al. (2012) used gasping as an indicator of respiratory distress and observed it occurring more when rabbits were exposed to 90\% CO\textsubscript{2} than the other concentrations examined. Feelings of breathlessness, such as gasping, are considered to correlate with negative affective states, and can occur during CO\textsubscript{2} exposure (Beausoleil and Mellor, 2015). Dalmau et al. (2016) measured distress in rabbits using vocalizations, and nasal discomfort, defined as head shaking and grooming. Nasal discomfort occurred frequently (50\%) across all concentrations. During exposure to CO\textsubscript{2} nasal discomfort can occur due to the formation of carbonic acid on mucus membranes (Danneman et al., 1997). Vocalizations occurred 20\% of the time at 70\% CO\textsubscript{2}, 50\% at 80\% CO\textsubscript{2}, 80\% at 90\% CO\textsubscript{2} and 50\% at 90\% CO\textsubscript{2}. Results from this research suggest that the majority of rabbits experienced distress at these CO\textsubscript{2} concentrations.

It can be concluded that a CO\textsubscript{2} concentration above 40\% causes insensibility and death in rabbits, and mixing nitrogen with CO\textsubscript{2} may help to limit distress. Lethal concentration and exposure time may differ from what is seen in other mammals, because rabbits are borrowing species with a potential higher CO\textsubscript{2} tolerance (Hayward and Lisson, 1978). Inability to study behaviour and time to insensibility in warren fumigation studies limits the usefulness of this research. The intention when stunning is to temporarily render the animal insensible, therefore making euthanasia conclusions less than ideal. Research is needed to identify the correct concentration and flow rate of CO\textsubscript{2} necessary to euthanize rabbits.
Carbon Monoxide

Carbon monoxide (CO) is readily absorbed through the lungs. Death following exposure is a result of lack of oxygen and reduced ATP production, inducing anoxia and leading to cardiac arrest (Ernst and Zibrak, 1998). The atmospheric content of CO is 0.001% (Ernst and Zibrak, 1998). Carbon monoxide is a colourless, odourless, toxic gas and is difficult to detect in the environment. Because CO is non-irritating to humans, hundreds of people die each year due to accidental CO poisoning (Ernst and Zibrak, 1998). The lethal properties of CO create a safety concern for operators. The Occupational Safety and Health Administration prohibits workers from being exposed to levels exceeding 35ppm during an 8-hour work day and 200ppm in 15min. This safety risk can be reduced by having appropriate ventilation in place and working in a well ventilated area, such as outdoors. Symptoms of low level inhalation in humans are nonspecific and are reported as dizziness, headaches, and weakness. Exposure to higher concentrations is reported by humans to progress from loss of visual clarity, nausea, confusion, and collapse to convulsions, coma and cardiac arrest (Lowe-Ponsford and Henry, 1989). Rabbits exposed to CO were observed to drop their heads, lose coordination and lose posture within 7min (Gigliotti et al., 2009). Closer to death, rabbits began convulsing and gasping. The majority of what is known about CO exposure is based on human literature, but to animals with heightened senses in general, CO could be perceived entirely differently. If CO is undetectable by animals this has a major welfare advantage, but still creates a safety concern for the human operator.

Use of CO for rabbits requires a minimal lethal concentration and exposure time to be determined. Information on the lethal CO concentration and exposure time for rabbits comes from research on warren fumigant for pest depopulation (Oliver and Blackshaw, 1979; Ross et al., 1998; Gigliotti et al., 2009). It is important to note when fumigating a warren the concentration of gas used differs from the actual peak concentration in the warren. Warren fumigation differs from experimental exposure in a chamber as the warren is blocked and animals are not checked for an extended period of time. Gigliotti et al. (2009) researched fumigation of a warren with 5% CO. When the entrances were blocked off a peak warren concentration of 3.8% was reached, resulting in death of 4 of 5 rabbits in 87min. In the same study, fumigation at 6% resulted in a peak concentration of 4.5%, killing 8 of 10 rabbits in 28min. Increasing the concentration by less than 1% resulted in death occurring 3x faster.
(Gigliotti et al., 2009). However, in both trials death rate was not 100%, suggesting that the lethal concentration of CO is higher than 4.5%. Warren fumigation by Ross et al. (1998) using cylinders containing 1% CO produced varying concentrations of 2 to 20%, unequally distributed throughout the warren. A majority (10/12) of rabbits were discovered dead at the entrances where the concentration was recorded to be the highest. When exposing rabbits to the CO concentrations used in this study, mortality rates were 100% in an artificial warren made of plastic netting tunnels covered in top soil and sand, and 79% in a natural warren (Ross et al., 1998). This research suggests there are multiple challenges when fumigating a warren and that the data might not extrapolate to other uses of CO. Oliver and Blackshaw (1979) examined fumigation of a warren with exhaust from a choked engine run at full idle, as a source of CO. Exposure to CO from exhaust resulted in the death of all rabbits within 3-16min. However exposure of CO from a tail pipe is considered a contaminated source and should not be used (AVMA, 2013). These CO studies in rabbits do not provide clear information on lethal concentrations and exposure times.

Carbon monoxide is commonly used in the mink industry for euthanasia at pelting. Korhonen et al. (2013) reported CO concentrations of 4 to 6% to be effective inducing death in 3-6mins. Concentrations below this (1.2-3%) were too low to cause death. Hansen et al. (1991) reported that exposure of mink to 4% CO mixed with air resulted in loss of balance in 64s. Electroencephalogram recordings from the Korhonen et al. (2012) study showed insensibility occurred within 4-7mins when mink were exposed to the same concentration of CO, leading to inconsistent data. The lethal dose needed to euthanize mink could be used as a starting point for research on rabbits.

Behavioural responses to an inhaled gas can be affected by flow rate. A gradual flow versus use of a prefilled chamber can have a different effect on response and distress. In studies exposing rabbits to CO, the flow rate was only mentioned by Gigliotti et al. (2009) and was 400L/min to fill a warren. The AVMA advises that CO be introduced at a flow rate that allows a uniform concentration of 6% to be reached immediately after introduction. There is no available research on an appropriate flow rate to use on rabbits for minimal distress.

Reasons for aversion to CO could include adverse odour, dyspnea, and hypoxia. Mink induction behaviour was compared between CO and CO₂ with no difference noted in behaviour,
even though CO$_2$ can be readily detected in the environment and is a respiratory irritant (Hansen et al., 1991). The averseness of CO was explored in a study on rats by Makowska and Weary (2009). Rats were tested in a 2-level cage connected by a tunnel and were exposed to CO in the bottom cage while being fed a highly rewarding treat. Aversion was judged by the decision to leave the treat and escape exposure to CO. Rats did not remain in the cage until loss of sensibility, but two remained until becoming ataxic (loss of muscle coordination and balance) and one until recumbent (loss of posture) at 7% CO. Under forced exposure with no possibility of escape to the other cage, rats exposed to 3%, 6% and 7% CO displayed the same pattern of escape behaviour; increased activity, scratching at the exit and corners, and running in circles. Carbon monoxide was the first inhalant that was tolerated until recumbency by a rat, but was still aversive to most rats. The authors concluded that higher flow rates might lead to less distress as animals choose to remain in the cage until the initial signs of loss of sensibility. In rabbits observed during exposure to 3.8% and 4.5% CO, 2 of 15 rabbits showed arousal (Gigliotti et al., 2009). Varying observations from these studies make it hard to conclude whether CO can be detected to the point that it causes distress.

Both CO$_2$ and CO gas exposure has been minimally evaluated in rabbits, and the research to date does not extrapolate well to on-farm euthanasia. Specific research needs to be conducted to determine the appropriate concentration, flow rate, and exposure times for euthanizing rabbits. In addition, behavioural observations need to be examined to determine whether animals are distressed. To ensure operator safety chamber and ambient air gas concentrations should be closely monitored. In ability to source accurate monitoring equipment for CO eliminated this gas from further investigation in this body of work.
Research Goals and Objectives

There are currently no validated on-farm euthanasia methods or guidelines available for commercial meat rabbit producers and their veterinarians to use when making end of life decisions. This study aims to validate methods of on-farm euthanasia for commercial meat rabbits. Validating methods will result in rabbit producers being able to create a euthanasia action plan and have confidence in their methods.

1. Objective: To evaluate BFT, assisted manual CD (AMCD) and a NPCB device for their effectiveness in euthanizing pre-weaned kits, growers and adult rabbits. Effectiveness was determined by onset of rapid and irreversible insensibility and the degree of brain damage induced (Chapter 2).

Hypothesis: Of the physical methods tested, the NPCB device was predicted to be the most effective. Reasons for this include that it is already being used to stun rabbits in Ontario abattoirs and it is a validated on-farm euthanasia device for other livestock species. Use of a NPCB device eliminates the need for operator physical strength that other physical methods require and could reduce psychological effects on the operator.

2. Objective: To determine time to insensibility and death, and to evaluate the welfare of commercial meat rabbits (pre-weaned to adult) when exposed to gradual-fill and fast-fill rates of CO₂. Behavioural responses before loss of sensibility were used to assess welfare (Chapter 3).

Hypothesis: Both fill rates would cause a period of distress for the rabbits, but at a lower intensity during gradual-fill. Reasons for this prediction included anecdotal reports and aversion to CO₂ in other species.
Chapter 2: Efficacy of blunt force trauma, assisted manual cervical dislocation and a non-penetrating captive bolt device for on-farm euthanasia of pre-weaned kits, growers, and adult commercial meat rabbits

2.1 ABSTRACT

The commercial meat rabbit industry is without validated on-farm euthanasia methods, potentially resulting in inadequate euthanasia protocols. The objective of this research was to evaluate blunt force trauma (BFT), assisted manual cervical dislocation (AMCD) and a pneumatic-powered non-penetrating captive bolt (NPCB) device as euthanasia methods for pre-weaned kits, growers and adult rabbits. Euthanasia procedures were conducted on 3 commercial meat rabbit farms over 28 trial days on a total of 170 cull rabbits of different ages. Technique effectiveness and quality of death were determined by the ability to induce immediate and irreversible insensibility, as well as induction of permanent damage to the brain and spinal cord. Immediate and irreversible insensibility was assessed using brainstem and spinal cord reflexes, absence of rhythmic breathing, and absence of vocalizations, after each method was applied. A subsample of rabbits (n=12) were radiographed after death to assess location and degree of damage prior to dissection. All rabbits were assessed and scored for gross brain hemorrhage and skull fractures. Specific brain regions (frontal cortex, thalamus and mid-brain, and cerebellum) from a subsample of rabbits (n=38) were scored microscopically for subdural and parenchymal hemorrhage. Method failure was associated with euthanasia method (p<0.001). All 63 rabbits euthanized by the NPCB device were immediately and irreversibly rendered insensible. Assisted manual cervical dislocation failed to induce immediate and irreversible insensibility in 3 of 49 (6%) rabbits. Method failure was highest for BFT with 13 of 58 (22%) applications requiring re-application or application of a different technique. The NPCB device caused significantly more gross hemorrhage than the other 2 methods (p<0.001). Microscopically, brain sections from rabbits killed with the NPCB device scored significantly higher than those killed with BFT for subdural (p=0.001) and parenchymal hemorrhage (p=0.005). We conclude that BFT is neither humane nor effective method for meat rabbits due to the high method failure rate and minimal associated brain damage. Assisted manual cervical dislocation, with the device used in this study, is an accurate and reliable euthanasia method causing clean dislocation at the base of the skull.
and immediate irreversible insensitivity. The non-penetrating captive bolt was 100% effective and reliable, required minimal animal restraint, was easy to use, and esthetically acceptable euthanasia method.
2.2 INTRODUCTION

All livestock producers must have effective methods in place to euthanize sick, injured, and cull animals in a timely matter. Globally, there are minimal guidelines and reference resources available to assist meat rabbit producers and their veterinarians in making on-farm euthanasia decisions, including determining which methods are most appropriate for each age group. A recent survey of Canadian commercial meat rabbit producers established that the most common on-farm euthanasia method is blunt force trauma (BFT) (Walsh et al., 2016). The use of BFT on rabbits has never been scientifically evaluated for effectiveness as a euthanasia method. Manual BFT was used historically in Ontario abattoirs to stun rabbits prior to slaughter, but was formally discontinued in 2005 due to concerns surrounding efficacy and operator fatigue (OMAFRA, 2016). The American Veterinary Medical Association (AVMA) discussed the use of manual BFT across all species in their 2013 euthanasia guidelines, and encourages all those actively using this method to seek alternative methods (AVMA, 2013). The AVMA guidelines recognize that while manual BFT may be an effective and humane killing technique there is a large margin for error and operator fatigue, in addition to issues associated with observer esthetics. One possible alternative euthanasia method is a non-penetrating captive bolt (NPCB) device. The NPCB device was designed to deliver lethal brain trauma without penetrating the skin, removing potential biosecurity concerns. This is the method now used for stunning meat rabbits in Ontario provincial abattoirs and a slightly different design NPCB device has been validated for euthanasia of turkeys and piglets (Erasmus et al., 2010a; Casey-Trott et al., 2014).

Cervical dislocation (CD) is another method commonly used on-farm for rabbit euthanasia (Walsh et al., 2016). Guidelines for euthanasia of laboratory rabbits suggest that this method is inappropriate for rabbits weighing >1kg due to their large neck muscle mass (CCAC, 2010; AVMA, 2013). Various mechanical CD devices have been used in other livestock species, such as poultry and mice, to assist with the technique. There is concern about the ability of these devices to cause a clean dislocation between the skull and first cervical vertebrae, and their effectiveness for inducing rapid loss of sensibility (Erasmus et al., 2010a; Carbone et al., 2012). There is no specific research to date evaluating CD or the use of a device to assist with CD in meat rabbits.
The goal of any euthanasia method is to rapidly kill an animal in a way that minimizes pain and distress. Pain and distress can be minimized if the animal is rendered rapidly insensible (unconscious) and if it remains so until death occurs (CVMA, 2014). Insensibility should be confirmed by checking for brain activity using an EEG, brainstem and spinal cord reflexes or assessing damage to the brain (Erasmus et al., 2010b; Verhoeven et al., 2015a). On-farm euthanasia methods should also be safe for the operator to use and take into consideration practicality, operator and observer esthetics, and cost.

The objective of our research was to evaluate the effectiveness of several physical methods for euthanizing adult and juvenile rabbits, including BFT, assisted manual CD (AMCD) and a NPCB device. Effectiveness was determined by onset of rapid and irreversible insensibility and degree of brain damage induced. We hypothesized that the NPCB device would be the most effective method because of its precision and ease of use.
2.3 MATERIALS AND METHODS

Animals

The procedures and protocol for this research were reviewed and approved by the University of Guelph Animal Care Committee (AUP3366). The University of Guelph holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care and is registered under the Ontario Animals for Research Act. All rabbits used in this study were ones targeted for euthanasia by producers and euthanasia was conducted on-farm. Reasons for euthanasia included sick or injured rabbits, unthrifty rabbits that were unlikely to survive to market weight, and rabbits reaching the end of reproductive utility. Three rabbit farms (Farm 1=500 does, Farm 2=150 does, Farm 3=600 does) from across southwestern Ontario were recruited to participate in this study. Producers were experienced in rabbit production having been raising meat rabbits full-time for at least 8 years. A total of 170 rabbits were euthanized on 28 trial days by the following methods: BFT (n=58), AMCD (n=49), and a NPCB device (n=63). Live weights were collected prior to euthanasia and body length was measured post mortem from the tip of the nose to the tail to calculate body mass index \[\text{BMI} = \frac{\text{weight (kg)}}{\text{length (m)}^2}\]. All methods were tested on New Zealand white-like rabbits across 3 age groups: pre-weaned kits (0 to 5 wk), growers (6 to 12 wk), and adults (>12 wk).

Euthanasia Techniques

All 3 farms used BFT as a euthanasia method. Blunt force trauma was the primary method for all age groups on Farms 2 and 3, and was used on pre-weaned kits only on Farm 1. The BFT method used by producers varied, but in all cases the rabbit was suspended by its back legs prior to striking the animal on the head with a heavy object or against a hard surface.

Assisted manual cervical dislocation was the primary method used by Farm 1. It was accomplished through use of a homemade or purchased (Rabbit Wringer,
http://www.rabbitwringer.com, West Grove, PA) stainless steel V-shaped wall-mounted device (Fig. 2.1) that secures the rabbit’s head prior to the operator applying a downward dislocating force. The device was mounted to the wall at approximately operator shoulder height. The commercial design of the device allows for alteration according to size with an adjustable neck plate.

The NPCB device (Zephyr-E, Bock Industries, Philipsburg, PA) used was a modified pneumatic nail gun weighing 750g, with a mushroom-shaped nylon bolt head that extends 2cm from the end of the barrel (Fig. 2.2). The device attaches to a standard air compressor, set to the desired pressure, which was validated in an initial trial. The device was discharged twice in rapid succession in the same location based on recommendations from Erasmus et al. (2010a). The NPCB device was used exclusively by trained research personnel during this trial. When the NPCB device was used, rabbits were restrained in a plastic container with non-slip flooring, with the operator’s hand resting on the shoulder blades, and thumb and forefinger around the neck of the rabbit.

**NPCB Device Validation Trial**

An initial pilot study was conducted at the University of Guelph on 22 donated rabbit cadavers of varying sizes and ages, weighing between 0.9kg to 4.7kg, to determine the appropriate placement and required pressure for the NPCB device. The correct location and pressure was determined by dissection of the head following application of the device looking for maximum brain damage without breaking the skin or injuring other anatomical sites, such as the eye or frontal sinuses, which might contribute to poor esthetic outcome as well as increased biosecurity risk. From this initial cadaver work, and based on preliminary observations and discussions with abattoir personnel who use this device routinely for rabbit stunning prior to slaughter, it was determined that the NPCB device should be positioned on the frontal and parietal bones, in the center of the forehead, with the barrel placed in front of the ears and behind the eyes (Fig. 2.2C). The required pressure setting for the air compressor was determined through dissection assessing degree of skull fracture. Kickback from the device was a concern
for operator safety as well as inhibiting speed of delivery of the second discharge. Too high of pressure also resulted in external bleeding from skin penetration. For this reason, the appropriate pressure was determined based on skull development and thickness rather than applying a maximum pressure to all animals. The appropriate pressure for sufficient brain trauma and skull fractures was determined to be 621kPa (90psi) for adult rabbits (>12 weeks of age), 483kPa (70psi) for growers (6 to 12 wk), and 379kPa (55psi) for pre-weaned kits (150g and larger, ≤ 5 weeks of age). A minimum pressure of 345kPa (50psi) is needed for the NPCB device to discharge.

Assessment and Scoring of Euthanasia Outcome

Immediately after each euthanasia method was applied, an observer assessed insensibility by evaluating the pupillary reflex (cranial nerves II and III), palpebral reflex (cranial nerves V and VII), toe and ear pinch withdrawal reflex (pain responses), nostril poke (pain response), and corneal reflex (cranial nerves V and VII). These reflexes were tested first on sensible animals to ensure a reliable response. Nostril poke required a pointed tool to be applied to the inner medial surface of a nostril and elicited a variable response in sensible rabbits. Because of this unreliability, it was eliminated from euthanasia trials. The palpebral reflex was assessed by gently tapping the skin around the eye and was considered present if the rabbit blinked in response. The pupillary reflex was evaluated by shining a white light-emitting disposable penlight (Safe Cross First Aid LTD, Toronto, ON, Canada) into the rabbit’s eye and was considered present if the rabbit tried to avoid the bright light or if the pupil constricted in response. The toe and ear pinch withdrawal reflexes were assessed by gently pinching the fold of skin between the rabbit’s toes or the rabbit’s ear and was considered present if the rabbit moved its foot or head away in response. The corneal reflex was assessed by touching the surface of the cornea and was considered present if the rabbit blinked in response. If the animal was not immediately insensible or it returned to sensibility, as determined by onset of rhythmic breathing, purposeful vocalizations or reflex response, the euthanasia technique was deemed a failure, and the same method or an alternative method was immediately applied. Rhythmic breathing was defined as nostril flaring, differentiating it from gasping. Vocalizations were
defined as a high pitch squeal, differentiating it from sounds made as air passively escaped from the lungs.

Time to death was recorded based on complete loss of reflexes, lack of movement, and onset of cardiac arrest. The occurrence and duration of involuntary movements were timed and recorded. Involuntary movements were classified as tonic convulsions (rigid extension of limbs) and clonic convulsions (leg paddling). Cardiac arrest was determined when a heartbeat could no longer be palpated or auscultated with a stethoscope. For consistency, personnel acting as the observer, the recorder, and the NPCB device operator were constant throughout all trials. Prior to beginning trials, personnel trained and practiced using these reflexes and physiologic responses to judge insensibility and death at an abattoir that used the NPCB device on rabbits.

Rabbit cadavers were taken to the University of Guelph for dissection and assessment of damage. Brain damage assessments were conducted only on rabbits that were successfully killed on a first attempt by any method (Table 2.1).

**Survey Radiographs**

Survey radiographs were conducted at the OVC Health Sciences Centre on 4 randomly selected growers from each euthanasia technique, except in the BFT group where 1 rabbit (5 wk) taken for radiographs was classified as a pre-weaned kit. Both dorsoventral and lateral views were taken and radiographs were qualitatively assessed for location and degree of skull injury.

**Macroscopic Assessment of Tissue Damage**

Dissections and macroscopic examinations scoring for gross hemorrhage (GH) were conducted on all rabbits euthanized on first attempt. All macroscopic assessments were conducted by the same 2 people who trained and scored together, to ensure consistency. A semi-
quantitative scoring system was developed based on Casey-Trott et al. (2014) and Veltri and Klem (2005) and was used to assess the severity of skull fractures, subcutaneous GH and subdural GH (Table 2.2). After removing the scalp, the degree of subcutaneous GH was scored from the top of eyes to the base of the skull. Similarly, the neck was excised exposing the first 4 cervical vertebrae to score perispinal hemorrhage. Skull fractures were scored for severity (number of fractures and degree of fracture displacement). Vertebral dislocations and fractures were assessed (present or absent) through palpation and dissection. Following superficial scoring, the skull was opened with a Stryker saw (Mopec. MI), the dura removed, and the amount of hemorrhage on the dorsal and ventral surface of the brain was examined and scored. The total subdural GH score was calculated as the average of the dorsal and ventral score for each rabbit brain.

**Microscopic Brain Hemorrhage Scoring**

After macroscopic analysis, all brains were placed in 10% neutral buffered formalin, placed on a shaker for 72 h and stored prior to trimming. Thirty-eight brains were selected for microscopic hemorrhage (MH) scoring as follows: 11 brains from the growers that were radiographed, 3 randomly selected brains from pre-weaned kits for each euthanasia method (1 pre-weaned kit that was radiographed) and 6 randomly selected brains from adult rabbits for each euthanasia method (Table 2.1). Three sections were trimmed from each brain by the same person to represent the hindbrain (cerebellum), midbrain (thalamus), and frontal cortex. Brain sections were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (Animal Health Laboratory, University of Guelph). Sections were randomized and assessed by a veterinary pathologist (Dr. P.V. Turner) blinded as to euthanasia method and rabbit age. The degree of subdural and parenchymal MH was scored for each brain section using a scale of 0 to 4 (Erasmus et al. 2010b) based on the relative area of the brain section affected: (0) 0%, (1) <5%, (2) 5% to 10%, (3) 11% to 30%, (4) >30%. Differences in damage among the 3 brain regions were assessed by comparing scores from each region. Overall degree of subdural and parenchymal
MH damage to the brain was determined using the highest score for subdural and parenchymal MH in any of the 3 regions from the same rabbit.

**Statistical Analyses**

Statistical analyses were performed using SPSS (SPSS Statistics for Windows, Version 23.0. 2014. Armonk, NY: IBM Corp.), p <0.05 was accepted for significance. All values are reported as mean ± SE. Dependent variables were checked for normality using the Shapiro-Wilk test, and transformed if not normally distributed. An independent samples t-test was used to compare body weight and BMI between the replacement and breeder age groups. A Chi-square test for independence was used to test for associations between method, age, and method failure rate. Cramer’s V test was run to determine the strength of the association. Duration of clonic and tonic convulsions were combined for total time convulsing for analysis of involuntary movements. Time convulsing was log transformed and analysed via a 1-way ANOVA Tukey test with treatment as the independent variable. All hemorrhage scores (subcutaneous GH, subdural GH, subdural MH, and parenchymal MH) and skull fractures were rank transformed. Macroscopic scoring of damage was analysed using a Kruskal Wallis 1-way ANOVA with method and age group as the independent variables. Microscopic brain hemorrhage scores were analysed using a Kruskal Wallis 1-way ANOVA with method, age group, and brain section as independent variables. Data was spilt by treatment to allow for comparisons within treatment groups.
2.4 RESULTS

Assessment and Scoring of Euthanasia Outcome

Euthanasia methods were evaluated for 170 rabbits across the 3 age groups. Pre-weaned kits weighed $0.2kg \pm 0.03$ with an average BMI of $10.6 \pm 2.4$, and growers weighed $1.4kg \pm 0.1$ with an average BMI of $12.7 \pm 0.7$. Replacement rabbits did not significantly differ in body weight ($3.3kg \pm 0.2$) compared to breeding rabbits ($3.5kg \pm 0.1$), $t(40) = -0.69$, $p=0.50$. Similarly there was no significant difference in BMI for replacement rabbits ($15.9 \pm 2.0$) compared to breeding rabbits ($16.1 \pm 2.1$), $t(40) = -0.23$, $p=0.82$ and the 2 age groups were combined for the adult age group. Rabbits in the combined adult age category weighed on average $3.4kg \pm 0.1$ with an average BMI of $16.0 \pm 0.3$.

A euthanasia method was judged to have been effectively applied if the rabbit demonstrated immediate and irreversible insensibility, based on no vocalizations, no response to reflex testing and lack of rhythmic breathing until cardiac arrest. Of 170 rabbits in the study population, 154 or 91% were euthanized successfully (Table 2.1). A Chi-square test was performed and a medium strength association (Cramer’s $V=0.33$) was found between method used and probability of method failure, $\chi^2 (2, n=170) = 18.67$, $p<0.001$. Probability of method failure was highest for BFT (Table 2.1). BFT data collection was discontinued on Farm 2 after 5 of 8 rabbits failed to achieve insensibility and death after the first application. The highest probability of method failure across all 3 farms occurred when adult rabbits were euthanized with BFT, with a 43% chance of incorrect application and unsuccessful euthanasia. The NPCB device was 100% effective across all age groups in all trials. Assisted manual cervical dislocation failed for 1 pre-weaned kit and 2 adults (Table 2.1). These animals were outside the weight range with which the operator had experience and required technique and force to be altered. The minimal size and age evaluated for AMCD was 150g or 2 weeks. Although it did not occur in this study, the AMCD operator cautioned that too much force is commonly used when first learning the method resulting in decapitation. There was no association between age and probability of method failure when evaluated across all methods, $\chi^2 (2, n=170) = 2.66$, $p=0.27$. When analysed within each method, there was also no association between age and...
probability of method failure (BFT, $\chi^2 (2, n=58) = 4.66, p=0.1$; AMCD, $\chi^2 (2, n=49) = 5.70, p=0.06$).

For rabbits successfully euthanized on a first attempt, all reflexes were absent when first checked immediately after method application. While the pupillary reflex was used throughout, assessment was done outdoors (brightly lit environment) limiting its usefulness. Rhythmic breathing was a reliable indicator of return to sensibility, predicting the return of other reflexes. Vocalizations occurred when a method was incorrectly applied and the rabbit was still sensible, or occasionally when hanging a rabbit by its back legs prior to application of BFT.

The average total convulsion time for BFT, the NPCB device, and AMCD was 50s ± 7, 62s ± 4, and 58s ± 7, respectively. There was a difference between groups as determined by one-way ANOVA [$F(2,140) = 5.03, p=0.008$]. A post hoc Tukey’s test indicated that total time convulsing following BFT was shorter than for the NPCB device ($p=0.01$) and AMCD ($p=0.03$). Clonic and tonic convulsions in rabbits did not follow a clear pattern or transition. Convulsion strength seemed to depend on the health status of the animal, with healthier animals having more aggressive convulsions, although this was not specifically scored for in this study. A pattern predictive of a return to sensibility was convulsions suddenly stopping versus slowly fading out. No convulsions occurred when rabbits responded to tests for sensibility. Cardiac arrest occurred 160s ± 5 after the method was applied with a range across all successful euthanasia of 70s to 388s. Heartbeat was challenging to palpate and auscultate for young pre-weaned rabbits, emaciated rabbits, and for rabbits euthanized by BFT.

After application of the NPCB device, swelling under the skin could be seen forming immediately. No distal vertebral fractures or hip luxations were noted after application of ACMD, and dislocations were only slightly noticeable with palpation. Rabbits were difficult to restrain for BFT and this difficulty in safely restraining them could have attributed to method failure. Rabbits were easily restrained for application of the NPCB device and the relatively large bolt and barrel of the device to the size of the head of the rabbit allowed for easy alignment.
Survey Radiograph Findings

Survey radiographs of the 4 growers euthanized by the NPCB device demonstrated depressed cranial bones, some with skull fragments embedded in the brain and all rabbits had noticeable swelling from subcutaneous GH (Fig. 2.3A). Those euthanized by BFT varied in the degree of damage to the cranial bones. Two rabbits demonstrated limited, focal fractures, one rabbit had depressed fragments (i.e., compression fracture), and one rabbit (Fig. 2.4A) had 2 significant cranial fractures. The radiographs of the 4 growers euthanized by AMCD showed consistent trauma resulting from complete dislocation between the base of the skull and the first vertebrae with no vertebral fractures or other dislocations (Fig. 2.5A).

Macroscopic Assessment of Tissue Damage

Macroscopic evaluations consistently demonstrated marked brain hemorrhage for rabbits euthanized with the NPCB device. Swelling of the tissue above the skull (subcutaneous hematoma) resulted in high subcutaneous GH scores (Fig. 2.3B). Rabbits euthanized by AMCD had a small dislocation gap but a large amount of neck hemorrhage resulting from ruptured blood vessels (Fig. 2.5C). Some rabbits euthanized by AMCD had minor skull fractures of the occipital bone. This is the location the device applies pressure during application while holding the rabbit’s head in place, and in such cases a clean dislocation still occurred. Macroscopic damage resulting from BFT varied and was often not obvious. Damage, including fractures, was found in areas other than the targeted location for BFT, such as the back, shoulder blades and nasal cavity. There was a statistically significant increase in brain hemorrhage score for the NPCB device compared to the other 2 methods, \( \chi^2 (2, n=154) = 120.82, p<0.001 \) for subcutaneous GH and \( \chi^2 (2, n=154) = 82.36, p<0.001 \) for subdural GH, respectively. There was a statistically significant difference between all 3 methods for skull fractures, \( \chi^2 (2, n=154) = 93.10, p<0.001 \), with the NPCB device scoring the highest, followed by BFT then AMCD.
Comparing between age groups, skull fracture scores were higher for pre-weaned kits compared to adult rabbits across all methods, \( \chi^2 (2, n=154) = 9.60, p = 0.008 \), whereas no differences were noted between the other age groups. Subcutaneous and subdural GH were not significantly different between age groups across all methods. Comparisons for age within treatment groups indicated that within the BFT group, pre-weaned kits scored significantly higher than adults for subcutaneous GH, \( \chi^2 (2, n=45) = 8.19, p=0.02 \), and for skull fractures, \( \chi^2 (2, n=45) = 9.81, p=0.006 \). Within the NPCB group, adults had a significantly lower skull fracture score than pre-weaned kits and growers, \( \chi^2 (2, n=63) = 9.81, p=0.001 \). Within the AMCD group there was less subdural GH hemorrhage in the growers than the adults, \( \chi^2 (2, n=46) = 8.32, p=0.02 \). These were the only statistically significant differences among age groups.

**Microscopic Brain Hemorrhage Scores**

Histological analysis of the 3 sections from each brain indicated that rabbits euthanized with the NPCB device had the highest average subdural and parenchymal MH scores across brain sections, except for parenchymal MH score in the hindbrain (Table 2.3). Comparing brain sections from the hindbrain, BFT scored significantly lower than other methods for subdural MH hindbrain scores, \( \chi^2 (2, n=38) =9.06, p=0.01 \). There were no significant differences in hindbrain parenchymal MH scores between methods. Comparing brain sections from the mid-brain, the NPCB device scored significantly higher than other methods for mid-brain subdural MH, \( \chi^2 (2, n=38) =15.03, p=0.001 \), and scored significantly higher than BFT for mid-brain parenchymal MH, \( \chi^2 (2, n=38) = 9.22, p=0.010 \). Comparing brain sections from the cortex, there was no significant difference in cortex subdural MH scores between methods, but NPCB cortex parenchymal MH scores were significantly higher than other methods, \( \chi^2 (2, n=38) = 19.957, p<0.001 \).

Within the AMCD group, comparing all brain section scores, there was significantly more parenchymal MH damage to the hindbrain than to the cortex, \( \chi^2 (2, n=39) = 12.39, p=0.002 \) (Table 2.3). Within the NPCB group, comparing all brain section scores, there was significantly
more parenchymal MH damage to the cortex than to the hindbrain, χ² (2, n=39) = 9.584, p=0.008. There were no other significant differences within groups for brain section scores.

The highest overall subdural and parenchymal MH score for each brain regardless of brain section origin indicated that all brains had some form of hemorrhage. Average highest overall subdural and parenchymal MH scores were significantly higher for the NPCB device than BFT, χ² (2, n=38) = 12.31, p=0.001, χ² (2, n=38) = 10.94, p=0.005 respectfully (Table 2.3). There were no significant differences between other methods or between highest overall scores and age groups across all methods. The most common highest subdural MH score across methods was 2 for BFT, 4 for the NPCB device, and 3 for AMCD. The most common highest parenchymal MH score was 0 for BFT, 3 for the NPCB device, and 2 for AMCD. Comparisons made within treatment groups indicated that the only significantly different overall MH scores between age groups were within the BFT group. Growers euthanized by BFT had significantly higher subdural MH scores than adults euthanized by the same method, χ² (2, n=12) = 6.72, p=0.04.
2.5 DISCUSSION

Our results demonstrated that the NPCB device was 100% effective for euthanizing all age groups of commercial meat rabbits tested and that AMCD was also a highly effective technique with an overall 6% failure rate, largely attributable to inexperience with specific weight range. Blunt force trauma had the highest failure rate with 13 of 63 animals (22%) unsuccessfully euthanized, including a 43% failure rate (8 of 14) in adult rabbits. Based on these findings we cannot recommend blunt force trauma as a euthanasia method for commercial meat rabbits.

Several reasons may have accounted for BFT failures, one of which is insufficient force. Li et al. (2012) found that a difference of 150kPa of force applied during machine operated BFT altered the chance of mortality in 2kg rabbits. Rabbits in Li et al. (2012)’s mild injury group were also less likely to die from their brain injuries with only 10% mortality compared to 60% mortality in the marked injury group. Although this may have been a factor in our study, based on our observations, a more likely cause for method failure is a lack of accuracy in applying the method to the correct anatomic location. Erasmus et al. (2010a) found that it is more challenging to apply BFT accurately to broiler turkeys than adult turkey toms and attributed this to a smaller target area. However, our research found more MH BFT damage to growers than adult rabbits. Difficulty safely restraining the rabbits was observed to be the cause limiting accuracy when applying BFT. The NPCB device has been termed a controlled form of BFT because of the similar trauma goal. Use of a device such as the NPCB device greatly improved accuracy. Reasons for high accuracy for the NPCB device include minimal restraint needed, easy alignment and application.

The device used for AMCD also resulted in a high degree of accuracy, observed from the radiographs showing a clean dislocation at the base of the skull. Dislocations were only slightly noticeable through palpation, limiting the ability to check for correct application other than through assessment of sensibility. Gap distances in poultry after application of manual cervical dislocation are significantly larger (~5cm) than those found in this study making them easier to palpate (Martin et al., 2016). A small gap distance for rabbits could be related to their large neck muscle mass. However, Martin et al. (2016) found that heavier birds had a larger dislocation than
lighter birds, and this was not correlated with age. Gap distance is more likely related to species anatomy differences. This device allowed cervical dislocation to be possible on rabbits weighing >1kg, for which manual cervical dislocation would not have been appropriate (CCAC, 2010; AVMA, 2013).

The immediate insensibility noted following CD in rabbits in this study is quite different from findings in poultry, in which brainstem reflexes are reported to persist for several seconds after application (Erasmus et al., 2010a; Martin et al., 2016). In Erasmus’ et al. (2010b) study turkey hens were not immediately insensible after cervical crushing, corresponding to lower MH scores (scored using a similar method) than the findings in this study. The mean MH scores for rabbits euthanized successfully by AMCD in this study were 2.1 (± 0.2) for subdural and 0.9 (± 0.2) for parenchymal, compared to 0.5 (± 0.5) for subarachnoid and 0 (± 0) for parenchymal MH for turkey hens after cervical crushing (Erasmus et al., 2010b). Immediate insensibility following CD may be a species-specific response or a response specific to the device. This is the first study to assess a wall-mounted device for AMCD in any species. It has been suggested that a concussive force is needed during CD to cause immediate insensibility (Sparrey et al., 2014). Within the AMCD group of rabbits, microscopic scores of parenchymal hemorrhage were higher in the hindbrain than the cortex, as expected based on the area the device applies pressure. This suggests that sufficient force is applied to cause immediate and significant vessel disruption and hemorrhage at a critical area of the brain responsible for sensibility.

There was a significant difference between all 3 euthanasia methods for the amount of skull fractures. No skull fractures were predicted to occur during AMCD in accordance with proper technique. Although there were no fractures of the vertebrae some rabbits had minor occipital bone fractures caused by pressure of the device during downward application force. It was not predicted that there would be a difference in skull fractures resulting from the NPCB device compared to BFT. A difference in skull damage between BFT and NPCB rabbits is in line with our other BFT findings of little damage to brain, even in successful euthanasia cases. Across all euthanasia methods pre-weaned kits had more skull fractures than the adults. Skull thickness correlates with age and this finding might have been due to the younger rabbits’ thinner skulls fracturing more easily. This supports our reasoning for altering pressure settings for the NPCB device in accordance with age in line with skull development. Finnie et al. (2003)
used a different model of the NPCB device and compared the effects on pig and lambs, finding that less skull damage occurred to pigs than lambs, relating this to skull thickness.

The NPCB device caused significantly more subcutaneous GH than the other 2 methods. The strength of subcutaneous GH findings is minimal compared to the other measurements, based on research evaluating cervical crushing in turkeys (Erasmus et al., 2010b). Sensible turkeys with no direct damage to the brain still had a significant amount of subcutaneous GH. The predicted reason was ruptured carotid arteries causing blood flow under the scalp. This might be the case for both AMCD and BFT in this study and not reflective of damage to the brain. Unlike subcutaneous GH hemorrhage, scores for subdural GH, subdural MH, and parenchymal MH have been used as indicators of traumatic brain injury that are likely to be associated with insensibility and mortality (Erasmus et al., 2010b; Finnie et al., 2003; Li et al., 2012; Casey-Trott et al., 2013). Specifically in rabbits, parenchymal MH was linked to mortality after BFT (Li et al., 2012). Rabbits euthanized with the NPCB device had more parenchymal MH in the cortex than in the hindbrain. Piglets euthanized with the NPCB device similarly had significantly higher parenchymal MH in the cerebral cortex than other brain sections (Casey-Trott et al., 2013; Casey Trott et al., 2014), which is the area of the brain the device directly targets. Overall rabbits euthanized with the NPCB device received a mean parenchymal MH score of 2.1 ± 0.2 which is comparable to the mean parenchymal MH score for other species euthanized with the NPCB device (Casey-Trott et al., 2013; Casey Trott et al., 2014; Erasmus et al., 2010b). The NPCB device had significantly higher subdural GH, subdural MH and parenchymal MH scores than the other methods. Within the BFT group, scores for parenchymal MH and subdural MH were significantly higher for the growers than the adults. This could be associated with the higher chance of method failure in the BFT adult group. It can be concluded that parenchymal and subdural MH scores are associated indicators of insensibility.

Clonic and tonic convulsions in rabbits did not follow any clear pattern or transition as reported for other species, such as piglets, where clonic convulsions begin first, and are followed by tonic convolution (Casey-Trott et al., 2013; Casey Trott et al., 2014). In broiler chickens, a correlation was found between the end of convulsions and time of brain death, as indicated by a flat line EEG (Dawson et al., 2009). In the current study there was a significant difference between euthanasia methods with rabbits euthanized by BFT convulsing for the shortest
duration. However the significance of such a correlation is limited to esthetic versus animal welfare concerns, if the animal remains insensible until time of brain death.

Esthetics are an important consideration when evaluating euthanasia methods as euthanasia can have detrimental mental effects on the operator and observers (Whiting and Marion, 2011). Increasing the esthetics of a method also increases the operator’s comfort and confidence with euthanasia, and may result in euthanasia being applied in a more timely fashion on-farm (Walsh et al., 2016). The NPCB device requires minimal animal restraint, is associated with minimal external bleeding, and distances the operator from the task, eliminating the need for physical force to induce cerebral trauma. Operators using a NPCB device for piglets rated the device highly (8.7/10), indicating that it was easy to use and more esthetically pleasing than BFT (Casey-Trott et al., 2013). In rabbits, BFT is associated with a high failure rate, difficult restraint, significant physical force needed and marked external bleeding, making it unpleasing esthetically. Assisted manual cervical dislocation still requires physical force, and when learning how to use, might result in decapitation or method failure. Despite this, AMCD can be rapidly applied, results in immediate insensibility, no external bleeding and is improved with experience.

Among the three euthanasia methods evaluated in this study, there were differences in the training and experience operators had with their methods. Operators using BFT had never been formally trained in the method, but had been using the technique for 8 or more years. The operator for the AMCD had been using this method for 6 months prior to this study and was self-taught based on an online video. The operator of the NPCB device had received training from an experienced NPCB device operator and had practiced the technique on rabbit cadavers during the pilot study. With any physical method of euthanasia there is a learning curve and this is the thought to be the reason for the 3 AMCD failures. These animals were outside the weight range that the operator had experience with and required the technique and force to be altered. Our study suggests that the NPCB device requires the least training for proficiency; however, training should occur for all euthanasia methods applied to meat rabbits. Limitations of this study include that rabbits were not randomly assigned to methods. Instead operators conducted the methods they were experienced with on their own animals. This study design also meant that scoring for insensibility and macroscopic damage was not blinded, although microscopic assessments were blinded.
Cost and operator safety were also evaluated to ensure euthanasia methods validated are practical. Blunt force trauma does not require a specific tool to be purchased, but safety is a concern as difficulty to restrain could result in self injury. External bleeding and possible brain tissue discharge are also biosecurity safety concerns for BFT. The AMCD can be homemade or purchased commercially for ~$120 (Rabbit Wringer, [http://www.rabbitwringer.com](http://www.rabbitwringer.com), West Grove, PA). This device is accommodating to different breeds and sizes of rabbits with an adjustable neck plate. The device does not pose a safety risk to the operator, such as physical harm or biosecurity. The AMCD can be homemade or purchased commercially for ~$120 (Rabbit Wringer, [http://www.rabbitwringer.com](http://www.rabbitwringer.com), West Grove, PA). This device is accommodating to different breeds and sizes of rabbits with an adjustable neck plate. The device does not pose a safety risk to the operator, such as physical harm or biosecurity. The NPCB device used was the Zephyr-E, which can be purchased for ~$1,200 (Bock Industries, Philipsburg, PA). Restraint of pre-weaned kits increases the proximity of the device to the hand leading to a potential safety risk. The manufacturer of the device proposes that accidental misfire of the device to the hand would result in bruising. Results on operation of the device are specific to its design and the size of the rabbit. Re-evaluation would be required if modifications to the device were made, if it was applied to rabbits of a breed other than the New Zealand white-like rabbits and outside the weight range of 0.2 to 3.3kg. All methods should be tested first on cadavers before trying on live rabbits.

In conclusion, the results of this study suggest that blunt force trauma is neither a humane nor an esthetic method for meat rabbits of any age and it is not recommended for on-farm euthanasia of rabbits. Both a non-penetrating captive bolt device and the assisted manual cervical dislocation method provided effective, humane single step methods for euthanasia of rabbits >150g. Both techniques require operator training and there is a significant difference in costs for these 2 techniques.
Figure 2.1. Device (Rabbit Wringer, http://www.rabbitwringer.com, West Grove, PA) employed for assisted manual cervical dislocation (top) and correct application (bottom).
Figure 2.2. The non-penetrating captive bolt device (Zephyr-E, Bock Industries, Philipsburg, PA). (A) The device with the safety pin removed. (B) The bolt at its full extension from the barrel. (C) Correct location and handling for applying the non-penetrating captive bolt device to a rabbit’s head.
Figure 2.3. Eight week-old grower rabbit euthanized with the non-penetrating captive bolt device. (A) Survey radiograph shows depressed cranial bones (arrow). (B) Marked subcutaneous hemorrhage completely covers the area from the eyes to the base of the skull (gross score of 4/4). (C) More than five complete fractures are present leaving the brain exposed (gross score of 4/4). (D) Marked subdural ventral hemorrhage with 76% coverage (gross score of 4/4). (E) Marked subdural dorsal hemorrhage (gross score of 4/4). (F) Photomicrograph (20x magnification) of marked subdural hemorrhage (histologic score of 4/4) and moderate parenchymal hemorrhage (histologic score of 3/4) in the mid-brain.
Figure 2.4. Six week-old grower rabbit euthanized by blunt force trauma. (A) Survey radiograph shows two fractures (arrows) in the cranial bones. (B) Mild subcutaneous hemorrhage covers less than 25% of the area from the eyes to the base of the skull (gross score of 1/4). (C) More than one single depressed fracture is present (gross score of 3/4). (D) Mild subdural dorsal hemorrhage covers less than 25% of the brain (gross score of 1/4). (E) Mild subdural ventral gross hemorrhage covers 26 to 50% of the brain (gross score of 2/4). (F) Photomicrograph (20x magnification) of mild subdural hemorrhage (histologic score of 2/4) and no parenchymal hemorrhage (histologic score of 0/4) in the mid-brain.
Figure 2.5. Eight week-old grower rabbit euthanized by assisted manual cervical dislocation. (A) Survey radiograph shows a clean dislocation between the base of the skull and the first vertebrae (arrow). (B) No subcutaneous hemorrhage is present (gross score of 0/4). (C) Marked perispinal hemorrhage (gross score of 4/4). This rabbit also had a large abscess on its neck. (D) No subdural dorsal hemorrhage is noted (gross score of 0/4). (E) No subdural ventral hemorrhage is noted (gross score of 0/4). (F) Photomicrograph (20x magnification) of brain demonstrating a lack of subdural hemorrhage (histologic score of 0/4) and mild parenchymal hemorrhage in the mid-brain (histologic score of 1/4).
TABLES

**Table 2.1.** Number of rabbits per age group euthanized by different methods included in each stage of the trial.

<table>
<thead>
<tr>
<th>Method</th>
<th>Age group¹</th>
<th>Total euthanized</th>
<th>Body weight, kg (mean ± SE)</th>
<th>No. successfully euthanized</th>
<th>No. radiographed</th>
<th>No. gross scored</th>
<th>No. microscopic scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt force trauma</td>
<td>Pre-weaned kits</td>
<td>23</td>
<td>0.1 ± 0.03</td>
<td>20 (87%)</td>
<td>1</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Growers</td>
<td>21</td>
<td>1.6 ± 0.1</td>
<td>17 (81%)</td>
<td>3</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>14</td>
<td>3.6 ± 0.3</td>
<td>8 (57%)</td>
<td>0</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>58</td>
<td>1.5 ± 0.2</td>
<td>45 (78%)</td>
<td>4</td>
<td>45</td>
<td>12</td>
</tr>
<tr>
<td>Non-penetrating captive bolt device</td>
<td>Pre-weaned kits</td>
<td>17</td>
<td>0.2 ± 0.04</td>
<td>17 (100%)</td>
<td>0</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Growers</td>
<td>26</td>
<td>1.4 ± 0.1</td>
<td>26 (100%)</td>
<td>4</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>20</td>
<td>3.3 ± 0.2</td>
<td>20 (100%)</td>
<td>0</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>63</td>
<td>1.7 ± 0.2</td>
<td>63 (100%)</td>
<td>4</td>
<td>63</td>
<td>13</td>
</tr>
<tr>
<td>Assisted manual cervical dislocation</td>
<td>Pre-weaned kits</td>
<td>9</td>
<td>0.4 ± 0.1</td>
<td>7 (78%)</td>
<td>0</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Growers</td>
<td>25</td>
<td>1.3 ± 0.1</td>
<td>25 (100%)</td>
<td>4</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>15</td>
<td>3.3 ± 0.2</td>
<td>14 (93%)</td>
<td>0</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>49</td>
<td>1.7 ± 0.2</td>
<td>46 (94%)</td>
<td>4</td>
<td>46</td>
<td>13</td>
</tr>
<tr>
<td>Grand total</td>
<td></td>
<td>170</td>
<td>1.6 ± 0.1</td>
<td>154 (91%)</td>
<td>12</td>
<td>154</td>
<td>38</td>
</tr>
</tbody>
</table>

¹Pre-weaned kits = 0 to 5 wk, Growers = 6 to 12 wk, Adults = >12 wk
Table 2.2. Scoring system for grading skull fractures, macroscopic subcutaneous gross hemorrhage, and subdural gross hemorrhage in rabbits euthanized by different methods.¹

<table>
<thead>
<tr>
<th>Score²</th>
<th>Fracture score description</th>
<th>Hemorrhage score description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No fracture, intact skull</td>
<td>No hemorrhage</td>
</tr>
<tr>
<td>1</td>
<td>Hairline fracture, no separation of bone</td>
<td>&lt;25% of surface area covered</td>
</tr>
<tr>
<td>2</td>
<td>1 to 2 complete fully separated fractures or single depressed fracture</td>
<td>26 to 50% of surface are covered</td>
</tr>
<tr>
<td>3</td>
<td>More than just a single depressed fracture, 3 to 5 complete fractures</td>
<td>51 to 75% coverage</td>
</tr>
<tr>
<td>4</td>
<td>&gt;5 complete fractures</td>
<td>76% to complete coverage</td>
</tr>
</tbody>
</table>

¹ Adopted from Casey-Trott et al. (2014) and Veltri and Klem (2005).
² Mild = score of 0 or 1, moderate = score of 2, marked = score of 3 or 4
Table 2.3. Subdural (SD) and parenchymal (P) microscopic hemorrhage scores (± SE) across method and brain sections.

<table>
<thead>
<tr>
<th>Method</th>
<th>Hindbrain</th>
<th>Mid-brain</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>P</td>
<td>SD</td>
</tr>
<tr>
<td>Blunt force trauma</td>
<td>1.4&lt;sup&gt;a&lt;/sup&gt; ± 0.4</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt; ± 0.3</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt; ± 0.4</td>
</tr>
<tr>
<td>Non-penetrating captive bolt</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt; ± 0.3</td>
<td>1.5&lt;sup&gt;a,bb&lt;/sup&gt; ± 0.3</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt; ± 0.2</td>
</tr>
<tr>
<td>Assisted manual cervical dislocation</td>
<td>2.8&lt;sup&gt;b&lt;/sup&gt; ± 0.3</td>
<td>1.6&lt;sup&gt;a,aa&lt;/sup&gt; ± 0.2</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt; ± 0.3</td>
</tr>
</tbody>
</table>

Mean scores:

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt force trauma</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt; ± 0.2</td>
<td>0.7&lt;sup&gt;b&lt;/sup&gt; ± 0.2</td>
</tr>
<tr>
<td>Non-penetrating captive bolt</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt; ± 0.1</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt; ± 0.2</td>
</tr>
<tr>
<td>Assisted manual cervical dislocation</td>
<td>2.1 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Different single letter subscripts denote a significant difference between methods for column comparisons of average score, a ≠ b, p <0.05. Different double letter subscripts denote a significant difference for a type of brain hemorrhage between brain sections for row comparisons of average score, aa ≠ bb, p <0.05.

<sup>1</sup>Each brain was divided into 3 coronal sections, n=38 brains (blunt force trauma = 12, non-penetrating captive bolt = 13, assisted manual cervical dislocation = 13). 114 brain sections (hindbrain = 38, mid-brain = 38, cortex = 38)
Chapter 3: Euthanasia of commercial meat rabbits with carbon dioxide: Behavioural and physiologic responses to gradual- and fast-fill chamber exposure

3.1 ABSTRACT

Euthanasia requires there be minimal distress prior to loss of sensibility. Use of carbon dioxide (CO₂) as a euthanasia method has not been evaluated for rabbits using different chamber fill rates. The objective of this study was to determine time to insensibility and death, and to evaluate the behavioural and physiologic responses of commercial meat rabbits (pre-weaned to adult) when exposed to gradual-fill and fast-fill rates of CO₂. Eighty-one rabbits from 3 different age categories (pre-weaned kits n=28, growers n=28, and adults n=25) were randomly assigned to 2 different treatment groups: gradual-fill (n=42) and fast-fill (n=39) CO₂ exposure. Chamber CO₂ and oxygen concentrations were monitored and manually recorded every 10s. A hand input was created in the side of the chamber to allow reflex checking for insensibility. Sensibility was lost in significantly shorter time (p<0.001) during fast-fill (40s ± 1) than gradual-fill (99s ± 3). There was significant difference (p = 0.02) in the CO₂ chamber concentration the rabbits were exposed to before they lost sensibility, 20.7% ± 0.9 for gradual-fill and 16.5% ± 1.5 for fast-fill. Rabbits were left in the chamber for 360s to 420s. The rabbits took their last breath at significantly different times (p<0.001), and concentrations (p<0.001) depending on fill rate, 328s ± 11, 55.2% ± 0.8 for gradual-fill and 116s ± 4, 65.6% ± 1.1 for fast-fill. Fast-fill rate resulted in a more rapid loss of sensibility and a faster death. Carbon dioxide, regardless of fill rate was 100% effective in achieving death for all rabbits. There were minimal differences in behavioural responses between fill rates with no obvious signs of distress. These findings indicate that CO₂ is suitable for commercial meat rabbit euthanasia using a displacement rate of ≥28% volume change/min exposing rabbit up to 40% CO₂ in the first minute.
3.2 INTRODUCTION

Protocols identifying the best method to use when an animal needs to be culled should be in place for all livestock species, so that animals are killed in a prompt and effective manner. Euthanasia is a humane death achieved through immediate irreversible insensibility (unconsciousness), and subsequent death. When insensibility is not immediate, it is recommended that the period before insensibility should not cause avoidable anxiety, pain or suffering (AVMA, 2013).

No validated methods exist for on-farm euthanasia of commercial meat rabbits. Although physical euthanasia methods are most commonly used on-farm by commercial meat rabbit producers in Canada, concerns for esthetics and dissatisfaction with current methods suggests gassing methods might be a favorable alternative option (Walsh et al., 2016). Producers indicated they would be willing to use gassing methods if operator safety could be ensured. Carbon dioxide (CO₂) gassing is a common method chosen and considered for emergency depopulation in other livestock species because it can be applied easily to multiple animals at once and limits animal handling. However, CO₂ has its limitations. It does not cause immediate insensibility and can potentially cause distress prior to insensibility. Carbon dioxide leads to death by hypercapnia and hypoxia. Atmospheric air consists of 20.9% oxygen (O₂), and CO₂ is considered a trace gas at 0.04%. Elevated CO₂ levels provoke physiologic reactions, such as increased respiration and formation of carbonic acid on mucus membranes (Danneman et al., 1997; Sadler et al., 2014a). Once the animal is insensible the method is assessed based on efficacy. An efficient euthanasia method is one that achieves irreversible insensibility and subsequent death.

The use of CO₂ on rabbits has been evaluated for warren fumigation (Hayward and Lisson, 1978) and stunning before slaughter (Llonch et al., 2012; Dalmau et al., 2016), but not as a euthanasia method. Currently the European Food Safety Authority and American Veterinary Medical Association (AVMA) do not recommend use of CO₂ for euthanasia of sensible rabbits (AVMA, 2013; EFSA, 2013). There is concern for the humane application and effectiveness of CO₂ use on rabbits. While used effectively on other animals, rabbits are a burrowing species exposed to CO₂ level above atmospheric while underground (Hayward and Lisson, 1978). As a result, it is proposed that they have a higher CO₂ tolerance, leading to a prolonged and
potentially more adverse period to insensibility and death. Gradual-fill, defined by the AVMA as a displacement rate of 10% to 30% chamber volume change/min, has been deemed as a less adverse form of CO₂ exposure for rodents (AVMA, 2013). This gradual-fill rate has not been evaluated for use on rabbits. Displacement rate (volume change/min) is multiplied by the chamber volume (L) to calculate the flow rate (L/min) of gas entering the chamber controlled by a flow meter. To determine exactly what the animals are experiencing during gas exposure equipment should be used to measure the gas concentration change (%) in the chamber.

The objective of this research was to determine time to insensibility and death, and to evaluate the welfare of commercial meat rabbits (pre-weaned to adult) when exposed to gradual-fill and fast-fill rates of CO₂. Welfare was judged based on behavioural responses before loss of sensibility. It was hypothesized that both fill rates would cause a period of distress to the rabbits, but at a lower intensity during gradual-fill.
3.3 MATERIALS AND METHODS

Study Animals

Eighty-one New Zealand white and California mix rabbits (11 d to 25 mo old) were used in this study. Rabbits were obtained from an Ontario commercial meat rabbit farm, identified for cull by the producer based on: unthrifty, injured, sick (enteritis), and end of reproductive utility. Any rabbits with clinically obvious respiratory disease were rejected from the study. Rabbits from 3 different age categories (pre-weaned kits n=28, growers n=28, and adults n=25) were randomly assigned to 2 different treatment groups: gradual-fill (n=42) and fast-fill (n=39) CO₂ exposure (Table 3.1). Age categories were divided into, pre-weaned kits 11 d to 5 wk old, growers 6 to 12 wk, and adults >12 wk. Young pre-weaned kits less than 11 d were evaluated, but included in a separate study on the difficulties euthanizing rabbits at this age. Pre-weaned kits and growers were a mix of both sexes and adults were does at the end of production cycle. The procedures and protocol for this research were reviewed and approved by the University of Guelph Animal Care Committee (AUP3366). The University of Guelph is in compliance with the Animals for Research Act of Ontario (OMAFRA, 1990) and the Guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Experimental Apparatus

To facilitate behavioural observations, the 14.3L induction chamber (VetEquip, Inc., Livermore, CA) (internal dimensions; 38.5cm length x 19.4cm width x 19.1cm height) was made of clear acrylic and modified to allow for testing of reflexes. Following a similar design to Moody et al. (2015), a 7.5cm diameter hole was drilled in the center of the front chamber wall 4cm from the floor bottom. The hand input was created using a clear poly boot cover (Fisher Scientific, Ottawa, ON), a wire diameter ring for structure, and sealed with duct tape (Fig. 3.1A).
Industrial grade CO\textsubscript{2} was supplied from a 22.7kg cylinder (Praxair Canada, Mississauga, ON) equipped with a single stage regulator (Harris, Gainesville, GA) and connected to a M3000 table-top veterinary anesthesia machine (Supera Anesthesia Innovations, Clackamas, OR). The anesthesia machine was modified to solely utilize the flow meter and was connected to the induction chamber inflow port. The inflow and outflow ports were located on the same wall of the chamber 15cm from floor bottom and 3cm from the side walls. The outflow tube was 6m long and emptied downwind outside of the building. An overview of the setup is shown in Figure 3.1A.

Chamber gas concentrations were monitored and manually recorded every 10s using the X-am 7000 multi-gas monitor (Draeger Safety Canada Ltd., Mississauga, ON) configured to measure CO\textsubscript{2} concentration in the range of 0\% to 100\% and O\textsubscript{2} concentration 0\% to 25\%. The gas monitor sat outside the induction chamber with the pump connected to a 0.7cm diameter 14cm long tube entering the chamber through a 1cm diameter hole drilled at grower rabbits nose height (9.3cm from the floor bottom) centered on the same chamber wall as the inflow and outflow ports (Fig. 3.1B). Design and testing of the equipment was conducted under the guidance and assistance of a mechanical engineer (John Van de Vegte).

**Gas Concentration Analysis – Dry Run Chamber Testing**

Before starting the animal experiments, 24 CO\textsubscript{2} trials without any animals were completed to test for any safety risks for personnel working near the chambers and to determine the equipment settings needed to achieve the desired chamber concentration fill rates. Chamber CO\textsubscript{2} and O\textsubscript{2} concentrations were recorded every 10s from the gas monitor. A second identical monitor was placed immediately outside the chamber for assessment of the ambient air, to check for leaks and to ensure personnel safety. The gradual-fill rate desired was based on the AVMA recommendation of 10\% to 30\% chamber volume change/min displacement rate. The fast-fill flow rate was established based on the flow meter’s maximum capacity. The flow rate and displacement rate for fast-fill was calculated from the average CO\textsubscript{2} concentrations assuming
similar chamber gas losses (gas inhaled by the rabbit and gas lost through the exhaust and leaks) as gradual-fill.

**Gas Concentration Analysis - Rabbit CO₂ Exposure Trials**

Chamber temperature during CO₂ filling was recorded for the first 17 trials conducted on the first research trial day and discontinued afterwards due to minimal fluctuation. Temperature was monitored using an indoor/outdoor thermometer (RadioShack, Fort Worth, TX) with a remote sensor probe inserted through a 1cm diameter hole (9.3cm from the floor bottom) on the opposite chamber wall as the inflow and outflow ports.

Animal experimental trials were conducted on a large (n=600 doe) farm in Southwestern Ontario over 7 research trial days from July 14 to August 9, 2016 in a drive-in shed open to the outdoors, allowing for good air flow from a natural breeze. Daily weather observations and fluctuations throughout the day were recorded. Order of treatment was assigned randomly the day before using free software (random.org). Rabbits were euthanized individually while the other rabbits waited in holding crates.

**Behavioural and Physiologic Analyses**

An ethogram (Table 3.2) was developed based on predicted behaviours and modified after the first day of rabbit exposure trials. Behavioural and physiologic responses were constantly observed during the period of exposure and recorded every 10s. The first time a behaviour was observed during exposure, the time and concentration was recorded. Reflexes were checked and when a response was found absent, time and concentration was recorded. Behavioural observations were made both directly and via video recording using a GZ-E200 full HD Everio Camcorder (JVC, Yokohama, Japan). For direct observation the observer stood at eye level to the chamber and used the hand input to check for reflexes. Behavioural and physiologic
observations were spoken out-loud and recorded by the personnel recording and monitoring the chamber gas concentration. Video recordings were used to confirm and check behavioural and physiologic observations when inputting data. Chamber CO\(_2\) and O\(_2\) concentration was recorded every 10s from the gas monitor. All rabbits, regardless of treatment group, were exposed to CO\(_2\) for 360s to 420s. Heartbeat was palpated upon removal from the chamber to confirm death and post-mortem weights were taken. Rabbits were observed for return to breathing or sensibility for a period of time (5 to 19min) after removal from the chamber and a non-penetrating captive bolt device (Zephyr-E, Bock Industries, Philipsburg, PA) was used as a secondary method. The induction chamber was disinfected in between use and time allowed for ambient air displacement.

**Statistical Analysis**

Statistical analyses were conducted using SPSS (SPSS Statistics for Windows, Version 23.0. 2014. Armonk, NY: IBM Corp.), and p<0.05 was accepted for significance. All values are reported as mean ± SE. Dependent variables were checked for normality using the Shapiro-Wilk test. To check for differences in time and concentration between fill rate treatments, a non-parametric independent sample Mann-Whitney U was used for variables not normally distributed and an independent samples t-test was used for variables that were normally distributed. The Kruskal Wallis 1-way ANOVA was used to check for differences between age categories within treatment. Data was split by treatment to allow for comparisons within treatment groups, age category as the independent variable. To determine if behaviours such as urination, open mouth breathing and convulsions occurred before a loss of sensibility, time of behaviour occurrence was subtracted from time of loss of righting reflex. Behaviours compared for their occurrence were gasping, head shake, chewing, head bob, urination, vocalizations and open mouth breathing. They were first categorized as occurring or not occurring for each animal during exposure. A Chi-square test of independence tested for associations between occurrence of a behaviour and treatment. Column properties were compared using a Bonferroni correction for multiple analyses. Cramer’s V test was run to determine the strength of the association.
3.4 RESULTS

Gas Concentration Analysis – Dry Run Chamber Testing

From the 24 dry CO$_2$ runs, it was determined that a cylinder regulator output pressure of 30psi was needed to overcome the resistance of the tubing and maximize air flow per flow rate. The gradual-fill displacement rate used was 28% chamber volume change/min and was within the AVMA recommended range (10% to 30% chamber volume change/min). This displacement rate was most accurately achieved with the flow meter set to a flow rate of 4 L/min. The gas monitor recorded a mean chamber CO$_2$ concentration increase of 10% ± 0.3 (range of 6.8% to 15.5%) in the first min during gradual-fill. The fast-fill rate was established based on the flow meter’s maximum capacity calculated at 8.3L/min with a calculated displacement rate of 58% chamber volume change/min. The gas monitor recorded a mean chamber CO$_2$ concentration increase of 39% ± 0.7 (range of 32% to 54%) in the first min during fast-fill. During the fast-fill trials, maximum CO$_2$ chamber concentration was met within 3min. The flow rate was turned down to 4L/min at 3min, which allowed the CO$_2$ concentration to be held constant.

Measurements of the ambient air outside the chamber determined that there were minimal safety risks for personnel working near the chamber. The most common location for leaks was at the hand input. Increasing the thickness of the material for the hand input from a plastic grocery bag to a poly boot cover reduced leaks. Ambient air CO$_2$ levels remained below the gas monitor’s safety alarm level of 3%.
Gas Concentration Analysis - Rabbit CO₂ Exposure Trials

Chamber temperature monitored during CO₂ filling of the first 17 trials determined minimal temperature change. The greatest temperature fluctuation inside the induction chamber was 3.9°C during a gradual-fill trial (Fig. 3.3). The outdoor temperature during this research trial day ranged from 24.6°C to 28°C. During the other 6 research trial days the temperature was between 19°C to 30°C, with high humidity, and a natural breeze.

Chamber gas concentrations were consistent across all trials of the same treatment, forming tight gradual-fill curves and fast-fill curves (Fig. 3.2). Average CO₂ and O₂ concentrations at each time point were calculated for treatments (Fig. 3.4; Fig. 3.5). The maximum concentration achieved for gradual-fill was 58.4% CO₂ concentration, reached at 360s. The fast-fill rate achieved higher concentrations of CO₂. The maximum fast-fill CO₂ concentration was 73.6% reached at 230s. Oxygen concentration mirrored the CO₂ concentration curve, lowering as CO₂ increased. The lowest O₂ concentration during gradual-fill was 5.2% at 420s (end of exposure), and 1.1% in 180s during fast-fill.

Behavioural and Physiologic Analyses

Rabbits progressed from a loss of balance to loss of posture to loss of righting reflex (Table 3.2). Loss of righting reflex occurred within seconds (9s ± 1) of loss of posture and was recorded for all but 1 rabbit (lack of space in the chamber prohibited checking). Loss of righting reflex (inability to right itself after being turned on its side) was used as the primary variable to judge loss of sensibility. Loss of righting reflex occurred statistically earlier (p<0.001) during fast-fill (40s ± 1) than gradual-fill in 99s ± 3. When analysed within each fill rate, time until loss of righting reflex significantly differed (p<0.001) between pre-weaned kits and growers during gradual-fill. Pre-weaned kits lost sensibility earlier (84s ± 3) compared to growers (113s ± 5). There was a significant difference (p=0.02) in the mean overall CO₂ chamber concentration the rabbits were exposed to before they lost sensibility, 20.7% ± 0.9 for gradual-fill and 16.5% ± 1.5
for fast-fill. The maximum CO₂ concentration any rabbit was exposed to before loss of sensibility was 36% for gradual-fill and 39% for fast-fill.

The palpebral reflex was found absent upon inspection after loss of righting reflex in all rabbits. Toe pinch and ear pinch varied in which reflex disappeared first. The corneal reflex was the last reflex to disappear for all rabbits. Corneal reflex was absent at statistically significant different times per fill rate; 142 ± 4 for gradual-fill and 50 ± 2 for fast-fill (p<0.001). Carbon dioxide concentration did not vary significantly between fill rates (p=0.11) when corneal reflex was found absent; 30.5 ± 0.8 for gradual-fill and 27.3 ± 2.0 for fast-fill. When analysed within gradual-fill, time to loss of corneal reflex significantly differed between pre-weaned kits and growers (p=0.01), and adults and growers (p=0.001). The corneal reflex was absent for adults at 129 ± 7, 164 ± 8 for growers and 132 ± 3 for pre-weaned kits.

Respiration rate changed during the process of euthanasia from an increased respiration to open mouth breathing to decreased respiration to last breath (Table 3.2; Fig. 3.6). Increased respiration occurred at significantly different times (p<0.001), and concentrations (p=0.002) per treatment. Increased respiration occurred at 35 ± 3, 4.2% ± 0.7 for gradual-fill, and 15 ± 1, 1.8% ± 0.3 for fast-fill. Breath holding (lack of nostril flaring or open mouth breathing while sensible) was not observed in any rabbits. When open mouth breathing did occur, 65% of the time it started after the rabbit was already insensible. Distribution of open mouth breathing across age groups and fill rates is shown in Table 3.3. There was no association found between open mouth breathing before loss of sensibility and fill rate treatment, χ² (1, n=81) = 0.48, p=0.49. Last breath was taken at significantly different times (p<0.001), and concentrations (p<0.001) depending on fill rate; 328 ± 11, 55.2 ± 0.8 for gradual-fill and 116 ± 4, 65.6 ± 1.1 for fast-fill.

Chewing motions made with the mouth and tongue was the only behaviour that was not independent of treatment, χ² (1, n=81) = 5.56, p=0.02, and had a medium strength of association (Cramer’s V=0.3). More rabbits displayed this behaviour during gradual-fill (Table 3.3). Chewing occurred at 71 ± 8, 13.5% ± 2.2 CO₂ for gradual-fill (n=19), and 27.5 ± 4, 8.3% ± 3.6 for fast-fill (n=8). Other behaviours observed, but with no findings of associations between occurrence and treatment were, gasping (n=33), head shake (n=16), head bobbing (n=24), urination (n=28), coughing (n=3), vocalizations (n=6), defecation (n=7), and head lifting (n=56).
Table 3.3 shows the distribution of these behaviours across age groups. Urination was most commonly observed before loss of righting reflex (n=18). Vocalizations were observed occurring in both fill rates but more frequently during fast-fill, and occurred evenly across age groups (Table 3.4). Five rabbits vocalized during fast-fill at a mean time of 12s ± 2, and mean concentration of 1.5% ± 0.3. One rabbit vocalized during gradual-fill at 140s, 30%. The rabbit that vocalized during gradual-fill started making vocalizations after loss of righting reflex and stopped shortly before the corneal reflex was lost. During this time spent vocalizing the rabbit was considered insensible based on no response to toe pinch, ear pinch and palpebral reflex. For one of the rabbits from the fast-fill group, vocalizations (starting before loss of balance) persisted for a short period of time after loss of the righting reflex. Response to CO₂ varied amongst individuals with some rabbits (n=14) turning around upon first exposure while other rabbits stuck their faces directly in the inflow experiencing 100% CO₂ with no obvious behaviour response.

A constant pattern was observed where rabbit’s pupils would constrict during exposure to CO₂ and dilate after the rabbits became insensible. Rabbits became cyanotic after loss of sensibility, noted by purple colouration of the lips and sometimes the ears. Some rabbits (n=10) were observed squinting during the period of sensibility. After death and removal of the rabbits, it was observed that some rabbits had red colouration of the nictitating membrane and occasional slight bleeding from around the eyes and nostrils. During exposure, there was never any discharge from the eyes, nose or mouth, and no cleaning of the face.

Clonic (n=24) and tonic (n=11) convulsions never occurred before loss of righting reflex. All rabbits remained in the chamber for 360s to 420s. Three rabbits were left in the chamber beyond this time period due to a delay in last breath or movement. Time frame of exposure was based on observations from pilot studies and on AVMA recommendations to expose animals during gradual-fill for at least 1 minute after respiratory arrest (AVMA, 2013). No rabbits had a heartbeat present when removed from the chamber, and none regained breathing or sensibility during the time they were observed afterwards.
3.5 DISCUSSION

The objective of this study was to determine time to insensibility, time to death, and to evaluate welfare during CO₂ exposure. Exposure to CO₂ at a gradual-fill rate (10% ± 0.3 chamber CO₂ increase in the first min, displacement rate of 28% volume change/min) caused insensibility, judged by a loss of righting reflex, in 99s ± 3. This occurred significantly earlier during fast-fill (39% ± 0.7 chamber CO₂ increase in the first min, 58% volume change/min) in 40s ± 1. Rabbits stopped breathing and were determined dead in 328s ± 11 during gradual-fill and in 116s ± 4 during fast-fill. Irreversible insensibility, judged by a lack of heartbeat upon removal and no return of breathing or response to reflexes, was achieved for all rabbits regardless of treatment. There were minimal differences between fill rates for behaviour and physiologic responses. Our hypothesis that both fill rates would cause a period of distress for the rabbits, but at a lower intensity during gradual-fill, was not supported by the results.

Identifying exact times for loss of sensibility when exposed to CO₂ has important welfare implications, contributing to rabbit euthanasia research. Loss of sensibility, defined by loss of righting reflex, acts as an end point limiting the duration of potential distress. Sensibility was lost significantly earlier when applying the fast-fill rate. In comparison to the work of Hayward and Lisson (1978) insensibility occurred quickly during both treatments in the current study. Hayward and Lisson (1978) found that 30% CO₂ was not effective inducing insensibility in rabbits, even after 180min of exposure. It was concluded that a concentration greater than 40% was needed for loss of sensibility and death. This exposure time and concentration differs from the current study. Insensibility occurred in less than 2min and in a concentration range of 5% to 40%. Reasons for this difference is unknown as the flow rate used in Hayward and Lisson’s study was 2 to 3L/min, only slightly lower than the current experiment at 4L/min.

Current AVMA (2013) recommendations for use of CO₂ as a euthanasia method for other species suggests use of a gradual-fill displacement rate (10% to 30% volume change/min). Whether gradual-fill does limit distress is unclear in the literature. Niel and Weary (2006) found that rats with the option to avoid exposure did so quicker as displacement rate increased. Hickman et al. (2016) observed signs of distress in rats at displacement rates as low as 7% volume change/min, and found 30% volume change/min to minimize pain and distress.
piglets, faster displacement rates were advantageous for quicker onset of insensibility, lower frequency of behaviours indicative of distress and more effective at inducing death (Sadler et al., 2014a). Bolvin et al. (2016) found that for mice, displacement rate did not cause a difference in level of pain or distress. These results support our lack of behavioural and physiologic differences between gradual- and fast-fill.

The experimental design in this study was the first of its kind to allow interaction with the rabbits to test reflexes, contributing to the body of knowledge on loss of sensibility. The righting reflex was the primary variable used to judge insensibility in this study, used in combination with other reflexes. Loss of posture was validated by Dalmau et al. (2016) who equipped rabbits with an EEG, and found a correlation between neurological indicators of insensibility and loss of posture. Chisholm and Pang (2016) addressed the lack of consistency in using the same reliable indicator of insensibility in the field. Their work determined that loss of righting reflex in rats was the most sensitive measure of insensibility and use of loss of posture was incorrectly estimating when an animal was insensible. Dalmau et al. (2016) was unable to use loss of righting reflex as observations were conducted from the top of the gassing pit not allowing interaction. In the current study loss of posture occurred immediately before loss of righting reflex. These findings support our use of loss of righting reflex as the primary measure of insensibility.

Similar to other studies, the corneal reflex was the last reflex to disappear in our study. The corneal reflex has been validated for use by other studies to judge insensibility in rabbits (Dennis et al., 1988; Anil et al., 1997; Anil et al., 2000; Maria et al., 2001; Li et al., 2012). The late disappearance of the corneal reflex compared to loss of the righting reflex does not indicate a discrepancy for time to loss of sensibility but a progression to complete loss of sensibility. The corneal reflex disappears at a deeper stage of anaesthesia, past the point of being able to perceive pain (Cors et al., 2015). Therefore, the righting reflex and corneal reflex should be used in combination to determine irreversible insensibility. Telemetry is needed to confirm our findings for insensitivity and death.

Within the gradual-fill rate group there was a significant difference in time to loss of righting reflex between the pre-weaned and grower rabbits. The pre-weaned rabbits became insensible faster than the grower rabbits. Time to insensitivity during fast-fill did not vary
amongst age categories. Previous studies exposing rabbits to CO₂ used only grower rabbits at market weight (Hayward and Lisson, 1978; Llonch et al., 2012; Dalmau et al., 2016). This is the first study to determine time to insensibility across different age groups of rabbits. In other species, neonates have been observed to be more tolerant than adults to CO₂ exposure, taking longer to lose sensibility and die (Pritchett-Corning, 2009). Piglets also seem to be an exception to this pattern. Sadler et al. (2014a) identified pre-weaned piglets succumbing to the effects of the gas earlier than grower piglets, without an explanation. One explanation for our finding is that because CO₂ is denser than air, it exposed the younger animals, with less vertical height, to higher concentrations initially than the adults. Niel and Weary (2006) examined the concentration difference in different areas of a similar sized chamber and found that during the first few seconds while filling the chamber, there was a difference in concentration between the top and bottom of the chamber. After 20s the gas seemed to mix more thoroughly and no difference was observed.

Use of carbon dioxide for euthanasia purposes requires it to effectively achieve irreversible insensibility and subsequent death. While examining CO₂ for the purpose of stunning rabbits, Dalmau et al. (2016) evaluated different exposure times in a pre-filled chamber of 70, 80, 90 or 98% CO₂ for ability to sustain insensibility for up to 5min after removal. In that study, none of the tested concentrations and exposure times were 100% effective. In our study, CO₂ was 100% effective at achieving irreversible insensibility and death. None of the rabbits had a palpable heartbeat upon removal, and none returned to breathing or response to reflexes during the observational period (5 to 19min) afterwards. All rabbits in the present study took their last breath within 328s during gradual-fill and 116s for fast-fill. Last breath for fast-fill was within the exposure times Dalmau et al. (2016) tested. Oxygen concentrations were recorded, but not reported in Dalmau et al. (2016). Perhaps the O₂ concentration was not sustained low enough, such as in the present study, to result in death.

One mechanism of action and potential cause of distress, stimulated by CO₂, is air hunger indicated by a change in respiration rate (Beausoleil and Mellor, 2015). Activation of both the central and peripheral chemoreceptors by elevated CO₂ levels increases respiratory rate (McKeegan et al., 2007). Increased respiration was a physiologic response observed in the current study, occurring at a low CO₂ concentration shortly after the gas was turned on. Labored
breathing at low concentrations of is controlled by lower airway receptors capable of detecting small changes in air quality. It is debatable as to whether labored breathing at low concentrations is a conscious experience and causes pulmonary pain (McKeegan et al., 2005; McKeegan et al., 2007; Beausoleil and Mellor, 2015). Respiration rate in the current study turned into more visible labored breathing when open mouth breathing began. The majority of the time (65%) open mouth breathing occurred after loss of righting reflex, and did not correlate with fill rate. Gasping occurred 41% (n=33) of the time overall with no correlation to fill rate. Labored breathing might not be painful, but is likely fear inducing (McKeegan et al., 2007; Sadler et al., 2014a). Since open mouth breathing commonly began after other signs indicated a loss of sensibility this is likely not a concern for distress or pain. Increased respiration without open mouth breathing is not predicted to cause distress. Gasping could indicate that the rabbits were in a negative affective state (Beausoleil and Mellor, 2015).

Carbon dioxide bonds with water to form carbonic acid, making it mildly acidic, lowering intercellular pH and bonding to mucus membranes. This may create an acidic taste and irritation of the mucus membranes, including the eyes, nose and mouth, as is the case in rats (Danneman et al., 1997). Based on previous studies of rabbits exposed to gasses other than CO₂, the behaviours predicted for rabbits experiencing irritated mucus membranes were rapid blinking, vigorous cleaning of the face, nasal and eye discharge (Oliver and Blackshaw, 1979; Gigliotti et al., 2009), none of which were observed in the current study. However, some rabbits had red colouration of the nictitating membrane and occasional slight bleeding from around the eyes and nostrils. However, this was noted only after the animal was removed from the chamber at the end of exposure and not observed during the period before signs of loss of sensibility. It is possible that this red discolouration and slight bleeding was from irritation of the mucus membranes after the rabbit was insensible.

The formation of carbonic acid has the potential to cause pain by direct stimulation of the trigeminal nerve found in the nasal, oral and ocular epithelium, sending nociception and chemoreception information to the central nervous system (Anton et al., 1992; McKeegan et al., 2005). Research by McKeegan et al. (2005) evaluated the chicken’s trigeminal sensitivity to a range of irritants including carbonic acid and CO₂. Head shaking and rapid beak movements were observed and peaked at a concentration of 20% CO₂. This was below the threshold for
trigeminal innervation and thought to be a gustatory response (McKeegan et al., 2005). Although McKeegan’s et al. (2005) work was with poultry, 20% (n=16) of rabbits in the current study displayed head shaking. Head shaking occurred independent of treatment, but chewing was dependent on treatment, differing between fill-rate and is comparable to rapid beak movement in chickens. Chewing motions were seen in 33% of 81 rabbits and was significantly more common during gradual-fill. Squinting is another behaviour observed that could be due to irritation of the mucus membranes. These behaviours could be in response to aversive properties of carbonic acid, however lack of a control group limits interpretation.

In humans, pain is reported to be experienced in the nasal mucosa upon exposure to CO₂ concentrations above 40% (Anton et al., 1992). Human nociceptors are considered activated at this concentration. Signs of loss of sensibility were achieved in the current study before this concentration was reached. The maximum concentration in the chamber across all trials before signs of loss of sensibility was 39%. Rabbits in extreme pain or distress will vocalize (Mayer, 2007). Vocalizations were a rare occurrence with no correlation to fill rate, and happened at low CO₂ concentrations. There is some uncertainty in how to interpret vocalizations during this study as one rabbit began vocalizing during the period where righting reflex had been lost and the corneal reflex persisted. Unresponsiveness to other reflexes (toe pinch, ear pinch, palpebral) suggested that this rabbit was insensible. Future work should use an EEG to confirm that in such cases the rabbit is insensible and not in pain. Vocalizations demonstrate the variability in individual experience. Some rabbits were observed sticking their face directly into the inflow and chewing the plastic piece. Pure 100% CO₂ would have been blowing directly into their face and stimulating the trigeminal nerve, but these animals did not vocalize nor rapidly back away. Lack of vocalizations should not be used as a sign of good welfare. The rabbits might still be in distress, but not at a level that stimulates vocalizations. The concentration needed to stimulate nociceptors is based on human research, and a rabbit’s threshold for pain could differ. Moving forward in assessing pain in rabbits upon exposure to CO₂ future work could use the Rabbit Grimace Scale developed by Keating et al. (2012). Squinting was one of the behaviours observed in this study and is a facial action unit used in the Rabbit Grimace Scale.

Due to difference in relative space per age group and lack of a control group this study was limited in its ability to assess behavioural signs of distress. The adult commercial meat
rabbits were restricted to head movements. Breeding adult males were not used due to chamber size and low cull rates. The growers were able to turn around, but could not perform vertical movement such as standing on their hind legs. In comparison, the pre-weaned kits had enough room for a full range of movement such walking and crawling the sides. This difference in relative space effects the different behaviours the rabbits could perform. During induction, space is commonly restricted to allow for rapid inhalation. Perhaps space should have been restricted based on body size to allow for behaviour comparisons across age groups. A non-treatment group would have allowed for baseline comparisons, and would have controlled for other experimental variables causing fear. The motivation behind a behaviour is limited in correct interpretation if a control group is not used. Multiple aspects of the experimental design could have stimulated fear in both treatment groups and offset the behavioural observations. Fear could be stimulated by the novel environment, noise of the equipment, air flow and intense direct observation of a prey species. A comparison could be made to rabbits exposed to isoflurane and sevoflurane for anesthesia. The impact on welfare is the same regardless of the outcome of euthanasia or anesthesia for surgery. Flecknell et al. (1999) observed rabbits exposed to isoflurane and sevoflurane avoid the gas by raising their noses to the lid, breath-hold and violently struggle. In comparison with methods known to cause overt distress in rabbits, CO₂ exposure did not cause behavioural signs of distress.

Personnel safety was assessed in this study and found not to be a concern. Equipment set up for this experiment allowed CO₂ to empty outside of the drive-in shed. Small leaks were found near the hand input, however due to good airflow, safety levels of CO₂ in the ambient air were not breached. There are minimal concerns for personnel safety when using CO₂ on-farm for euthanasia if conducted in a well ventilated area.

As far as the authors are aware, this is the first study to evaluate the use of CO₂ for rabbit euthanasia. We determined how long it takes for a rabbit to become insensible, based on loss of righting reflex, during both gradual-fill and fast-fill, as well as the concentration at which this occurred. Signs of loss of sensibility and last breath were unjustifiably prolonged during gradual-fill, occurring in a significantly shorter period of time during fast-fill CO₂ exposure. Both methods were highly effective in that all animals were verified dead after removal from the chamber, and none returned to sensibility. Continued exposure to high concentrations of CO₂ and
low levels of O₂ after loss of sensibility resulted in a faster death during fast-fill, based on time to observed last breath. There were minimal differences between gradual- and fast-fill for the behaviours observed prior to insensibility. Based on the behaviours observed the taste of carbonic acid may be aversive to rabbits during gradual-fill CO₂ exposure. Both fill rates could be used for euthanasia, noting that fast-fill (39% ± 0.7 chamber CO₂ increase in the first min, 58% volume change/min) reduces the time to loss of sensibility. Further research is needed to assess the level of distress prior to insensibility. The requirements to meet the recommendations of this study are to match the chamber CO₂ concentration and displacement rate, and maintain exposure for 420s.
Figure 3.1. Equipment setup for carbon dioxide gassing euthanasia study. (A) Overview of setup. (B) Gas monitor (Draeger Safety Canada Ltd., Mississauga, ON) placement for measuring chamber concentrations of carbon dioxide and oxygen.
Figure 3.2. Chamber gas concentration curves during rabbit gassing trials. Chamber concentrations of carbon dioxide and oxygen were recorded every 10s. (A) Carbon dioxide curves for the gradual-fill trials (n=42). (B) Carbon dioxide curves for the fast-fill trials (n=39).
Figure 3.3. Change in chamber temperature across the first 17 gradual- and fast-fill rabbit gassing trials. Temperatures were recorded every 10s. The average ambient outside temperature across these trials is indicated by the arrow.
Figure 3.4. Mean concentration of carbon dioxide (CO$_2$) and oxygen (O$_2$) in the chamber during gradual-fill experiments (n=42). SE across time points ranged from 0.02% to 0.8%. Concentration was recorded every 10s. Median time and CO$_2$ concentration at onset of euthanasia stages: A. Increased respiration 30s, 2.4%. B. Loss of righting reflex 100s, 20.5%. C. Loss of corneal reflex 135s, 29%. D. Last breath 315s, 56%. F. Gas off and rabbit removed from chamber 420s.
**Figure 3.5.** Mean concentration of carbon dioxide (CO$_2$) and oxygen (O$_2$) in the chamber during fast-fill experiments (n=39). SE across time points ranged from 0.09% to 1.8%. Concentration was recorded every 10s. Median time and CO$_2$ concentration at onset of euthanasia stages: A. Increased respiration 10s, 1.4%. B. Loss of righting reflex 40s, 14.3%. C. Loss of corneal reflex 50s, 27%. D. Last breath 120s, 66%. E. Gas flow rate reduced to 4 L/min to maintain CO$_2$ levels at 70%, 180s. F. Gas off and rabbit removed from chamber 420s.
**Figure 3.6.** Timeline of progression of physiologic responses (median time to occurrence) during gradual-fill (n=42) and fast-fill (n=39) carbon dioxide exposures.
TABLES

Table 3.1. Number of rabbits per age group randomly assigned to different treatments.

<table>
<thead>
<tr>
<th>Method</th>
<th>Age category</th>
<th>No.</th>
<th>Body weight, kg (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradual-fill</td>
<td>Pre-weaned kits</td>
<td>14</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Growers</td>
<td>15</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>13</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Fast-fill</td>
<td>Pre-weaned kits</td>
<td>14</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Growers</td>
<td>13</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>12</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>

1Gradual-fill = 10% ± 0.3 chamber CO₂ increase in the first min, Fast-fill = 39% ± 0.7 chamber CO₂ increase in the first min

2Pre-weaned kits = 11 d to 5 wk, Growers = 6 to 12 wk, Adults = >12 wk, Pre-weaned kits and growers were mixed sex, and adults were female only.
**Table 3.2.** Ethogram for evaluating rabbit behavioural and physiologic responses during carbon dioxide gas exposure.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal reflex</td>
<td>Blink response to touching the surface of the eye</td>
</tr>
<tr>
<td>Palpebral reflex</td>
<td>Blink response to touching the area around the eye</td>
</tr>
<tr>
<td>Ear pinch reflex</td>
<td>Pinching the rabbit’s ear looking for voluntary movement, i.e. pulling head away</td>
</tr>
<tr>
<td>Toe pinch reflex</td>
<td>Pinching the skin in between the rabbit’s toes looking for voluntary movement, i.e. pulling foot away</td>
</tr>
<tr>
<td>Clonic convulsions</td>
<td>Leg paddling</td>
</tr>
<tr>
<td>Tonic convulsions</td>
<td>Rigid extension of the limbs</td>
</tr>
<tr>
<td>Righting reflex</td>
<td>Turning the animal on its side and seeing if it can right itself</td>
</tr>
<tr>
<td>Loss of balance</td>
<td>Uncoordinated movement or falling over</td>
</tr>
<tr>
<td>Loss of posture</td>
<td>Rabbit is no longer able to hold itself up; falls to the side, head sags, belly on the ground</td>
</tr>
<tr>
<td>Open mouth breathing</td>
<td>Mouth opening to breathe</td>
</tr>
<tr>
<td>Increased respiration</td>
<td>Increased speed of nostril flaring</td>
</tr>
<tr>
<td>Decreased respiration</td>
<td>Long pauses in between breaths</td>
</tr>
<tr>
<td>Last breath</td>
<td>Nostril flaring and open mouth breathing stop occurring</td>
</tr>
<tr>
<td>Gasping</td>
<td>Deep full body breath through a wide open mouth</td>
</tr>
<tr>
<td>Head shake</td>
<td>Rapid movement of the head</td>
</tr>
<tr>
<td>Head lift</td>
<td>Attempts to get above chamber concentration; stretching neck and lifting head up, nose pointed up, small rabbits crawl sides</td>
</tr>
<tr>
<td>Head bob</td>
<td>Moves head up and down and side to side</td>
</tr>
<tr>
<td>Turn around</td>
<td>Rabbit switches direction facing</td>
</tr>
<tr>
<td>Chewing</td>
<td>Moving mouth and tongue in pattern, not in attempt to manipulate or eat material</td>
</tr>
<tr>
<td>Defecation</td>
<td>Visible stool is released from the body</td>
</tr>
<tr>
<td>Urination</td>
<td>Visible urine is released from the body</td>
</tr>
<tr>
<td>Vocalizations</td>
<td>High pitch scream</td>
</tr>
<tr>
<td>Grunts</td>
<td>Undefined sounds emitted by the animal</td>
</tr>
</tbody>
</table>
Table 3.3. Frequency of behavioural response, grouped by age and fill-rate.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Fill Rate¹</th>
<th>Total</th>
<th>No. of pre-weaned kits²</th>
<th>No. of growers²</th>
<th>No. of adults²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open mouth breathing</td>
<td>Gradual-fill</td>
<td>42</td>
<td>14</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>14</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>42</td>
<td>28</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>28</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Open mouth breathing before loss of righting reflex</td>
<td>Gradual-fill</td>
<td>42</td>
<td>57</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>107</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>97</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Chewing</td>
<td>Gradual-fill</td>
<td>42</td>
<td>14</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>13</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>28</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>28</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Gasping</td>
<td>Gradual-fill</td>
<td>42</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Head shake</td>
<td>Gradual-fill</td>
<td>42</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>11</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>11</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Head Bob</td>
<td>Gradual-fill</td>
<td>42</td>
<td>14</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>24</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>24</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Urination</td>
<td>Gradual-fill</td>
<td>42</td>
<td>18</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>28</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>28</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Coughing</td>
<td>Gradual-fill</td>
<td>42</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Defecation</td>
<td>Gradual-fill</td>
<td>42</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Head lifting</td>
<td>Gradual-fill</td>
<td>42</td>
<td>11</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>39</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Gradual-fill</td>
<td>81</td>
<td>56</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Fast-fill</td>
<td>78</td>
<td>56</td>
<td>22</td>
<td>14</td>
</tr>
</tbody>
</table>

¹Gradual-fill = 10% ± 0.3 chamber CO₂ increase in the first min, Fast-fill = 39% ± 0.7 chamber CO₂ increase in the first min

²Pre-weaned kits = 11 d to 5 wk, Growers = 6 to 12 wk, Adults = >12 wk, Pre-weaned kits and growers were mixed sex, and adults were female only.
Table 3.4. Time, concentration, and age of rabbits (n=6) vocalizing during exposure to carbon dioxide.

<table>
<thead>
<tr>
<th>Method†</th>
<th>Age category</th>
<th>Carbon dioxide concentration</th>
<th>Time of vocalization</th>
<th>Duration of vocalization</th>
<th>Loss of righting reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradual-fill</td>
<td>Grower</td>
<td>30%</td>
<td>140s</td>
<td>30s</td>
<td>100s</td>
</tr>
<tr>
<td>Fast-fill</td>
<td>Pre-wean</td>
<td>1%</td>
<td>10s</td>
<td>10s</td>
<td>30s</td>
</tr>
<tr>
<td>Fast-fill</td>
<td>Adult</td>
<td>1.2%</td>
<td>10s</td>
<td>50s</td>
<td>40s</td>
</tr>
<tr>
<td>Fast-fill</td>
<td>Grower</td>
<td>0.6%</td>
<td>10s</td>
<td>10s</td>
<td>40s</td>
</tr>
<tr>
<td>Fast-fill</td>
<td>Pre-wean</td>
<td>2.4%</td>
<td>10s</td>
<td>10s</td>
<td>30s</td>
</tr>
<tr>
<td>Fast-fill</td>
<td>Adult</td>
<td>2.2%</td>
<td>20s</td>
<td>10s</td>
<td>60s</td>
</tr>
</tbody>
</table>

†Gradual-fill = 10% ± 0.3 chamber CO₂ increase in the first min, Fast-fill = 39% ± 0.7 chamber CO₂ increase in the first min

‡Chamber concentration when vocalizations began
Chapter 4: General Discussion

Despite on-farm euthanasia being a common task for commercial meat rabbit producers, there are no published, validated methods. To the best of our knowledge, this research is the first to evaluate methods for on-farm euthanasia of commercial meat rabbits. Euthanasia methods researched in this thesis were compared for effectiveness, humaneness, operator safety, cost, practicality, and esthetics acceptability, allowing for recommendations to be made.

Effectiveness of an euthanasia method was judged by its ability to cause insensibility, as per AVMA requirements (AVMA, 2013). The gold standard for confirming insensibility in most animals is a flat line on an electroencephalogram (EEG) (Verhoeven et al., 2015a). Electroencephalogram equipment is expensive, tracings are challenging to interpret and the technique is impractical for physical euthanasia methods and for use on-farm. Thus, it was necessary to develop reliable reflexes for confirming insensibility and death of meat rabbits. Reflexes can be validated with use of an EEG through correlation to loss of neurological function. Loss of posture of rabbits during exposure to CO\(_2\) was validated in such a way (Dalmau et al. 2016). Loss of righting reflex has been shown in rats to be more reliable indicator of insensibility than loss of posture (Chisholm and Pang, 2016). However on-farm it is not practical to check for righting reflex during euthanasia as it requires a hand input, but use of a clear tote as a chamber would allow for observation of loss of posture during CO\(_2\) gassing.

The literature supported the use of other reflexes in combination to confirm insensibility (Dennis et al., 1988; Schutt-Abraham et al., 1992; Hellebrekers et al., 1997; Anil et al., 1997; Anil et al., 2000; Maria et al., 2001; Nodari et al., 2008; Li et al., 2012; Cors et al., 2015; Dalmau et al., 2016). We used the corneal reflex in combination with the palpebral reflex, pupillary reflex and withdrawal reflex, as well as disappearance of rhythmic breathing and lack of vocalizations. These reflexes worked best in combination as some circumstances did not allow for assessment of certain reflexes. For example, eye injuries or infections prevented the use of eye reflexes to be used, as did trials on young kits with unopened eyes. Pupillary reflex could only be checked in dim lighting, whereas a number of trials occurred outdoors in broad daylight. Withdrawal reflexes were sometimes hard to differentiate from convulsions and in some young animals, as pinching the toe stimulated movement. Movement can be stimulated by withdrawal
reflexes and not be associated with sensibility (Saposnik et al., 2009). Rhythmic breathing was a reliable indicator as found in other rabbit experimental trials (Anil et al., 1997; Maria et al., 2001). Nostril flaring was clearly absent when the animal was insensible, and its reappearance was indicative of other reflexes returning shortly, as the rabbit regained sensibility. We agree with Nodari et al. (2008) that it can be difficult to observe rhythmic breathing if there is bleeding from the nose and mouth as was the case with some rabbits euthanized by BFT.

Vocalizations were used to judge sensibility in our research as it has been suggested that rabbits rarely vocalize unless in pain or experiencing significant fear (Mayer, 2007). Keating et al. (2012) correlated rabbit vocalization with pain when evaluating New Zealand White rabbits being ear tattooed. In our studies, vocalization was an obvious indicator of an incorrectly applied BFT, but its utility to identify a conscious animal in distress was questionable in our CO₂ studies. Vocalizations were a rare occurrence during CO₂ gassing, leading us to conclude that a majority of the rabbit were not experiencing distress. However, the only rabbit to vocalize during gradual-fill did so after it had lost its righting reflex but during the time when corneal reflex was still present. The rabbit did not respond to tests of withdrawal reflexes. We concluded that this rabbit was insensible but vocalizing, which suggests that vocalization may not always be a conscious action. Use of indicators to measure the subjective state of an animal is limited in their accuracy as they are left up to human interpretation. Vocalizations are recommended in other species as indicators of a sensible animal but need to be distinguished from sounds made as air escapes the lungs (Niel and Weary, 2006; NFACC, 2014; NFACC, 2016).

Based on our research, we would recommend that the palpebral and corneal reflex be used to confirm insensibility on-farm and that loss of posture be used when observing onset of insensibility during CO₂ gassing. These are practical measures that do not rely on special equipment and need minimal training to use. Due to their practicality the palpebral and corneal reflex are recommended as on-farm measures of sensibility for other species (NFACC, 2014; NFACC, 2016).

It is critically important that producers confirm an animal is dead before leaving or disposing of it. Commercial meat rabbit producers are generally unaware of key signs to check to confirm death (Walsh et al., 2016). Involuntary movements can be confusing to interpret and through our research we had hoped to be able to gain information on their occurrence. A clear
pattern of progression from tonic to clonic convulsions found in other species, such as piglets (Casey-Trott et al., 2013), was not found with rabbits. Convulsions can be expected to last up to 1 min in duration after application of a physical method (Chapter 2), although they do not occur in every rabbit. If convulsions occur before loss of sensibility they are thought to be associated with escape attempts (EFSA, 2013; Lonch et al., 2012). Convulsions never occurred before loss of righting reflex during any of our trials, concluding that they are involuntary movements not indicative of distress. We concluded that convulsions occur too late to be a useful indicator of insensibility in rabbits, consistent with Chisholm and Pang’s (2016) findings. A clear predictable pattern of pupillary dilatation was observed in all rabbits regardless of euthanasia method used. A fully dilated unresponsive pupil can be used to indicate that the animal is dead, and is used in the Pig Code of Practice (NFACC, 2014). We recommend checking for death by assessing lack of breathing and palpable heartbeat, as well as a fixed, dilated pupil.

Effectiveness of physical euthanasia methods were judged by immediate sustained insensibility and subsequent death. For CO₂ gassing, effectiveness was judged by time to insensibility, time to death, and a sustained death after removal from the chamber. Based on these criteria, the NPCB device was 100% effective. This method’s high level of efficacy in rabbits is consistent with similar findings in piglets and turkeys (Erasmus et al., 2010a; Casey-Trott et al., 2013; Casey-Trott et al., 2014). Blunt force trauma was effective 78% of the time and resulted in damage to areas other than the targeted location, such as the nasal cavity, shoulder blades and ocular bone. Assisted manual cervical dislocation caused immediate insensibility 94% of the time and was limited by training (Chapter 2). The high level of efficacy for the AMCD was unexpected based on challenges using manual CD on rabbits greater than 1kg (AVMA, 2013; CCAC, 2010) and lack of success inducing immediate insensibility (Erasmus et al., 2010; Martin et al., 2016; Limon et al., 2016). Carbon dioxide exposure at both fill rates resulted in a relatively rapid loss of sensibility, was 100% effective in inducing death after exposure for 7 mins in rabbits greater than 11 d of age, and death was sustained in all animals after removal from the chamber (Chapter 3). Blunt force trauma was the only method that did not meet the efficacy expectations, and for that reason, we are unable to recommend it as a routine euthanasia method for commercial meat rabbits.
A humane death requires pain and distress to be eliminated or minimized. Distress is measured by duration and intensity, and includes the period of time up to when the method is applied. All euthanasia methods studied require handing prior to application. Restraint during application needs to be safe for the operator, allow for the method to be properly applied and cause minimal stress and fear to the rabbit. Blunt force trauma, as performed in this study, involved suspending rabbits by their back legs prior to applying force, which caused the rabbits to squirm and in some cases, vocalize. Based on these observations this restraint method is strongly aversive, limiting the humaneness of the euthanasia method. The device used for AMCD was mounted on the inside barn wall requiring rabbits to be carried a short distance to it after being identified for euthanasia. There was a short period of stress (<5s) while the rabbit’s head was being aligned within the device. When mechanical devices are used to assist in CD there is concern for pressure being placed on the trachea and esophagus, causing the animal to choke (Sparrey et al., 2014). Anecdotally, some larger rabbits would attempt to push against the wall during this period, but overall application and alignment was a rapid smooth process, requiring <5s of alignment. Rabbits euthanized by the NPCB device were placed in a non-slip container and backed against the container wall, limiting the restraint needed. Alignment of the device on the head required minimal adjustment and was applied in <5s. We recommend that rabbits be restrained in a non-slip container/surface, and never suspended during application of the NPCB device. Similarly, a portable sling was designed for use when applying the NPCB device to piglets to improve worker safety and animal welfare (Casey-Trott et al., 2014).

Prior to initiating the CO₂ gassing trials, distress was predicted as a response to hypoxia and/or mucosal acidosis. At both fill rates, no obvious signs of distress were observed. The gradual-fill rate (10% ± 0.3 chamber CO₂ increase in the first min, 28% chamber volume change/min) was within the AVMA recommended displacement rate of 10% to 30% volume change/min. The fast-fill rate (39% ± 0.7 chamber CO₂ increase in the first min, 58% volume change/min) resulted in earlier loss of sensibility than the gradual-fill rate, limiting the duration of sensible CO₂ exposure and potential distress. Niel and Weary (2006) found that rats with the option to avoid exposure to CO₂ left quicker as displacement rate increased. In our study we did not give the rabbits an option to avoid the gas. It is possible that the rabbits would choose to leave at a lower displacement rate than what was used in this study, but based on behaviour and physiologic responses the rabbits were not in obvious distress at any concentration. Rabbit CO₂
preference testing should be evaluated in future studies. We recommend using the fast-fill rate over gradual-fill as gradual-fill was unnecessarily slow, providing no obvious advantage. We conclude that humaneness does not vary significantly between methods, except for BFT, and that it depends on operator skill and confidence in gently restraining the rabbit.

Operator safety should always be considered when evaluating euthanasia methods. The euthanasia methods used in this research were selected over other possible methods based, in part, on safety (Chapter 1). Thirty-five percent of Canadian turkey producers rated safety as their primary concern when choosing a euthanasia method (Erasmus, 2009). Safety can be improved by training and refining skills. Rabbit producers rank themselves highly on skill despite never being trained on their methods and not being able to identify an insensible or dead rabbit (Walsh et al., 2016). Rabbits are challenging animals to restrain and the proximity of the operator’s hands to the target area is a safety concern, particularly for BFT and the NPCB device. Safety risks with use of CO₂ can be limited by using a sealed gassing chamber with chamber outflow directed downwind and away from the operator, and by conducting the procedure outside or in a well ventilated area. These safety recommendations are in line with what is recommended in the Mink Code of Practice for use of carbon monoxide, which poses more operator safety risks (NFACC, 2013). We feel confident recommending the NPCB device, AMCD and CO₂ as operator-safe methods for rabbit on-farm euthanasia.

The euthanasia methods validated are not equal in terms of producer financial outlay. The AMCD device, termed the Rabbit Wringer®, can be purchased commercially for $120. Its simple design also means that it could be made on-farm, likely for less than half this cost. The NPCB device validated for use on rabbits in this study was the Zephyr-E, manufactured by Bock Industries for $1,200. An air compressor is needed to operate the Zephyr-E making the total investment cost around $1,260. A 23kg CO₂ cylinder can be purchased for $29 and can be used for euthanasia of approximately 90 rabbits, depending on the fill-rate and chamber size. The chamber size should be slightly larger than what was used in this study (14.3L, 38.5cm length x 19.4cm width x 19.1cm height) to allow for euthanasia of large breeding bucks. The chamber can be constructed from household items such as a clear plastic bin (or at least with a clear lid allowing for inspection) for $20 and tubing for inflow and outflow ports for <$10. Outflow tubing should be of a significant length to allow emptying of the gas away and downwind from
the work space. The CO₂ cylinder will need to be equipped with a regulator (~$160) and flow meter (~$380). In total, set up for on-farm CO₂ euthanasia will cost ~$570 plus $29 for a new cylinder every 90 rabbits. Commercial meat rabbit producers indicated that they preferred investing less than a $100 on a new euthanasia method (Walsh et al., 2016). The AMCD device can be purchased or made within this financial restriction. The cost of the NPCB device is above this range but is a one-time investment. Set up for CO₂ gassing is a one-time cost but requires an additional $0.32 per rabbit to purchase the gas. Financial restriction was not found to correlate with size of production for commercial meat rabbit producers, indicating there must be another reason underlying money willing to spend, as turkey producers did not rank cost as very important when choosing a euthanasia method (Erasmus, 2009; Walsh et al., 2016). Based on cost alone, the AMCD device is the most economical option for on-farm euthanasia.

Practical euthanasia methods are ones that are easy for the producer to implement. Cost and portability are factors that limit the use of the specific NPCB device validated in this research. Commercial meat rabbit producers listed cost and the need for an air compressor as major concerns when surveyed about the use of a NPCB device for rabbit euthanasia (Walsh et al., 2016). Piglet stock personnel also commented on the NPCB device’s limited portability as an issue when considering implementing use (Casey-Trott et al., 2013). Blunt force trauma and AMCD are more practical methods as they use tools the producer already has on-farm or can cheaply make. These methods can be performed at or close to the location of the sick or injured animal, whereas the NPCB device needs to be attached to an air compressor to operate. Carbon dioxide is relatively inexpensive and a gassing chamber can be easily designed out of common materials. Of the 3 methods evaluated for rabbit euthanasia and found to be suitable, the NPCB device is least practical for on-farm use, but utility could be improved with a long air compressor hose or with a self-contained charge, as is available for the Turkey Euthanasia Device (Sparrey et al., 2014).

The euthanasia method used affects operator and observer well-being as well as rabbit welfare (Whiting and Marion, 2011). Esthetics was reported as one of the primary concerns for commercial meat rabbit producers in a survey of satisfaction with methods used and opinions of novel techniques (Walsh et al., 2016). If a method is esthetically displeasing a producer may be reluctant to use the method. Due to lack of alternative methods some commercial meat rabbit
producers elected to leave sick and injured animals to die on their own. Anecdotal observations and operator comments provided information on the esthetics of the euthanasia methods evaluated in this research. Blunt force trauma required the operator to apply physical force to the rabbit, and resulted in significant bleeding. Blood loss and tissue discharge are a biosafety and esthetic concern, and is the reasons why exsanguination is not appropriate on-farm (AVMA, 2013). When incorrectly applied, vocalizations, physical damage to other areas of the body, and a sense of failure resulted, and were distressing to the operator and the observer. Assisted manual cervical dislocation also requires the operator to apply physical force, and for this force to be adjusted based on the size of the animal. An incorrect amount of force can result in decapitation. This was not observed during our research trials but was mentioned by the operator. Except for rare cases in which this might occur, there is minimal bleeding when AMCD is used. The NPCB device has the esthetic advantage of partially removing the operator from the physical task, requiring the pull of a trigger versus striking the rabbit. The device was rated 8.7 out of 10 for performance appeal by swine stock personnel who switched to using this method (Casey-Trott et al., 2013). Set to the appropriate pressure, which is based on the size of the rabbit, there is minimal external bleeding, instead swelling occurs under the skin above the skull at the site of application. Carbon dioxide inhalation removes the operator one step further from the task of euthanasia. Once the animal is placed in the chamber, the operator no longer has to physically interact with the animal and only has to turn on the gas. Behavioural observations, such as vocalizations or escape behaviours can be disturbing to watch; however, exposure of rabbits at the fill rates used in this study rarely resulted in such behaviours. If euthanasia methods are ones that the operator knows are humane and effective and they have been trained to perform the method efficiently and with confidence, euthanasia is easier to do and can be conducted in a more timely manner.

The current study was restricted to using cull rabbits for ethical reasons and excluded rabbits with clinically obvious respiratory disease from use in the CO₂ trials. Adult male breeding rabbits are present at lower numbers than other age groups on-farm and are rarely euthanized. We were unable to evaluate them in this study, although there is no reason to suspect that results would be significantly different, based on identical physiology. Male rabbits have a thicker skull, which might require a higher NPCB operating pressure. Rabbits with respiratory disease might have altered respiration rates prolonging CO₂ induction time. However, Sadler et
al. (2014b) found that piglets with a respiratory disease lost sensibility sooner than piglets euthanized for other reasons, opposite of what was predicted. Respiratory disease did not result in a difference in behaviour or physiologic response upon exposure, and duration of open-mouth breathing was shorter.

Blinding was challenging during our research trials as the same personnel were involved in data collection and analysis. Blinding was used for histologic analysis of brain sections however, and these results correlated well with other findings. Blinding could have been used in the gassing studies if the trials were filmed, coded, and scored by a third party. Another limitation of our study is the lack of an air-only control group for the CO\(_2\) gassing research. A control group would have allowed for unbiased assessment of various behaviours and physiologic responses. Future research should be mindful of these limitations.

Based on the research from this thesis we have multiple recommendations for the commercial meat rabbit industry. We recommend that all rabbit producers work with their herd veterinarians to develop a euthanasia action plan (Turner and Doonan, 2010). Those using BFT are recommended to switch to a different validated euthanasia method. This recommendation is in line with AVMA recommendation and along with the euthanasia recommendations in the Pork and Poultry Code of Practices (AVMA, 2013; NFACC, 2014; NFACC, 2016). We recommend AMCD as a humane method but encourage those learning to use this method to train on cadavers before live rabbits to reduce the chance of method failure. This is good practice when learning to conduct any physical euthanasia method. Backup methods should be identified and ready in case of method failure (National Pork Board, 2008). The NPCB device must be used at an appropriate pressure setting, according to the size of the rabbit to ensure efficacy [i.e., 90psi for adults, 70psi for growers, and 55psi for pre-weaned kits, (>150g)] and we recommend discharging twice in rapid succession as per Erasmus et al. (2010a). Carbon dioxide should be used within the chamber fill rates evaluated in this study and should not exceed a 40% CO\(_2\) chamber concentration increase/min. Our recommended fill rates are based on measured chamber CO\(_2\) and calculated displacement rates. For duplication, exact rates based on equipment should be determined first. Any follow-up research should measure the chamber concentration to allow continued analysis of the concentration to behaviour correlation in rabbits. Carbon dioxide should be from a purified source without contaminants, ideally from a commercially supplied
It is important that the chamber be sealed to prevent leaks and exposing operators to CO₂. After application of any euthanasia method, insensibility should be checked for using the corneal and palpebral reflex and death confirmed via palpation of a heartbeat as per other species’ Code of Practice (NFACC, 2013; NFACC, 2014, NFACC, 2015).

In conclusion this study met both research objectives of evaluating the effectiveness and welfare of BFT, AMCD, a NPCB device and CO₂. The hypothesis that the pneumatic-powered NPCB device would prove to be the most effective physical method was supported. The NPCB device caused immediate insensibility and subsequent death in all animals, and significant traumatic brain damage. The hypothesis of both fill rates of CO₂ causing a period of distress for the rabbits, but at a lower intensity during gradual-fill was not supported. Fast-fill (39% ± 0.7 chamber CO₂ increase in the first min, 58% volume change/min) resulted in a significantly earlier loss of sensibility and death than gradual-fill, potentially limiting distress. Overall, AMCD, the NPCB device and CO₂ were validated as effective, minimally distressing, practical, safe and esthetic euthanasia methods.
Future Work

- Evaluate euthanasia methods on male breeding stock, breeds other than New-Zealand mixed, and rabbits outside of the weight ranges studied.
- Verify that rabbits with a respiratory disease do not respond differently to CO$_2$ exposure
- Use preference or motivation testing to determine level of aversion to CO$_2$ gassing during concentration increases on rabbits
- Confirm the exact displacement rate needed to match our fast-fill chamber CO$_2$ concentration increase/min
- Determine how rabbits react to being placed in an empty chamber with atmospheric air blowing in at the fill rates evaluated in this study to control for noise and observer effect.
- Evaluate EEG changes in response to euthanasia technique application to confirm findings for time to insensibility when exposed to CO$_2$
- Determine whether a shorter total period of time can be used for CO$_2$ exposure, while still maintaining 100% euthanasia efficacy
- Test if stock person affects physical method success.
- Validating vocalizations as an indicator of pain, distress or fear in rabbits during CO$_2$ exposure.
- Test if applying the NPCB device once instead of twice in rapid succession has the same effectiveness and causes the same degree of injury.
- Survey producers who switch to these new methods to compare how their attitude and approach towards euthanasia has changed.
References


Erasmus, M. 2009. Examining physical methods for on-farm killing of turkeys. MSc Diss. Univ of Guelph, Canada


Llonch, P., P. Rodriguez, A. Velarde, V. Abreu de Lima, and A. Dalmau. 2012. Aversion to the inhalation of nitrogen and carbon dioxide mixtures compared to high concentrations of carbon
dioxide for stunning rabbits. Anim Welf 21(S2): 123-129. doi: 10.7120/096272812X13353700593923


Rau, J. Phase II Research Project – Special topics elective development of a non-penetrating captive bolt device for stunning rabbits at slaughter. Personal communications


Appendix A: On-farm euthanasia practices and attitudes of commercial meat rabbit producers in Canada

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ABSTRACT

Appropriate and timely on-farm euthanasia is the responsibility of the producer, working together with their herd veterinarian. Unfortunately, validated methods for euthanasia of commercial meat rabbits are lacking and there are few educational materials available for producer training. Because euthanasia must be performed in a timely fashion to minimize suffering, it is critical to ensure that methods used are esthetic, humane, and effective. We surveyed Canadian meat rabbit producers for current on-farm euthanasia practices as well as attitudes towards the methods they employed and thoughts on novel euthanasia techniques. Surveys were distributed with a response rate of 26% (n=26). Blunt force trauma was the most common euthanasia method used (54%) followed by assisted manual cervical dislocation (31%). Half of producers admitted to not having a euthanasia method in place for all age groups of rabbits, instead electing to let sick and injured rabbits die on their own. While some producers reported feeling highly skilled and satisfied with their current euthanasia method, 58% reported concerns with their current method and 42% desired alternative methods to be developed. Responses to additional questions on training and awareness of euthanasia resources indicated that veterinarians are not part of on-farm euthanasia planning for meat rabbits.
INTRODUCTION

Many livestock producers are faced with the task of euthanasia on a regular basis. On-farm euthanasia differs substantially from euthanasia conducted in the veterinary clinic and slaughterhouse settings. Herd veterinarians are encouraged to guide their clients in making end of life decisions, but euthanasia is rarely solely their decision, and for some species of animals, such as poultry and meat rabbits, veterinarians rarely visit the farm (CVMA, 2014). Thus producers are tasked with the responsibility of making euthanasia decisions on a daily basis as well as conducting the procedure. There are limited methods available to producers for conducting euthanasia, for example, controlled drugs that are used commonly in companion animal practices are restricted to veterinary use only. Instead producers must often rely on physical methods for humanely killing sick or diseased animals in their care. Ensuring that appropriate on-farm euthanasia methods are available to all livestock and poultry producers and that methods are conducted correctly has been identified as a priority issue by the Canadian government. This is important for meeting ethical obligations towards farmed animals, addressing consumer expectations for providing good animal care and welfare, and for meeting Canada’s animal welfare obligations to the World Organization for Animal Health (OIE) and to international markets (OIE, 2016).

Euthanasia methods should be validated as humane, effective, species-appropriate, and ideally, methods result in immediate and sustained insensibility (AVMA, 2013). There are currently no validated euthanasia methods for use on commercial meat rabbits. The American Veterinary Medical Association (AVMA, 2013) and Canadian Council on Animal Care (CCAC, 2010) address euthanasia methods for companion and laboratory rabbits, which permits some information to be extracted; however, most are not practical or appropriate for use in a farm setting. Before validating an on-farm euthanasia method it is important to know what methods are currently being used and what new methods are likely to be adopted. Addressing this knowledge gap is the first step towards ensuring validated on-farm euthanasia methods are available for commercial meat rabbit producers and for subsequent development of appropriate training materials.
Because certain techniques are used for euthanasia on-farm does not mean that they are preferred and it is important to determine how producers feel about the methods they use and if they desire alternate methods to be developed that may be better suited to their personal feelings or to the age and size of the animal. A strong dislike of conducting a particular procedure may mean that animals are not euthanized in a timely fashion because the task is perceived as being unpleasant. Increased knowledge of factors that producers consider when euthanizing animals may direct research in procedures that producers are more likely to implement. Garforth (2015) researched factors influencing producer attitudes toward implementing changes. This study demonstrated that it is a mistake to assume the particular set of goals and priorities that producers consider when making decisions without directly asking them. For consumers and producers alike, motivation for change is not simply economical, but instead is more deeply rooted in values (Swanson et al, 2011; Garforth, 2015). Producer decisions are influenced by their values, attitudes, and the level of trust they have in the person or institution providing advice (Garforth, 2015). Gathering producer opinions and understanding their rationale increases the likelihood of the desired outcome.

A survey administered to Canadian meat rabbit producers was developed in response to a lack of awareness of industry euthanasia practices for meat rabbits as well as the general dearth of training materials about euthanasia methods for commercial meat rabbits. The purpose was to determine current euthanasia practices used by experienced meat rabbit producers and their attitude towards these methods as well as novel euthanasia methods. The ultimate goal of this research is to ensure humane, effective methods are being used on-farm and that appropriate training materials are available for producers to conduct this important task.
MATERIAL AND METHODS

Human Ethics Approval

The study survey was reviewed and approved by the University of Guelph Research Ethics Board (15MR006). The cover letter provided a definition of euthanasia, outlined participant rights, and the purpose for collecting the information. Participants were informed that involvement was voluntary, responses were anonymous, and that skipping a question was permissible. Options were provided to complete the survey by telephone or to return it by mail. A low value incentive gift card was offered to encourage participation.

Study Population

To participate in the survey, respondents were required to be the primary person responsible for euthanasia of rabbits on their farm and only one survey could be submitted per farm. Surveys were completed between March 31 to April 30, 2015 and copies were distributed at a spring meeting for Ontario commercial rabbit producers, and were left at two feed mills and a rabbit processing plant in southwestern Ontario for potential completion and mail-in. Ontario represents over 65% of the Canadian commercial meat rabbit industry with approximately 100 large commercial meat rabbit farms (AAFC, 2014).

Survey

The survey consisted of 34 questions with fixed- and open-ended responses, and required approximately 20 minutes to complete. Fixed-response questions consisted of ‘yes/no’ responses, 5-level ‘Likert-like’ ranking scale responses, and ‘check all applicable boxes’ responses with
room for other responses to be noted. Part One of the survey enquired about current on-farm euthanasia methods and attitudes, as well as self-reported skill, satisfaction, and effectiveness of methods. For many questions more than one response could be selected. Part Two introduced two novel methods for on-farm euthanasia used in other livestock species; the non-penetrating captive bolt device and carbon monoxide inhalation. These methods were introduced in a brief written description providing details of application, required equipment, cost, and safety concerns. Following this description were questions on the theoretical use of these methods in meat rabbits and concerns that influenced the respondent’s opinion. Respondents were then asked what they thought was the best method of euthanasia for meat rabbits in an unrestricted response. The last questions addressed financial concerns for adopting new euthanasia methods on their farm.

**Statistical Analyses**

Returned surveys were examined and responses tallied. Open-ended questions were reviewed and categorized. Farms were grouped according to size (small ≤50 does, medium 51-199, large ≥200) to determine whether the size of the farm impacted the euthanasia methods used (Table A.1). Ranked responses (1-5) for level of satisfaction and level of skill with current method were grouped into high (4-5) and low (1-2) with middle responses (3) eliminated for comparison purposes. Statistical analyses were performed using SPSS (SPSS Statistics for Windows, Version 23.0. 2014. Armonk, NY: IBM Corp.). Descriptive statistics were calculated as the mean (±SD) response rate. Thirteen key questions were selected to run a Pearson chi-square test ($\chi^2$) of association, to test for correlations between questions. Column properties were compared using a Bonferroni correction for multiple analyses. Cramer’s V test was run to determine the strength of the association. The significance level was set at $p< 0.05$. These tests of association required the number of responses to be greater than 5. To meet this requirement, similar responses were grouped together and responses that were too few in number were not examined for association with other questions (indicated in the Results section).
RESULTS

Completed surveys were received from 26 Ontario rabbit producers, representing approximately 26% of the provincial industry.

Part 1: On-Farm Euthanasia Methods and Producer Attitudes

Reported farm size ranged from 3 to 600 does with an average size of 155 (± 34) does. The majority of producers (65%) raised other food animal species in addition to rabbits, the most common species being poultry (31%); however, methods of euthanasia for other species were not pursued.

Reasons selected for euthanizing rabbits on farm included sickness (88%), animal injury (58%), family or companion animal consumption (i.e., home slaughter) (31%), and large litter size (i.e., litter balancing) (4%). When producers were asked how often they euthanized sick or injured animals, the majority (46%) indicated that it was on a monthly or longer basis with no producer reporting that they conducted euthanasia on a daily basis. A significant strong association (Cramer’s V = 0.500) was observed between farm size and how often producers needed to euthanize rabbits $\chi^2 (6, 26) = 12.984$, p = 0.043. On small farms significantly more producers euthanized animals on a monthly versus weekly basis. Farm size was not noted to have a significant association with any of the other question responses.

The most common on-farm euthanasia method used by producers was blunt force trauma (54%). Device assisted cervical dislocation methods (for example, a broomstick or foot applied to the head before stretching the animal for cervical dislocation) and purchased, wall mounted, stainless steel v-shaped devices for cervical dislocation were combined as ‘assisted manual cervical dislocation’ to increase group size for comparison purposes. Pellet gun and rifle were eliminated from association comparisons because of low response rates. Euthanasia methods
used were tested for relationships with other responses and no significant associations were found.

A summary of responses to questions aimed at addressing rabbit euthanasia training and knowledge is provided in Table A.2. Most producers ranked themselves as highly skilled at their current euthanasia methods for kits (pre-weaned rabbits) and for older, adult rabbits (Figure A.1). Similarly, in general, producers reported being highly satisfied with their current euthanasia methods (Figure A.2). Despite a high self-reported level of skill and satisfaction, 58% of producers indicated that they had concerns with their current preferred euthanasia method. Specific concerns identified included dislike in performing euthanasia (50%), lack of confidence that the method was applied properly (23%), uncertainty as to whether the method is humane (19%), uncertainty as to whether animals are effectively killed by their preferred method (8%), and concerned with what the public might think of their preferred euthanasia method (4%). Most producers believed that death should occur immediately after a method was applied (77%).

The level of satisfaction of producers with their primary euthanasia method was associated with responses to other questions. There was a significant strong association (Cramer’s V = 0.707) with level of satisfaction with current method used on older rabbits and willingness to spend money on a new euthanasia device, $\chi^2 (2, 23) = 11.512$, $p = 0.003$. When highly satisfied with the method used on older rabbits significantly less producers were willing to spend greater than $100$ on a new hypothetical euthanasia device. Similarly, there was a significant strong association (Cramer’s V = 0.603) between satisfaction with the current euthanasia method used on older rabbits and desire for a new method, $\chi^2 (6, 24) = 17.431$, $p = 0.008$. When highly satisfied with the current method used on older rabbits significantly less producers desired a new method.

Self-reported skill level with on-farm euthanasia of rabbits correlated with other producer perspectives. There was a significant and strong association (Cramer’s V = 0.643) between reported skill level of current method used on older rabbits and desire for a new method, $\chi^2 (6, 23) = 19.221$, $p = 0.004$. All producers who reported a low skill level for the current method used on older rabbits desired a new method. Similarly, there was a significant and strong association (Cramer’s V = 0.574) between reported skill level of current method used on older rabbits and concern for current method, $\chi^2 (6, 22) = 7.238$, $p = 0.027$. When high skill level was reported for
the current euthanasia method used on older rabbits significantly less producers had concerns with their methodology.

**Part 2: Consideration of Alternate Euthanasia Methods**

Less than half of producers surveyed (42%) thought there was a need for new methods to euthanize rabbits on-farm, 35% did not, with the remaining respondents indicating uncertainty. When presented with information about a non-penetrating captive bolt device, 54% thought it would work to euthanize rabbits on their farm. Reasons listed for why it might not work included cost and time requirement (35%), that it was not needed (23%), lack of an air compressor (8%), and inability for the device to be used on kits (4%). Further, 27% of respondents felt this method would be effective while 20% had concerns with the use of the non-penetrating captive bolt on rabbits. Concerns listed included cost, training, only stunning the rabbit, weight of the device, and ease of operation.

When presented with information on controlled carbon monoxide gassing in a chamber, 35% thought it would work to euthanize rabbits on their farm and 50% had concerns with it. Reasons given for why they thought it might not work were small farm size (15%) and safety (15%). Safety was listed as a reason for CO not working on their farm and was also the most prominent concern identified (42%).

When respondents were asked what they thought was the “best” method of on-farm euthanasia for rabbits, blunt force trauma and carbon monoxide tied for most votes (19%), with the reasoning that it was a quick method. Half of producers preferred investing less than a $100 on a new euthanasia method. Financial restriction on a per animal basis was less than a dollar to euthanize a kit for 77% of producers and less than a dollar to euthanize an older rabbit for 65% of producers.

When blunt force trauma was chosen as the “best” method of euthanasia, significantly fewer producers were willing to spend more than a $100. There was a significant strong association (Cramer’s V = 0.832) between selecting blunt force trauma as the “best” euthanasia
method and money willing to spend on a new method $\chi^2 (5,17) = 11.782, \ p = 0.038$. There was no significant association between those producers whose main method was blunt force trauma and selecting it as the “best” method. No other significant associations were found.
DISCUSSION

The most common euthanasia technique employed by Ontario commercial meat rabbit producers was blunt force trauma (BFT). Blunt force trauma can be a humane euthanasia method, if applied correctly, and the technique is approved for euthanasia of animals with a thin cranium, such as nursery piglets (NFACC, 2014; AVMA, 2013). However, the AVMA Guidelines on Euthanasia also recommend that an alternative method be sought for BFT because of esthetic concerns for the operator and potential observers, the potential for operator fatigue to lead to errors, and because of biosecurity issues arising from blood and tissue dispersion during and after BFT application (AVMA, 2013). The results of this survey support this concern for esthetics, as producers generally indicated that they did not like performing euthanasia, particularly when BFT was their method of choice. They also indicated a concern for what the public might think when witnessing BFT being applied to a rabbit. In 2005, the Ontario government banned the use of BFT to stun rabbits prior to slaughter in abattoirs for animal welfare and esthetic reasons (OMAFRA, 2016). It is unknown whether the unease that producers express for this method is directly related to esthetics or efficacy in killing the animal after a single strike. Further research is needed to determine the actual effectiveness of BFT when used on-farm for rabbit euthanasia.

The second most common rabbit euthanasia technique was reported to be cervical dislocation (CD). This was presented as 2 different techniques in the survey; manual CD, described as stretching or breaking the neck by hand, and assisted manual CD, in which a broomstick or commercially available device is used to stabilize the head and neck prior to pulling. The AVMA and CCAC euthanasia guidelines indicate that manual CD is an inappropriate method to use on rabbits weighing more than a kilogram because of their large muscle mass (AVMA, 2013; CCAC, 2010). This would seem to preclude the use of manual CD on growing rabbits older than 5 wk of age. The use of a device to hold the animal’s head in place does permit the operator to use both hands to assist in a full body pull, giving the operator more strength in application. However, in some species use of a device does not assist in a clean dislocation at the correct location, creating a risk of fracturing or luxating cervical vertebrae before the animal loses consciousness (Carbone and others, 2012; Bader and others, 2014).
Further work is needed to determine the efficacy of this technique when used for rabbit euthanasia on farm.

A full half of the experienced rabbit producers surveyed admitted to not having a euthanasia plan in place for all of their rabbits and instead leaving some sick or injured rabbits to die on their own. This is an unacceptable practice that highlights the critical need for veterinary support of rabbit producers to enhance their animal care and welfare practices. On-farm euthanasia plans are needed for all species, and action plans should include training from veterinarians on recognition of sick animals, assessment for fitness to transport, development of end-point algorithms, and training in appropriate euthanasia techniques (Turner and Doonan, 2010; CVMA, 2014). Whether this practice results from esthetic issues arising from the act of euthanasia, distaste for employing their specific euthanasia technique on a specific age group of rabbits, a lack of understanding of the pain and distress that may exist in a sick or injured animal, some other reason or a combination of any of these is unknown. This is a significant and concerning industry gap that needs to be addressed through producer training and education as well as increased veterinary support.

Despite producers reporting that they felt highly skilled and satisfied with their current euthanasia methods, a majority subsequently reported concerns with their method and a desire to adopt new euthanasia techniques. In general, producers felt slightly less skilled and satisfied with euthanasia of older rabbits than pre-weaned kits. This suggests that there are more complicating factors when euthanizing older rabbits, possibly resulting in reduced efficacy or increased esthetic concerns. That farm size did not alter the primary euthanasia method used or the willingness to spend money on a new method of euthanasia, suggests a lack of awareness of alternative euthanasia methods for meat rabbits. Training and education about techniques other than BFT and CD may help to reduce producer concerns with euthanasia, which may also lead to more timely euthanasia decisions.

It is essential that animals be evaluated for loss of sensibility and death following application of a euthanasia technique. This survey did not differentiate between unconsciousness and death due to concern that producers do not distinguish between them. This hypothesis was confirmed when producers were questioned on how long it should take for a rabbit to die and 77% indicated that it should be immediate (that is, that rabbits were dead immediately after they
applied their euthanasia method). A majority of producers reported that 80% to 100% of the rabbits euthanized by their primary method experienced immediate death, and similarly, almost all producers indicated that they check to ensure that the rabbit is dead after euthanizing it. However, the variability of responses selected for how to verify death suggested that producers were unsure what signs to look for. The most common method selected to confirm death was to look to see if the animal was alive; a vague response. Some specified that they check for a heartbeat; however, this idea conflicts with the concept that death is immediate as a heartbeat does not disappear immediately after a physical method is applied. Some producers reported using some version of the corneal reflex or toe withdrawal response, but others interpreted this to mean simply looking at the animal’s eye, with no indication of what they were looking for. An effective euthanasia method should result in immediate insensibility followed rapidly by death (CVMA, 2014). It is important that producers are trained to confirm that euthanasia attempts are successful each and every time.

A key conclusion from this survey is that producers do not have adequate training or education about effective euthanasia methods, how and where to correctly apply these methods, and how to check for insensibility and death. Producers do not receive hands-on euthanasia training and are unable to identify what training is available to them. For food animal species for which it is impractical for a veterinarian to euthanize all animals, veterinarians must provide producers with the necessary skills to perform euthanasia independently (CVMA, 2014). Training can make the task of euthanasia more tolerable, generally resulting in more timely action. It is concerning that only one commercial meat rabbit producer identified their veterinarian as a euthanasia resource.

Producers indicated a desire for alternative euthanasia methods to be developed. Before validating any method, it is crucial to gain information on what influences producer decisions and might restrict them from adopting a new method. Descriptions of two methods; the non-penetrating captive bolt (NPCB) device and carbon monoxide inhalation (CO), both validated for use in other agriculture species (Erasmus and others, 2010; NFACC, 2012; Casey-Trott and others, 2014), were provided. More producers thought that the NPCB device was a better fit for their farm. They had more concerns with CO, largely related to safety. In contrast, CO was deemed the best method for on-farm euthanasia, suggesting that if safety could be ensured, it
would be a widely accepted method. The main concern listed for the NPCB device was cost. Economics was not the sole concern with this method as ease of operation, effectiveness across age groups, and need for additional equipment (that is, an air compressor) were also listed concerns. This survey identified potential producer concerns with these methods so that they can be taken into consideration if research is pursued for their use on commercial meat rabbits.

While limited to Ontario rabbit producers, based on other informal discussions with animal welfare inspectors and producers outside of Ontario, we believe that the results obtained are representative of producers across Canada. This survey was the first of its kind to formally explore common on-farm euthanasia practices in the commercial meat rabbit industry. The fragmentation of the commercial meat rabbit industry in Canada, which is very similar to the industry in the United States, and other parts of the world, and lack of an organised marketing body made survey distribution challenging. Answers relying on self-report should always be interpreted with caution as biases can alter the strength of response. That responses were completely anonymous in this study may have helped to overcome this limitation. It is also important to note that fixed-response questions have the potential to trigger responses that would not be given to open ended questions.

The purpose of this survey was to determine current euthanasia practices used on-farm by commercial meat rabbit producers and their attitudes towards their current methods as well as novel methods. It was determined that the two most common practices of euthanasia were BFT and assisted manual CD. Both of these methods require further research to validate their use. Commercial meat rabbit producers are in need of veterinary support to assist them in developing euthanasia action plans that cover all age groups. Animal welfare could also be improved by providing hands-on euthanasia training to increase producer confidence and comfort. Through this survey, we have identified areas needing attention and improvement within the commercial meat rabbit industry to ensure rabbits are killed by humane, effective methods.
Acknowledgements

This project was supported by Farm and Food Care Ontario through Agriculture Agrifood Canada. We appreciate the support of Ontario Rabbit in distributing the surveys and thank Ms. Lucia Costanzo for statistical support.
References


Figure A.1. Distribution of producer’s self-reported level of skill (on a scale of 1 to 5, low to high) for their current preferred euthanasia method for (a) pre-weaned kits, and (b) adult rabbits (n=26 producers).
Figure A.2. Distribution of producer’s self-reported level of satisfaction (on a scale of 1 to 5, low to high) for their current preferred euthanasia method for (a) pre-weaned kits, and (b) adult rabbits (n=26 producers).
## Table A.1. Current euthanasia methods used and distribution by relative rabbit herd size (n=26)

<table>
<thead>
<tr>
<th>Euthanasia method</th>
<th>Overall(^1)</th>
<th>Small farm ≤50 does</th>
<th>Medium farm 51-199 does</th>
<th>Large farm ≥200 does</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt force trauma</td>
<td>14 (54%)</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Manual cervical dislocation</td>
<td>6 (23%)</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Assisted manual cervical dislocation</td>
<td>8 (31%)</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Gun</td>
<td>3 (12%)</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Leave rabbits to die on own</td>
<td>13 (50%)</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Relative herd size of participants(^1)</td>
<td>10 (38%)</td>
<td>9 (35%)</td>
<td>7 (27%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)N (%), note –more than one euthanasia method could be selected for this question
Table A.2. Responses to questions examining producer training in rabbit euthanasia on-farm (n=26)

<table>
<thead>
<tr>
<th>Questions and responses</th>
<th>Type of question</th>
<th>¹No. selecting response</th>
<th>¹No. of non-responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>How did you learn your primary euthanasia method?</td>
<td>Open</td>
<td>20 (77%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>- Experience and peers</td>
<td></td>
<td>5 (25%)</td>
<td></td>
</tr>
<tr>
<td>- Just knew from growing up on a farm</td>
<td></td>
<td>7 (35%)</td>
<td></td>
</tr>
<tr>
<td>- Continuing education seminar</td>
<td></td>
<td>3 (15%)</td>
<td></td>
</tr>
<tr>
<td>- Internet</td>
<td></td>
<td>4 (20%)</td>
<td></td>
</tr>
<tr>
<td>- Books or other written materials</td>
<td></td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Did you receive hands-on training to perform euthanasia on rabbits?</td>
<td>Fixed</td>
<td>26 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>- Yes</td>
<td></td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td></td>
<td>23 (88%)</td>
<td></td>
</tr>
<tr>
<td>What euthanasia training materials would you recommend to new commercial rabbit producers?</td>
<td>Open</td>
<td>18 (69%)</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>- I do not know/none</td>
<td></td>
<td>14 (77%)</td>
<td></td>
</tr>
<tr>
<td>- Veterinarian</td>
<td></td>
<td>1 (6%)</td>
<td></td>
</tr>
<tr>
<td>- Provincial government website</td>
<td></td>
<td>2 (11%)</td>
<td></td>
</tr>
<tr>
<td>How many rabbits that you euthanize experience immediate death?</td>
<td>Fixed</td>
<td>24 (92%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>- &lt;50%</td>
<td></td>
<td>3 (13%)</td>
<td></td>
</tr>
<tr>
<td>- 60-70%</td>
<td></td>
<td>3 (13%)</td>
<td></td>
</tr>
<tr>
<td>- 80-90%</td>
<td></td>
<td>9 (38%)</td>
<td></td>
</tr>
<tr>
<td>- 100%</td>
<td></td>
<td>9 (38%)</td>
<td></td>
</tr>
<tr>
<td>Do you routinely check to see if the rabbit is dead after euthanasia?</td>
<td>Fixed</td>
<td>24 (92%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>- Yes</td>
<td></td>
<td>22 (92%)</td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td></td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>How do you confirm death of the rabbit?</td>
<td>Fixed</td>
<td>24 (92%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>- Check if it is alive</td>
<td></td>
<td>17 (71%)</td>
<td></td>
</tr>
<tr>
<td>- Look for breathing</td>
<td></td>
<td>13 (4%)</td>
<td></td>
</tr>
<tr>
<td>- Feel for a heartbeat</td>
<td></td>
<td>7 (29%)</td>
<td></td>
</tr>
<tr>
<td>- Look at the eyes</td>
<td></td>
<td>8 (33%)</td>
<td></td>
</tr>
<tr>
<td>- Pinch the foot</td>
<td></td>
<td>3 (13%)</td>
<td></td>
</tr>
</tbody>
</table>
- Touch the eye  
  2 (8%)
- Other: limp posture, nasal bleeding, trust method  
  3 (13%)

\(^1\)N (%) – Proportion of respondents per selection choice. Note – more than 1 response could be selected for each question.
SUPPLEMENTAL I: 2015 Rabbit Producer Survey – On-Farm Euthanasia Methods

General Farm Information

1) On average, how many does do you have on your farm? : _________________

2) Do you own other livestock? YES □ NO □
   a) If so, what kind(s)? (list all)________________________________________
   b) On the same property as the rabbits? YES □ NO □
   c) Same barns? YES □ NO □

3) a) Do you perform euthanasia on rabbits that need to be culled? YES □ NO □

   b) Who else performs euthanasia of rabbits on your farm (e.g., spouse, employee)?
       ________________________________________________________________

   c) Do you have to euthanize other livestock on your farm from time to time?
       YES □ NO □

Current Method of On-Farm Euthanasia for Rabbits

4) What methods do you use to euthanize rabbits on your farm (circle all that apply considering the different sizes and ages of rabbits that may need to be killed)?
   a) Blunt trauma (hitting on head or hitting rabbit on floor or other hard surface)
   b) Manual cervical dislocation or fracture (stretching or breaking the neck with hands)
   c) Broom handle/other device for cervical dislocation/fracture
   d) Rabbit Wringer (a commercially available device for cervical dislocation)
   e) Carbon dioxide (CO₂) gas
   f) Other gas. Please specify: _______________________________
   g) Non-penetrating captive bolt device (e.g., Zephyr or TED gun)
   h) Penetrating captive bolt device
   i) Other method. Please specify: _______________________________
   j) I let nature take its course (let the rabbit die on its own)

5) For what reasons are rabbits euthanized on your farm? (Check all that apply)
   Sick □  Injured □  Personal consumption □  Depopulation □
   Other □  (Please specify)____________________
6) a) How satisfied are you with your main method of euthanasia for kits (circle the best answer)?
   Dissatisfied  1  2  3  4  5  Satisfied

   b) How satisfied are you with your main method of euthanasia for older rabbits (circle the best answer)?
   Dissatisfied  1  2  3  4  5  Satisfied

7) a) How skilled do you feel you are at performing your main method of euthanasia on kits (circle the best answer)?
   Unskilled  1  2  3  4  5  Skilled

   b) How skilled do you feel you are at performing your main method of euthanasia on older rabbits (circle the best answer)?
   Unskilled  1  2  3  4  5  Skilled

8) In general, for rabbits being euthanized using your main method, what percentage of animals experience immediate death (circle the best answer)?
   None  20%  30%  40%  50%  60%  70%  80%  90%  100%

9) a) How did you learn how to kill rabbits that needed to be euthanized? __________
   ____________________________

   b) Did you receive hands-on training to perform euthanasia?: YES  ☐  NO  ☐

10) What training is available for producers getting into rabbits for on-farm euthanasia?
   __________________________________________________________
   __________________________________________________________

11) On average how often are sick/injured rabbits euthanized on your farm?
   At least daily  ☐  every 2-4 days  ☐  1x/week  ☐
   1 to 4 weeks  ☐  monthly or longer  ☐
   Other  ☐ (Please specify) ________________________________

12) How do you dispose of culled or found dead rabbits on your farm?
   Compost  ☐  Bury  ☐  Deadstock  ☐  Manure pile  ☐
   Other  ☐ (Please specify) ____________________

13) Do you have any concerns with your main method of rabbit euthanasia?
14) What are your concerns with your main method of euthanasia (circle all that are correct)? *(Please skip this question if you answered NO to question 13)*
   a) I do not like doing it
   b) I'm not sure if I'm doing it right
   c) I'm not really sure if the animal is dead when I do it
   d) I do not think the method I/we use is humane
   e) Other (please specify): ________________________________

15) Do you routinely check to see if the rabbit is dead after euthanizing it?
   YES □   NO □
   If yes, please indicate how you check that the animal is dead (include all methods):
   I look at the animal to see if it is alive □
   I look for breathing □
   I feel for a heartbeat □
   I look at the eyes □
   I pinch the foot (withdrawal reflex) □
   I touch the eyes (corneal reflex) □
   Other □ (Please specify) ________________________________

16) In your opinion, how long should it take a rabbit to die that must be killed on-farm?
   It should be immediate □  Less than 1 minute □  1-5 minutes □
   >5 minutes □  Other □ (Please specify) ______________

17) Do you feel there is a need for new methods to euthanize rabbits on farms (circle the best answer)?
   No □  Maybe □  Yes □  Not sure
Other On-Farm Euthanasia Methods Used

Below are descriptions of 2 different methods for on-farm euthanasia of rabbits. These are methods that are being used to euthanize other livestock on-farm. Read the information provided on each the method and consider if this method might work on your farm to euthanize rabbits. Survey questions to gather your opinion of the method will be presented after the description of the method.

The Zephyr

The Zephyr is a non-penetrating captive bolt gun that costs about $900 and requires compressed air to run. It resembles a nail gun and is powered by compressed air, so must be connected to an air compressor or portable tank. The bolt in the barrel makes contact with the animal's head but does not penetrate the skull. This force causes injury to the brain making them insensible while killing them at the same time. This method is currently used in the swine industry to euthanize suckling piglets on-farm. It is also used by abattoir personnel to stun rabbits before bleeding them. The human safety concern with using the Zephyr is to ensure that it is not used on humans, whether accidental when the animal moves at the last minute or on purpose with the intent to harm. Human fatigue from the weight of the Zephyr and the force of administering could also cause loss of accuracy and become a safety concern.

16) Have you ever used the Zephyr for euthanizing rabbits?
   YES □      NO □

17) Have you ever seen the Zephyr being used for euthanasia?
   YES □      NO □

18) Do you think the Zephyr would work to euthanize rabbits on your farm?
   YES □      NO □

19) Please explain your answer to question 18: __________________________________________
    __________________________________________
    __________________________________________
    __________________________________________
    __________________________________________
    __________________________________________
    __________________________________________
    __________________________________________
    __________________________________________

20) Do you have any concerns regarding the on-farm use of the Zephyr for rabbits?
   YES □      NO □
21) What are your concerns with the Zephyr?
(Please skip this question if you answered NO to question 20)
Carbon Monoxide Gassing

Carbon monoxide (CO) is a tasteless, colourless, odourless gas that when given to an animal at a certain concentration for a certain amount of time will kill them. This method is used by mink producers to euthanize mink on-farm for pelting. Mink producers use a wooden gassing chamber that is built on top of a trailer pulled by a small tractor. This allows the gassing chamber to be mobile and to be brought directly to the mink, in well ventilated sheds, that are to be euthanized, generally. Multiple animals are placed in the chamber at once and then the gas is turned on for 10 seconds. Death is fast and painless. The biggest safety concern with using carbon monoxide is the risk of the operator inhaling it, as it can lead to death if it is not used properly. When used on mink farms it is used in open-sided barns with good ventilation. Another safety concern with CO is proper storage because the tanks may leak over time. It is best to store the tanks outdoors in a protected area, to ensure they will not be knocked over. It costs $240/cylinder and will euthanize ~ 4,000 mink (6 cents/mink).

22) Have you ever used carbon monoxide for euthanizing rabbits?
   YES □    NO □

23) Have you ever seen carbon monoxide being used for euthanasia?
   YES □    NO □

24) Do you think carbon monoxide would work to euthanize rabbits on your farm?
   YES □    NO □

25) Please explain your answer to question 24: ________________________________
__________________________________________
__________________________________________
__________________________________________
__________________________________________

26) Do you have any concerns regarding the use of carbon monoxide? YES □    NO □

27) What are your concerns with CO gassing?
   (Please skip this question if you answered NO to question 26)
   ________________________________
   __________________________________
   __________________________________
   __________________________________
   __________________________________
28) What do you think is the best method of on-farm euthanasia for rabbits? (You can pick a method not yet mentioned in the survey):

_______________________________________________________________

29) What is your reasoning for your answer to question 28? ________________________________

______________________________________________________________________________

______________________________________________________________________________

30) How much would you be willing to spend on a new euthanasia method?

☐ under $100
☐ $100-$300
☐ $300-$500
☐ $500-$700
☐ more than $700

31) How much money would you be willing to spend per kit that needed to be euthanized?

☐ less than $1
☐ $1 - $5
☐ $5 - $10

32) How much money would you be willing to spend per rabbit (growers and older breeding animals) that needed to be euthanized?

☐ less than $1
☐ $1 - $5
☐ $5 - $10

Are there any other notes or comments about euthanasia methods that you would like to make?: _________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________

______________________________________________________________________________