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FSVO/UFAW/HSA Online Symposium – Humanely Ending the Life of Animals

3rd-4th November 2020
#HELA2020

UFAW SCIENCE IN THE SERVICE OF ANIMAL WELFARE

Humane Slaughter Association

Introduction

Welcome to this online symposium on Humanely Ending the Life of Animals. Finding the most humane way to end the life of animals slaughtered for food or killed after they have been used in biomedical research is a fundamental problem in animal welfare which is also highly contentious. There is a lack of consensus on which methods cause the least possible suffering. One of the most commonly used methods to kill both livestock (pigs and poultry) and laboratory rodents, Carbon Dioxide (CO_2) gas, is particularly contentious. Whilst it is widely accepted that exposure to CO_2 causes some welfare compromise, the severity of this and the improvement offered by alternatives is hotly debated. The viability of alternatives is also a contentious issue - many potentially humane alternatives are regarded by many as impractical.

The Swiss Federal Veterinary Office (FSVO) has organised two previous meetings to examine the current scientific evidence regarding the humaneness of CO_2 and potential alternatives, and to map out the future research priorities necessary to fill the current gaps in our knowledge. This strategy formed the basis of a paper - *Humanely Ending the Life of Animals: Research Priorities to Identify Alternatives to Carbon Dioxide*[1].

In this third meeting the FSVO has partnered with two animal welfare charities – the Universities Federation for Animal Welfare (UFAW) and the Humane Slaughter Association (HSA) to provide an update on the latest scientific developments in the field which we hope will contribute to filling some of the knowledge gaps.

We are very grateful to the speakers for their participation and for adapting their talks to an online format at short notice as well as to the organising committee (Daniel Weary, Robert Meyer, Claudio Zweifel, Thom Gent and Ngaio Beausoleil) for their dedication to bringing this meeting to fruition. We thank you for registering for the meeting and hope you find the two days informative.

Ingrid Kohler

Federal Food Safety and Veterinary Office FSVO, Switzerland

Huw Golledge

Chief Executive Officer – UFAW and HSA, United Kingdom

[1] *Animals* 2019, *9*(11), 911; https://doi.org/10.3390/ani9110911

Day 1 - Livestock

Session Chairs: Claudio Zweifel (*Federal Food Safety and Veterinary Office, Switzerland*) and Ngaio Beausoleil *(Massey University, New Zealand)*

Session 1

11.00 Welcome
Kaspar Joerger (*Federal Food Safety and Veterinary Office, Switzerland*)

11.05 Introduction and background to the meeting
Huw Golledge *(UFAW and HSA, United Kingdom*) and Aline Steiner (*University of Zürich, Switzerland*)

11:30 Keynote: Where there's will, there is a way
Mohan Raj *(Independent, United Kingdom)*

12:00 Asking chickens about feelings under exposure to CO_2 and alternatives to CO_2. Novel animal model using broiler breeders
Sabine Gebhardt-Henrich, Abdulsatar Abdel Rahman, Thom Gent and Michael Toscano *(Universities of Bern and Zurich, Switzerland; Swedish University of Agricultural Sciences, Sweden)*

12:25 A novel stunning method of pigs with high expansion nitrogen foam: An initial study
Cecilia Lindahl *(Agriculture and Food, RISE Research Institutes of Sweden, Sweden)*

12:50 Lunch

Session 2

14:00 CO_2-stunning of pigs. An example of behaviour during induction and overview of gas concentration and other key parameters during routine slaughter of pigs in modern low stress group stunning devices
Karen Von Holleben and Martin Von Wenzlawowicz *(Training and consultancy institute for animal welfare, bsi Schwarzenbek, Schwarzenbek, Germany)*

14:25 On-farm euthanasia of commercial meat rabbits. Evaluation of physical methods compared to gradual and fast-fill carbon dioxide gas exposure
Jessica Walsh, John Van de Vegte, Brianne Mercer, Aaron Percival and Patricia Turner *(University of Guelph, Ontario Ministry of Agriculture, Food and Rural Affairs and Charles River Laboratories, Canada)*

14:50 Low atmospheric pressure stunning in pigs: Insights from analgesic and anxiolytic interventions
Jessica Martin, Emma Baxter, Marianne Farish, Julian Sparrey, Peter Tennant, Adrian Ritchie and Dorothy McKeegan *(The Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh and University of Glasgow, United Kingdom)*

15.25 Panel discussion and wrap up

16:00 End

NB: All times GMT

Day 2 - Laboratory

Session Chairs: Huw Golledge (*UFAW, United Kingdom*) and Thom Gent (*University of Zürich, Switzerland*)

Session 1

10.15 Introduction to the day

10:30 Keynote: Connecting values and data for ethical euthanasia practices in the animal laboratory
Larry Carbone (*Independent, USA*)

11:15 CO_2 exposure as translational cross-species experimental model for panic
Nicole Leibold *(University of Maastricht, The Netherlands)*

11:40 Nitrogen gas as an alternative euthanasia agent for laboratory rodents
Ellen Deelen and Esther Langen *(Utrecht University. The Netherlands)*

12:05 A systematic review of the welfare impact of CO_2 euthanasia on research mice and rats
Patricia Turner *(Charles River Laboratories, Canada)*

12: 30 Lunch

Session 2

13:30 Challenges in the evaluation of the well-being of laboratory rodents during euthanasia
Debra Hickman *(Indiana University, USA)*

13: 55 Variation in rat CO_2 sensitivity
Lucia Amendola and Daniel Weary (*University of British Columbia, Canada)*

14:25 The use of an automated captive bolt for euthanasia of laboratory rats
Stephanie Yam, Catherine Schuppli and Daniel Weary *(University of British Columbia, Canada)*

14:50 Assessment of environmental factors affecting the well-being of rats euthanized with carbon dioxide
Debra Hickman *(Indiana University, USA)*

15:15 Break

Session 3

15:45 Understanding rat emotional responses to CO_2
Lucia Amendola and Daniel Weary *(University of British Columbia, Canada)*

16:05 Attitudes of European and Canadian laboratory animal professionals and researchers towards carbon dioxide euthanasia for rodents and perceptions of barriers for implementation of refinements
Michael Brunt, Lucia Améndola and Daniel Weary *(University of British Columbia, Canada)*

16.30 Panel discussion and wrap up

17:00 End

NB: All times GMT

WHERE THERE'S WILL, THERE IS A WAY

Mohan Raj
Independent, United Kingdom

Phylogenic studies reveal that peripheral carbon dioxide chemoreception evolved prior to central chemoreception during vertebrate evolution. Therefore, all the vertebrates have biological predisposition to detect elevated carbon dioxide levels in the atmosphere and, several species (fish, poultry, rodents, pigs) of them have been shown, given a free choice, to avoid such atmospheres.

Background scientific literature concerning humans suggests that breathlessness (dyspnoea) can occur due to changes in the blood oxygen and/or carbon dioxide levels. In humans, a blood carbon dioxide tension increase of 5 mm Hg above normal will stimulate respiration, whereas the blood oxygen tension has to decrease by about 60 mm Hg from the normal level before hypoxia stimulates the respiratory centres in the brain. Evidently, hypercapnoea is a more potent respiratory stimulant than hypoxia. It is therefore evident that animals will have to inhale acidic and pungent carbon dioxide gas endure the ensuing respiratory distress until rendered unconscious.

In spite of all the scientific knowledge and understanding of the effects of exposure to carbon dioxide, millions of pigs are killed each year by exposing them to this gas. Prof. John Webster wrote, 26 years ago, that 'the best of the existing stunning systems based on CO_2 undoubtedly cause more distress at the point of stunning than the best of the high voltage electrical stunning systems. However, the aim is to minimise all the stresses likely to be expected by pigs in the abattoir and the best of the CO_2 systems (group stunning) do permit free, minimally-stressed movement of pigs right up to the point that they enter the gas chamber'. Yet, there is very little evidence of wanting to change to using pure anoxia induced with argon or nitrogen. Research has shown that pigs do not show any aversion to the inhalation of 90% argon in air (2% residual oxygen) and they do not show escape attempts. They showed no hesitation to re-enter the chamber to obtain a reward of apples, implying recovery from anoxia is not stressful either.

It is worth mentioning that the trout and salmon industry have developed electrical and captive bolt stunning methods and stayed away from using carbon dioxide. It is also reassuring to note that a mixture of 20 to 25% argon in nitrogen is being used to stun / kill chickens and turkey, whilst they are in transport containers, in the UK. This gas mixture can be contained easily within the concrete pits used for gas stunning pigs, and therefore, there is no need for any structural changes.

It is very much hoped that stakeholders will find a way to achieving humane death in millions of pigs slaughtered for human food using a non-aversive anoxic gas mixture.

ASKING CHICKENS ABOUT FEELINGS UNDER EXPOSURE TO CO_2 AND ALTERNATIVES TO CO_2 - NOVEL ANIMAL MODEL USING BROILER BREEDERS

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Animal welfare is about the feelings of animals which, due to their subjective nature, can only be quantified to a limited extent. We suggest a novel animal model using a particular genetic line of chickens in a place preference test. The aim was to have trained chickens in the same chamber that could be filled with either: CO_2, N_2, or receive low atmospheric pressure and their aversion to the stimulus evaluated. Broiler breeders (BB) are the parental generation of broilers, i.e. chickens for meat production. Broilers have been selected for fast growth and are slaughtered long before reaching sexual maturity. In order to obtain fertilized eggs from the parent generation (breeders) BB have to be subjected to severe feed restriction, which induces long-term hunger. These characteristics make these animals easy to train for a task to indicate that a condition is worse than profound hunger. For the test procedure, a cylinder shaped arena was divided into half by a barrier with a passage at one side wide enough for a chicken to pass. On one side feed was present. Eighty-eight individually marked BB (Ross 308, about 35 weeks of age) were singly placed into the area between both halves of the arena and were allowed to feed briefly when they found the feed. Training was performed during 3 – 4 days a week. Following acclimation to the procedure, when a hen was feeding, a negative stimulus (compressed air or dripping water) was introduced on the side with the feed and could be avoided by going to the other side of the arena that contained no feed but also no negative stimulus. A trial was successful when the hen approached the feeder fast and went to the other half after encountering the negative stimulus. After 14 weeks of training 66% of the hens approached the feed within 45s (mean: 5.6s, STD: 9.3). BB left the side with the feed during the water faster than during the compressed air (water: 5.6s, STD: 15.3, air: 22.8s, STD: 20.0, Wilcoxon test $\chi^2_1 = 19.3$, $P < 0.0001$). Applying the negative stimulus too often slowed down the approach time to the feed, so it was only applied once a week. After the majority of trials was successful, animals were habituated to the test chamber and during one day to the sight and sound of the closing lid, and the compressor. We conclude that this animal model is useful for measuring the aversiveness of exposure to different methods of euthanasia in chickens.

A NOVEL STUNNING METHOD OF PIGS WITH HIGH EXPANSION NITROGEN FOAM – AN INITIAL STUDY

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Carbon dioxide gas stunning is currently the most commonly used stunning method for commercial slaughter of pigs. However, the high concentration of carbon dioxide used for stunning pigs has been shown to cause a high degree of aversion and animal distress prior to loss of consciousness. Nitrogen gas is approved for stunning and is reported to have less negative effects than carbon dioxide but is not used, due to technical difficulties administering nitrogen gas. By using a high expansion foam filled with nitrogen gas to effectively eliminate oxygen from a container and to quickly create an anoxic atmosphere these technical problems can be avoided. However, proof of concept for the N_2 gas filled foam stunning method on pigs and piglets has been lacking.

The objective of this study was to assess the behavioural and physiological response of pigs to air filled and nitrogen filled foam respectively, and to assess the stunning process when pigs are exposed to nitrogen filled foam.

The experimental study included 60 pigs (20 per treatment; 9 weeks old and 27.8 ± 4.4 kg) and was conducted at SLU's pig research facilities at Lövsta, Sweden. Pigs were exposed to either air-filled foam, nitrogen-filled foam or no foam (Control). By comparing the pigs' responses to nitrogen-filled and air-filled foam, responses related to the nitrogen gas could be differentiated from responses related to the foam itself.

The pigs did not show any strong aversive behaviours when exposed to foam, regardless if it was air-filled or nitrogen-filled foam. However, they seemed to avoid putting their heads and snouts into the foam and the rate of escape attempts through the lid increased when foam levels became high. Heart rate and respiratory rate increased when pigs were exposed to nitrogen filled foam and oxygen levels decreased. Mean time to loss of posture was 57.9 s. Loss of posture was followed by vigorous convulsions (i.e. kicking, body convulsions, gasping) which went over to more irregular, milder movements. Mean time to last observed convulsion was 131.2 s. Five minutes after the start of the nitrogen foam production, the pigs were assessed to be in deep unconsciousness or dead.

This study offers proof of concept for using nitrogen-filled foam for the stunning of small pigs. However, further studies are needed to evaluate the method for stunning of lager sized slaughter pigs, the potential for group-stunning (two or more pigs), and to determine the adequate time of exposure and the maximum stun-stick interval to ensure unconsciousness throughout the subsequent killing procedures at slaughter.

CO_2-STUNNING OF PIGS. AN EXAMPLE OF BEHAVIOUR DURING INDUCTION AND OVERVIEW OF GAS CONCENTRATION AND OTHER KEY PARAMETERS DURING ROUTINE SLAUGHTER OF PIGS IN MODERN LOW STRESS GROUP STUNNING DEVICES

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Alternatives to CO2- stunning of pigs should result in veritable improvements with regard to animal welfare. To date, studies from which many judgements with regard to animal welfare were developed, were not performed in modern low stress group stunning devices, where pre-slaughter stress can be significantly reduced and many technical features have improved. This brings into question the validity of the results in relation to modern practices. We therefore present a behavioural study of pigs during the induction to CO2 stunning. To integrate these results and to give an idea of the conditions pigs encounter during modern CO2 gas stunning we will show the results of gas measurements and other key parameters, gathered during inspection at site in 25 slaughter plants between 2010 and 2020. A behavioural study was performed in 2016 under practical conditions (slaughter speed 290/h) on 110 pigs, stunned in a device type Backloader 5 (Butina®) in groups of (2 to) 4. For remote video observation we used GoPro® cameras in 30 gondolas. The space in the gondola was 2.45 m2 = 0.61m2 /pig. Gas concentrations (CO2- and O2) were continuously measured using a PBI Dansensor A/S, Ringsted Denmark (CheckMate III O2 (ZrOx) / CO2), in combination with a pump (Type PM13421-NMP30, Fa. Neuberger, Freiburg, Germany) connected to a hose (diameter 4 mm) let down at the side of gondola, at the same height of the pigs nose, entering into the gas atmosphere. Additionally we measured at the level of the monitoring sensors of the stunning device (>80% CO2 was reached after 6 seconds at 30 cm below floor level; the pre-set concentration of 95% CO2 at the first stop 249 cm below floor level was reached after 23 seconds, while the gondola stopped there for 27s). Video recordings were analysed for “start of deep breathing through open mouth”, “start of signs of agitation i.e. back or forward movements, quick lateral head movements”, “time of inability of the animal to remain in an initial standing position (e.g. stumbling, tumbling = LOP)” each sign if visible at the first pig of the group after start of gondola movement and “time of definitely lying (first / all pigs of the group)”. Agitation occurred in 10 of the 30 groups. In 20 groups the pigs showed deep breathing, then tumbled and fell. As an approach to calculate a critical time interval of potentially negative experience we calculated roughly the time interval between the first pig of the group showing tumbling or – if so - agitation to all pigs lying. This was on average 10s (range 6-18s; 50% interval 8-12s) and thus shorter than presumed to date. 1 The data presented on modern CO2 stunning were recorded in 25 plants using group stunning devices of different manufacturers (Butina, Marel, Banss, Banss Austria) mostly type Backloader or similar on behalf of retailers, authorities and slaughter industry. We will focus on conditions during induction, such as time to reach > 80% CO2, time to reach the first stop and CO2 concentration at the first stop. Additional information will comprise design, number and size of gondolas including space per pig, dwell time in > 80% CO2, gas concentration at deeper stops, and stun to stick interval. Our measurements as well as volumetric calculation show that in most of the plants concentration of oxygen falls below 2% within 10 seconds or less, thus suggesting an anoxic besides the hypercapnic component during the induction phase. We conclude that technological progress has modified conditions pigs experience during CO2-stunning and refinement has the potential to improve animal welfare during stunning of millions of slaughter pigs until better alternatives are available. If studies on alternatives to CO2-stunning include comparative studies the actual best practice and technological standards of CO2 stunning should be represented.

ON-FARM EUTHANASIA OF COMMERCIAL MEAT RABBITS: EVALUATION OF PHYSICAL METHODS COMPARED TO GRADUAL AND FAST-FILL CARBON DIOXIDE GAS EXPOSURE

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The commercial meat rabbit industry was without any validated on-farm euthanasia methods or guidelines. We approached this gap by first surveying Ontario commercial meat rabbit producers (n=26) on their current euthanasia methods, practices, and attitude towards euthanasia methods. We then evaluated several methods of on-farm killing of cull rabbits of different ages to determine which were most effective and humane. Physical methods evaluated on 170 cull rabbits included blunt force trauma, a device to assist in manual cervical dislocation, and a non-penetrating captive bolt device. Method efficacy was determined by time to loss of sensibility and subsequent cardiac arrest. Survey radiographs were taken of a subsample of rabbit heads to assess the degree of skull damage and/or cervical dislocation. Further evaluation included macroscopic scoring of gross hemorrhage, and microscopic analysis by a veterinary pathologist blinded to killing method. Blunt force trauma had the highest chance of method failure (22%) and resulted in less microscopic damage than the non-penetrating captive bolt device, which was 100% effective at causing immediate and sustained unconsciousness. The device used for manually assisted cervical dislocation resulted in high accuracy with a clean dislocation at the base of the skull. From our survey, concern for aesthetics seemed to be linked to 42% of producers electing to let rabbits die on their own versus performing their euthanasia method. Producers indicated an interest in using gassing method if human safety could be assured. Carbon dioxide was evaluated at two different fill rates, 28% volume change/min (n=42) and 58% volume change/min (n=39), assessing the rabbit's behavioural and physiological response, and time to onset of unconsciousness and cardiac arrest. A hand input into the chamber allowed for reflexes and righting response to be assessed and death to be confirmed. The fast-fill rate of 58% volume change/min limited potential distress by reducing time to loss of consciousness (40 ± 1s). Human safety was not found to be a concern. No obvious signs of anxiety or escape attempts were observed. We conclude that the non-penetrating captive bolt, the device for manually assisted cervical dislocation and carbon dioxide inhalation at the rates evaluated were acceptable forms of euthanasia for commercial meat rabbits.

LOW ATMOSPHERIC PRESSURE STUNNING IN PIGS: INSIGHTS FROM ANALGESIC AND ANXIOLYTIC INTERVENTIONS

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Low atmospheric pressure stunning (LAPS) is associated with acceptable welfare outcomes in poultry and is emerging as a novel approach to controlled atmosphere stunning. There is growing concern about the negative welfare impacts of carbon dioxide (CO_2) stunning systems for pigs, and it has been suggested that LAPS could provide a humane alternative. LAPS involves gradual decompression of animals inside a sealed chamber leading to hypobaric hypoxia, loss of consciousness and a non-recovery state. The aim of this study was to assess the welfare impact of a candidate LAPS decompression curve, a commercially relevant CO_2 treatment and a sham stun, as part of a wider project to systematically evaluate the potential of LAPS to be a humane method of stunning pigs. Female pigs (n=216, 30 Kg bodyweight) were randomly assigned to treatments applied in a 3 x 3 factorial design with three primary treatments (LAPS, SHAM and CO_2) and three secondary treatments (analgesia – Buprenorphine; anxiolytic – azaperone; control – saline; 24 pigs per 2nd level treatment). The study involved monitoring of brain, physiological and behavioural responses; only behaviour is reported here. Pigs were exposed to the stunning treatments in pairs in a specially designed clear poly carbonate crate that allowed visual and auditory contact. The LAPS treatment was biphasic with an initial phase lasting 95 s with a mean pressure change of -0.86 kPa s^{-1} and a second phase lasting 505 s with a mean pressure change of -0.14 kPa s^{-1}. These rates were chosen based on a previous experiment with unconscious pigs and estimates of maximum commercially relevant cycle durations. The CO_2 treatment was applied in a containerised gassing unit and was designed to mimic gas exposure rates in commercial dip/lift systems (duration 150 s, with 80% CO_2 reached within 40 s). The SHAM treatment consisted of being placed in the LAPS chamber for 5 min without any decompression taking place. A range of behaviours associated with aversion were observed in all three treatments, but were most pronounced in the stunning treatments. Loss of posture (LOP) occurred on average at 36.2±0.6 s in CO_2 and 118.4±1.8 s in LAPS. Higher counts of head shaking, head tilts and facial grimace were seen in LAPS compared to CO_2 or SHAM, but more pigs tried to escape in CO_2. We also saw the highest frequency of escape attempts per pig in CO_2, despite the shorter cycle time. High-pitched vocalisations were greater (in terms of counts and proportion of pigs) in LAPS compared to CO_2, and occurred on average around the point of LOP in both stunning methods. There were more gasps with CO_2, indicating more air hunger, however the frequency of gasping was significantly reduced by the anxiolytic treatment in both LAPS and CO_2. Analgesic treatment was associated with less head tilt in LAPS and less high-pitched vocals in CO_2. This suggests that head tilting may be related to pain (most likely ear pain) during LAPS and gasping is related to anxiety in response to air hunger (both treatments). The different durations of the LAPS and CO_2 cycles make direct comparison difficult, but more time was spent exhibiting negative behaviours in the

conscious phase with LAPS. We conclude that both LAPS and CO_2 stunning are associated with several indicators of poor welfare, and the drug interventions provide evidence for pain and anxiety with both treatments. This study does not support the notion of LAPS as a viable stunning method for pigs at rates that would result in loss of consciousness and death in a commercially feasible timeframe.

CONNECTING VALUES AND DATA FOR ETHICAL EUTHANASIA PRACTICES IN THE ANIMAL LABORATORY

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Effective refinement of research animal euthanasia procedures requires the best possible data on how procedures affect animals, laboratory workers and research outcomes. Our knowledge is presently quite limited, and animal welfare scientists, social scientists and scientists using animal models are working to fill in the many knowledge gaps. Data alone will not lead to consensus on how best to euthanize our animals; for that we need to clearly label and discuss often-competing values, to derive an ethical consensus on best practices. I review some cases from some euthanasia controversies to illustrate this ethics-value-fact interplay. In one, rodent decapitation results in persistent brain waves. No one disputes the reality of the brain waves, though what they tell us about rodent brain function is controversial. Moreover, we make ethical decisions about how many seconds of potential consciousness should matter, and how to ethically proceed if we really do not know what the brain waves signify. Similarly, in the face of uncertain facts about carbon dioxide, we make ethical decisions about how to prioritize potential pain against potential non-pain distress. In all of this, ethics committees, policy makers and others make ethical choices on animals' behalf in the face of uncertain data, and this uncertainty must figure into responsible discussions of animal euthanasia practices.

CO_2 EXPOSURE AS TRANSLATIONAL CROSS-SPECIES EXPERIMENTAL MODEL FOR PANIC

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Exposure to 100% carbon dioxide is commonly used for killing animals or for stunning them prior to killing. This is likely due to practical and economic reasons: it is cheap, easy to handle and allows killing of several animals at the same time. The method remains popular in spite of reports showing that inhalation of carbon dioxide causes considerable distress. However, exactly how aversive CO_2 is for animals remains difficult to quantify.
In humans, a long tradition exists of using carbon dioxide in the study of panic attacks. Specifically, high dosages (between 5 and 35%) of CO_2 are used to experimentally induce panic attacks in the laboratory. We have repeatedly demonstrated a dose-response relationship between the dosage of CO_2 and the level of evoked fear in humans. Moreover, using the same physiological outcome measures in a translational study in humans and mice, we were able to quantitatively compare the level of distress in these species, induced by inhaling different concentrations of CO_2. The primary aim of this study was to validate an experimental murine model for the study of the molecular mechanisms of panic. However, from the results it can be directly inferred that inhaling of increased concentrations of CO_2 (but far below 100%) causes a level of distress in animals that is labeled by humans as intense fear. Thus, these data may help to convince the community that the use of 100% CO_2 is to be considered as inhumane and that there is a pressing need to adopt alternative methods, in order to improve animal welfare.

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NITROGEN GAS AS AN ALTERNATIVE EUTHANASIA AGENT FOR LABORATORY RODENTS

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Exposure to high levels of carbon dioxide (CO_2) is a common method of euthanasia for laboratory rodents. However, the use of CO_2 as a euthanasia agent has become a matter of discussion, because studies have reported that exposure to high levels of CO_2 might induce fear responses, aversion, or even pain. This has inspired a search for alternatives that cause less distress than CO_2 and are equally safe and effective as a method of euthanasia. Amongst other gasses, nitrogen (N_2) has been suggested as an alternative. However, there has been some controversy regarding the effectiveness of N_2 as a euthanasia agent for rodents. Moreover, it remains unclear what the welfare impact of N_2 euthanasia is in rodents. More research is therefore needed to assess both components. In this proposed study, we plan to compare CO_2 and N_2 as euthanasia agents for laboratory rodents, investigating the effects of both gases on behavioural and physiological parameters. Amongst others, we plan to review the effectiveness of both methods, but also to investigate their welfare impact.

A SYSTEMATIC REVIEW OF THE WELFARE IMPACT OF CO_2 EUTHANASIA ON RESEARCH MICE AND RAT

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Increased concern and discussion have occurred about the suitability of CO_2 as a euthanasia method for laboratory rodents. Concerns have revolved around the potential for discomfort, pain or distress that animals may experience prior to loss of consciousness; time to loss of consciousness; best methods for CO_2 use; and the availability of more humane alternatives. These discussions have been useful in providing new information, but have resulted in significant confusion regarding the acceptability of CO_2 for rodent euthanasia. In some cases, researchers and veterinarians have become uncertain as to which techniques to recommend or use for euthanasia of laboratory mice and rats. A taskforce was convened by the International Association of Colleges of Laboratory Animal Medicine (IACLAM) to examine the evidence for adverse welfare indicators in laboratory rats and mice undergoing CO_2 euthanasia using a SYRCLE-registered systematic review protocol. Of 3772 papers identified through a database search from 1900 to 2017, 37 studies were identified for detailed review (some including more than one species or age group), including 15 in adult mice, 21 in adult rats, and 5 in neonates. Experiments or reports were included if they assessed at least one parameter directly related to animal welfare during CO_2 induction and/or euthanasia. Study design and outcome measures were highly variable and there was an unclear to high risk of bias in many of the published studies. Changes in the outcome measures evaluated were inconsistent or poorly differentiated. It is likely that repeated exposures to CO_2 inhalation are aversive to adult rats and mice, based on avoidance behavior studies; however, this effect is largely indistinguishable from aversion induced by repeated exposures to other inhalant anesthetic gasses. There is insufficient evidence to permit an unbiased welfare assessment of the effect of CO_2 inhalation during euthanasia in research mice and rats. Additional well-designed studies are needed to accurately assess the well-being of rodents undergoing CO_2 gas euthanasia.

CHALLENGES IN THE EVALUATION OF THE WELL-BEING OF LABORATORY RODENTS DURING EUTHANASIA

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Comparisons of well-being of laboratory rodents during euthanasia is challenged by the diversity of methods that are employed and the available tools of assessment, especially as all methods of euthanasia involve some pain or distress to the rodent. In this presentation, we'll evaluate how some of the commonly used tools have been used to assess well-being of common and uncommon laboratory rodents using a variety of methods of euthanasia.

VARIATION IN RAT CO_2 SENSITIVITY

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Cumulative evidence indicates that CO2 induces negative emotions in rats, but conflicting conclusions have emerged from studies using inescapable exposure (forced exposure) reporting great variability in behavioural responses to CO2. In a series of experiments, we assessed if rats vary in CO2 sensitivity. Our results show considerable individual differences in rat aversion to CO2. Variability in aversion to CO2 is not related to rat personality but rather to differences in sensitivity. We also found that rats experience anxiety when exposed to lower CO2 concentrations and that variation in rat CO2 sensitivity is driven by individual differences in the onset of these feelings. All rats avoided CO2 before losing consciousness, even less-sensitive rats when treated with an anxiolytic. We conclude that the magnitude of the negative emotional experience of rats exposed to CO2 varies among individuals, likely due to differences in the onset of CO2-induced anxiety.

THE USE OF AN AUTOMATED CAPTIVE BOLT FOR EUTHANASIA OF LABORATORY RATS

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Captive bolts are used to stun livestock, poultry, and rabbits prior to slaughter, but the feasibility of using captive bolts for euthanizing laboratory rats has yet to be explored. Here we report the results of a pilot study assessing the efficacy of the Goodnature A24 trap as a humane euthanasia method.

The Goodnature A24 is a CO2-powered device certified for humane pest control by New Zealand's Animal Welfare Advisory Committee. The captive bolt is activated when the animal's head is in place while she is receiving a food reward at the end of a feeding tube. The captive bolt is intended to crush the skull and brain, thus killing the animal.

In this study, 5 Sprague Dawley were individually trained to enter a disarmed trap to receive the food reward. Once trained, rats were allowed to freely enter an armed trap. All 5 rats entered the device but only one was rendered unconscious immediately, established by the absence of corneal and strong pedal withdrawal reflexes and cessation of breathing and heartbeat in less than 60 seconds. For this animal, post-mortem examination and histology showed clear cranial fracture, widespread brain bleeds, and bone shards and skeletal muscle present in the brain. Two other rats triggered the device but the bolt did not render these animals immediately unconscious. They were immediately removed from the test arena, anesthetized with isoflurane and euthanized with CO2. Post-mortem examinations revealed fractured left zygomatic arches with minimal brain hemorrhage in both cases. The two remaining animals repeatedly entered the device but never activated the trigger.

To study the cause of these failures, we conducted video studies and retested the two surviving animals with an unarmed trap. We found that these rats positioned their heads sideways to access the food reward without activating the trigger. We speculate that the ability to perform this behavior was associated with our training period. Future work is now planned with untrained rats.

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ASSESSMENT OF ENVIRONMENTAL FACTORS AFFECTING THE WELL-BEING OF RATS EUTHANIZED WITH CARBON DIOXIDE

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When euthanizing rats, regulatory and guidance documents provide recommendations of techniques that can be used to minimize the potential pain and distress experienced during the euthanasia procedure. However, the effectiveness of these recommendations have been inconsistently evaluated, and there is research that suggests that the recommendations may actually be contraindicated if the goal is to minimize the distress experienced by animals. In this presentation, we'll explore common recommendations regarding the environment in which the rat is euthanized with carbon dioxide. Specifically, we'll explore social interactions during euthanasia and how the chamber design affects the well-being of rats euthanized with carbon dioxide.

UNDERSTANDING RAT EMOTIONAL RESPONSES TO CO_2

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Most laboratory rodents are killed with carbon dioxide (CO_2). Within the euthanasia literature, several behavioural studies indicate that CO_2 elicits negative responses and is aversive to rats; however not all studies agree. Paradoxically, rodent exposure to CO_2 is a well-accepted translational model for the understanding of fear, anxiety, dyspnoea (feeling of breathlessness), and panic in humans, but the findings from this literature are seldom considered within the euthanasia literature. The aim of this talk is to review the available evidence from the euthanasia and translational models literature regarding rat emotional experiences during CO_2 exposure. The studies reviewed show that CO_2 exposure is aversive to rats, and that rats respond to CO_2 exposure with active and passive defence behaviours. All rats avoid CO_2 at concentrations far lower than those required to render them ataxic or recumbent (>33% CO_2). When motivated some rats tolerate concentrations up to 18% CO_2. Exposure to concentrations over 35% have anxiogenic effects and produce strong conditioning. Plasma corticosterone and bradycardia increased in rats exposed to CO_2. As with anxiogenic drugs, responses to CO_2 are counteracted by the administration of anxiolytics, SRIs, and SSRI's. Human studies reviewed indicate that, when inhaling CO_2, humans experience feelings of anxiety fear and panic, and that administration of benzodiazepines, serotonin precursors, and SSRIs ameliorate these feelings. In vivo and in vitro rat studies reviewed show that brain regions, ion channels, and neurotransmitters involved in negative emotional responses are activated by hypercapnia and acidosis associated with CO_2 exposure. On the basis of the behavioural, physiological, and neurobiological evidence reviewed, we conclude that CO_2 elicits negative emotions in rats.

ATTITUDES OF EUROPEAN AND CANADIAN LABORATORY ANIMAL PROFESSIONALS AND RESEARCHERS TOWARDS CARBON DIOXIDE EUTHANASIA FOR RODENTS AND PERCEPTIONS OF BARRIERS FOR IMPLEMENTATION OF REFINEMENTS

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Cumulative evidence indicates that Carbon Dioxide (CO_2) inhalation induces negative affective states (i.e. anxiety, fear and distress) in laboratory rodents. This method is still commonly used to kill these animals despite the availability of refinements such as pre-exposure to inhalant anaesthetic. To better understand the views of laboratory animal professionals and researchers about the use of CO_2 and alternative methods for rodent euthanasia we surveyed 209 Canadian and 383 European participants. Very few responders had completely favourable views of CO_2, but approximately half of the participants used this agent. The attitudes of participants around the use of CO_2 centered around four themes: 1) the animal's experience, 2) practical considerations, 3) technical description, and 4) compromise. Approximately 50% of the participants stated that there are alternatives available that could be considered an improvement over CO_2, but perceived barriers to implementing identified alternatives. Perceived barriers fell into five main themes: 1) financial, 2) institutional culture, 3) research, 4) safety, and 5) regulatory. The availability of scientific evidence regarding the suitability of refinements was not a predominant theme. In summary, participants expressed concern about the effects of CO_2 on the animal's experience, but perceived important barriers, often related to operational limitations, to adopting alternatives. We suggest that many of these perceived barriers are surmountable and that future efforts should focus on institutional barriers to the adoption of refinements.