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An independent animal welfare assessment of mass destruction methods for poultry on-farm

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Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

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Introduction

The purpose of this review is to provide a critical assessment of the methods available for mass depopulation of poultry on-farm in an emergency animal disease outbreak response. It also aims to provide a scientific opinion on methods that are suitable for inclusion in AUSVETPLAN documents. The information within could also help to inform future iterations of the Australian Animal Welfare Standards and Guidelines regarding the humane killing of poultry. This review has been prepared for the Department of Agriculture, Fisheries and Forestry (DAFF).

Australian governments and industry have identified a critical need to update and revise the national guidance on humane destruction of poultry in an emergency animal disease response. The AUSVETPLAN destruction manual was last updated in 2006 and is due for revision in the 2022-2023 financial year. The relevance of this work is highlighted by recent poultry depopulation events in Australia, including outbreaks of Highly Pathogenic Avian Influenza (HPAI) in Victoria in 2020 and Salmonella Enteritidis in NSW and Victoria in 2018/2019. Overseas, outbreaks of HPAI in the United States and United Kingdom have created a heightened awareness of the need for additional humane destruction methods for poultry in the future. Humane destruction methods, including those utilising new and emerging technologies, require expert assessment of the associated animal welfare considerations and hazards.

It is important to note that the humane killing of poultry on-farm for reasons other than emergency animal disease events is subject to relevant state and territory animal welfare legislation, subordinate regulations, and the Australian Animal Welfare Standards and Guidelines. This includes routine situations, such as the culling of spent layer hens and non-routine situations, such as in the event of natural disasters, building collapses or other unpredictable events.

1 Project objectives

The project addresses the following objectives:

- A review of the methods currently recommended for the mass destruction of poultry as documented in national and international standards and guidelines
- An evidence-based animal welfare assessment of the listed methods when applied for the purpose of on-farm depopulation
  - Inhalational methods: Air-filled (water-based) and gas-filled foam administered to birds held in containers
  - Inhalational methods: Air-filled (water-based) and gas-filled foam administered in-house (floor-reared poultry)
  - Ventilation shutdown
  - Low atmospheric pressure stunning (LAPS) and other novel methods (if identified during the review)
- A proposed scientific opinion on whether these methods are suitable for large-scale destruction of poultry, and acceptable for inclusion in Australian standards and guidelines for the humane destruction of poultry
- Development of evidence-based, best practice guidelines on the parameters required for the correct use of each suitable method to mitigate animal welfare risks, ensure acceptable animal welfare outcomes and achieve depopulation objectives.

2 Methodology

Poultry at different stages of production may have to be killed on-farm for reasons other than slaughter for human consumption. This may include both individual animals and large-scale (mass) destruction (for example, for disease control).

2.1 Project statement of requirement

The first part of the exercise involved the identification and review of the methods currently recommended for the mass destruction of poultry, as is documented in national and international standards and guidelines.

2.2 Selected standards and guidelines

The researchers identified the listed national and international documents for consideration in the review:

- AUSVETPLAN Operational manual: Destruction of animals (AUSVETPLAN), and associated technical documents
- Australian Animal Welfare Standards and Guidelines: Poultry
- Model Code of Practice for the Welfare of Animals: Domestic Poultry (superseded)
- OIE Terrestrial Animal Health Code Chapter 7.6 Killing animals for disease control purposes
- AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
- AVMA Guidelines for the Depopulation of Animals: 2019 Edition
- Code of Welfare: Meat Chickens (2018), Ministry for Primary Industries, New Zealand
- Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing (endorsed by the Farm Animal Welfare Council Opinion on the Welfare of Animals Killed on Farm, 2017)
- The Code of Practice for the Care and Handling of Hatching Eggs, Breeders, Chicken and Turkeys, NFACC 2016, Canada
- The Code of Practice for the Care and Handling of Pullets and Laying Hens, NFACC 2017, Canada
- RSPCA Australia - Mass Euthanasia of Poultry Guidelines
- RSPCA Assured Standards, UK - Meat Chickens and Laying Hens.

Table 1 shows a simple summary of the methods referenced in the identified documents, that were reviewed as part of the literature review.

Table 1 Methods described by the reference documents for the mass destruction of poultry

Method Category	Method	AUSVET	MCOP	AUS S&G	OIE	AVMA[1]	CODE NZ[2]	EU Reg[3]	CODE CAN	RSPCA AUS	RSPCA UK[4]
Inhalational	Carbon dioxide	✓		✓	✓	✓		✓	✓	✓	
	Inert gases			✓	✓	✓		✓	✓		
	CO_2-inert gas mix			✓	✓	✓	✓	✓		✓	
	Water-based foam	✓				✓				✓	
	Gas-filled foam					✓				✓	
Injectable	Barbiturate	✓		✓	✓	✓		✓	✓		
Mechanical	PCB NPCB[5]			✓	✓	✓		✓	✓	✓	✓
	MBFT[6]			✓		✓		✓	✓		
	Neck dislocation	✓	✓	✓	✓	✓	✓	✓	✓		
	Decapitation				✓	✓			✓		
	Gunshot			✓	✓	✓		✓			
Electrical	Head-only stunning			✓		✓	✓	✓			✓
	Water-bath			✓	✓	✓		✓			✓
Oral agent	Anaesthetic	✓				✓					
Other	LAPS[7]			✓	✓						
	VSD[8]					✓					

1 AVMA Guidelines for the Euthanasia of Animals: 2020 Edition and AVMA Guidelines for the Depopulation of Animals: 2019 Edition. **2** Specifies any other methods used for humane destruction of chickens (referred to in the OIE Terrestrial Animal Health Code) and can be used in an emergency situation. **3** FAWC Opinion on the Welfare of Animals Killed on Farm, 2017 recognises the methods approved under EU regulation. **4** RSPCA Assured Standards cover on-farm killing, though without clear guidelines on methods deemed suitable in an emergency situation. **5** Penetrative captive bolt, non-penetrative captive bolt. **6** Manual blunt force trauma. **7** Low atmosphere pressure stunning. **8** Ventilation shutdown.

2.3 Literature search strategy

The literature search utilised the CSIRO library and Adelaide University database subscriptions. The electronic literature databases included were:

- Web of Science - Peer-reviewed/Conference proceedings
- Scopus - Peer-reviewed/Conference proceedings
- Agricola - Industry and technical reports/Patents
- Derwent Innovations Index – Patents.

The search was completed between 1st -15th December 2021. Articles identified during the search were uploaded to EndNote reference manager and duplicates automatically detected and removed, followed by manual removal of any additional duplicates (for example, publications published in more than one format or indexed in more than one database). Articles identified in the search were also exported to an Excel spreadsheet for sorting, alignment, and synthesis.

Primary searches of the publication title were conducted using the listed species/type name (or variants of):

- Poultry
- Chicken (Broiler, meat)
- Hen (Layer, pullet)
- Turkey (Poult)
- Duck
- Geese
- Pheasant
- Quail
- Partridge.

Secondary searches of the publication title included the listed key words:

- Depopulation
- Killing (Kill)
- Euthanasia (euthanise/euthanase)
- Destruction
- Culling (Cull).

To exclude publications related to 'Turkey' - the country, the term 'poultry' was used as a qualifying statement in the search. The primary search was restricted to the years 2015-2021. This returned 155 hits based on the presence of the key words in the title of the publication. A series of secondary searches were then undertaken, using the species/farmed bird type name in the primary search and combining it with additional key terms related to specific methods outlined in Table 1.

2.4 Article screening and selection

The 155 publications identified in the initial search were made up of 91 articles (with the other 64 publications being made up of newspaper articles, patents, reports, dissertations and newsletters). These were subject to a further screening process, through evaluation of each title and abstract, to identify 48 target documents for critical appraisal. This was performed using the inclusion/exclusion criteria for article screening. The following were included:

- Papers relevant to scope detailed previously
- Date of publication: Articles published between 2015-2021

- Geographic focus: Worldwide
- Reviews and book chapters (to be included if relevant).

The following were excluded:

- Papers that focused exclusively on meat quality, food safety and disease control with no reference to animal welfare
- Philosophical/opinion papers
- Publications not written in English.

Full text versions of the target documents were obtained. During this phase, approximately 25 additional publications were also identified and located. These included seminal works (published before 2015) and older papers covering the minor poultry species. Relevant data from the target documents were extracted and collated in an excel spreadsheet. In the excel file, the information was arranged to allow sorting and tabulation by field and focus areas.

2.4.1 Geographical location

The literature search methodology protected against unintentional bias in the selection of papers for inclusion in the review. The papers that have been included are primarily from Europe and North America. In reading this review it should be acknowledged that factors relating to Australian conditions, environments and established farming practices may not be fully represented in the literature.

2.4.2 Overview of the literature identified

The majority of literature reviewed was focused on the use of inhalational agents and mechanical methods. There were fewer studies on the use of ventilation shutdown and LAPS. Many studies were based on a small sample size in research, or ‘laboratory-scale’ setting and assumed a scalable process if mass destruction was to be considered. The studies on ventilation shutdown were proof-of-concept studies on a small number of birds.

There was considerable variation in research methodologies as well as methods used for assessment of unconsciousness and a degree of subjectivity around the determination of death. Only a few studies focused on the induction period and the time to loss of consciousness; those that did tended to be carried out in the proposed context of commercial slaughter (for human consumption) as opposed to on-farm killing or mass destruction.

3 Introduction to mass destruction methods for poultry

3.1 Definitions

Before determining which methods were suitable for the humane mass destruction of poultry, it was necessary to first establish the meaning of the term ‘humane’. Fundamental to the concept of humane is the meaning of ‘consciousness’ and ‘unconsciousness’.

There are many definitions of consciousness, but in general it is associated with the awake state and the ability to perceive, interact and communicate with the environment and others (Zeman, 2006). Unconsciousness (the opposite of consciousness) is defined as: ‘a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence of this disruption, the unconscious animal is unable to respond to normal stimuli, including pain’ (EFSA, 2006). If an animal is conscious or if it regains consciousness, pain, fear and distress can be experienced.

Humane destruction methods should ideally induce an immediate state of general unconsciousness that lasts until death occurs (EFSA, 2004). Under practical conditions, EFSA (2006, 2004) has defined immediate (or instantaneous) as “unconsciousness occurring within 1 second” of the intervention being applied. For methods that do not induce immediate unconsciousness, any alternative procedure should ensure: 1) the absence of pain, distress and suffering until the onset of unconsciousness, and 2) that the animal remains unconscious and insensible until death. Methods using inhalational, oral and injectable agents fall into this category as unconsciousness is induced gradually (Gerritzen and Raj, 2009). The method must either kill the animal whilst it is unconscious or result in a duration of unconsciousness that is longer than the time needed for a secondary (or terminal) procedure to kill the animal (Gerritzen and Raj, 2009).

Therefore, the assessment of mass destruction methods focuses on the ability of the identified methods to produce a state of unconsciousness, without the animal feeling pain, fear or distress and which lasts until the animal is dead. To achieve this in a practical situation and to align with existing literature and guidelines, the following needs to be considered:

- The type of handling and restraint required to perform the method
- The time to loss of consciousness and likelihood that poultry experience pain and distress prior to loss of consciousness, and
- Ability to maintain a state of unconsciousness until the animal is dead.

The killing methods that have been identified (through the statement of requirement and in the source documents) as relevant for poultry can be grouped into five categories: (i) Inhalational agents; (ii) Oral agents; (iii) Injectable agents; (iv) Mechanical; and (v) Electrical. A sixth category, called ‘Others’ includes Low Atmospheric Pressure Stunning (LAPS) and ventilation shutdown (VSD). For the electrical methods, head-only electrical stunning (followed by a killing method, such as cervical dislocation) and electrocution (using a water-bath and head-to-cloacal electrical stunning) are considered. Mechanical methods include penetrative captive bolt, non-penetrative captive bolt, manual blunt force trauma, neck dislocation, decapitation and gunshot. Inhalational agents include gases and foams administrated in containers or in the housing environment. Injectable agents include barbiturates and barbiturate derivatives, whilst oral agents study the use of alpha-chloralose and sodium nitrite. Most of the methods will result in the death of the bird, whilst others will need to be followed by a secondary (terminal) procedure to ensure death.

The review provides a scientific opinion on methods that are acceptable or unacceptable on welfare grounds and their suitability for inclusion in Australian standards and guidelines for the humane mass destruction of poultry. Interestingly, many of the articles that study the suitability of methods for humane killing on-farm tend to focus on the capacity to result in 100% lethality as an indicator of efficacy, rather than whether the method induces immediate unconsciousness. Consequently, some of the methods recommended in the literature and source documents would not satisfy the criteria for being 'humane' (inducing unconsciousness without causing pain, fear or distress). The ultimate decision regarding the selection of methods for mass destruction requires scrutiny from an animal welfare perspective, but also the consideration of additional process requirements such as biosecurity, cost, aesthetics and safety.

3.2 Mass destruction requirements

When considering methods for the mass destruction of poultry, it is important to remember that it may be an atypical situation (such as an emergency disease outbreak), where the ideal choice of method may not be available or may be affected by the prevailing conditions. Achieving an acceptable animal welfare outcome is a multi-faceted challenge, particularly when trying to balance it with the safety of personnel, biosecurity and environmental requirements and production conditions. EFSA (2019) provides a scientific opinion on the killing of poultry on-farm (other than for commercial slaughter) and describes the different scenarios where large-scale killing (mass destruction) may be required. Mass destruction for disease control involves the killing of all birds in at least one biosecure area, such as a poultry house. More often, and depending on the nature of the disease, it involves the killing of all birds at the premises involved or at several farms in an area with the aim of preventing the spread of the disease (Berg, 2009; EFSA, 2008).

The general principles for the welfare of livestock during humane destruction for disease control are detailed in the OIE Terrestrial Animal Health Code (WOAH, 2022) and summarised in a review of killing animals for disease control purposes (Thornber et al., 2014). Animal welfare considerations during the selection of methods for the mass destruction of poultry, based on existing literature and guidelines (Berg, 2012; AVMA, 2020; WOAH, 2022; Galvin, et al 2005; EFSA, 2019) include:

- Suitability for poultry type - Influence of bird age, size and type on effectiveness
- Required competencies - Knowledge and skill requirements
- Handling and restraint - Degree of handling and restraint required for application
- Induction and immediacy - Efficacy of induction of unconsciousness in a mass destruction scenario
- Confirmation of death - Ease of confirming death prior to disposal

- Disease control objectives - Impact of the method on biosecurity and spread of disease. The chosen methods should facilitate carcass (and disease) containment and minimise contact with animals and infectious material
- Animal welfare concerns if applied inappropriately - The primary objective of a selected method is to kill the animals without causing undue pain or distress. The consequences of inappropriate use or ineffective application may be more significant for some methods compared to others.

Process considerations during the selection of methods for mass destruction include (Berg, 2012; AVMA, 2020; WOAH, 2022; Galvin et al., 2005; EFSA, 2019):

- Human safety - Physically safe with low psychological impact for the human operator or other bystanders. Minimising human-animal interaction is advisable, particularly during zoonotic disease outbreaks, therefore methods which limit contact with animals are preferred. The impact of mass destruction activities on mental health is also important
- Suitability for production system - Ease of application and carcass removal after killing. Differences in farm size, location and housing type will influence the choice of method. In some production systems, it may be necessary to move birds out of their housing to perform the killing process, as housing design may influence the efficacy of the killing process
- Availability - Access to equipment, personnel and any additional necessary resources. The availability of equipment and resources will influence how quickly mass destruction can be completed and consequently influence the associated animal welfare outcome. Some methods may require specialist restraint or container systems which may not be readily available in the locality
- Efficiency of the process - Ability to complete the whole process in a timely manner. Methods for mass destruction should result in the death of a large population of birds in a quick and effective manner. Delays in the time taken to complete the process can have numerous welfare implications. For example, in diseased poultry, delays can lead to further suffering as the disease progresses
- Environmental impact - Specific impact of disposal of carcasses and process waste on the environment. The environmental impact of chemicals must be considered in terms of the final disposal method on a locality basis
- Aesthetics - Acceptability for operators, the public (media) and community impact. Killing of any animal can be confronting, particularly when on a large scale. Particular aspects that are aesthetically challenging are behavioural responses such as vocalisation, escape attempts (wing flapping) and gasping as well as visible blood or carcass damage. Methods performed outside poultry housing may, if possible, need to be screened from onlookers, including from the air (drones)
- Cost - Capital and operating costs.

The methods reviewed were considered against these objectives and conditions of mass destruction to identify a group of methods that are not only acceptable in relation to animal welfare but are also appropriate during a large-scale destruction under different field conditions. The preferred methods are presented in

Table 2. Conditions of use and assessment criteria for the application of the recommended methods under field conditions were also considered and detailed in

Table 2. However, the development of detailed methodologies (for example, work instructions or standard operating procedures) for the application of the recommended methods for large-scale destruction was outside the scope of this project. It is recommended that this is undertaken in the future.

For each method, a description on how it is technically and practically carried out in the context of mass destruction (for example, applied when birds are in house, in containers or in a restraint device) is provided. In addition, for each process, scientific information on the welfare hazards and the relevant welfare consequences that can occur, are also reported.

3.2.1 Required competencies

During the mass destruction of poultry within a production environment, the activities around handling, restraining, stunning and killing are broadly comparable with the equivalent operations used by qualified stock people on-farm and in a processing plant environment. The need to handle and restrain individual birds will vary between methods. Appropriate skill, attitude and knowledge is essential in performing effective humane killing (Thornber et al., 2014; WOAH, 2022), with some methods requiring more complex skills to undertake. For all methods it is essential that competent, responsible and accountable personnel are present to confirm signs of death before carcass disposal (EFSA, 2013). In a report on the on-farm killing of poultry (EFSA, 2019), 29 hazards related to killing processes were identified and characterised, with ‘personnel’ identified as the origin for 26 hazards, and 24 hazards being attributed to lack of the appropriate skill set needed to perform the task, or fatigue.

3.2.2 Handling and restraint

Mass destruction activities may involve handling individual birds and sometimes moving them from production sheds to a central killing point. The duration and nature of handling will affect animal welfare outcomes, due to the potential to cause pain and fear. Potential methods of mass destruction can be divided simply into two categories: those that require handling of individual birds and those that do not. To reduce the likelihood of pain and fear related to moving and handling, selecting a killing method that requires less handling and restraint could be favourable. Animal welfare hazards and animal-based measures associated with the handling and restraint process are discussed in the context of on-farm destruction of poultry in the EFSA review (EFSA, 2019). Movement of poultry during an infectious disease outbreak is discouraged and, in many cases, prohibited for biosecurity reasons, therefore methods that allow birds to remain inside the production environment could be preferable in a mass destruction situation. If birds must be handled prior to killing, compassionate and professional handling techniques should be practiced (EFSA, 2019).

4 Inhalational agents

The use of inhalational agents, in particular carbon dioxide (CO_2), for the purpose of killing poultry has been studied mainly in the context of processing for human consumption. Commercially, CO_2 systems are used for stunning poultry before slaughter, however for the purpose of mass destruction, there may be opportunities to use other gas mixtures and alternative methods of delivery. In the context of this review, inhalational agents include:

- Carbon dioxide (CO_2)
- Inert gases (for example, nitrogen and argon)
- Gas mixtures (inert gases + CO_2)
- Low-medium expansion water-based foams (air and gas-filled)
- High expansion foam (gas-filled).

The advantages of different inhalational agents as well as the different gassing methods have been reviewed previously (Gerritzen, 2006; Gerritzen et al., 2006; Raj et al., 2006; Raj, 2008a; Sparks et al., 2010; McKeegan et al., 2011). Under conditions of mass destruction, gases have been used in containers and introduced into the whole shed/house (whole-house gassing).

From the literature review, it appears that carbon dioxide (CO_2) is the primary gas used for mass destruction of birds (applied as whole-house gassing and in containers), whilst inert gases are used less frequently. Carbon dioxide and inert gases do not induce unconsciousness immediately, so possible aversive reactions and respiratory effects in the conscious phase are important animal welfare considerations (McKeegan et al., 2007; 2011). When CO_2 is used under commercial processing conditions (when processing poultry for human consumption), the predominant method involves exposing poultry to a rising concentration of the gas. Birds are first exposed to a relatively low concentration of CO_2 (<40% CO_2 by volume in air), and then, once the birds are unconscious, the concentration is increased (approximately 80% - 90% CO_2 by volume in air) to ensure unconsciousness that lasts until death. Gradual exposure in this way avoids the aversive reactions observed in conscious poultry when exposed to high CO_2 concentrations. During exposure to a concentration of <40%, loss of consciousness is indicated by loss of posture. However, as poultry have chemoreceptors which are sensitive to carbon dioxide, they will react in the form of headshaking and gasping, to the presence of CO_2 at relatively low concentrations (McKeegan et al., 2007). This is not thought to be a sign of aversion (EFSA, 2019).

Much of the research into the use of CO_2 and inert gases involves the application to meat chickens in a commercial setting. The use of CO_2 methods for other poultry species is limited, although EFSA (2019) states that a residual oxygen (O_2) of 5% by volume or less created using a mixture of 80% by volume of argon and 20% by volume of carbon dioxide will cause death in pheasants, quails, chickens and turkeys within 2 minutes. Ducks and geese require residual O_2 of 2% by volume or less to cause death within 2 minutes of exposure to this gas mixture (Raj et al., 2008a). When using CO_2, Van den berg and Houdard (2008) suggested that waterfowl, such as ducks and geese, required longer exposure times and concentrations of above 70% CO_2 to ensure an effective killing process.

4.1 Whole-house gassing methods

Using CO_2 or gas mixtures to kill birds inside their production system is often termed whole-house gassing (WHG). It was developed to kill large numbers of birds in a short period of time, whilst avoiding individual bird handling and contact with infectious material (Raj et al., 2006).

The chemical properties of CO_2 (for example, it is heavier than air) allow it to be effectively held in a sealed building (even when absolute sealing is difficult to achieve) (Gerritzen et al., 2006). It can be gradually introduced into the house, thereby inducing unconsciousness before a high concentration is reached (Gerritzen et al., 2007). Exposure to a final concentration of 45% CO_2 in air is sufficient to kill chickens (Gerritzen et al., 2004), although death can be brought about more rapidly if birds are exposed to concentrations above 55% (applied once birds are unconscious) (Raj and Gregory, 1990). During whole-house gassing, the carbon dioxide concentration should be raised from 0% to at least 45% in the air at bird height (EFSA, 2019). The time taken to reach the final concentration of CO_2 will vary according to several factors, for example, the size of the house (cubic space to be filled), injection rate of the gas or the extent of sealing and leakage from the building. In a commercial trial, Sparks et al (2010) evaluated the use of liquid CO_2 delivered through a single injection point into a shed containing 12,000 pullets and found that it took around 5 minutes to reach a concentration of 45% at bird level.

Gerritzen et al (2004) examined the suitability of different gases and gas mixtures for the whole-house gassing of meat chickens. They found the optimum method to be a source of 100% CO_2 (giving a concentration of around 40% at bird level), which killed all the birds in the shed. In addition to the lethality of a method, the time taken to induce unconsciousness and death is also important, with research showing that meat chickens die within 2-3 min when exposed to 45% carbon dioxide in air (EFSA, 2019). For whole-house gassing, it is important to ensure that all birds are dead before entering the house or evacuating the gas, therefore it is recommended that the birds are left undisturbed for at least 20 minutes after gas application (EFSA, 2019).

The method by which inhalational agents are introduced to the birds can strongly influence the welfare outcome (Raj, 2008a). During whole-house gassing, CO_2 is often injected as a liquid which vaporises inside the house (EFSA, 2019). Using a single injection point may result in the uneven dispersal of gas throughout the house, meaning that birds close to the injection site are likely to be exposed to a high concentration of CO_2, compared to others located elsewhere in the house. The injection of liquid CO_2 can also cause a substantial drop in temperature within the poultry shed. One study measured the temperature at -85℃ at bird level within 6 minutes of liquid CO_2 injection (Sparks et al., 2010). However, Sparks et al (2010) estimated that the time to unconsciousness was around 38 seconds, at which time the temperature was not below 0℃, therefore they concluded that the extreme drop in temperature was not a welfare issue. Other researchers (McKeegan et al., 2011; Turner et al., 2012) also measured the physiological response of chickens to the use of liquid CO_2. Both studies confirmed the results of Sparks et al (2010), concluding that it was unlikely that the birds had died of hypothermia. Despite these reassurances, EFSA (2019) still do not recommend the direct injection of liquid CO_2 into the building. The use of multiple injection points, operating under high pressure, leads to a gradual increase of the carbon dioxide concentration in the whole building, which decreases the risk of birds being chilled or exposed to high concentrations of CO_2 (EFSA, 2019). A method of pre-heating the liquid CO_2 has also been developed to heat and vaporize the gas before it is injected into the building. This prevents a significant drop in temperature and allows even distribution of the gas throughout the house (EFSA, 2019; Livetec, 2022).

Whole-house gassing systems are now commercially available in the UK and Ireland (Livetec, 2022). The commercial systems utilise multipoint CO_2 injection and gas monitoring (recording gas concentrations at 10 locations within the shed), temperature sensors and CCTV (with low light/infrared cameras) to remotely observe the behaviour of the birds.

4.2 Application of gas in containers

Inert gases (argon and nitrogen) induce unconsciousness by displacing O2 from air inside a container. Unlike high concentrations of CO_2, the inhalation of inert gases is not aversive to birds, nor does it induce signs of respiratory distress. Raj et al. (2006), in a review of gaseous methods of killing poultry on-farm, concluded that mixtures of inert gases are preferable to direct exposure to a high concentration of CO_2. Unfortunately, the use of inert gases to create an anoxic environment is not suitable for whole-house gassing, as poultry buildings cannot generally be sealed to a degree that allows the creation of an atmosphere with less than 2–4% of oxygen (EFSA, 2019). There is however an opportunity to mix inert gases with CO_2 for whole-house killing. This approach has been described, together with appropriate concentrations and exposure times, by McKeegan et al (2006) and Sandilands et al (2011). The development of systems that involve the use of alternative gases is increasingly important as CO_2 becomes difficult to source.

The use of CO_2 or inert gases in containers, as an alternative to whole-house gassing, provides a greater choice of suitable inhalational agents, however it often requires birds to be handled individually. The use of different container designs has been described previously for different poultry species (Turkeys: Kingston et al., 2005; Meat chickens: Gerritzen et al., 2006; Layers: Webster and Collett, 2012). The early use of containers (for example, skips and waste bins) was criticised due to issues with the management of gas concentration and the methods used to introduce the birds into the vessels. An inherent problem with the simpler containers is that it is difficult to apply CO_2 as a rising concentration, and as such, birds are usually placed into a prefilled container. This means that the CO_2 concentration at the bottom of the container is usually around 100%, which poultry would find highly aversive and potentially painful (EFSA, 2004). It is also difficult to introduce birds into the container in a controlled manner and they are often dropped through a small opening. To avoid compression and suffocation, it is important that each batch of birds dropped into the container is allowed sufficient time to die before adding the next batch of birds (Webster and Collett, 2012). Welfare can be compromised if there is an insufficient interval between the introduction of batches of birds. Furthermore, when CO_2 is used in containers, birds can be loaded as a steady stream without the need to seal the container for gassing, however when using inert gases, birds have to be loaded and killed in batches to ensure that the container remains sealed and residual O_2 is maintained below 2%. The stress associated with individual bird handling can be ameliorated by placing birds into transport crates immediately after catching and then placing the crate into the gas container (Raj et al., 2008a).

To eliminate some of the potential issues identified in the previous paragraph, containerised systems have been developed which allow for improved bird handling and the controlled use of less aversive gas mixtures. The commercially available Containerised Gassing Units (CGUs) (Livetec, 2022) can process up to 10,000kg per hour and allow birds to remain in standard transport crates after catching. The system uses a gas mixture of argon and 20% CO_2, which is known to be less aversive to poultry than high concentrations of CO_2 (Raj et al., 2008a). Chickens and turkeys are exposed to the gas mixture for around 3 minutes to ensure death. Longer dwell times (around 5 minutes) have also been used with this system to effectively kill ducks and geese.

4.3 Low and medium expansion water-based foams

Foams are defined by their expansion ratio, which is the ratio of the volume of foam produced relative to the volume of solution used to generate it. Low and medium expansion foams have expansion ratios of 2-20:1 and 20-200:1 respectively. Early research into the use of foams to depopulate poultry houses focused on water-based (air-filled) low expansion foams (modified fire-fighting foam). Water-based foams are produced using specialised equipment to mix foam concentrate, water and atmospheric air (and less commonly, an alternative gas when gas-filled foams are being used). The method involves covering floor reared birds with a blanket of foam, with bubble sizes suitable to occlude the airways and asphyxiate the birds (Thornber et al., 2014; Benson et al., 2007). Occlusion of the airways caused by air-filled water-based foam is not recognised as a humane killing method in the guidelines for killing animals for disease control purposes by the World Organisation for Animal Health (WOAH) (WOAH, 2022). The use of low-medium water-based foams is not included as a euthanasia method in the AVMA Guidelines for the Euthanasia of Animals (2020), however it is recognised by the AVMA as a suitable method for mass depopulation (AVMA, 2019). The USDA therefore allow its use under certain conditions, for example, disease control and containment.

Low-medium expansion water-based foams have been trialled in meat chickens (Dawson et al., 2006; Benson et al., 2007), laying hens (Benson et al., 2012; Gurung et al., 2018a), turkeys (Benson et al., 2012; Rankin et al., 2013), ducks (Benson et al., 2009; Caputo et al., 2012), partridges (Benson et al., 2009) and quail (Benson et al., 2009). Application of the foam effectively killed all species, although not all the studies examined the time to loss of consciousness. Caputo et al (2012) examined the physiological response of ducks to the application of foam, to investigate the capacity of waterfowl to hold their breath when submerged. The results of the experiment demonstrated that apnoea and bradycardia, as a result of the diving reflex, occurred after submersion in the foam. This may have an impact on the time it takes to cause unconsciousness followed by death in ducks ($P < 0.001$; foam mean, X = 142 seconds compared to mean X=77 seconds in CO_2) and therefore should be considered during water-based foam depopulation. Benson et al (2007) measured the time taken to kill meat chickens in the growing shed when low expansion air-filled foam was used. Cessation of heart activity (via ECG measurement) took an average of 274 seconds from application. Post-mortem results indicated that birds had lesions in their respiratory system consistent with physically-induced hypoxia. Dawson et al (2006) used an accelerometer to assess time to cessation of movement and concluded that time to death in meat chickens was around 174 seconds.

Evaluations in meat chickens, have indicated that low-medium expansion water-based CO_2-filled foam is not more effective than air-filled foam (Benson et al., 2007; Alphin et al., 2010). Alphin et al (2010) used EEG monitoring (time to an isoelectric EEG) to compare low-medium water-based air-filled and CO_2-filled foam. An isoelectric EEG, indicative of brain death, was produced in 134 and 120 seconds respectively. The likely reason for no measurable difference between air-filled and CO_2-filled foam is that in low-medium expansion foams death is brought about by physical occlusion of the airways, rather than by inhalation of CO_2 (which is held inside the small intact bubbles).

A further study on hens by Gurung et al (2018b) examined the use of CO_2 and N_2 infused medium expansion water-based foams on physiological stress (via measurement of serum serotonin and corticosterone) and time to death (assessment of cessation of movement). The results showed that the reaction of hens to foam, with and without gas infusion, did not differ significantly. However, hens exposed to foam filled with nitrogen died earlier compared to birds exposed to both air and CO_2 infused foams. The authors concluded that N_2-filled foam gave a better foam quality (with a higher expansion ratio), thereby shortening time to death compared to CO_2-filled foam (which has a lower expansion ratio).

When compared with CO_2 gas systems, foam systems provided a shorter time to unconsciousness in three studies (Rankin et al., 2013; Benson et al., 2007, 2018), but a longer time to cessation of movement in a third study (Gurung et al., 2018a). Reasons for the conflicting outcomes are not clear but may be attributable to differences in the research methodologies.

4.4 High expansion gas-filled foam

High expansion foams are categorised as those which have an expansion ratio of >200:1 and are sometimes referred to as dry foams. Their use was developed in response to concerns over the application of low-medium expansion foams (Raj et al., 2008b).

The mass destruction method involves administration of the high expansion gas-filled foam into the poultry house to create an atmosphere depleted of oxygen which then kills the birds. The foam effectively acts as a gas delivery system, assisted by the movement of the birds which bursts the bubbles and releases the gas (EFSA, 2019). Foam with an expansion ratio of between 250:1 and 350:1 appeared to be the optimum compromise between foam stability, water content, bubble size and wetness, so that the airways are not occluded and suffocation does not occur (Gerritzen and Sparrey, 2008; Raj et al., 2008a, b; McKeegan et al., 2013a; Gerritzen and Gibson, 2016). EFSA (2019) recommends that the foam has an expansion ratio of at least 250:1. High expansion foams containing nitrogen or carbon dioxide have been considered for mass destruction of poultry in their sheds.

A study by McKeegan et al (2013a) demonstrated that meat chickens, hens, ducks and turkeys could be humanely killed when N_2- and CO_2-filled high expansion foams were used. Post-mortem examination of the birds confirmed that they died from anoxia and the foam did not occlude the airways. During induction, birds did not display behaviours indicative of aversion or distress. The time to loss of consciousness was less than 30 seconds for all poultry species tested. The researchers concluded that the gas-filled high expansion foam produced rapid, humane euthanasia due to the anoxic conditions produced (<1% O_2 inside the foam). As observed with low-medium expansion foams, the use of CO_2 in the foam did not appear to provide a welfare benefit over N_2, and it was more challenging to deliver through foam generators due to the extreme cold produced (McKeegan et al., 2013a).

One important disadvantage of high expansion foam is that the large bubble size increases the fragility of the foam. This means that any wing flapping and anoxic convulsions can burst the bubbles, thereby potentially reducing exposure time and efficacy. Therefore, foam production capacity is required to be greater than the rate of foam breakdown and dilution of the gas, which may not always be possible in large poultry houses (EFSA, 2019). This may also have implications for the application of this method in systems with complex infrastructure, such as cages. Further work is required in this area.

McKeegan et al (2013a) found that infusion with N_2 produced a better quality and more consistent foam than CO_2 and birds find the inhalation of nitrogen less aversive than CO_2. For these reasons, the commercial development of whole-house high expansion foaming systems has focused on the use of N_2-filled foam (Livetec, 2022). The commercial system (Nitrogen Foam Delivery System – NFDS) generates N2-filled foam with an expansion ratio of 350:1. The foam generator, when supplied with the correct pressure and flow rates of gas and foam solution, can generate up to 50m^3 (1765 CU ft) of high expansion nitrogen foam per minute. This can fill a 30,000-bird meat chicken shed up to a height of around 5 metres in approximately 1 hour (Ranger Magazine, 2020). This type of commercial system has sufficient capacity to overcome some of the earlier concerns around ensuring sufficient foam generation to maintain efficacy.

High expansion foams can also be administered in containers. Birds are usually placed in a container prior to it being filled with foam. The flow of foam should be sufficient to keep the birds covered during the wing flapping and convulsion phase that will occur due to rapid induction of the anoxic situation (EFSA, 2019).

4.5 Summary

Inhalational agents can be introduced into the poultry shed to kill birds in situ or can be used in containers into which birds are placed. Whole-house gassing represents a highly practical and effective method for killing birds while they remain in their production system. The main advantages of killing birds within their production system is that handling or restraint of birds is not required, there is the potential to kill very large numbers of birds at the same time, and there is almost no contact between humans and infected birds (Sparks et al., 2010). The disadvantage of killing poultry within their production system is that the procedure is often more difficult to control. Buildings need to be effectively sealed to minimise gas leakage, especially when gas mixtures or inert gases are used (Galvin, et al 2005). Gas concentration or residual oxygen (when inert gases are used) therefore needs to be monitored at bird height from commencement through to completion of the process. Welfare issues that have been reported when using this method (Berg, 2014), include:

- Failure to reach the required gas concentration
- Extended time between ventilation shut down and gas application, resulting in heat stress, and
- Significant gas leakage (sometimes affecting non-target birds on the same site).

These welfare issues can be resolved by the effective management of resources, implementation of effective operating procedures and confirmed operator competency. To optimise the welfare outcome, whole-house gassing requires system parameters that can produce an outcome equivalent to that achieved in commercial Controlled Atmosphere Stunning (CAS) poultry processing systems.

When using containers to hold the gas or gas mixture, limits in processing volume and the need to repeatedly load and empty the container incurs both logistical and operator safety considerations. Use of containers usually requires birds to be manually caught and placed into the container of gas. The distance birds need to be carried will depend on the placement of the containers and the design and arrangement of the poultry housing. Laying hens are arguably the poultry type most susceptible to handling damage, due to bone weakness and fragility related to osteoporosis (Gregory and Wilkins, 1989). Removing hens from laying systems, especially cages, and carrying them to the container of gas can expose them to injuries, including bone fractures. An advantage of containerised systems is that they can be used under a range of conditions where whole-house gassing may not be suitable, for example, for poultry in open-sided or multi-level housing or when buildings cannot be effectively sealed. The main disadvantage of containerised systems is the need to handle birds, not just from a welfare perspective, but also from an increased risk of exposing personnel to infectious agents (Gerritzen et al., 2004). Containerised systems can also be quite labour-intensive and may not provide sufficient capacity to cope with a disease outbreak on a large production site. The use of CO_2 in containers can only be recommended as a preferred method if the gas can be introduced to the birds as a rising concentration (exposing birds to less than 40% CO_2 in air until unconscious) and birds are allowed sufficient time to die before subsequent batches of birds are added (Webster and Collett, 2012). When inhalational methods are used, inappropriate flow rates or poorly sealed containers can lead to failure to euthanise poultry (if the gas concentration is too low or the residual oxygen is too high), or birds being exposed to aversive concentrations of gas (for example, when CO_2 gas concentration is increased too quickly). A short dwell time in the gas can also lead to poultry being unconscious, but not dead, and at risk of recovery. When gas is delivered in a foam, insufficient foam production rates or short dwell times can lead to failure to euthanise birds. It is important that inhalational agents are supplied in purified form without contaminants, preferably from a commercially supplied source, as contamination can increase aversion during induction or reduce efficacy.

One of the challenges in drawing firm conclusions or ranking various gas mixtures lies in the large variety of research methodologies and assessment parameters used in published literature. There is no standardized protocol, so comparisons between published data are difficult. Most of the published research is in the context of the commercial use of controlled atmosphere stunning (CAS) in the processing plant. There have been fewer welfare assessments on the use of inhalational agents for mass destruction on the farm, however similar physiological and behavioural responses in the birds are observed.

There are established animal-based indicators to assess the effectiveness of inhalational agents (EFSA, 2013). However, under conditions of mass destruction, it may be difficult to monitor these animal-based measures during their use, for example, when birds are covered with foam. Therefore, important parameters such as gas concentration and exposure time should be monitored to ensure that a sufficient exposure period is maintained. When inhalational methods are used, poultry can remain in the system until death is achieved. During application, operators need to be able to monitor gas concentration and adjust flow rates, and subsequently confirm death in individual birds. When using gas-filled foam, operators will require additional knowledge of foaming equipment and the ability to monitor and adjust foam production rates. Specific competencies required will depend on the complexities of the system and equipment used.

Experimentally, the exposure to inert gases is less aversive as it causes less pain, fear and respiratory distress compared with gas mixtures containing CO_2 at high concentrations. However, during mass destruction events, inert gases need to be used in containers. It is recommended that ongoing work continues to identify suitable alternatives to high concentrations of CO_2. To-date, a variety of alternative gas mixtures, multi-stage processes (using rising concentrations of CO_2) and use of gas-filled foams have been investigated in a research context, but no clear optimal process for practical application in all situations has emerged and the selection of a method is ultimately determined by the features of the poultry housing and the resources available. Relatively easy access to pure CO_2 in cylinders and its low cost compared to inert gases makes it an obvious choice for mass destruction of poultry. If parameters and delivery methods can be effectively controlled on-farm, using a rising concentration of CO_2, this will deliver a welfare outcome equivalent to that achieved in a commercial controlled atmosphere stunning (CAS) system in poultry processing plants.

An advantage in the use of foam over CO_2 or gas mixtures is that it can be applied in poorly sealed buildings and naturally ventilated accommodation. It also only affects birds that are immersed so there is no risk of affecting non-target birds. Using low-medium expansion air-filled water-based foam is logistically simpler than gas-filled foams, however, it still requires large volumes of water and foam generating equipment (Benson et al., 2012). Welfare assessments of low-medium expansion foams show that they effectively produce unconsciousness and eventual cardiac arrest (Benson et al 2012), however, available information indicates that physically-induced hypoxia due to occlusion of the airways is not acceptable from a welfare perspective. The use of high expansion gas-filled foams is a more humane alternative as it does not cause physical obstruction to the airways. It also facilitates the use of inert gases in poultry houses where whole-house gassing with inert gases is not possible. High expansion foam systems may be more technically demanding than the low-medium water-based foam systems, however, the welfare advantages make this a preferred solution.

5 Oral agents

5.1 Oral anaesthetics: Alpha-chloralose

The AVMA (2020) guidelines suggest that oral anaesthetics may be used to sedate very reactive animals such as game birds before killing using an appropriate method. For example, pheasants, partridges, guinea fowl, and quail or other species of domestic poultry reared in free-range systems could be sedated with alpha-chloralose in feed or water and then handled for killing. Alpha-chloralose has been used successfully in food and water as a sedative for poultry prior to killing by neck dislocation (Raj, 2008). At concentrations of 3% or more it tastes bitter and consequently, birds may not consume a lethal dose.

5.2 Sodium nitrite

Sodium nitrite ingested at high concentrations prevents the transport of oxygen in the blood and thereby renders an animal unconscious and then dead. The efficacy of sodium nitrite relies on the timely consumption of a toxic dose. In Australia, sodium nitrite was first identified in the 1980s as a possible oral euthanasia agent for feral pigs. In December 2019, Animal Control Technologies (Australia) Pty Ltd registered HOGGONE microencapsulated sodium nitrite (MeSN) with the Australian Pesticides and Veterinary Medicines Authority (APVMA) as a bait for the reduction of feral pig populations. The use of sodium nitrite is recognised as a conditional method of euthanasia for pigs by the AVMA (2020) under constrained circumstances. However, its use in domestic pigs in Australia is relatively undeveloped, despite showing promising results in limited published and unpublished trials. A model for assessing the relative humaneness of pest animal control (Edition 2) was published in 2011 (Sharp and Saunders, 2011) and recognises the use of sodium nitrite for feral pigs but not for pest birds.

Lay and Enneking (2020) investigated the use of sodium nitrite for the euthanasia of hens. Laying hens (n=8 per treatment, 18 weeks of age) were subjected to 1 of 4 treatments: A, 75 mg/kg BW; B, 150 mg/kg BW; C, 300 mg/kg BW; or D, 600 mg/kg BW of sodium nitrite in feed. The treated feed caused hens to become lethargic and eat and drink less. The reduced feed intake was probably due to sedation or the aversive taste of the sodium nitrite. Only one hen died during the experiment, therefore the researchers could not confirm that the application of sodium nitrite was a humane method of euthanasia for poultry. It was suggested that to improve intake of treated feed, future research should investigate feeding sodium nitrite in an encapsulated form. It should be noted that microencapsulation was the key factor in supporting palatability and stability and hence, effective lethal outcomes in feral and domestic pigs.

5.3 Summary

The efficacy of an oral agent relies on the timely consumption of a lethal dose. On this basis, there is no evidence to support the use of sodium nitrite and alpha-chloralose. Theoretically, the use of lethal oral agents would be best suited to poultry that are readily consuming feed and have a good appetite, and unsuitable where adequate feed or water consumption cannot be assured (for example, with sick birds). If an appropriate lethal oral agent became available, a secondary, terminal procedure, such as neck dislocation, may also need to be implemented to kill birds that have not died within the required timeframe. The use of an appropriate oral agent could potentially allow for large numbers of poultry to be killed without the need for individual handling and whilst remaining in the production system, which could provide an important welfare advantage over other available methods. The use of oral agents is unlikely to be affected by housing design. Consumption by non-target species would not be a concern for indoor housing systems, however it would need to be considered if using the product in outdoor systems.

Presently, the use of oral agents is not recognised under AUSVETPLAN, which states that: ‘there is no justification for using a poison on managed stock’. The ESFA report on methods for the on-farm killing of poultry (EFSA, 2019) also states that methods which involve the administration of toxic substances to feed or water should not be used.

In summary, there are currently no suitable oral agents that are effective in terms of lethality, welfare impact and palatability. It is recommended that the use of alpha-chloralose, and sodium nitrite for mass depopulation of poultry be reconsidered after further studies have been completed, published and reviewed.

6 Injectable agents

6.1 Injection of barbiturate

In the context of this review, injectable agents are barbiturates or their derivatives. Barbiturates depress the central nervous system, resulting in anaesthesia. With an overdose, deep anaesthesia progresses to apnoea due to depression of the respiratory centre, followed by cardiac arrest (AVMA, 2020). Within Australia, anaesthetic compounds are scheduled substances under the Poisons Standard 2021 (Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) No. 33) and regulatory requirements specify that these agents can only be administered under the authority of a registered veterinarian.

When injectable agents are administered, the route of administration will affect the outcome. Intravenous (IV) delivery is usually preferred (EFSA, 2019) as it achieves more rapid distribution of the agent whilst placement in other areas (for example, intraperitoneal) may reduce speed and efficacy as well as increase the likelihood of experiencing irritation or pain. Difficulties around the administration of injectable agents to birds also need to be effectively managed. When injecting into a vein, a small gauge needle should be used as avian blood vessels are more fragile than those of mammals and susceptible to haematoma formation. When the intraperitoneal route is used, it is important to position the needle carefully to avoid misdirection into the air sacs. According to manufacturer instructions, the doses, rates and routes of administration that cause rapid loss of consciousness followed by death should be used. Birds should be monitored to ensure the drugs have been effectively administered and death must be confirmed before carcass disposal (Berg, 2012). Death can be confirmed by the complete absence of movements, breathing and a heartbeat. Barbiturates are known to heavily suppress respiration and the breathing interval can also be quite long in birds that are still alive (EFSA, 2019).

Injectable barbiturates can persist in animal carcasses. When carcasses have been insufficiently buried or left uncovered, these can cause secondary toxicosis (sedation and death) in animals that consume the remains. Compared with other methods of mass destruction, the use of injectable agents potentially has the highest cost per bird, associated with veterinary involvement and drug costs (EFSA, 2019). For example, a barbiturate for euthanasia (such as Lethabarb - 325 mg/mL solution of pentobarbitone) administered at 1ml/2kg body weight currently costs approximately $150 for 450ml.

Administration of injectable agents requires handling of individual birds and therefore may not be suitable for killing large numbers of poultry during a mass destruction event. Despite this limitation, the use of an injectable agent can be a useful adjunct method for birds that have not been killed effectively by a primary method such as whole-house gassing or foam application.

6.2 Summary

Handling of some chemicals will require specific Chemical Safety competency or certification and others may require veterinary registration to enable procurement and administration (Galvin et al., 2005). Injectable agents usually need to be administered by a vet or under direct veterinary supervision only.

When injectable agents are used, there is also a risk of a non-lethal dose being administered, an inappropriate route of administration being used, or failed administration, all of which may inflict pain on the animal involved.

For the killing methods that require poultry to be handled individually, the use of an injectable agent (administered intravenously) provides a humane option, resulting in a short time to death. However, the use of injectable agents for poultry in a mass destruction event is not practical, therefore it should be reserved for use as a back-up procedure or for small numbers of birds.

7 Mechanical methods

In the context of this review, mechanical methods used for poultry include:

- Penetrative and non-penetrative captive bolt devices
- Manual blunt force trauma
- Cervical dislocation
- Decapitation, and
- Firearm (gunshot).

7.1 Penetrative and non-penetrative captive bolt devices

Penetrative captive bolt devices are designed to fire a retractable bolt through the cranium and into the brain of the animal. Penetrative captive bolts deemed suitable for poultry are normally powered by a blank cartridge or spring. Non-penetrative captive-bolt devices were developed as a humane method of killing poultry for use on-farm (Hewitt, 2000). The desired outcome for both penetrative and non-penetrative devices is for the impact of the bolt on the skull to result in concussion and the associated immediate loss of consciousness (EFSA, 2004). Bolt diameter, velocity (and penetration depth when penetrative devices are used) are important determinants of stunning outcome (EFSA, 2004).

In Europe, the use of captive bolts is stipulated in Council Regulation (EC) No. 1099/2009 as a potentially reversible (termed 'simple') stunning method, however, the structural damage to the brain may lead to rapid death of the animal (AVMA, 2020). In poultry, it has been demonstrated that when applied correctly, the force of impact and physical damage to the brain is sufficient to kill the bird (Hewitt, 2000; Raj and O'Callaghan, 2001; Erasmus et al., 2010a, b; Gibson et al., 2018). Raj and O'Callaghan (2001) suggested that a bolt diameter of at least 6mm driven at an air pressure of 827kPa was necessary to kill chickens. Other researchers corroborated these parameters when using similar equipment to kill turkeys, ducks and geese (Erasmus et al., 2010a,b; Sparrey et al., 2014; Gibson et al., 2018).

A variety of non-penetrative captive bolt devices, developed specifically for poultry, are commercially available. The Turkey Euthanasia Device (TED) is a mobile device powered by a mini propane Paslode canister that is used to kill poultry (chickens, turkeys, geese and ducks) in a range of weights from 3.5kg chickens to 20kg turkeys. It produces immediate unconsciousness followed by brain death (Hulet et al.,2013; Gibson et al., 2018). A detailed manual for use of the Turkey Euthanasia Device is available on-line from Bock Industries (2016). In addition, a series of videos are available addressing TED use and troubleshooting (Bock Industries, 2019). A similar device, the Zephyr-EXL, runs off a compressed air power source (Bock Industries, 2016). Baker-Cook et al (2021a) examined the use of the Zephyr-EXL for killing meat chickens and found that the loss of consciousness was quickest in mechanically stunned birds when compared with birds killed using manual and mechanically assisted neck dislocation. The use of the Zephyr-EXL produced skull fractures in all birds examined, even though the device is regarded as non-penetrative device. The conical-shaped bolt head was shown to partially penetrate the head of the bird. The Cash Poultry Killer (CPK) is another mechanical device that effectively kills poultry (Sparrey et al., 2014; HSA, 2021). Two types of CPK are currently available; an air-powered device which was initially developed for use on the production line in slaughterhouses and a cartridge-powered tool for on-farm use where an independent power source is essential (Hewitt, 2000). Although the cartridge-powered device is suitable for on-farm use, it would not be practical for killing large numbers of birds during mass destruction, as it can overheat when used continuously over extended periods of time. During a mass destruction event, the air-powered CPK would be a better alternative.

Penetrative captive bolts will usually require individual handling and restraint of birds for correct application (Boyel et al., 2020). Some non-penetrative captive bolt devices are operated without pressing them firmly against the head of the bird, and therefore could be used on free-standing birds (Hewitt, 2000). Boyel et al (2020) described the development of a mobile bird euthanasia apparatus (MBEA) that could be used to restrain birds for mechanical stunning and enable effective euthanasia to be performed by a single operator. In addition to minimizing movement, securing the bird may also help to improve personnel safety (Erasmus et al., 2010).

7.2 Manual blunt force trauma

Manual blunt force trauma involves the application of a physical blow to the head of the bird. It is performed by holding a bird by its legs, placing its head on a hard surface and delivering a manual blow to the back of the head with a hard object (European Commission, 2018). A percussive blow of sufficient force and accuracy will lead to brain concussion and death.

Manual blunt force trauma is an approved stunning method for poultry (up to 5kg) in Europe (EU, 2009), however, it cannot be used as a routine killing method on-farm and its use is restricted to 70 birds per person per day. Cors et al (2015) concluded that a single, sufficiently strong hit placed in the frontoparietal region of the head led to a reduction or loss of the auditory evoked potential (indicative of unconsciousness) in all categories of poultry tested, including broilers, broiler breeders and turkeys (<16kg). This method has been reported by Erasmus et al. (2010a, b) to be effective when performed by a trained operator. However, the welfare risk associated with the use of manual blunt force trauma is the opportunity for operator error, resulting in inaccurate placement or a blow of insufficient strength.

7.3 Cervical dislocation

Cervical dislocation can either be performed manually or mechanically (with the use of equipment). EFSA (2019) describes it as a killing, but not stunning, method and there are differences in opinion regarding the effectiveness of cervical dislocation and its ability to result in immediate brain dysfunction. Several authors have concluded that manual cervical dislocation results in rapid loss of brain function and onset of brain death (Brainstem reflexes: Martin et al., 2018a; Jacobs et al., 2019; Musculoskeletal movements: Jacobs et al., 2019). The Humane Slaughter Association do advocate the use of cervical dislocation without prior stunning but stipulate that it should only be used in an emergency or for the slaughter of very small numbers of birds where preferred methods are not available (HSA, 2021).

Other studies have shown that cervical dislocation may not lead to immediate brain death in turkeys or chickens and the researchers express concern over its use as a killing method (EFSA, 2004; Gregory and Wotton, 1990b; Erasmus et al., 2010a, b; Carbone et al., 2012; Bader, et al., 2014; Baker et al., 2017).

Successful manual cervical dislocation is dependent on the ability of the operator. Repeated success is also influenced by operator fatigue, bird size and bird type (Martin et al., 2018a, b). For example, performing manual cervical dislocation can be difficult in birds approaching 3kg or more. In Europe (EU, 2009), manual cervical dislocation may only be used for birds less than 3kg and is limited to 70 birds per person per day. The limit on number is likely to be directly associated with the concern that the ability of the operator to produce a consistent stun will diminish with repeated applications (Jacobs et al., 2019), although this concern is not shared by all researchers (Martin et al., 2018b). In the study by Martin et al (2018b), evaluation of manual cervical dislocation showed that there was no evidence of reduced performance with time or increasing bird number (up to 100 birds).

Mechanical cervical dislocation is often used for larger birds, where manual manipulation is likely to be more difficult. Mechanical devices dislocate by stretching or crushing. The equipment used for mechanical cervical dislocation by stretching typically consists of a restraining cone with hinged parallel bars below the apex of the cone attached to one of the legs. The bars are placed either side of the neck just behind the head of the bird. They are then gripped tightly together, and a sudden downward movement dislocates the bird’s neck. Several variations on the killing cone have been produced for different poultry species (Hewitt, 2000). In 2019, the Livetec NEX®, a hand-held mechanical neck dislocation assistance device, was commercially designed (Livetec, 2022) to improve the consistency of manual neck dislocation in poultry and gamebirds.

Mechanical cervical dislocation devices that crush the neck are sometimes used for killing broiler chickens, though they are more commonly used for larger birds or game birds. The operator applies the pliers to the neck of the bird, just behind the head and squeezes the handles tightly so that the jaws meet. Jacobs et al (2019) compared the latency to the onset of brain stem death between manual neck dislocation and the use of the Koechner euthanasia device (KED) in slaughter age broiler chickens. The use of the KED was manipulated in some birds by extending the bird’s head at a 90o angle after application of the device (termed KED+). Onset of brain death was assessed using the time to loss of nictitating membrane reflex, gasping reflex and musculoskeletal movements. Manual cervical dislocation resulted in a quicker loss of reflexes and movement compared to KED and KED+ treatment groups. Reflexes were seen to return in 0-15% of birds in the manual cervical dislocation group, 50-55% of birds in the KED group and 40-60% of birds in the KED+ group, indicating a possible return to consciousness. Based on these results, manual neck dislocation was considered to be the preferred method of cervical dislocation for meat chickens. Stewert et al (2021) examined three neck dislocation procedures (manual, broomstick and KED) in turkeys. Birds were assessed for a loss of brainstem reflexes indicating euthanasia success. Use of the KED resulted in a longer latency time for the loss of pupillary and nictitating membrane reflexes compared to manual neck dislocation and broom-assisted neck dislocation. Manual neck dislocation caused less crushing damage to the neck, with a more visible separation of the vertebra.

The AUSVETPLAN Operational manual: Destruction of animals (AUSVETPLAN) and associated technical documents (AHA, 2015) recognise cervical dislocation as an approved killing method for poultry. The current RSPCA Approved Farming Scheme Standard for Meat Chickens (2020) refers to the use of manual cervical dislocation for birds on-farm. However, it does not permit the use of killing pliers (or other equipment that crushes the neck) or methods of cervical dislocation that require spinning or flicking of the bird by the head. EFSA (2019) recommend that methods which cause cervical dislocation by crushing should not be used.

7.4 Decapitation

Decapitation is usually performed using a knife whilst birds are restrained on a shackle or in a cone. This practice involves the separation of the head from the body (Close et al., 1996) causing death through anoxia of the central nervous system and blood loss. The blade should be positioned high on the neck, ideally at the level of the first vertebra, and the head should be severed using one cut (EFSA, 2019). It is not a commonly used method for diseased birds because of the risk of infection from any blood spillage.

The effect of decapitation on brain activity has been studied using neurophysiological studies (Cartner et al., 2007) which have shown that the resulting brain activity post-decapitation is not indicative of immediate unconsciousness. When evaluating the welfare impact of decapitation, the effect of head severance on oxygen tension in the brain is also an important consideration. Derr (1991) calculated the time required for the oxygen tension in a decapitated rat brain to decline to a level at which unconsciousness occurred. He estimated it to be approximately 2.7 seconds and concluded that decapitation was therefore a humane method of dispatching rats. Conversely, the nervous tissue in reptiles can withstand comparatively long periods of anoxia and hypotension. A study in alligators showed that brain activity (assessed using a corneal reflex test) continued for 54 minutes (range: 34 to 99 minutes) after spinal cord severance. Avian tolerance to anoxia is thought to be somewhere in between that of mammals and reptiles and after decapitation, brain activity in chickens was seen to persist for up to 3 minutes, with the waveform being virtually unchanged for the first 30 seconds (Gregory and Wotton, 1986). Therefore, loss of consciousness may not be immediate, and birds may feel pain due to afferent stimuli from the trigeminal nerve (EFSA, 2004).

The use of decapitation as a killing method (without stunning) is not permitted by the current RSPCA Approved Farming Scheme Standard for Meat Chickens (2020). The AUSVETPLAN (AHA, 2015) does not permit the decapitation of conscious poultry and only includes it as a permitted terminal procedure for unconscious animals.

7.5 Firearm (gunshot)

The use of a firearm (with free projectile) involves the passage of one or more projectiles into the cranium causing immediate unconsciousness and extensive damage to the brain, ultimately resulting in death (HSA, 2017a). The physical principle behind killing with free projectiles is the transfer of high levels of kinetic energy in an extremely short time from the projectile to the animal's brain. The free projectile may be a bullet (used in a rifle or handgun) or a charge of lead (used in shot guns). Although gunshot is a recommended method for killing poultry by the AVMA (2019, 2020), there are few scientific studies on the use of different firearms for killing poultry in the field and their suitability from a practical perspective is questionable. While all mass destruction methods require skilled personnel, the use of firearms raises even more operational and safety concerns (AVMA, 2019). Reference to the use of firearms, in the context of mass destruction of poultry, is usually in relation to the dispersal of wild birds (during depopulation activities) and for killing larger farmed birds, such as ratites (AVMA, 2019).

7.6 Summary

Non-penetrative devices provide a practical alternative to gunshot and penetrative captive bolts for all bird types. The use of manual blunt force trauma does not require specialised equipment; however, it requires skill and confidence to apply it successfully and repeatedly. Individuals need to be able to apply the physical blow accurately and with sufficient force to kill the bird. It is recommended that non-penetrative devices are used to replace manual blunt force trauma, which should only be used when other more suitable methods are not available. The UK Farm Animal Welfare Committee (2017) also recommend that non-penetrative devices should ultimately replace cervical dislocation for most poultry. Penetrative and non-penetrative captive bolt devices for poultry are available at a range of prices between $700 for a basic model and $5000 for a full euthanasia kit, with cartridge costs around 40-50c per cartridge depending on the manufacturer and shipping costs.

Killing large numbers of birds using mechanical methods is likely to be a protracted process, requiring multiple operators to restrain, kill and confirm death, and to manage personnel fatigue. Repeated firing of cartridge-powered captive bolts (for example, the CASH Poultry Killer) in quick succession will lead to overheating and failure of the device (Gibson et al., 2015), therefore there must be a sufficient number of devices on-hand to allow for rotation. The risk of inappropriate application of mechanical methods increases with operator and equipment fatigue and the difficulty of the task (for example, number of birds involved, environmental conditions, nature of restraint). This increases the chance of ineffective application or birds regaining consciousness before death.

Mechanical methods result in the bird displaying physical convulsions (even after death), which can be challenging psychologically for operators and observers. The application of blunt force trauma and decapitation are aesthetically unpleasant for both operators and observers, and as such, are often regarded as being unacceptable by the general public.

8 Electrical methods

In the context of this review, electrical methods include:

- Head-only electrical stunning
- Electrocution using a water-bath
- Electrocution using head-to-cloaca application.

8.1 Head-only electrical stunning

The aim of head-only electrical stunning is to pass an electrical current across the brain of the bird, resulting in unconsciousness (EFSA, 2004, 2006). It is usually used to stun small numbers of poultry on-farm or in small throughput processing plants, although its use in Australia is relatively uncommon. Electrical stunning requires the bird to be individually handled and restrained, usually in a cone, on a shackle or held manually by the legs. The electrical stunning current is delivered by a pair of adjustable tongs or fixed electrodes applied across the head. Head-only electrical stunning does not usually kill the bird, but results in a recoverable state of unconsciousness. Therefore, this method must always be followed by a secondary (terminal) procedure, such as exsanguination. Under mass destruction conditions, when biosecurity is paramount, a preferred terminal method would be neck dislocation.

The recommendation for a minimum current varies between sources of information. Gregory and Wotton (1990c) recommend that 240mA for chickens should be applied to the head for at least 7 seconds (using a constant voltage stunner (110 V 50Hz AC), with neck cutting performed within 15 seconds from the end of the stunning current application). The Humane Slaughter Association (HSA) (2021) refers to a minimum head-only current of 300-400mA for chickens, but do not specify the frequency of the current. Raj and O' Callaghan (2004a) studied the effects of frequency on the minimum current to stun chickens and concluded that minimum currents increase with increasing frequency (from 100mA for 50 Hertz and 150 to 200mA for 400 and 1500 Hertz sinusoidal alternating currents respectively) and need to be applied for 4 seconds with neck cutting occurring within 15 seconds. Lambooij et al., (2010) studied an alternative approach to commercial electrical stunning, where the current was passed across the head of the bird instead of the body. The researchers concluded that head-only single bird stunning with a minimum current of 250 mA induced unconsciousness in broiler chickens and recommended that neck cutting be performed within 10 seconds of the end of the stun to prevent recovery.

The EU regulation does not specify the frequency of the applied stunning current, though requires a minimum head-only current of 250mA for chickens and 400mA for turkeys (EU, 2009). This is in-line with scientific studies, however it is lower than that recommended by the Humane Slaughter Association (2021), who recommend 300-400mA for chickens and 400mA for turkeys. A minimum current of 600mA delivered using a 50 Hz sine wave AC is recommended for ducks (EFSA, 2006).

The exposure time should be long enough to ensure that birds show recognised signs of unconsciousness, such as tonic seizure activity (rigidly extended legs), wings folded tightly around the breast and muscle tremors. After removal of the electrodes, the eyes will remain wide open (no blink reflex when touched) and rhythmic breathing will be absent. Return of eye reflexes and normal breathing precedes a return of consciousness (EFSA, 2004, 2013). Ideally, head-only electrical stunning should be performed using a constant current source, where the required current is assured. However, most of the head-only electrical stunning equipment used around the world is supplied with a constant voltage, where the current achieved is determined by the resistance of the bird. One problem following head-only electrical stunning is the occurrence of severe wing flapping, which can impede prompt neck cutting. This can be addressed by utilising appropriate restraint (Boyel, 2020). Head-only electrical stunning of poultry requires individual bird handling and restraint and is labour-intensive. Therefore, its suitability as a method for mass destruction is questionable.

8.2 Electrical stunning using a water-bath

Globally, electrical water-bath stunning is the most widely used stunning technique in commercial poultry abattoirs. Birds are inverted and suspended, with their feet restrained in metal shackles, for conveyance through the system. The shackling of conscious birds has been associated with welfare issues (Gentle and Tilston, 2000). Stunning is achieved by the passage of an electrical current from the electrode in the water-bath through the bird to the shackle line. The contact of the head and neck with the water or electrode completes the electrical circuit between the water (positive electrode) and shackle (which acts as the ‘earth’ or negative electrode), so that an electric current passes through the bird’s head and body. The aim of electrical stunning is to pass sufficient current through the brain of the bird to induce generalised epileptiform activity (epilepsy) which is deemed to be incompatible with consciousness (Opdam, 2002). The production of generalised epilepsy depends upon the amount and frequency of the current applied (EFSA, 2004). In multiple bird water-bath stunning systems, all the birds passing through the water-bath will be exposed to a constant voltage. This means that the flow of electrical current through the bird is dependent on the resistance of each bird to current flow, such that birds with a low resistance will receive more current than birds with a higher resistance (EFSA, 2012). The problem of birds receiving different amounts of current in a constant voltage water-bath is made even more complicated by the array of different electrical parameters (for example, current, frequency and waveform) that are used. The interaction between the different variables is complex (EFSA, 2004). For example, it has been shown that higher frequencies require higher currents to induce an effective stun (EFSA, 2004, 2006; Raj, 2004b; Raj, 2006; Raj et al., 2006b). Studies also indicate that a sine wave alternating current (AC) is more effective than a pulsed direct current (pDC) in terms of inducing epileptiform activity in the brain of chickens (Raj and O’Callaghan, 2004; Raj et al., 2006 a, b; Raj, 2006; EFSA, 2012).

The type of electrical parameters used determine whether the stun produces unconsciousness only (termed ‘simple’ electrical stunning in EU legislation) or produces unconsciousness and cardiac arrest (termed ‘electrocution’). In poultry abattoirs, high and low frequency electrical currents, ranging from 50 to 1500Hz are used in water-bath stunning systems. High frequency systems do not stop the heart, whilst low frequency (50Hz) systems, with an applied current of 120mA per bird, will cause cardiac arrest in the majority of chickens resulting in death during unconsciousness (Gregory and Wotton, 1990a). A recommended minimum stun duration of 15 seconds is necessary to induce epilepsy (EFSA, 2014). The optimal electrical parameters to produce an irreversible stun (unconsciousness and cardiac arrest) in the majority of birds have been described as low frequency (sinusoidal AC waveform of 50Hz) using a minimum current of 120 mA (for meat chickens; Schutt-Abraham et al., 1983) or 150mA (for turkeys; Mouchonière et al., 1999). These combinations result in the abolition of brain activity and the onset of a quiescent EEG, indicating death post-stun.

Systems that induce effective stunning followed by cardiac arrest have been used for the mass destruction of poultry (Gerritzen et al., 2006; Scheibl, 2008). The application of this method involves manually catching and transferring birds outside their housing, and then hanging the birds on a moving shackle line that carries them through an electrified water bath (EFSA, 2019). The Humane Slaughter Association (HSA, 2017b) in the UK recommends, in their guidelines for on-farm killing for disease control purposes, using a minimum current of 400mA and waveform of 50 Hz (AC) to induce cardiac arrest in chicken, guinea fowl, duck and geese. This current application is higher than those shown to be effective experimentally and used commercially (described in the previous paragraph). However, a higher recommended current helps to overcome the complexities of water-bath stunning, ensuring that the majority of birds are effectively killed, in a situation where carcass quality is not important. EFSA (2019) agree with this recommendation and stipulate that the duration of exposure to the current should be at least 4 seconds. As a minority of birds may not receive sufficient current to induce cardiac arrest, neck cutting or cervical dislocation at the exit of the stunner should be employed.

8.3 Electrocution: Head-to-cloaca application

Head-to-body electrocution is a method used to induce immediate unconsciousness followed or accompanied by cardiac arrest resulting in death (EFSA, 2019). One type of head-to-cloaca stunning is a variation of conventional water-bath stunning, where the head of the bird is placed in a water-bath and the second electrode is automatically applied to the cloaca of the bird (Lambooij et al., 2008, 2012), thereby allowing individual current application.

An adapted head-to-cloaca stunning system for 31 individually restrained birds has also been designed and is available commercially. A current of sufficient magnitude (400 mA; HSA, 2017b) delivered using AC with a frequency of 50 Hz should be applied when using this equipment.

The Top Equipment H2H Euthanizer is a mobile device that kills individual poultry using a head-to-cloaca application. The bird is restrained inverted in a flexible cone with its head held in position by two electrode plates. An electrode is simultaneously placed on the cloaca to complete the electrical circuit and a 220V/50Hz electrical current is applied to kill the bird.

8.4 Summary

When electrical methods are employed, operators need to understand the appropriate electrical parameters (for example, the applied electrical current, electrode application, duration of application and the necessary cleaning and maintenance of equipment). As with all the other methods, they must also be able to recognise the signs of effective stunning and death. When electrical methods are used, incorrect application may increase the chances of experiencing pain associated with a pre-stun shock or result in an ineffective stun (EFSA, 2012). The induction of cardiac arrest without unconsciousness is also a risk when electrocution methods are used (EFSA, 2006).

When appropriate parameters are utilised, irreversible electrical stunning with the induction of cardiac arrest using a water-bath has been demonstrated to be effective for the majority of poultry species (EFSA, 2004, 2006). Electrocution equipment (head-to-cloaca application) is also available for killing individual meat chickens. The use of an electrical water-bath would allow large numbers of birds to be killed during a mass destruction event, however, all electrical methods require poultry to be individually handled and restrained.

9 Other methods

In the context of this review, other methods include:

- Low atmosphere pressure stunning (LAPS)
- Ventilation shutdown.

9.1 Low atmosphere pressure stunning (LAPS)

Low atmosphere pressure stunning (LAPS) operates by removing air from a sealed chamber containing animals. Unconsciousness is brought about by a gradual reduction of oxygen tension in the chamber, leading to progressive hypoxia (Vizzier et al., 2010; Vizzier, 2015; McKeegan et al., 2013b) and as with CAS systems, it is not immediate. The LAPS system allows for birds to be stunned in containers, thereby removing the need to shackle conscious birds. The LAPS system has been highlighted as a potential method that can be utilised for whole-flock culling.

The summary of LAPS provided by the Farmed Bird Welfare Science Review (Nicol et al., 2017) states that:

> LAPS has the potential to offer significant welfare benefits for poultry slaughter. Birds remain in their transport crates during LAPS stunning so there is no need for conscious birds to be shackled or positioned. Its effectiveness is relatively insensitive to variations in bird size and conformity, so it does not underperform when presented with flocks with a large variance in bird size. No aversive gas is used to displace oxygen, and stunning is irreversible. Concerns surrounding LAPS centre around spasms and wing flapping induced by hypoxia, as well as the potential for hypobaric injury.

Since the completion of the Farmed Bird Welfare Science Review in 2017, the use of LAPS has been approved for use in the EU, after assessment by the ESFA Panel on Animal Health and Welfare (EFSA, 2017). The EFSA Panel produced a detailed scientific evaluation of the key parameters required when LAPS is used to stun broiler chickens. It was concluded that under certain conditions (for example, rate of decompression, weight of chickens, exposure time and ambient conditions) the LAPS system was found to be able to provide a level of animal welfare at least equivalent to that provided by at least one of the currently allowed methods (for example, electrical water-bath stunning or controlled atmosphere stunning) (Purswell et al., 2007; Holloway and Pritchard, 2017; Martin et al., 2016 a, b). For effective operation, the pressure time curve should be adjusted to ensure that all birds are irreversibly stunned and killed within the cycle time (EFSA, 2017). Deviations from these conditions might have different consequences for animal welfare, and this was not assessed by EFSA. Therefore, the conclusions from this assessment cannot be extended to other types of chicken (layers and breeders) and if LAPS methodology is intended to be used for the stunning of layers, further studies would be required to determine the effect of decompression on intra-abdominal shell eggs. Martin et al (2020) investigated the possible effects of gas expansion in body cavities during a commercial LAPS procedure. Birds were subjected to postmortem examination to detect and score haemorrhagic lesions or congestion in the major organs and cavities (for example, air sacs, joints, ears and heart). The results were compared to a control group that had been euthanised with pentobarbital sodium. The findings were used to provide evidence that LAPS did not result in visible changes, consistent with distension, to the air sacs and intestines. The researchers also noted that there was no evidence of barotrauma in the ears and sinuses.

Further research into the effects of LAPS on different sized birds, different species and potential for aversion was recommended by EFSA. A study on the aversion of LAPS compared with gaseous methods of stunning (CO_2 and nitrogen) was completed by Gent et al (2020). Broiler breeders indicated aversion to a particular environment by relinquishing a food reward to seek a preferable environment. The researchers found that cessation of feeding occurred most rapidly in the CO_2 environment, whereas in the low atmospheric pressure and nitrogen environments, birds continued to eat for longer. Behavioural indicators of possible aversion were also more pronounced in the CO_2 treatment, with gasping and headshaking occurring earlier and at a greater frequency. Gasping did not occur in the nitrogen and LAPS treatment groups. Additional research on other species and types of poultry is still required, however post-2017 research in meat chickens to investigate possible aversion has demonstrated that LAPS is likely to be less aversive than the use of CO_2.

As with CAS systems, the ability to observe birds in the container is important for welfare monitoring. In the first LAPS research units, a viewing window was used. However, the influx of light through the window caused increased activity in the birds. Commercial LAPS equipment utilises infrared video cameras (with a wide-angle view) to effectively view birds, whilst keeping them in the dark (Thaxton, 2018).

A mobile LAPS system is available in the US and has been used for on-farm killing (EFSA, 2019). The current AUSVETPLAN destruction manual states that 'decompression' is an unacceptable method for killing poultry (AHA, 2015), however this should be reviewed.

9.2 Ventilation shutdown (VSD)

The term ventilation shutdown (VSD) refers to a procedure that involves sealing poultry within their housing environment, shutting down the ventilation and introducing supplementary heat. The body heat from the birds, combined with the added heat, raises the temperature in the shed until poultry die from hyperthermia or suffocation. Heat stress (hyperthermia) has long been associated with reduced welfare status, and temperature-humidity combinations that are high enough to cause death are also known to cause severe stress and suffering. In broilers, heat stress has been documented to increase serum concentration of corticosterone (a marker of stress and negative welfare in birds). It can therefore be argued that killing via inducing hyperthermia fails to meet the criteria for an acceptable killing method, since the birds do not experience a rapid loss of consciousness or loss of brain function, with minimal pain or distress, prior to death.

The Royal Society for the Protection of Animals (RSPCA) (Australia and UK) are both strongly opposed to the use of VSD. The UK RSPCA state that “with proper planning, ventilation shutdown should never need to be used” (RSPCA, 2008). The AVMA (2019) permit the use of VSD in constrained circumstances for floor-reared and caged poultry, if it is applied with supplemental heat or CO_2 to produce 100% mortality. The AVMA do not recommend VSD alone. In the UK, the Welfare of Animals (Slaughter or Killing) Regulations 1995 (as amended) (WASK, 1995) was amended to allow a derogation for ventilation shutdown as a killing method for poultry in certain disease control situations if authorised by the Secretary of State, following advice from the Farm Animal Welfare Council. The Department for Environment, Food, & Rural Affairs (DEFRA) (2009) stated that it was only permitted to use VSD when:

- All other permitted killing methods had been explored and discounted
- There was serious and heightened concern over human and animal health
- Resources were stretched beyond capacity, making the use of other methods impossible (for example, multiple outbreaks of infectious disease)

DEFRA also developed instructions on the use of VSD. They can be summarised as follows:

- Sealing of the building including gaps in the structure of the building and ventilation outlets/inlets. Effective sealing will be influenced by age, size and design. Buildings need to be sealed from the outside
- Use of monitoring equipment – including temperature sensors at bird height
- Available power supply and back-up to run supplementary heating equipment
- Drinking system operational and water not withheld during VSD
- Available trained personnel to kill any birds that remain alive after completion of VSD process
- Sufficient personnel available for clean-up operation to disinfect and remove birds expediently, particularly as the elevated temperatures involved cause rapid decomposition
- Placement of heaters for adequate heat distribution (capacity for at least 3 hours of heating)
- Strategies for reducing temperature stratification (particularly in multilevel systems), such as air-mixing should be used with VSDH and $VSDCO_2$.

The aim of the DEFRA protocol was to quickly raise the temperature inside the house to 40°C within 30 minutes and maintain it for 3 hours (DEFRA, 2009) whilst attempting to minimise the welfare impact. The instructions were based in-part on modelling to predict the time taken for the core body temperature of a 2kg meat chicken to reach 45°C (given that the normal core body temperature is 41.4°C). The model assumed leakage from the building of no more than 2 air exchanges per hour, an ambient temperature of 10°C and humidity of 70%. Under these conditions and with supplementary heating supplied, the time taken to reach a core body temperature of 45°C was found to be 35 minutes (Zhao et al., 2021). The model was validated during VSD in a turkey breeder house. It was concluded that the model can be used to predict supplemental heat requirements under different environmental conditions to ensure effective VSD application, however, building designs that result in temperature stratification (for example, multilevel housing) are unlikely to fit the model in its current form.

Only two studies have examined the effects of VSD in an experimental setting. A proof-of-concept study (Eberle-Krish et al., 2018) was designed to evaluate the effectiveness of VSD, VSD with supplemental heat (VSDH), and VSD with CO_2 ($VSDCO_2$) as alternative mass destruction methods in a multi-level caged system for laying hens. Assessment parameters included ambient and core body temperatures, time to death, and survivability. Time to death for VSD, VSDH, and $VSDCO_2$ were 3.75, 2, and 1.5 hr, respectively. The goal of any depopulation is 100% mortality, and this remains true for VSD. Survivability in VSD did not meet the flock depopulation standard of 100% lethality as 2.8% of hens survived. When supplemental heat or CO_2 was added, 100% lethality was achieved, however, it is likely that time to loss of consciousness was protracted. Based on time to death, VSD with supplemental heat and VSD with CO_2 proved equivalent.

In another proof-of-concept study, the effectiveness of VSD and VSDH for killing turkey breeder hens was studied (Krish, 2018). Time to death for VSD and VSDH was 360 and 181 minutes respectively. For VSD alone, the ambient temperature needed to cause hyperthermia was not reached, leading to high survivability (34.4%). Ventilation shutdown with supplemental heat was successful in producing 100% lethality, with ambient temperatures reaching over 54.4 °C. The researchers concluded that the turkey breeder hens were able to withstand high temperatures and relative humidity, which needs to be considered when employing ventilation shutdown with supplemental heat. The loss of heat production as mortality increases presents serious issues for VSD as a depopulation method in turkey breeder hens. They also noted that the high ambient temperatures needed to cause lethality in ventilation shutdown with supplemental heat should be closely monitored as they could potentially cause damage to the equipment within a turkey breeder hen facility.

While the data from the studies emulates commercial poultry production environments, application of the techniques have not been evaluated in commercial egg-laying and turkey breeder facilities. However, given its negative welfare implications, it is not a recommended method for mass destruction except in the most extreme emergency situations.

10 Recommendations

In general, on-farm killing involves the killing of animals that are injured, diseased (and unlikely to recover) or for disease control purposes, on their production site. Poultry might also be killed for economic reasons, deteriorating husbandry conditions or in the event of other unforeseen emergency situations, for example reduced slaughtering capacity due to the COVID-19 pandemic or other supply chain disruptions (Grandin, 2021; Marchant-Forde and Boyle, 2020). Large-scale (or mass) destruction refers to the killing of large numbers of poultry and may not only include animals affected by disease, but also healthy animals of varying ages in different production systems. The efficacy of the methods for large-scale destruction may not always reflect that observed when the same methods are used in processing plants or when they are applied to individual or small numbers of birds. This can be due to the lack of specialist handling, restraining and killing infrastructure and equipment in an on-farm situation.

When the decision has been made to humanely kill poultry, the method employed should result in the rapid loss of consciousness (or induce unconsciousness without pain, fear and distress) followed by cardiac or respiratory arrest and ultimately the loss of brain function. In addition, handling and restraint should aim to minimise any pain, fear and distress experienced by the bird prior to unconsciousness.

It was evident from the review that the ability of each method to deliver an acceptable animal welfare outcome is largely dependent on the type of poultry and the production systems used. There is not one individual method that is optimal for all types of poultry in all situations. All methods require the appropriate conditions for their use in a production environment or as part of an emergency response. The ideal method for the mass destruction of poultry would allow for large numbers of birds to be killed in-situ in their production system, without individual handling, whilst still resulting in an acceptable animal welfare outcome.

Potential methods have been categorised in a similar format to that used by the AVMA Guidelines for the Depopulation of Animals (2019). The categories used are:

- Preferred methods are given the highest priority and should be used preferentially when circumstances allow reasonable implementation during emergencies.
- Not recommended methods should only be considered when the circumstances preclude the reasonable implementation of any of the preferred methods and when the risk of doing nothing is deemed likely to have a reasonable chance of resulting in significantly more animal suffering than that associated with the proposed depopulation method.

Under the conditions of mass destruction, the preferred methods described in

Table 2 could deliver acceptable animal welfare outcomes when appropriate operational parameters are implemented.

Table 2 Preferred methods for the mass destruction of poultry

Method Category	Method	Bird Species	Application	Operational parameters required for effective use
Inhalational	Carbon dioxide	• Chickens • Turkeys • Ducks • Geese • Pheasants • Quail	Whole-house gassing	• Correct timing of ventilation shutdown to avoid heat stress • Water supply maintained • Compressed CO_2 vaporised or heated before introduction (temperature monitoring throughout process) • Birds protected from jet stream to avoid exposure to high concentration of CO_2 prior to unconsciousness • Gradual displacement filling to ensure that birds are not exposed to >40% CO_2 before they are unconscious • Maintenance of correct gas concentration • Visual observation possible during gas exposure • Birds exposed to a lethal dose of gas • Death confirmed and back-up method used if necessary
			Containers – rising concentration of CO_2	• Catching, handling and restraint performed competently and with care • Birds introduced in single layer; no further birds added until previous birds are dead • Compressed CO2 vaporised or heated before introduction (temperature monitoring throughout process) • CO2 introduced as a rising concentration. Birds exposed to <40% until unconscious • Maintenance of correct gas concentration • Birds exposed to a lethal dose of gas (monitoring equipment to monitor dwell time) • Visual observation possible during gas exposure • Death confirmed and back-up method used if necessary
	Inert gases (including inert gas + CO_2)	• Chickens • Turkeys • Ducks • Geese • Pheasants	Sealed containers	• Catching, handling and restraint performed competently and with care • Birds introduced in single layer; no further birds added until previous birds are dead • Birds restrained in transport crate where possible • Maintenance of correct gas concentration

Method Category	Method	Bird Species	Application	Operational parameters required for effective use
		• Quail		• Birds exposed to a lethal dose of gas (monitoring equipment to monitor dwell time) • Visual observation possible during gas exposure • Death confirmed and back-up method used if necessary
	High-expansion gas-filled foam	• Chickens • Turkeys • Ducks	Whole-house foam application	• Correct timing of ventilation shutdown to avoid heat stress • Water supply maintained • Foam expansion ratio of at least 250:1 • Use of N2-filled foam is preferred over CO_2-filled foam • Sufficient volume of foam to completely cover the birds and reach a height above the birds, such that they remain covered even after wing flapping • Foam production capacity should be larger than the breakdown speed of the foam by the birds • Visual observation possible during gas exposure • Birds exposed to a lethal dose of gas • Death confirmed and back-up method used if necessary
			Containers	• Catching, handling and restraint performed competently and with care • Birds introduced in single layer or held in transport containers • Birds exposed to a lethal dose of gas (production of foam continues until the birds are dead) • Monitoring equipment to monitor dwell time • Death confirmed and back-up method used if necessary
Mechanical	Non-penetrative captive bolt	• Chickens • Turkeys • Ducks • Geese • Pheasants • Quail	Restrained birds – equipment or manual	• Catching, handling and restraint performed competently and with care • Manufacturer's instructions are followed • Death confirmed and back-up method used if necessary
	Neck dislocation	Birds <3kg • Chickens • Turkeys	Restrained birds – equipment or manual	• Catching, handling and restraint performed competently and with care • Neck crushing methods not permitted • Death confirmed and back-up method used if necessary

Method Category	Method	Bird Species	Application	Operational parameters required for effective use
		• Ducks • Geese • Pheasants • Quail		
Electrical	Electrocution	• Chickens • Turkeys • Ducks • Geese • Quail	Water-bath – restrained on a shackle	• Catching, handling and restraint performed competently and with care • Sufficient number of competent shackling operatives • Shackle line designed to minimise struggling and wing flapping • Use of breast comforting apron • Limit duration of shackling • Wet shackles to improve conductivity • Avoidance of pre-stun shocks • Sufficient stun duration (head in contact with electrode/water) - for at least 4 seconds • Stunning current of at least 400mA at 50Hz AC • Death confirmed and back-up method used if necessary
	Electrocution	• Chickens	Head-to-cloaca application – restrained in the equipment	• Catching, handling and restraint performed competently and with care • Manufacturer’s instructions are followed • Sufficient stun duration • Stunning current of at least 400mA at 50Hz AC • Death confirmed and back-up method used if necessary
Others	Low atmosphere pressure stunning (LAPS)	• Chickens Validated for broilers and male layer chicks	Mobile LAPS equipment	• Catching, handling and restraint performed competently and with care • Manufacturer’s instructions are followed • Sufficient exposure (correct specifications for time/target pressure) • Death confirmed and back-up method used if necessary

The methods detailed in Table 3 cannot currently be recommended for killing poultry under conditions of mass destruction, due to the reasons described in the table.

Table 3 Methods that are not recommended for the mass destruction of poultry

Method Category	Method	Application	Reasons for omission
Inhalational	Carbon dioxide	Pre-filled containers	• Exposes birds to a high concentration of CO_2 which is aversive
	Inert gas	Whole-house gassing	• Difficult to ensure an anoxic environment • Requires poultry house to be completely sealed which is practically difficult
Oral agent	Alpha-chloralose	Whole-house	• Absence of supporting science
	Sodium nitrite	Whole-house	• Absence of supporting science
Injectable	Barbiturates and barbiturate derivatives	Individually restrained birds	• Labour and skill intensive therefore more suited to small numbers of birds
Mechanical	Penetrative captive bolt	Individually restrained birds	• Labour intensive therefore more suited to small numbers of birds • Non-penetrative devices provide a more suitable alternative • Operator safety risk
	Manual blunt force trauma	Individually restrained birds	• Aesthetically unacceptable • Risk of inconsistent application
	Gunshot	Individual birds	• Impractical • Operator safety risk
	Cervical dislocation	Individually restrained birds	• Not suitable for birds >3kg • Absence of supporting science for the use of mechanical crushing methods
	Decapitation	Individually restrained birds	• Absence of supporting science • Aesthetically unacceptable • Biosecurity risk
Electrical	Electrocution	Head-only Individually restrained birds	• Requires a terminal procedure • Requires restraint of individual birds • Labour intensive therefore more suited to small numbers of birds

Method Category	Method	Application	Reasons for omission
Others	Ventilation shutdown (VSD), VSDH and VSDCO_2		• Severe welfare concerns • Absence of supporting science

Note: these methods should only be considered when circumstances preclude the reasonable implementation of any of the preferred mass destruction methods for poultry

11 Conclusion

The majority of studies identified during the literature review describe the results of controlled experiments under laboratory conditions, using small sample sizes and assuming scalable processes. There was also a range of research methodologies, methods used for the assessment of unconsciousness and a degree of subjectivity around the determination of death. There were even fewer studies focusing on the induction period and the time to loss of consciousness. Studies that did focus on the induction period tended to be carried out in the proposed context of commercial slaughter processing for human consumption as opposed to on-farm killing or mass destruction.

Current readily available equipment allows for mass humane destruction, however, it is clear that all methods investigated have advantages and disadvantages. Selection of the most appropriate method(s) for use in any particular situation will require a case-by-case evaluation of factors such as poultry type, design of poultry housing, availability of the required equipment, operational capabilities, environmental and disposal considerations and personnel safety. Many of the studies suggest that competent and conscientious personnel are the single most important factor in assuring a humane death. Risk of failure is likely to increase with operator fatigue and time pressures, therefore we need to focus on methods that remove the need for individual handling of poultry.

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