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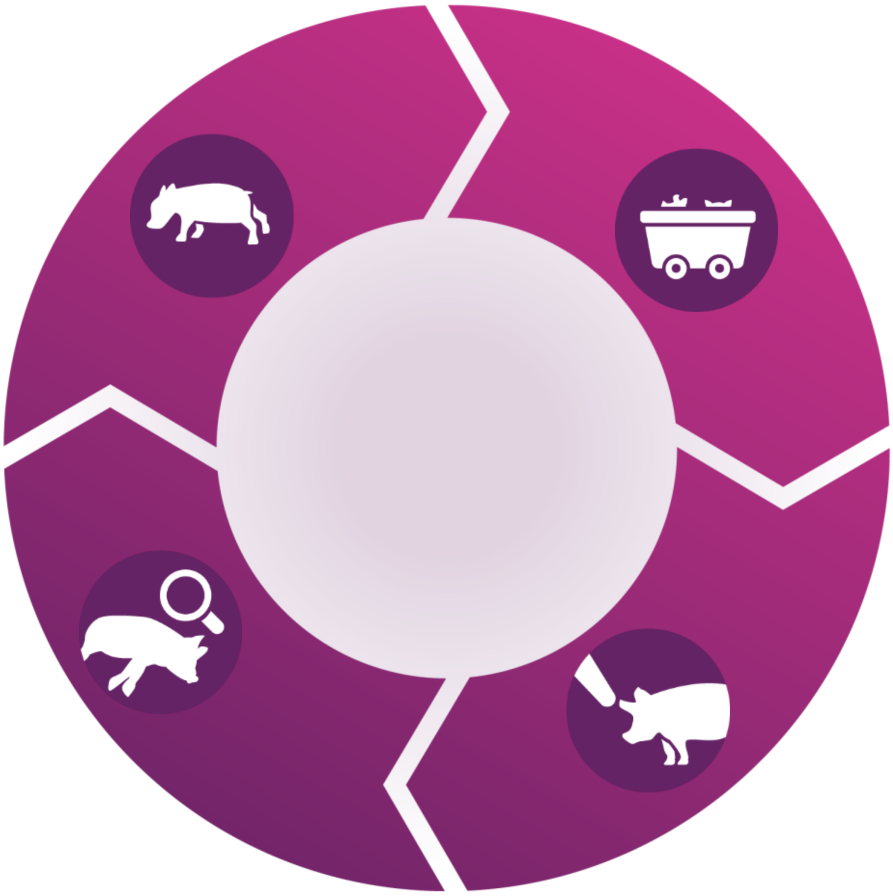
Centre for Animal Welfare *Pigs*

Review on euthanasia of suckling piglets on farm

Inga Wilk, Isa Kernberger-Fischer, Marien A. Gerritzen, Hanne Kongsted, Lars Schrader



info.pigs@eurcaw.eu

www.eurcaw-pigs.eu

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Inga Wilk[1], Isa Kernberger-Fischer[1], Marien A. Gerritzen[2], Hanne Kongsted[3], Lars Schrader[1]

[1] Friedrich-Loeffler-Institut, Germany
[2] Wageningen Livestock Research, The Netherlands
[3] Aarhus University, Denmark

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Each EURCAW-Pigs review provides background information on the biological relevance of the welfare topic. It then presents the most important key areas to focus on during welfare inspections, describes why welfare issues occur and lists specific animal-based indicators that can help official inspectors to identify these welfare issues. Finally, the review summarizes ways of good and better practices that can help to solve the previously described welfare issues, and deals with related legislative requirements.

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1 Executive Summary

The majority of animal losses in pig production occur during the first days postpartum, i.e. in new-born piglets. The necessity to euthanasia occurs particularly during this first period of pigs' life. The review will therefore focus on the euthanasia of suckling piglets. This group includes all such piglets until they are ready for weaning (up to 10 kg approximately). Euthanasia ("good death, in Greek "eu" meaning "good" and "thanatos" meaning death) includes ending the life of an individual animal in a way that minimizes or eliminates pain and distress (American Veterinary Medical Association [AVMA], 2020). This corresponds to the general requirement of the Council Regulation (EC) No 1099/2009 to protect animals at the time of killing such that they are spared any avoidable pain, distress or suffering (Article 3). The Regulation thus recognises that killing animals may induce pain, fear and distress even under the best available technical conditions. Therefore, any person involved in the stunning or killing of animals should take the necessary measures to avoid pain and minimise distress and suffering during the killing process, considering the best practices in the field. The main reasons for euthanizing a piglet are described below (including incurable diseases and injuries).

The killing procedure must spare animals any avoidable pain, distress or suffering. This implies that moving animals to the killing point and restraining them for the purpose of stunning and killing must be done without causing undue fear and distress. Animals may show the latter by vocalising and/or trying to escape. For killing animals, one-step procedures should be primarily used that lead directly to death without the application of any further subsequent killing procedure (e.g. bleeding). These one-step procedures are recommended from an animal welfare perspective to minimize the risk of application errors and from the user's point of view to reduce mental stress. The second step itself may additionally contribute to the mental stress of the person performing the procedure (Marahrens, 2014b). Only common methods that can be used by farmers are addressed. Basis is the EU legislation, but references are made to significant national differences. Additionally, new developed methods, not yet covered by the Regulation, are described.

Operators must affirm death of animals by confirming the absence of vital signs of cardiovascular, respiratory and neuronal functioning. Best practices maximally limit stress during handling and killing processes. This review should help to develop guides to good practice on operating and monitoring procedures for killing animals and to provide proper guidance on animal welfare for both, inspectors and business operators. It highlights the four key areas relevant for animal welfare at the time of stunning and killing, some of which can be subdivided further into appropriate decision-making on euthanasia, handling and moving piglets to the killing point, the killing process (physical and gas killing methods including restraint, if necessary), and post-mortem inspection of the carcass.

The review addresses the underlying scientific knowledge and key areas to focus on during welfare inspections related to on-farm euthanasia of suckling piglets. The review focuses on how to minimise welfare problems and facilitate improved practices, as well as the underlying legal requirements. This review will not deal with large-scale killing or depopulation.

2 Introduction

Keeping animals implies that owners and caretakers have a duty of care for all animals. When animals are sick, injured or are suffering and treatment is not effective or is no longer in the benefit of the animal euthanasia should take place to prevent unnecessary suffering. Methods and conditions for killing and thus for euthanasia are described in European legislation and in national regulations. Council Regulation (EC) No 1099/2009 requires that the animals are spared any avoidable pain, distress or suffering during killing (Article 3). The main killing methods for piglets applied on farm are mechanical methods, electrical methods and gas killing methods. These methods differ in important aspects. Mechanical and electrical methods (summarized as physical methods) provide an instantaneous stun but require individual restraint, whereas gas killing results in a gradual loss of consciousness but can be applied to groups of pigs resulting in a reduced sense of social isolation. Electrical killing methods include two-step procedures with head-only electrical stunning followed by a killing procedure. This subsequent killing method can be electrocution or bleeding for piglets over 5 kg body weight, and only bleeding for animals under 5 kg. Electrocution for use in piglets weighing less than 5 kg has recently been investigated and presented in a study (Husheer et al., 2020; Husheer, 2017), but is not yet ready for practical application.

To evaluate compliance with the detailed requirements in Council Regulation (EC) No 1099/2009 (see chapter 6), animal-welfare inspectors assess resource- and/or management-based indicators, e.g. device settings (e.g. pneumatic pressure, cartridges, gas concentration, exposure time). However, an enhanced focus on animal-based indicators (ABI) is recommended applying measurable and objective outcome-based criteria on animals.

Animal-based indicators should be used to assess animal welfare at the time of killing. In this review, the following four relevant key areas and corresponding animal welfare indicators are described to assess pig welfare in the killing process:

1. Appropriate decision-making in relation to euthanasia

2. Handling and moving to the killing point

3. Killing process (including restraint and confirmation of death)

4. Post mortem inspection of the carcass

Chapter 3 presents the underlying scientific knowledge. Chapter 4 identifies key areas for inspectors to focus on during welfare inspections, in particular animal-based indicators (ABIs) to assess proper handling and killing. Chapter 5 describes (suggestions for) improved practices if not already included in the method description. The final chapter specifies the legal requirements in Council Regulation (EC) No 1099/2009 related to the three key focus areas.

3 Scientific knowledge on killing methods

3.1 Deciding on euthanasia

As it is inevitable for a farm that a proportion of pigs are non-viable at birth or become ill or injured to a degree which makes recovery unlikely (Mullins et al., 2017), timely emergency killing or euthanasia might be necessary to avoid poor animal welfare. Council Regulation (EC) No 1099/2009, Art. 2, lit. d) and j) define the terms '**emergency killing**' as the *"[…] killing of animals which are injured or have a disease associated with severe pain or suffering and where there is no other practical possibility to alleviate this pain or suffering; […]"* and *"[…] '**slaughtering**' as the killing of animals intended for human consumption; […]"*. In contrast to growing pigs (> 30 kg) or fattening pigs an emergency slaughter is generally not considered as an option for killing acutely injured piglets (up to 10-12 kg). The necessity of euthanasia refers to cases where a humane death represents a better alternative for a pig compared to a future life under persistent pain and suffering (Mullins et al. 2017; National Pork Board [NPB], 2008; American Association of Swine Veterinarians [AASV] and NPB, 2016). Referring to Fraser et al. (2013), timely decisions on killing represents an acceptable means to eliminate pain and suffering associated with severe health impairments. As Council Regulation (EC) No 1099/2009 states within recital 12 "*It is an ethical duty to kill productive animals which are in severe pain where there is no economically viable way to alleviate such pain. In most cases, animals can be killed respecting proper welfare conditions.* Furthermore, in Council Regulation 1099/2009 EC which also applies with a few restrictions (Art. 1, paragraph 2) with regard to emergency killing, it is stated that "*In the case of emergency killing, the keeper of the animals concerned shall take all the necessary measures to kill the animal as soon as possible.*" (Art. 19 of Council Regulation (EC) No 1099/2009). The European Food Safety Authority [EFSA] (2020) mentioned different reasons for killing individual pigs on farm, which refer to severely injured or diseased animals for which there is no other practical possibility to alleviate pain or suffering as well as to runts, meaning very small non-viable piglets, which are not productive and therefore may be killed in order to prevent further suffering. However, Member States may have adopted stricter national regulations. For example, in Germany, the killing of so-called "surplus" piglets (more piglets per litter than the sow has teats) as well as weak but viable piglets is not permitted. In such a case management measures such as litter compensation, nurse sows or more intensive care must be taken (Pedersen et al., 2020). In other Member States other regulations concerning the killing of surplus piglets may exist, as reported in a scientific publication by Grist et al. (2018b).

The "AVMA Guidelines for the Euthanasia of Animals" (2020) provide an explanation why the death of an animal might be the appropriated way to relieve an animal from its unbearable burden. As an example, the AVMA (2020) cited that a veterinarian may recommend euthanasia for an animal that is suffering due to a terminal illness, because the loss of life is not relatively worse compared with a further life under prolonged illness, suffering, and duress. Edwards-Callaway et al. (2020) described euthanasia as an essential management tool to alleviate animal suffering.

Indications justifying the killing of non-viable suckling piglets

Referring to Article 3 of Council Directive 98/58/EC that highlights Member States' responsibility to ensure that the owners or keepers take all reasonable steps to safeguard the welfare of animals, which implicates that they are responsible to alleviate unnecessary pain, suffering or injury from animals under their care. This implicates the timely emergency killing if necessary. Therefore, competence for the ability of deciding about a necessity of euthanasia, choosing the appropriate method and the correct application of this method are required (Council Regulation (EC) No 1099/2009). In Art. 7 of the Council Regulation (EC) No 1099/2009 it is stated that killing and related operations should only be carried out by persons with the **appropriate level of competence**, thus without causing the animals any avoidable pain, distress or suffering. In Art. 2, lit. d) of the EU Regulation, the term 'emergency killing' is defined as the "[…] *killing of animals which are injured or have a disease associated with severe pain or suffering and where there is no other practical possibility to alleviate this pain or suffering.*" The "*keeper of the animals concerned shall take all the necessary measures to kill the animal as soon as possible*" (Art. 19). Within the Terrestrial Animal Health Code in Art. 7.13.27. on "*humane killing*" it is stated that […] "*allowing a sick or injured animal to linger unnecessarily is unacceptable. Therefore, for sick and injured pigs* ***a prompt diagnosis*** *should be made to determine whether the animal should be* ***treated or humanely killed***." […] Within an attachment of the decree of Ministry of Agriculture, Environment and Consumer Protection of Mecklenburg-Western Pomerania (2014) on "Animal Welfare - Keeping Pigs - Handling of Suckling Piglets" a guideline on the "Humane Handling of Suckling Piglets" was published that explains that animals must be killed if the further live of the animal "could" continue under pain, suffering that cannot be cured". The use of the subjunctive in the decree's attachment and the OIE-Guidelines indicates that, unlike required by Council Regulation (EC) No 1099/2009, pain and suffering do not have to be acute, but can also occur in the future.

The decision for or against the euthanasia of suckling piglets requires in some cases a **continuous surveillance and reassessment of the current status of the affected animals** via a profound investigation**.** In general, the **indication for euthanasia must be made individually for each animal**, which means it is always a case-by-case decision (Brase, 2014; Veterinary Association for Animal Welfare [in German: Tierärztliche Vereinigung für Tierschutz e.V., abbreviated as TVT), 2014; Baumgartner et al., 2015; Farm Animal Welfare Advisory Council, 2017; Meier and von der Aa-Kuppler, 2017; OIE, 2019; Woods et al., 2020). Several studies revealed that animals are often released from their suffering far too late (Baumgartner et al., 2014, 2015; große Beilage, 2017, 2021).

The general condition of the animal, its state of awareness, the food and water intake, urination and defecation, its body weight, its body temperature and the ability to move might be helpful indicators to measure the current or long-term welfare and health state of the affected animal (Meier and van der Aa-Kuppler, 2017).

The Terrestrial Code provides some guidelines in terms of humane killing (OIE - Terrestrial Animal Health Code, 2019). Indications for humane (emergency) killing are included in Art. 7.13.27.:

- *"[…] severe emaciation, weak pigs that are nonambulatory or at risk of becoming nonambulatory,*
- *severely injured or nonambulatory pigs that will not stand up, refuse to eat or drink, or have not responded to treatment,*
- *rapid deterioration of a medical condition for which treatment has been unsuccessful,*
- *severe pain that cannot be alleviated,*
- *multiple joint infections with chronic weight loss,*

- *piglets that are premature and unlikely to survive, or have a debilitating congenital defect, and*
- *as part of disaster management response. […]*

As emergency killing may prevent animals from excessive and prolonged suffering while treatment offers no prospect of recovery (AVMA, 2020) research into euthanasia was intensified and guidelines developed (Council Regulation (EC) No 1099/2009, recital 23), which provide criteria regarding the timepoint for euthanasia, advice on pre-euthanasia handling, available methods of euthanasia, their efficacy and safety, and their implications for both animal welfare and the disposal or use of carcasses (European Commission, 2017; FAWC, 2003; AASV and NPB, 2016; Woods and Shearer, 2021). There are a number of indicators that can help to make an ethical decision regarding the need to euthanize an affected piglet. However, there is little literature available which assist the person in charge concerning making a decision that is **justifiable from an ethical and animal welfare point of view**. In the following, some of the recent studies or guidelines are presented which will be helpful to interpret the recognisable indicators on the animal.

In the work of Balzer (2017) the following parameters for the characterization of non-viable suckling piglets are described: **low body weight**, **low body temperature**, **decreased mobility**, **reduced milk intake**, **impaired defecation and urination** and reduced awareness.

Previously, Baxter et al. (2008, 2009) suggested that other indicators of body conformation, such as **body mass index** and **ponderal index**, might also be important indicators of survivability. Furthermore, the **latency to suckle** (Baxter et al., 2008) or **rectal temperature at 1 h after birth** (Baxter et al., 2009), the **ability to regain heat and acquire colostrum** (Herpin et al., 2002), and piglets with **empty stomachs** (Hales et al. 2013) may explain greater variation than body mass index.

Meier and van der Aa-Kuppler (2017) described a so-called "**downward spiral of weak piglets**", in which the low birth weight together with a too low ambient temperature may lead to a decreasing body temperature in the affected piglets. The consequences are **trembling**, **apathetic behaviour** and **too little or no colostrum intake**. Such animals starve and show an **increased susceptibility to pathogens**, which often leads to death or to a necessary emergency killing by the animal owner.

Referring to Meier and van der Aa-Kuppler (2017), an **emergency killing is indicated** (in consultation with the veterinarian when in doubt) for:

- *a pig that cannot take in feed and water or cannot urinate and/or defecate, and therefore is not viable,*
- *a pig with a disease that do not respond to treatment, and*
- *a pig that suffers from untreatable disease, malformation or injury which severely limits the animal's ability to live without causing serious suffering and damage.*

Therefore, potential indicators for assessing the survivability of piglets can be (modified and translated from Marahrens, 2014a):

- *body weight (e.g. new-born < 700 g)*
- *behaviour (e.g. apathy)*

- *body temperature (e.g. new-born < 36 °C)*
- *lack of suckling reflex*
- *diseases (e.g. diarrhoea, respiratory diseases, polyarthritis), congenital malformations (e.g. atresia ani), injuries (e.g. severe tissue damage)*

In this context, in Germany, there is the recommendation that only the **presence of deviations in several of these indicators at the same time** or the **strong deviation in single indicators** may justify the need for euthanasia if simultaneously all measures for improvement have been taken beforehand and the animal's continued life is possible only with persistent pain and suffering. **Underweight and/or weakness alone do not constitute an indication for killing** (Marahrens, 2014a). The German Veterinary Association for Animal Welfare (TVT, 2014) states that euthanasia of suckling piglets is therefore indicated when the animals are incurably ill. Symptoms of incurably sick animals can be e.g. severe emaciation despite intensive care, in newborn piglets undertemperature, a non-ambulatory status, circulatory failure and lack of suckling reflex, congenital life-threatening malformations (e.g. Atresia ani) or unsuccessfully treated spreading of the hind legs.

For pig farmers, the decision concerning killing an animal is considerably challenging, but also an everyday-task in commercial pig farming and therefore requires a high level of knowledge and skills from staff (große Beilage, 2021; Meier and von Wenzlawowicz, 2017; Unterweger et al. 2015). The decision to euthanize a piglet must be made carefully, but also without unnecessary delay to spare the animals from prolonged suffering and pain (OIE - Terrestrial Animal Health Code, 2019).

Today there is only one decision support tool available which focusses on suckling piglets (Anoxia, 2018; see also Figure 3.1.1). Other support tools which were recently published do not address the special needs concerning emergency killing of suckling piglets (DLG, 2018; Chamber of Agriculture North Rhine-Westphalia, 2018).

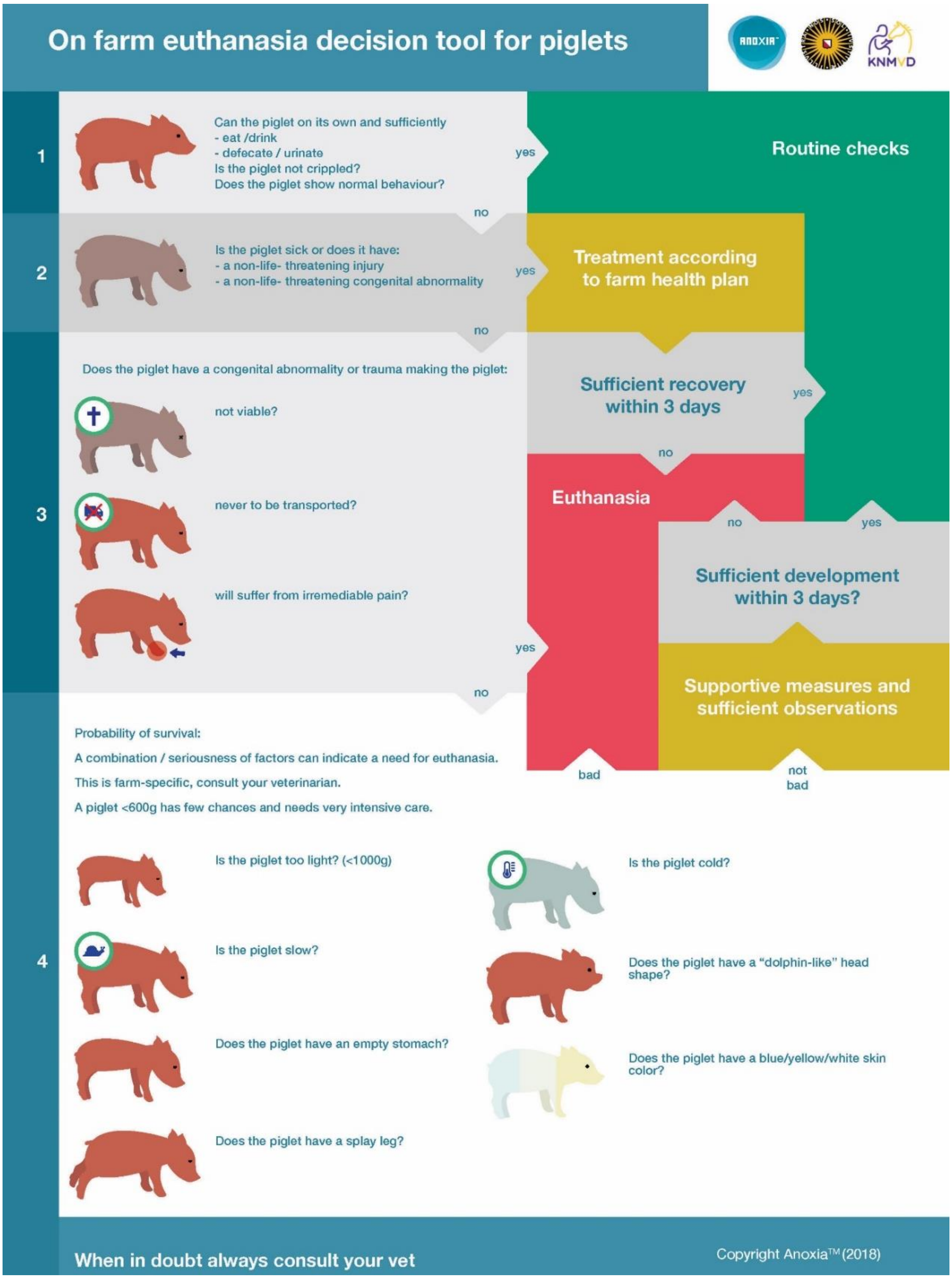

Figure 3.1.1. Decision support tool for piglets [Anette van der Aa, Anoxia BV, Tijs Tobias, Centre for Sustainable Animal Stewardship (CenSAS; as a collaborative project by Utrecht's Faculty of Veterinary Medicine and the Animal Sciences Group of Wageningen University & Research), Royal Netherlands Veterinary Association (RNVA/KNMvD), Dutch pig producers organization (POV), Dutch farmers organization ZLTO; 2018], used with permission from the copyright holder.

3.2 Killing methods for piglets

The methods will be described scientifically concerning desirable physiological effects, their aversive potential and risks of misuse resulting in distress and suffering and the assessment of the effectiveness. The farmer should be fully informed about the possible methods and supported in the selection of a suitable procedure for each specific age/weight category. Preferred methods are basically one-step methods. These methods result in death without the need of a second intervention (killing procedure) like bleeding or pithing. Permissible one-step methods according to the Regulation are the killing with gases with a sufficiently long exposure duration and the percussive blow to the head for piglets up to 5 kg. Since the blow to the head is not proven to lead to immediate death in all cases, EFSA (2020) requests a second intervention (e.g. bleeding). The manually applied blow to the head should be avoided and the use of mechanically operated devices should be implemented (EFSA, 2020). Table 3.2.1 gives an overview of the methods and their use in the different weight classes of piglets, including the application according to the Regulation.

Table 3.2.1. Methods for on-farm killing of piglets up to 10 kg and types of application (modified after EFSA, 2020).

Method		Number of steps • One step method (killing method) • Two steps (stunning and killing method)	Appropriate for body weight	Number of animals • Group killing • Individual killing	Further notes
Physical methods	*Mechanical methods*				
	Percussive blow to the head	**One step**; EFSA (2020) requests the use of this method as **two-step** procedure followed by a second intervention (e.g. bleeding) •	Less than 5 kg	individual	According to Council Regulation (EC) No. 1099/2009: not used as routine method but only where other methods are not available […]
	Penetrative captive bolt	**Two steps** • Blow to the head and penetration of the brain • Killing method (e.g. bleeding, pithing) required	No limitation (equipment must be appropriate for the animals' size)	individual	use suitable equipment (also for reasons of occupational safety), e.g. use a kind of hammock for restraint

	Non-penetrative captive bolt	**One step** (according to studies by Grist et al., 2017, 2018a)	Less than 10 kg	individual	Currently not yet allowed according to Council Regulation (EC) No. 1099/2009
	Electrical methods				
	Electrical stunning and killing	**Two steps** • Head-only electrical stunning • Killing method required	Restrictions on the selection of the subsequent killing method • Bleeding for piglets less than 5 kg • Electrocution for piglets with more than 5 kg	individual	
Gas killing methods	**Gas killing (gases and gas mixtures in containers)**	**One step**	No limitation (container must be appropriate for the size and number of animals)	Individual and Group-killing	
	Gas-filled foam	**One step**	No limitation (container must be appropriate for the size and number of animals)	Individual and Group-killing	The method applies the gas not alone, but enclosed in foam. The foam serves as a carrier for the gas. In Annex I of the Regulation, the application of gas-filled foam is not listed up to now.

Mechanical methods

Mechanical methods include percussive blow to the head using penetrative captive bolt guns and non-penetrating captive bolt guns.

Regardless of the type of application, mechanical methods in principle lead to a physical disruption of brain function with a coup-contre-coup effect. This coup-contre-coup effect occurs by the impact on the skull plate. The impact accelerates the skull against the brain mass and the brain hits the inner wall of the skull at the point of impact. If the blow is strong enough, the brain is also set in motion and, due to its inertia, collides with the opposite side of the skull. Depending on the severity of the impact, brain concussion thus occurs on both sides of the brain with or without haemorrhages, brain damage and immediate unconsciousness. Penetrating methods lead to prolonged unconsciousness, as the bolt enters the brain at high speed it causes structural damage to brain tissue and generates pressure waves within the fluid medium of the brain. This results in further damage at sites distant from the bolt trajectory (EFSA, 2004; Shaw, 2002; Daly and Whittington, 1989).

Widely reported concerns that the incomplete sutures in newborn piglets, which provide for deformation of the skull at birth, may provide elastic protection from the effects of blunt force and absorb the impact have been disproved. The cranial development of the piglet appears to be sufficient for the transfer of enough kinetic energy to the brain when mechanical methods are applied (Defra MH0116; cited by Grist et al., 2017). Moreover, newborn piglets seem to be particularly sensitive to brain injury (Armstead, 1999).

Percussive blow to the head (manually applied blunt force trauma)

The percussive blow to the head is only acceptable for use on piglets with a live weight up to 5 kg (EFSA, 2020; AVMA, 2020; Woods and Shearer, 2021; Council Regulation No 1099/2009; EFSA, 2004). A swift and single blow to the head of sufficient force and precision with a hard object (e.g. ball peen hammer, steel rods, wooden clubs, pipes) will result in concussion and immediate CNS depression and severe damage of brain tissue (EFSA, 2020; AVMA, 2020; HSA, 2016a; Woods and Shearer, 2021; EFSA, 2004). The blow needs to be delivered to the central bones of the skull at the highest point between the eyes and the ears (Woods and Shearer, 2021; Woods, 2012; Landwirtschaftskammer Niedersachsen [in English: Lower Saxony Chamber of Agriculture, abbreviated as LWK Nds.], 2018).

To perform the blow, the animals are held by the body and their head is placed on a hard surface (EFSA, 2020). Restraining of the animals for immobilising the piglets and their head may cause **pain and fear** (EFSA, 2020).

The other practice of holding the piglet with both hands around the hind legs and swinging the piglet's head against a hard surface (the animal is led to the object, called inversion) is not recommended from an animal welfare point of view. This way of performing leads to high stress and may have a higher probability of dislocated joints (e.g. hip) and broken legs (Woods and Shearer, 2021). The position and movement of the animals may cause **pain and fear** (EFSA, 2020). There is a potential risk of spinal disruption and/or brain concussion without loss of consciousness when there is an injury in the neck and the upper thoracic area (Blumbergs, 1997; Fong et al., 2009, cited by Dalla Costa et al., 2020). Spinal cord concussion without accompanying brain concussion would be recognizable by absent spinal functions (e.g. posture, cardiac and circulatory functions), while responses mediated via the cranial nerves would remain present (Dalla Costa et al., 2020). Such injuries without loss of consciousness and sensibility were not detected in the study by Dalla Costa et al. (2020) in very young piglets less than one week old. In EEG recordings in 13 neonatal piglets weighing up to 1.17 kg, an isoelectric EEG was visually detected after an average of 78.2 ± 6.5 s (means ± SE; range 18 to 115) when the animals were struck on the floor. This time corresponded to the time for EEG activity to become less than 10 % in comparison to baseline EEG activity in the pretreatment period before the blow (Dalla Costa et al., 2020). In the study by Dalla Costa et al. (2020), brain damage in animals strucked on the floor was concentrated in the frontal lobes. Hemorrhages were most common in the frontal, parietal, occipital lobes and the midbrain. Fractures concentrated on the occipital, frontal and the temporal bones.

The killing of the animals in this study was performed by a single, experienced person (Dalla Costa et al., 2020).

However, manually applied blunt force trauma is prone to errors in its application and highly dependent on the strength and skills of the operator (EFSA, 2020; Grist et al., 2017; Grist et al., 2018a; Whiting et al., 2011). Proper training of persons is crucial (AVMA, 2020; EFSA, 2020; Whiting et al., 2011; EFSA, 2004). For the operator, the method is unpleasant and physically exhausting (AVMA, 2020; Dalla Costa et al., 2019; Grist et al., 2018b; EFSA, 2004). Operator fatigue can lead to an inconsistent application, resulting in loss of effectiveness of the procedure (AVMA, 2020; EFSA, 2004). Therefore, the number of animals killed by a percussive blow to the head shall be limited per person per day (Regulation (EG) No. 1099/2009; EFSA, 2004). The method is in its effects less reproducible between animals (EFSA, 2020; Grist et al., 2017). Its effectiveness decreases with increasing age and weight of the animals (Walsh et al., 2017). The likelihood of obtaining an immediate and humane killing in all cases is low (Grist et al., 2018a). Because of the high risk of incomplete concussion, the procedure is not recommended as an on-farm killing method and should be avoided (EFSA, 2020). According to Council Regulation (EG) No. 1099/2009 the method is not intended for routine use and should only be used where other methods are not available [Council Regulation (EG) No. 1099/2009; EFSA, 2004]. AVMA (2020) recommends replacing the percussive blow to head with alternative methods as much as possible, EFSA (2020) advises to use mechanically operated devices instead. As the percussive blow to the head may not result certainly in death of the animals (EFSA, 2004), a second intervention is requested by EFSA (2020) or even mandatory in some Member States (e.g. Germany). This killing method needs to follow immediately afterwards, especially when there is any doubt that the animal has not been effectively killed (HSA, 2016a; EFSA, 2020). A killing method as a second intervention could be bleeding. Bleeding is to exsanguinate the animal by severing both carotid arteries and both jugular veins, the throat can be cut from ear to ear. Alternatively, the knife can be inserted into the base of the neck towards the entrance of the thorax to sever all major blood vessels as they emerge from the heart (HSA, 2016a). It leads to a lack of blood in the brain and to death via a rapid decrease of the blood pressure (AASV and NPB, 2016).

The percussive blow to the head, if done properly, leads to a rapid loss of consciousness (AVMA, 2020). Successful induction of unconsciousness is recognizable by an immediate **collapse** of the animal, onset of **apnea** and onset of a **tonic seizure** with an extended head and fixed eyes and hind legs rigidly flexed under the body (EFSA, 2020). Clonic spasms of varying intensity follow (EFSA, 2020; Grist et al., 2018a). Incorrect application of the blow to the head (e.g. wrong placement of the blow, wrong choice of tool, poor restraint, lack of skills of operators, operator fatigue) will fail to induce unconsciousness and will cause severe pain (EFSA, 2020). After an ineffective application of the method, the animals show **failure to collapse**, **breathing** (including laboured breathing) and in extreme cases, **vocalisations** (EFSA, 2020). These animals need to be immediately re-stunned by a back-up-procedure before applying the subsequent killing method. After application of the killing method, state of death needs to be monitored repeatedly and confirmed before disposal of the carcass (EFSA, 2020).

Penetrative captive bolt

A penetrative Captive Bolt (PCB) device fires a retractable steel bolt that penetrates the skull and enters the brain. After the shot, this bolt is retracted automatically or manually (EFSA, 2020). The impact of the bolt on the skull causes brain concussion (EFSA, 2004) with an immediate loss of consciousness. Penetrating methods lead to prolonged unconsciousness, as the bolt entering the brain at high speed causes structural damage to brain tissue and generates pressure waves within the fluid medium of the brain. This results in further damage at sites distant from the bolt trajectory (EFSA, 2020; Lambooij and Algers, 2016; EFSA, 2004). Since the physical damage to the brain does not necessarily lead to the death of the animals, a killing procedure (e.g. pithing) must follow (Council Regulation No. 1099/2009; EFSA, 2020; Lambooij and Algers, 2016). During pithing, a flexible wire or propylene rod is inserted into the head through the hole of the previously penetrated bolt and pushed forward to the brainstem or, if long enough, to the upper spinal cord in direction to the tail (EFSA, 2020; HSA, 2016b). Movements of the rod back and forward ("fiddling") lead to maximum mechanical destruction of the brain and upper spinal cord, the occurrence of convulsions is reduced (AASV and NPB, 2016; HSA, 2016b; EFSA, 2020). Pithing may be difficult to perform due to the convulsions that occur immediately after application of the captive bolt shot (Meier, 2020).

Cartridge-driven penetrative captive bolt guns with calibers 0.22 and 0.25 and in the in-line (cylindrical) and pistol grip (similar to a handgun) versions can also be used for on-farm killing (EFSA, 2020; EFSA, 2004). Until now, commercially available PCB guns have been designed for use on adult animals in terms of their dimensions and bolt exit length. As a result, the use on (suckling) piglets was not practicable and associated with a risk to the operator (e.g. exit of the bolt on the underside of the head; TVT, 2014; Marahrens, 2014b). Recently, penetrating captive bolt devices applicable to suckling piglets have been developed and their use on piglets may result directly in death according to a first study. In this study by Meier (2020), two penetrating captive bolt guns were modified for their use in piglets up to 30 kg body weight and investigated for their suitability as a single-stage killing procedure. By using a bolt that is relatively long in relation to the size of the head, as well as by determining the appropriate shooting position, the death of the animal should be induced after bolt firing by the destruction of the brain stem as a result of the entering bolt (similar to the effect of pithing) and without a following killing procedure. For piglets up to 5 kg the captive bolt gun "Drei Puffer" (now called "Ferkelblitz", bolt exit length: 5.3 cm, turbocut Jopp GmbH) was used. For heavier animals up to 30 kg, the device "Blitz Kerner" ("bolt exit length: 8.3 cm", turbocut Jopp GmbH) was applied, in both cases taking the green cartridges. The target shooting position for suckling piglets was approx. 1-2 cm, for larger piglets approx. 3-3.5 cm above eye level in the median with the shooting direction as parallel as possible to the longitudinal body axis in direction to the tail. Animals weighing up to 2.5 kg were restrained using a specially developed head restraint, while heavier animals were restrained in a commercially available load-securing net for vehicles (*Figure 3.2.1.*). It was necessary to be able to bend the piglet's head in the net so that there was a right angle between the forehead and the neck for the correct positioning of the captive bolt gun. Despite minor deviations in the targeted shot position in 24% of the animals, 98.5% of the animals (n = 198, weight: 0.48-39 kg) were successfully killed in one step. Only one animal was judged to be insufficiently stunned as a result of respiratory movements and, like the one of the two total animals classified as questionable, was re-shot in the study. Pathological examinations in 16 animals showed varying degrees of brainstem destruction. Disadvantages of using the method, according to Meier (2020), may be the strong convulsive activity after the shot for about two minutes (in 94.1% of piglets). Furthermore, the release of blood from the gunshot wound may contribute to the rejection of the method by users and bystanders. In the study of Meier (2020), most of the animals died directly after application of the penetrative captive bolt. However, according to Regulation (EC) No. 1099/2009, a killing procedure needs to follow afterwards up to now.

Figure 3.2.1. Fixation of a piglet with the help of a load securing net for correct head positioning and positioning of the penetrating captive bolt gun in the study of Meier (2020) (© C. Meier, Training and Consultancy Institute for animal welfare at transport and slaughter, Schwarzenbek)

Successful induction of concussion following a penetrative captive bolt shot is recognizable by an immediate **collapse** of the animal, onset of **apnea** and onset of a **tonic seizure** with an extended head and fixed eyes and hind legs rigidly flexed under the body (EFSA, 2020). The forelegs can be straightened out after initial flexion. Clonic convulsions with kicking movements follow (EFSA, 2020; HSA, 2016b).

Incorrect application of the penetrative captive bolt shot to the head (e.g. wrong placement, lack of skills of operators, operator fatigue, poor restraint) will fail to induce unconsciousness and will cause severe **pain** due to the impact of the bolt on the skull (EFSA, 2020). The same applies to incorrect captive bolt parameters (velocity, exit length, diameter of the bolt). When the characteristics of the used device are not suitable (e.g. low cartridge power, low bolt velocity, shallow penetration, too narrow bolt diameter), the energy transfer is not sufficient to trigger the concussion for loss of consciousness (EFSA, 2020; EFSA, 2004). The equipment and cartridges recommended by the manufacturer for the animal category should be used (HSA, 2016b). After an ineffective application of the method, the animals show **failure to collapse**, **breathing** (including laboured breathing), the absence of tonic and clonic seizures and in extreme cases, **vocalisations** (EFSA, 2020, HSA, 2016b). After application of the killing method, state of death needs to be monitored repeatedly and confirmed before disposal of the carcass (EFSA, 2020).

Restraint may cause pain in piglets but is decisive for the correct placement of the captive bolt shot. (EFSA, 2020). Training of personnel and their rotation on farm, the use of an appropriate equipment (device and adequate cartridges), that is ready to use and regularly maintained, and the delivery of an accurate and appropriate shot to the head are recommended measures to avoid incorrect application of the method due to an incorrect shooting position or incorrect captive bolt parameters. Operators need to be trained in adequate (un)consciousness monitoring procedures in order to prevent and correct potential stun failures. Stun failures must be corrected immediately by appropriate back up procedures (EFSA, 2020).

Sufficient backup devices must be kept on site (EFSA, 2020; AVMA, 2020). The cartridges are to be stored in a dry and cool place so that firing of the cartridges and sufficient force generated to drive the bolt remain guaranteed (EFSA, 2020).

Electrical methods

Two electrical killing methods are distinguished: One-step procedures of head-to-body application and two-step procedures with head-only stunning followed by a killing method.

One-step procedures of head-to-body application include a simultaneous electrical flow through the brain and heart. These procedures require a good restraint of the animal to ensure correct electrode placement and continuous contact of the electrodes (EFSA, 2020). More equipment is necessary for head-to-body-application of electric current. Therefore, it is more likely to be used in larger abattoirs (Grandin, 2020) or for the killing for disease purposes (i.e. Lambooy and van Voorst, 1986) and less likely to be available to the farmer for killing individual animals.

For piglets, the two-step method with head-only electrical stunning followed, without any delay, by a killing procedure is used. A sufficiently high current is used to disrupt normal brain function and to produce an epileptiform seizure accompanied by a loss of consciousness (AVMA, 2020; EFSA, 2004; Gregory, 1985, cited by Anil, 1991). The optimal electrode position for head-only electrical stunning is on both sides of the head between the eyes and the ears to apply a minimum current of 1.3 A for at least 3 s (EFSA, 2004; EFSA, 2020, HSA, 2013). There are disadvantages in using the method with piglets: Many of the commercially available stunning devices use too high voltages, which are associated with burns and can lead to uncertainties regarding the correct current path. In addition, the electrodes on the market are usually not adapted to the small piglet head. The duration of epileptiform activity can be shortened in weakened piglets or severely depressed piglets (Meier and von Wenzlawowicz, 2017; von Wenzlawowicz, 2014).

The subsequent killing methods are electrocution or bleeding for piglets over 5 kg body weight, and only bleeding for animals under 5 kg. The electrocution is the application of electric current through the heart during a second current cycle across the chest leading to ventricular fibrillation followed by cardiac arrest (AVMA, 2020; EFSA, 2020; HSA, 2013). Due to uncoordinated contractions of the heart muscle fibres, the blood flow stops and the death of the animal occurs (HSA, 2013). This second current cycle should only be applied after confirmed loss of consciousness and during tonic seizures, as it may be difficult to apply the electrodes to the chest during clonic seizures (AVMA, 2020; EFSA, 2020). A minimum current of 1.3 A should be used for at least 3 s (EFSA, 2020). The frequency of the alternating current should not exceed 50 Hz (EFSA, 2020) to 100 Hz (HSA, 2013) to induce ventricular fibrillation. Electrocution is not suitable as a killing method for piglets below 5 kg body weight, as the ventricular fibrillation - probably due to the small size of the heart - cannot be reliably induced (Meier and von Wenzlawowicz, 2017; TVT, 2014; von Wenzlawowicz, 2014). Other authors attribute this to the high heart rate of the piglets (Lambooy and van Voorst, 1986). Therefore, these smaller animals need to be killed by exsanguination after head-only electrical stunning (Meier and von Wenzlawowicz, 2017; von Wenzlawowicz, 2014).

Husheer et al. (2017, 2020) has examined the electrocution of suckling piglets (up to 2 kg) by using skin penetrating needle electrodes (1 x 2 cm^2) combined with electrode gel. After electrical stunning (electrode placement above the temples, 1.30 A, 50 Hz, 20 s), electrocution was effective in piglets when applying a current of 0.75 A at a frequency of 400 Hz first laterolateral (side-to-side) through the thorax for 5 s and after a break of 20-30 s dorsoventral through the thorax for 5 s. The method of Husheer (2017) and Husheer et al. (2020) has not yet reached practical maturity. Before making any further recommendations on the application of this method, it is necessary to wait until this electric killing of piglets is ready for practical application.

Electrical killing, if done properly, leads to an instantaneous loss of consciousness. Successful induction of unconsciousness is recognizable by an immediate **collapse** of the animal and onset of **tonic seizures** during the flow of the current. **Breathing** is absent, the **eyeballs** are **rotated in the socket**. **Clonic seizures** follow with subsequent relaxation and **loss of muscle tone**. At this stage, **responses to corneal, palpebral and external stimuli** (e.g. nose prick) are abolished.

Wrong placement of the electrodes (e.g. incorrect position of the head electrodes to span the brain, wrong application of the second cycle to span the heart, slipping of the electrodes) will fail to induce unconsciousness and can lead to painful induction of cardiac arrest. Poor electrical contact may arise if the operator fails to maintain the electrical contact when the animal collapses. The electrical contact is no longer sufficient to facilitate the current to induce loss of consciousness (head current) or cardiac arrest resp. electrocution (heart current). Too short exposure times or inappropriate electrical parameters (e.g. wrong choice of electrical parameters) also fail to induce consciousness and cardiac arrest resp. electrocution (EFSA, 2020). Lack of care or improper cleaning of the electrodes leads to a built-up of grease and dirt or to corrosion, which, by increasing the initial resistance, leads to the electrical parameters no longer being sufficient to induce unconsciousness and cardiac arrest (HSA, 2013).

After an ineffective application of the method, the animals show **failure to collapse**, **breathing**, the **absence of tonic and clonic seizures** and in extreme cases, **vocalisations** (EFSA, 2020). These animals need to be immediately re-stunned by a back-up-procedure (preferred by a penetrative captive bolt shot) before applying the subsequent killing method. After application of the killing method, death needs to be thoroughly confirmed before disposal of the carcass (EFSA, 2020).

Pain and fear during restraint and application of the electrical killing method should be prevented, when the restraining and killing equipment is adequately designed and maintained and the staff is trained. The application of the method in a group of pigs, as far as possible, will reduce fear as the animals are not separated and handled from their conspecifics. By limiting the space for the animals to move away (e.g. by using boards or walls), fear leading to escape attempts should be reduced and pain as a result of incorrect placement of the electrodes or a non-continuous electrical contact should be prevented (EFSA, 2020).

Gas killing methods (gas and gas mixtures)

The principle of gas killing is that one or more animals, individually or in a group, are placed in a container or gas chamber and they are killed in one step (without a subsequent killing method) by exposing them to gases, gas mixtures (e.g. Sutherland et al., 2017; Verhoeven et al., 2016) or gas-filled foam (see chapter 3.2.3.; Balzer, 2017; Lindahl et al., 2020; Pöhlmann, 2018; Sindhøj et al., 2021; Wallenbeck et al., 2020). Containerised gassing systems using carbon dioxide (CO_2), nitrogen (N_2), argon (Ar) and helium (He) have been studied and partly developed for killing of pigs on farm (e.g. Kells et al., 2018; Machold et al. 2003a,b, Machtolf et al. 2013; Rault et al., 2013; Sutherland et al. 2017; Velarde et al., 2007). Gradual filling is when a small group of pigs or a single pig is placed into a gassing container and subsequently a chosen gas or a gas mixture is administered to displace the atmospheric air in the container. Pre-filled or pre-charged gas killing methods include placing the animals in chambers that already contain gas of the desired target concentration. When placing the animals in the prepared gas atmosphere, there is a risk of significant reduction of effective gas concentrations on entry of the animals, what already has been described for poultry (Raj and Gregory, 1990). Gas killing procedures do not lead to an immediate loss of consciousness (EFSA, 2004; Raj et al., 1998). The induction of unconsciousness with gases or gas mixtures is a successive process (EFSA, 2004). Therefore, the goal must be to use as little aversive gas or gas mixture as possible and to minimize the time to loss of consciousness during gas exposure to reduce stress of the animals during the induction phase (EFSA, 2004; EFSA, 2019a; Gerritzen et al., 2000; Raj et al., 1998; Raj and Tserveni-Gousi, 2000). The duration of the induction phase depends on the gas concentration and the exposure time required to kill animals and vary depending upon the gas mixture (EFSA, 2020) as stated below:

- 90% CO_2 in air: 3 minutes
- Inert gasses (i.e. N_2, Ar) with less than 2% O_2: 7 minutes
- Mixtures of 30% CO_2 with inert gasses (N_2or Ar) with less than 2% O_2: 7 minutes.

Before the carcasses can be removed for disposal, at the end of the individual exposure time the gas must be evacuated and the death of each animal has to be ensured. If any animal shows signs of life (e.g. gagging), a back-up killing method (e.g. captive bolt) should be applied immediately.

In the following, scientific findings on the use of carbon dioxide, argon, nitrogen as well as different gas mixtures for stunning and killing pigs, and in this case especially piglets, are summarized.

Carbon dioxide (CO_2) is a narcotic gas that produces unconsciousness (Forslid, 1987). The minimum concentration of CO_2 to be applied is 80 % according to Council Regulation (EC) No. 1099/2009, preferably 90 %. Inhaling high concentrations of carbon dioxide leads to a hypercapnic hypoxia state (Dodman, 1977). The respiratory acidosis leads to a stimulation of the chemosensitive respiratory centre (Nattie, 1999; Troeger, 2008) and thus to a reflexive increase in respiratory frequency (hyperventilation) with the welfare consequence of **respiratory distress** during the induction phase prior to loss of consciousness (Gregory et al., 1990; Hartung et al., 2002; Llonch et al., 2012a). Respiratory distress includes the feeling of breathlessness, air hunger or chest tightness resulting in laboured breathing (Beausoleil and Mellor, 2015, EFSA, 2020). Not only the pH of the blood, but also the pH of the cerebrospinal fluid (Cantieni, 1977) decreases due to the respiratory acidosis (Guyton and Hall, 2001 cited in Mota-Rojas et al., 2012) and subsequently leading to a state of analgesia and anesthesia (Woodbury and Karler, 1960). However, the anaesthetic effect of CO_2 does not lead immediately to unconsciousness (Raj and Gregory, 1995; Rodriguez et al., 2008; Troeger, 2008). Therefore, as a result of the hyperventilation that begins at high concentrations of CO_2 (Gregory et al., 1990), animals are subjected to a sensation of respiratory distress for approximately 10-20 seconds during the induction phase before loss of consciousness (Troeger, 2008). CO_2 forms carbonic acid in reaction with water, resulting in irritation of the nasal and ocular mucosa as well as in the lungs (Peppel and Anton, 1993, as cited in Dalmau et al., 2010b; Dodman, 1977). This irritating effect (pungency) results in aversive responses in animals such **as retreating, escape attempts, vigorous head shaking, and vocalisations** (Dalmau et al., 2010b; EFSA, 2004; Llonch et al., 2012a,b; Machold et al., 2003a; Raj and Gregory, 1995, 1996; Rodriguez et al., 2008). The extent of aversive reactions increases with increasing concentrations of CO_2 (Dalmau et al., 2010b; Velarde et al., 2007). However, higher CO_2 concentrations are associated with a faster loss of consciousness compared to lower CO_2 concentrations or even to mixtures of CO_2 and inert gases (Verhoeven et al., 2016; Meyer, 2013; Velarde et al., 2007). Reduction of pre-slaughter stress and high CO_2 concentrations support faster onset of unconsciousness and shortening of the aversive induction phase (von Holleben und von Wenzlawowicz, 2020).

Nitrogen (N_2) and Argon (Ar) as inert gases are attributed to induce loss of consciousness and insensibility by hypoxia or anoxia and **without causing signs of aversion** (Machold et al., 2003a,b; Raj and Gregory, 1995) and **respiratory distress symptoms** (Llonch et al., 2012a; Machold et al., 2003a,b; Raj and Gregory, 1995) in slaughter pigs, due to the lack of stimulation of the respiratory centre (Llonch et al., 2012a) as neither mammals nor birds have chemoreceptors for inert gases (Berg and Raj, 2015).

Nitrogen is present in air at a concentration of almost 80 %, hence its extraction is significantly cheaper than, for example, for argon (Troeger et al., 2004a; Raj et al., 2008; Llonch et al., 2012b). Nitrogen is not stable in the atmosphere due to its lower relative density compared to air, where stability is defined as the ability of a gas to maintain a target concentration in the open system (pit) without being displaced by oxygen (Troeger et al., 2004b; Dalmau et al., 2010a,b,). Therefore, animal experiments with pure **gaseous nitrogen in high concentration** which are required for stunning (< 2% residual O_2 content) **cannot be carry out in open systems so far**. Hence, today only a few studies investigated the effects of nitrogen for stunning or killing purposes. It is known from the literature that high concentrations of pure nitrogen can lead to **a rapid and painless loss of consciousness** within 15 seconds (Ernsting, 1965), which is sometimes accompanied by euphoric effects (Haldane, 1947). As reported by Herin et al. (1978), recordings from EEG and arterial blood pressure showed that dogs which were euthanized in a chamber flushed with pure nitrogen were unconscious in an average time of 40 s and died within 204 s, without displaying detectable signs of pain before they lost consciousness. Zhang (2013) reported that hat the rapid introduction of N_2 (achieving < 2 % O_2 within 3 minutes) led to unconsciousness within less than one minute without recovery, which was accompanied by minimal irritating behaviour prior to unconsciousness and seizures for up to 4 minutes while the pigs of approximately 30 kg and smaller were unconscious.

Argon is heavier than atmospheric air and therefore easier to concentrate in open stunning or killing systems (Dalmau et al., 2010a; Sindhøj et al. 2021). As shown by Raj and Gregory (1995), pigs **did not show aversive behaviours, nor discomfort or fear** when they enter a box filled with 90% argon for a reward. Other studies also reported improved pig welfare when pigs were stunned with Ar compared to CO_2 (Machold et al., 2003a; Kells et al., 2018). In contrast, Dalmau et al. (2010b) observed **indications of aversion** for slaughter-weight pigs exposed to Ar (90%). Moreover, Machold et al. (2003a) observed a **longer duration of convulsions and time to loss of posture** for 90% Ar compared to high-concentrated CO_2. However, results for slaughter pigs cannot be directly transferred to piglets. Several studies on piglets also found no advantage of 100 % argon compared to other gases or gas mixtures. Kells et al. (2018) observed **more and longer vocalisations** (grunts) and **convulsions** and a **longer time to loss of posture** and the onset of a transitional and isoelectric EEG compared to 100% CO_2 or a mixture of Ar/CO_2 in piglets of approximately 17 days. Although loss of consciousness was delayed in the argon atmosphere compared to CO_2 and CO_2/Argon (Kells et al.,2018, Sadler et al.,2014 b,c), Kells et al. (2018) assumed that animals were less distressed under anoxic conditions, based on a decreased prevalence and duration of escape attempts and a decreased intensity of laboured breathings. Further, Kells et al. (2018) attributed the vocalisations (grunting before loss of posture and not during escape attempts) in argon and Ar/CO_2 to exploration behaviour under non-aversive environmental conditions. Sutherland (2011) found that the exposure of piglets (18 days) to 100 % Ar resulted in an increasing number and duration of vocalisations, a delayed onset of unconsciousness, as well as a longer time to cardiac arrest compared to an exposure to 100 % CO_2. Further, Sutherland (2011) reported that in 100% argon a transitional and isotonic EEG could be observed later than during exposure to 100% CO_2. Sadler et al. (2014c) showed that exposure in 100 % Ar **increased time to loss of posture** (**delayed onset of unconsciousness**) compared to 100 % CO_2, increased duration of **open mouth breathing** and **ataxia**, **increased the prevalence of righting attempts** and a **prolonged time to respiratory arrest** of suckling piglets determined for euthanasia. Furthermore, Sadler et al. (2014c) had to terminate the gradual-filling method for 100 % argon after only two repetitions as well as the prefilled argon method after six repetitions, due to ethical concerns regarding a high proportion of piglets displaying signs of sensibility after 10 minutes of exposure with the necessity to perform a secondary euthanasia step. In another study, Sadler at al. (2014b) also found that CO_2 exposure was associated with superior welfare of 3 to 10 weeks old pigs compared to the argon exposure (prefilled), due to a **shorter latency to loss of consciousness**, a **shorter duration of ataxia** and a **decreased intensity and duration of righting responses**.

Mixtures of nitrogen or argon with CO_2 exhibit an increased stability in the atmosphere (Dalmau et al., 2010a), so that in particular mixtures of N_2/CO_2 can be applied in open systems.

Mixtures of nitrogen and carbon dioxide with a residual O_2 content < 2%, (similar to mixtures of argon and CO_2) proved to be less aversive than carbon dioxide in high concentrations (Raj et al., 1997; Dalmau et al., 2010b; Llonch et al., 2012a). However, for the **same exposure duration**, the **induction of stunning is prolonged** and the **duration of unconsciousness is shortened** using **nitrogen mixtures compared to carbon dioxide** (Llonch et al., 2012b; Llonch et al., 2013). Moreover, in **gas mixtures of N_2/CO_2**, the **duration of muscular excitation** following anaesthesia is **longer than after anaesthesia using 90% CO_2** (Llonch et al., 2012b; Llonch et al., 2013). These convulsions result from the stronger **inhibition of the posterior brainstem** region (Ernsting, 1965) due to anoxia compared to hypercapnia (Raj, 1999). Longer lasting seizures up to four minutes in piglets (≤ 30 kg) killed with nitrogen at various introduction rates were also observed in the study of Zhang (2017), however the pigs were classified as unconscious at this time. The rapid N_2 introduction in parallel with the reduction of O_2 to < 2% within 3 minutes lead to unconsciousness within less than one minute (Zhang et al., 2013).

Mixtures of argon and carbon dioxide were studied for example by Raj et al. (1997), who revealed that hypercapnic anoxia using 30% CO_2 in argon led to a rapid loss of SEPs in the EEG within 11-20 seconds, comparable with CO_2 in high concentrations. In contrast to CO_2 in high concentrations, the majority of pigs (75%) did not show aversive reactions when CO_2 and argon were combined (Raj and Gregory, 1995). Following Raj and Gregory (1996), the order of preference of these gases would be **2 % O_2 in argon (anoxia)** which is accompanied by **minimal respiratory distress but a relatively slow induction of anaesthesia**, followed by **30% CO_2 in argon** with **2 % residual O_2** accompanied with an intermediate respiration score and rate of induction of loss of consciousness; and finally, **90 % CO_2 in air**, where **anaesthesia is rapidly induced but accompanied with short lasting, but severe respiratory distress**. Contradictory, Sadler et al. (2014a) could not achieve a reduction of the stress in neonatal piglets based on stress-related behaviour by applying a 50 % carbon dioxide/50 % argon mixture. This contradicts the results of the approach-avoidance tests of Rault et al. (2013), in which a lower aversion to a gas mixture of 30% CO_2/60% Ar compared to 90% CO_2 was found for piglets. Contrary to the authors cited above and although no significant differences were found between argon and argon/CO_2 mixtures, based on the calculation of a welfare index, Sutherland (2011) recommends the use of gases to induce hypercapnic anoxia over pure argon due to significantly more rapid loss of consciousness, faster onset of isoelectric EEG and respiratory arrest. Moreover, it should be noted that the use of inert gases in gradual filled chambers is to be rejected for ethical reasons, due to a prolonged induction phase and therefore only the use in prefilled chambers is generally recommended (Sadler et al. 2014c).

<u>**General remarks on condition and age of the piglets or stocking density:**</u>

The general condition of the piglets (severely depressed piglets vs. other piglets) is not a decisive factor for the effectiveness of gas stunning and killing with carbon dioxide (regardless of the way the gas is provided), but it does have a negative effect on the anoxia process using argon (Sadler et al., 2014c). In argon, the time to loss of consciousness as well as the duration of open-mouth breathing is significantly prolonged ($p<0.05$) in moribund piglets with decreased respiration rate and tidal volume compared to other piglets, while the time of last limb movements is faster and the duration of ataxia and righting attempts is shortened. Sadler et al. (2014c) attribute these differences in effectiveness to the different effect mechanisms of the two gases. CO_2 causes hypercapnia and affects multiple organ systems by lowering the pH value (even in the blood and interstitial fluid), so that the euthanasia process is equally possible regardless of the disease status of the animal. Therefore, the acidotic status of the moribund animal (Straw et al., 2009, cited by Sadler et al., 2014b) is not an inhibiting factor. Argon, on the other hand, produces a hypoxic state, which can additionally complicate euthanasia in animals with impaired lung function. Moreover, the assumption that euthanasia is more difficult to achieve in **neonatal compared to older weaned piglets** could be rejected by the studies of Sadler et al (2014 a,b). In neonatal piglets, with the exception of the duration of ataxia, an earlier loss of posture and earlier time of last movements during gas killing could be demonstrated while stress-indicating behaviour is less pronounced. As Fiedler et al. (2016) revealed, pigs (3–10 weeks) euthanized in groups of two or six in high-concentration argon paced less and made fewer escape attempts than pigs euthanized in the solitary treatment. This suggests that if euthanasia is indicated for more than one animal at the same time, it is better to do it in groups. Separate killing of individual animals should be avoided in this case.

<u>**General remarks and recommendations:**</u>

- **Exposure time** should be appropriate to the gas concentration at the animal level and long enough to induce death in all pigs. The lower the CO_2 concentration or higher the residual oxygen level in inert gases the **longer the time to death**. Vice versa, **too low CO_2 concentrations** or a **too high (residual) oxygen content** using inert gases **fail to induce loss of consciousness or death** at the given exposure time (EFSA, 2020). Therefore, it should be noted that as increased amounts of oxygen-rich ambient air enter the prepared gas atmosphere via air pockets between the animals, the **residual oxygen content** as decisive factor for the fast induction of unconsciousness and effectiveness of anoxia-based killing methods should nonetheless be **lower than 1%** (Raj et al., 1990). In addition, too low CO_2 concentrations will prolong the induction of unconsciousness, leading to prolonged **respiratory distress** (Raj and Gregory, 1996).
- **Exceeding the capacity** of the equipment by exposing too many piglets at the same time may lead to climbing behaviour and agonistic interactions (EFSA, 2020). Hence**, injuries**, **escape attempts** as well as **pain and fear** are possible consequences.

- **Administration of liquid or solid carbon dioxide** into the gassing chamber due to the lack of vaporization or injection of gas mixtures directly from high pressure cylinders on animals without preheating may lead to very low temperatures (even below 0 °C) of the gas or even freeze-burnings (Meyer and Morrow, 2005). The Inhalation of dry cold gas can cause **pain** to the pigs (EFSA, 2020). Therefore, the target gas temperature should be monitored and maintained preferred around the recommended ambient temperature of the animals continuously throughout the entire process.
- **Signs of aversion** like **escape attempts** (O'Malley et al., 2018), **high-pitched vocalisations** (Dalmau et al., 2009; Welfare Quality®, 2009), **head shaking** (EFSA, 2004; Velarde et al., 2007), **gasping**, **hyperventilation** (EFSA, 2004; Raj and Gregory, 1996) can be used as animal-based indicators related to pain, fear and respiratory distress as the three main welfare consequences during gas killing. As clarified by Velarde et al. (2007), a distinction should be made between breathlessness and gasping. Gasping is a physiological reaction associated with breathlessness during the inhalation of the gas, which can be observed as breathing through a gaping-open mouth (Lambooij et al., 1999; Raj and Gregory 1996). It corresponds to rudimentary respiratory activity based on medullary activity in the brainstem induced by hypercapnia (Gregory, 2008). Although it is not an expression of aversion, it is considered to compromise animal welfare before loss of consciousness. Furthermore, studies revealed that in some species under certain conditions of administration (e.g. gradual displacement) gaseous agents which were identified to be less aversive compared to CO_2 (e.g. Ar or N_2 gas mixtures) have still the potential to cause respiratory distress (e.g. hyperventilation and a sense of breathlessness) during the induction phase prior to loss of consciousness (Gregory et al., 1990 as cited in Dalmau et al., 2010b).
- **Effectiveness of stunning** can be monitored by **loss of posture**, no rhythmic breathing, **absence of corneal and palpebral reflex**, **no vocalisations**, **fixed eyes**, **rotated eyeballs** (EFSA AHAW Panel, 2013).
- At the end of the killing procedure, before the animal(s) are disposed the **absence of muscle tone**, **absence of breathing**, **absence of corneal and palpebral reflex**, **absence of heartbeat**, as well as **dilated pupils** (mydriasis) (EFSA AHAW Panel, 2013) indicated that the animal(s) died.

However, observation of these ABM's including assessment of unconsciousness may prove to be extremely difficult or impossible in gas containers without windows (e.g. Euthanex® AgPro) (Fiedler et al. 2014, 2016). In these cases, it is important that resource based measures as **gas concentration** (Rice at al., 2014), **gas temperature** (Meyer et al., 2014), **foam levels** (Pöhlmann, 2018) and **exposure time** are monitored thoroughly to maximise the efficiency simultaneously with decreasing possible shortcomings regarding animal welfare.

According to AVMA (2020), the use of carbon dioxide to kill small groups of suckling piglets as well as the application of Ar- or N_2-CO_2 gas mixtures for pigs, provided that animals could be directly placed into a < 2% O_2 atmosphere and exposed to the gas for more than 7 minutes, are methods "acceptable with conditions" for euthanasia. *"Methods acceptable with conditions are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure* *death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method can be met."* (AVMA, 2020). Therefore, carbon dioxide is acceptable in those species where aversion or distress can be minimized, for example by using a gradual-fill method. Further, the gases need to be used in pure form using commercially available gas cylinders or tanks and suitable equipment (e.g. pressure-reducing regulators, flow meters) in order to ensure an effective displacement rate and/or concentration in the gas-filled container. (AVMA, 2020).

New developments

The use of gas-filled foam and the application of the non-penetrative captive bolt are new developments that are currently not covered by Regulation (EC) No. 1099/2009.

Gas-filled foam

The immediate provision of nitrogen in high concentrations (> 98%) next to the animal without necessary excessive sealings of the container to avoid mixing with air has become possible for killing individual or small groups of pigs due to a new procedure using **highly expansive nitrogen-filled foam** (expansion rates > 1:250, > 1:300, up to 1:500) (van der Aa et al., 2020; Lindahl et al., 2020; McKeegan et al., 2013). To prevent animals from drowning, it is important that the **water content in high expansion foam is very low**, and that the **bubble size is large enough** (> 10-20 mm) to minimize the chance of bubbles entering/occluding the trachea (Gerritzen et al., 2010). Both can be assessed from the **size of the bubbles** (Gerritzen and Gibson, 2016; Gerritzen and Sparrey, 2008; McKeegan et al., 2013; Raj et al., 2008) preventing obstruction of the respiratory tract.

Recent studies have investigated the application of high expansion foam filled with N_2 gas for stunning and killing pigs (Balzer, 2017; Lindahl et al., 2020; Pöhlmann, 2018; Wallenbeck et al., 2020) and poultry (Gerritzen et al., 2010; Gerritzen and Sparrey, 2008; McKeegan et al., 2013; Raj et al., 2008). As Pöhlmann (2018) investigated for slaughter pigs, exposure times of 3.5 min to N_2 filled foam were **not sufficient to assure unconsciousness and insensibility**. In addition to aversive behaviours in form of **escape attempts** and **vocalisations** in 67% of the pigs, they observed **gasping** as well as pigs which **regain sensibility** shortly after stunning, which subsequently led to an extremely **high rate of re-stunning** (22%). Due to the fixation of pigs in a hammock, it was not possible to obtain the time to loss of posture. Similar results were found in the study by Balzer (2017), which was conducted on the killing of moribund, 3-day-old sucking piglets, although a nitrogen concentration of more than 98 % (residual O_2 < 1%) and bubble sizes of 15 mm were achieved. The **onset of apnea**, indirectly determined by the increase in pCO_2 to values above 20 mm Hg of the previously measured resting (baseline) values (Nagakawa et al., 2011), was late and occurred 4,1 ± 0,99 minutes after the animal was submerged in the foam. The latency period until the last movement was 296.8±155.33 s. The **confirmation of cardiac arrest** (i.e. cessation of electric activity of the heart) in case of N_2-anoxia for the determination of death (AVMA, 2020) was not evident in the study of Balzer (2017), but the **areflexia**, which is defined as the **complete absence of one or more reflexes**, present in each animal after 10 or 12 minutes of exposure, suggested the induction of brain death. A recently published study on pigs of approximately 30 kg which were exposed to air- (control) or nitrogen-filled foam (< 1% O_2) revealed that the method applied did **not lead to any strong aversive behaviours** of the pigs (Lindahl et al., 2020; Wallenbeck et al., 2020). However, pigs tried to **avoid to put their heads and snouts into the foam** and therefore the prevalence of **escape attempts** through the lid of the box increased as the foam levels became high. The

heart and respiratory rate of the pigs increased with decreasing O_2 levels during exposure to the nitrogen-filled foam, **loss of posture** occurred after a mean time of 57.9 s from starting the foam production followed by a period of **vigorous convulsions** of 132.5 s (period between loss of posture and last observed muscle contraction)**.** After 5 min from starting N_2-filled foam production, the pigs were removed and the status of the pig was classified either in **deep unconsciousness or dead**. The contrasting results of these studies can be attributed to the fact, that Balzer (2017) as well as Pöhlmann (2018) utilized an earlier prototype of the foam generator with lower capacity than those used by Lindahl et al. (2020) and operated in an open top container. Several structural adjustments to the technology were necessary during these studies. The technique initially did not yet consistently deliver the foam of the desired bubble size. Balzer (2017) and Pöhlmann (2018) reported both about spontaneously changing foam quality (formation of a small-bubble foam with bubbles smaller than 15 mm) due to longer running times of the foam generator as a result of liquid increasingly settling on the first grid in the nozzle, which was still installed at that time. Moreover, the lower capacity of the used foam generators especially in the study by Pöhlmann (2018) complicated the full coverage of the pigs' heads with foam as they simultaneously broke down the foam while moving. Technical modifications and supplements were made to ensure a sufficient amount of foam to cover the animal. To safely prevent the access of single animals to oxygen-rich ambient air, Pöhlmann (2018) recommended the construction of a closed system. Such a closed system was finally used in the study of Lindahl et al. (2020). The use of foam in closed systems with modification of the foam supply and the foaming agent showed promising results under practical conditions during stunning and killing of piglets. The foam generators were able to lower the O_2 concentrations to below 1% within 86 s (range 55–137 s) and maintained this controlled atmosphere throughout the exposure time. *Figure 3.2.2*.2.2 shows the current technical set-up of the foam technology. In this unit, after filling the container, the foam is destroyed by the influx of nitrogen to allow a view of the inside of the box (animal area) and to remove air pockets.

Figure 3.2.2 Foam system for piglets (© HEFT AB); after filling the box with nitrogen-filled foam (a), the foam is destroyed by the influx of nitrogen to allow a view of the inside of the box (animal area) and to remove air pockets (b).

<u>The risk factors during the killing of piglets with gas-filled foam are summarized in the following part with general remarks and additional recommendations.</u>

General remarks and recommendations:

- **Low foam production rates**, **destruction of the foam through vigorous movements** as well as **too short exposure times** may lead to an ineffective induction of unconsciousness, insensibility and death as well as high rates of pigs regaining consciousness (Gerritzen et al., 2010; McKeegan et al., 2013 Balzer, 2017; Pöhlmann, 2018; Lindahl et al., 2020). Therefore, as the method relies on the foam to burst open to release the gas, foam production rate should be sufficient to compensate for its destruction due to movements of the animals in order to maintain the required concentration of gases for induction of rapid unconsciousness and death (EFSA, 2020). Therefore, the **height of the foam layer above the animals** can serve as a suitable key parameter to control the process (McKeegan et al., 2013). The exposure time should be appropriate to the gas concentration at the animal level and sufficiently long to kill all the pigs. The higher the residual oxygen in the killing system, the longer the time to induce death (EFSA, 2020).
- **Exceeding the capacity of the equipment** (number of pigs per available floor space) may lead to injuries (Balzer, 2017; Pöhlmann, 2018; EFSA, 2020). Therefore, the capacity of the equipment must be monitored thoroughly during the entire killing process. During killing pigs in groups, a comparable animal size should be ensured to reduce the risk of injury during euthanasia (van der Aa et al., 2020).
- **Too small size of the foam bubbles** increases the risk of occluding the trachea as well as drowning of the animals (high water content) (Gerritzen et al., 2010; McKeegan et al., 2013). Bubble sizes of at least 15 mm (Balzer, 2017; EFSA, 2020, Pöhlmann, 2018; van der Aa et al., 2020) and an expansion ratio between 1:250 and 1:350 (EFSA, 2019b), better 1:500 (van der Aa et al., 2020) appeared to be the optimum compromise between foam stability, water content, bubble size and wetness (EFSA, 2019b).
- **Opaqueness of the foam** may impede behavioural observations (Gerritzen et al., 2010; McKeegan et al., 2013). Therefore, gas-filled boxes should always have a window or a transparent lid for observing the animals. Newly developed closed systems, in which the destruction of the foam is intended to facilitate the observation of the animals are recommended, since the foam here only enables the rapid displacement of the oxygen, but no longer prevents the nitrogen from escaping the chamber.
- **Signs of aversion** like **escape attempts** (O'Malley et al., 2018), **high-pitched vocalisations** (Dalmau et al., 2009; Welfare Quality®, 2009) **head shaking** (EFSA, 2004; Velarde et al., 2007), **gasping** and **hyperventilation** (EFSA, 2004; Raj and Gregory, 1996) can be used as animal-based indicators related to pain, fear and respiratory distress as the three main welfare consequences during gas killing.
- **Effectively stunned animals** can be indicated by **loss of posture**, **immediate onset of apnea**, **absence of corneal and palpebral reflex**, **no vocalisations**, **fixed eyes** and **obscured eyeballs** owing to rotation in the eye socket (EFSA AHAW Panel, 2013).
- **Death of the animal** at the end of the killing procedure, before the carcasses of the animals are disposed, must be confirmed by the **absence of body movement**, **absence of breathing**, **absence of corneal and palpebral reflex**, **absence of heartbeat**, as well as **dilated pupils** (mydriasis) (EFSA AHAW Panel, 2013). Due to the lack of detectability of cardiac arrest in case of N_2-anoxia for the determination of death (AVMA, 2020), which was shown in the study of Balzer (2017), the **areflexia** present in each animal after 10 or 12 minutes of exposure can be suggested as in indicator for the **induction of brain death**.

Please note, that for gas filled foam, the investigation of animals during the induction phase might be complicated due to the opaque foam covering the animal in the gassing chamber/box. Nevertheless, the animals should always be observed during the procedure, which is why a transparent lid or viewing window is urgently required. Therefore, features such as **gas concentration**, **foam levels** and **exposure time** should be monitored to guarantee sufficient exposure and to safeguard animal welfare as much as possible.

Non-penetrative captive bolt

Recent scientific research demonstrates the successful use of non-penetrating captive bolt to stun and kill piglets without an additional subsequent killing procedure (one-step procedure). There are cartridge driven and pneumatic non-penetrating devices that deliver the blow to the head with either blunt flat metal heads or mushroom-shaped bolts (Woods and Shearer, 2015). Up to now, the use of these devices on pigs is not permitted according to the Regulation (EC) No. 1099/2009.

According to the recommendations of the manufacturer, the "Cash Dispatch Kit" (Accles & Shelvoke, UK) equipped with non-penetrating captive bolt heads are recommended for use on piglets up to 5 kg. According to Woods (2012), these devices served as effective one-step euthanasia method for pigs up to 10 kg (Woods, 2012).

In the studies of Casey-Trott (2012) and Casey-Trott et al. (2013, 2014), the pneumatically powered non-penetrating captive bolt Zephyr-Euthanasia (Zephyr-E), using a two- or three-shot technique, resulted in immediate and sustained loss of consciousness and sensibility until death in neonatal piglets (< 72 h) without exception (Casey-Trott et al., 2013) and in suckling and weaned piglets in 98.6% of the animals (Casey-Trott, 2012; Casey-Trott et al., 2014). Severe skull fractures and widespread cerebral hemorrhages were found post mortem. For the application of a one-shot technique, the Zephyr E was further developed into the Zephyr EXL by increasing the velocity at 120 psi leading to a higher provided kinetic energy (at least 27.7 J; Grist et al., 2017).

The pneumatic non-penetrating captive bolt Zephyr-EXL (Accles & Shelvoke, UK) causes immediate loss of consciousness and death in piglets up to 10.9 kg body weight when applied once at 120 psi (Grist et al., 2017, 2018a). This has been verified in studies both, under laboratory conditions (based on the loss of visual evoked potentials immediately after firing (Grist et al., 2017) and under field conditions [based on behavioural indicators in piglets (Grist et al., 2018a)]. In addition to a correct frontal-parietal positioning of the device, good restraint of the piglet's head on a hard surface is crucial for the effective application of the method (*Figure 3.2.3*3.2.3). Massive traumatic damage to the cerebrum and hemorrhages as well as bone splinters up to the level of the corpus callosum were detectable post mortem in piglets euthanized by the one-shot-application of the Zephyr-EXL. (Grist et al., 2018a). Based on the rate of (one shot) killing success achieved with the Zephyr EXL in the study by Grist et al. (2018a) for all animals examined, and considering the underlying sample size, the proportion of animals not killed immediately ranges from 0 % to 1.2% with 95% confidence.

Figure 3.2.3. Necessary restraint of the piglets' head on a hard surface for the application of the non-penetrative captive bolt gun (Grist et al., 2018b; © A. Grist, University of Bristol, UK)

The cartridge-driven non-penetrating captive bolt "Cash Small Animal Tool" (Accles & Shelvoke, UK) results in immediate, reproducible one-shot stunning/killing in 147 neonatal piglets with a mean weight of 1.20 ±0.58 kg (average age: 5.8 days, median = 3 days) when restrained in a hammock and using the brown 1-grain cartridge (kinetic energy 47 J) and a frontal-parietal shot position (Grist et al., 2018b). Assessment was based on behavioural indicators and recovering (e.g., breathing, corneal and palpebral reflex, responses to painful stimuli). The result that 147 out of 147 animals were effectively stunned/killed provides a 95% confidence interval of 97.5-100% for the true percentage of animals that would be effectively stunned/killed with the CASH small animal device under the conditions of the current study (Grist et al., 2018b). Thus, the Cash Small Animal Tool leads to results comparable to the Zephyr EXL.

Application of the device using a more powerful 1.25-grain cartridge (kinetic energy: 107 J) also resulted in the successful killing of 55 additional neonatal piglets, but was associated with less nasal hemorrhages and greater laceration at the shooting position compared with the 1-grain cartidge. This fact and the excessive wear to gun components (esp. buffer) resulting in a shortening of its potential lifetime using cartridges with higher power without any benefits for animal welfare has led to the recommendation that the 1-grain cartridges, also sufficient for an immediate stun/kill in neonatal piglets should be used (Grist et al., 2018b).

Successful induction of concussion following a non-penetrative captive bolt shot is recognizable by an immediate **collapse** of the animal, onset of **apnea** and onset of a **tonic seizure** with an extended head and fixed eyes and hind legs rigidly flexed under the body (EFSA, 2020). Clonic convulsions of variable intensity follow (EFSA, 2020; Grist et al., 2018b).

Incorrect application of the non-penetrative captive bolt shot to the head (e.g. wrong placement due to lack of skills of operators, operator fatigue and poor restraint) will fail to induce unconsciousness and will cause severe pain due to the impact of the bolt on the skull (EFSA, 2020). The same applies to incorrect bolt parameters (velocity and bolt mass) leading to insufficient force and kinetic energy (when bolt energy is below 27 J; Grist et al., 2018a, b; EFSA, 2020). The colour of the cartridge informs about the bolt parameters and the suitability in terms of the animal weight class. Caution is needed because there may be differences between cartridges of the same colour of different manufacturers (see operating instructions of the manufacturer). After an ineffective application of the method, the animals show **failure to collapse**, **breathing** (including laboured breathing), the **absence of tonic and clonic seizures** and in extreme cases, **vocalisations** (EFSA, 2020, Grist et al., 2018b). After application of the killing method, death needs to be thoroughly confirmed before disposal of the carcass (EFSA, 2020).

Manual restraint for the immobilization of the piglet and its head is decisive for a correct positioning of the bolt shot, but may lead to **pain and fear** (EFSA, 2020). Staff training and their rotation on farm, the use of an appropriate equipment (device and adequate cartridges resp. power and bolt energy), that is ready to use and regularly maintained, and the delivery of an accurate and appropriate shot to the head are recommended measures to avoid incorrect application of the method due to an incorrect shooting position or insufficient force. Operators need to be trained in adequate monitoring procedures to assess (un)consciousness for prevention and correction of potential stun failures by appropriate back up procedures as well as signs of life to ensure death of animals before disposal of carcasses (EFSA, 2020).

3.3 Confirming death in euthanized animals

Operators must confirm the death of the animals before disposal of the carcass. Dead piglets show a **loss of muscle tone** leading to a **relaxed body**, **absence of breathing** (**apnea**), **absence of heartbeat and responses to corneal and palpebral stimuli** and **dilated pupils** (see section 4.2).

4 Key areas to focus on during animal-welfare inspections by use of animal-based indicators

In addition to the environment-based parameters required by law (Regulation 1099/2009) like e.g. device settings [e.g. electrical settings in case of electrical stunning and killing, (records of) gas concentrations in case of gas killing], this chapter deals with animal-based indicators inspectors should use to complement their welfare assessment.

We suggest to focus here on four key areas:

- **Appropriate decision-making in relation to euthanasia**
- **Handling and moving piglets to the killing point**
- **Killing process**
- **Post-mortem inspection of the carcass**

Pain and fear as well as **respiratory distress** are relevant welfare consequences that can occur in the context of killing piglets on farm. In the following, animal-based indicators (ABI) are identified to capture these animal welfare-relevant issues for each key area.

4.1 Appropriate decision-making in relation to euthanasia

Animals requiring euthanasia are subjected to a prolonged period of **pain and suffering** if euthanasia is not carried out at the appropriate time (große Beilage, 2017; OIE - Terrestrial Animal Health Code, 2019). Assistance in making the challenging decision about the need for euthanasia is provided in section 3.1.

The farmer should check the health of his animals at least once a day, or several times a day if necessary, on the basis of intensive animal observation. These inspections help identify such animals with severe, incurable injuries and diseases. By killing them as quickly as possible in an appropriate manner, animals are spared further and avoidable **pain and suffering** (DLG, 2018).

4.2 Handling and moving pigs to the point of killing

Moving animals by trolley is considered as the best option (EFSA, 2020). Effective and gentle handling can be checked by assessing animal movements and behaviour (no **slipping and falling**, no **escape attempts**, not being **reluctant to move** or **turning back, (high-pitched) vocalisations**) (see Section 3.1 and Table 3.2.1). For younger incurably injured or sick animals that do not walk independently to the point of killing but are moved there, **escape attempts and high-pitched vocalisations** can be used primarily as animal-based indicators to assess **pain and fear**.

4.3 Killing process

The killing process includes the necessary restraint before application of physical procedures (mechanical and electrical methods) as well as the application of the killing procedure itself.

According to EFSA (2020), pigs might experience **pain and fear** in the killing process in four cases:

1. during restraint before killing application (mechanical and electrical methods)
2. during gas exposure before loss of consciousness (during the introduction phase of gas killing)
3. during ineffective killing with persistence of consciousness (e.g. following an application failure)
4. during recovery of consciousness before death (re-awakening of animals, delayed or not properly done additional killing method)

During restraint (phase 1) and gas exposure (phase 2) ABI related to pain are escape attempts, high-pitched vocalisations and injuries. ABI related to fear are escape attempts, high-pitched vocalisations, reluctance to move and turning back (EFSA, 2020).

During the killing process itself (phase 3 and 4), the potential for pain and fear is linked to the s**tate of consciousness**. Consciousness is the capacity to receive, process, and respond to information from the internal and external environment, and thus the ability to feel emotions and be sensible to external stimuli that lead to pain and fear (Le Neindre et al.,2017; EFSA, 2020). Therefore, ABI for the state of consciousness serve to identify the potential for **pain and fear** in animals during the killing procedure. The outcomes of these ABI can either indicate consciousness (e.g. **presence of rhythmic breathing other than gasping**) or unconsciousness (e.g. **apnea**). Some of the ABI are specific for a killing method (e.g. **tonic-clonic seizures**) whereas others apply to several methods (see A.2 in the Annex of the Review). In two-stage procedures, these indicators serve to ensure that animals are unconscious prior to application of a subsequent killing procedure (EFSA, 2020). The control of the state of consciousness by using the ABI has already been discussed in detail in the description of the methods in subchapter 3.2. An overview of the ABI for the state of consciousness is given in A.2 in the Annex of this review, including a brief description of each indicator.

The application of physical (mechanical and electrical) killing methods will be limited to individual animals; the animal remains fully accessible to the inspector for the assessment of the ABI after application of the killing method. During gas killing in containers, the animals are not available to the observer, e.g., for performing stimulus-response tests or the control reflexes. Therefore, priority must be given to parameters that can be surveyed without further manipulation of the animal. The gas container should have an inspection window with a view on the animals. Under the mentioned circumstances, ABI for the state of consciousness during gas killing are vocalisations, posture and, as far as recognizable, rhythmic breathing other than gasping.

Respiratory distress is another welfare consequence that needs to be considered with gas killing methods during their induction, before animals have lost consciousness. It is mainly caused by an increased CO_2 concentration in the blood (Raj et al., 1997), but can also be induced, although less frequently reported, by a lack of oxygen or hypoxemia (Beausoleil and Mellor, 2015; EFSA, 2020). ABI referred to respiratory distress related to killing methods are **gasping** and **hyperventilation**.

Table A.1. (Annex) gives an overview of the ABI related to the welfare consequences pain, fear and respiratory distress (EFSA, 2020) including a brief description of each indicator. The group of animals to be killed at the same time should not be too large in order not to further complicate the surveillance of the ABI and to ensure that individual animals remain as much as easy to observe. When gas-filled foam is used in closed systems (e.g. boxes), the view on the animals may be even more limited due to opaque foam.

Due to the limited control possibilities when killing animals by gas in containers, the control of technical parameters (gas concentration, exposure time, temperature) becomes even more important to safeguard animal welfare (EFSA, 2020).

After application of the killing method, death must be confirmed before disposal of the carcass. An overview of ABI on the status of death is given in Table A.3 in the Annex of this review. The outcomes of these ABI can either indicate life (e.g. **body movements**) or death (e.g. complete **absence of muscle tone**).

For application of mechanical methods (e.g. penetrative captive bolt stunning followed by pithing) used for on-farm killing, EFSA (2020) provided a flowchart for monitoring ABI to assess the state of consciousness (step 1 after the shot) and death (step 2 after a subsequent killing procedure, see Figure). The indicators were not ranked according to sensitivity and specificity.

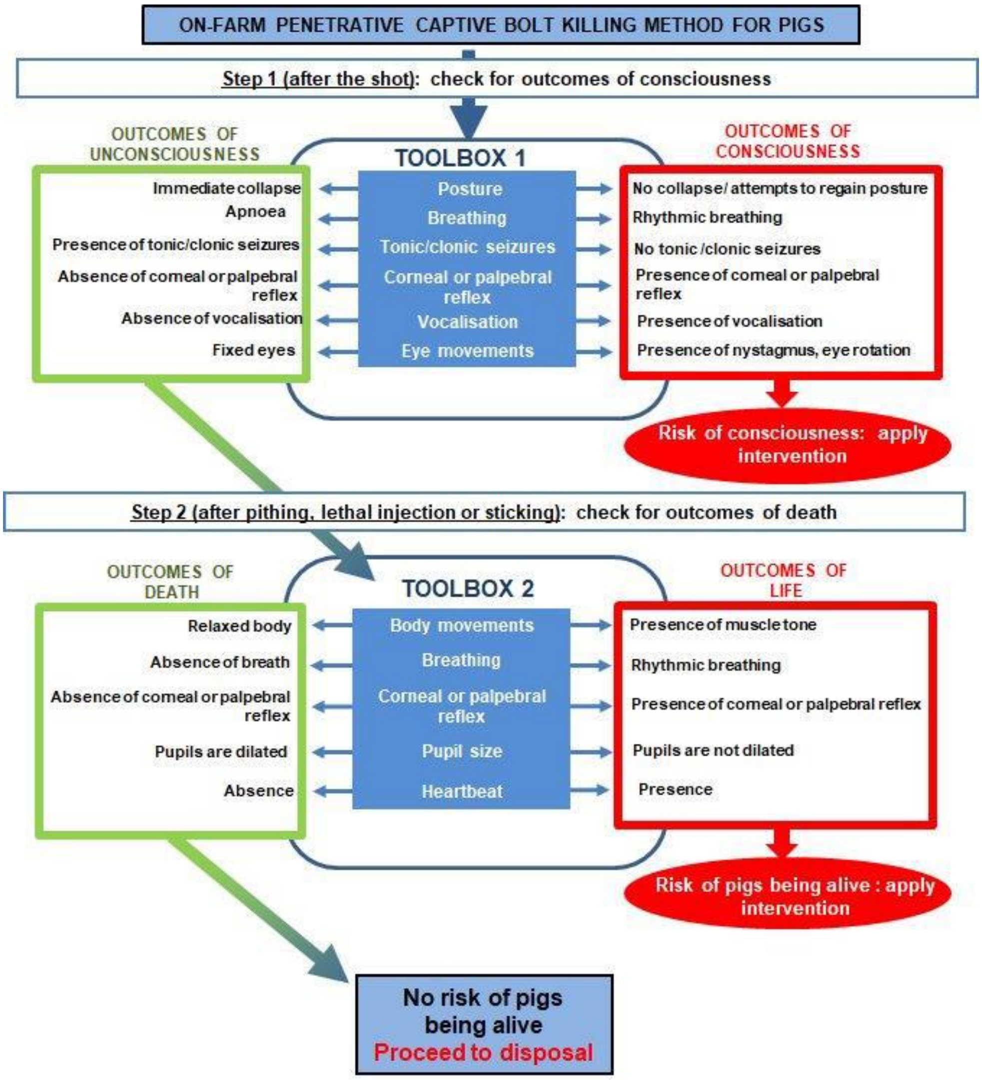

Figure 4.3.1. Flowchart of indicators for the monitoring of the state of consciousness and death of pigs following penetrative captive-bolt stunning and killing (Figure taken from EFSA (2020), p.37; © EFSA).

When outcomes of consciousness and death are observed, a back-up method needs to be applied. After re-stunning, the ABI for the state of consciousness and death must be repeatedly surveyed and checked in each case (EFSA, 2020).

4.4 Post mortem inspection of the carcass

The post mortem inspection of the carcass may reveal method application failures as well as a delayed killing of the animal following a prolonged period of pain and suffering before death. In the study of große Beilage (2017) in four animal by-products processing plants in Germany, deficiencies concerning the conduction of the stunning and killing method was obvious in 61.8 % of the investigated animals that were emergency killed. The emergency-killed animals (n = 165, of which 37 were suckling and weaning piglets) were identified by findings directly related to stunning and/or killing (e.g., skull injury after bolt shot, exsanguination cut). Findings were collected by examining the head, neck, and thorax of the animal on the outside and by palpation of the skull, jaw, occiput, and base of the neck. Deficiencies in the emergency killing of severely ill/injured animals, especially of the suckling and weaning piglets with corresponding findings related to stunning and/or killing included: absent or incorrect bleeding following the application of a penetrating captive bolt shot or a percussive blow to the head, incorrectly applied percussive blow to the head (destruction of the zygomatic bone and jaw, probably by a laterally directed blow e.g. against a wall) and without subsequent bleeding, bleeding without previous stunning, application of a percussive blow to the head to pigs weighing more than 5 kg, (große Beilage, 2017).

The carcass itself can still provide long after animal's death valuable information as to whether the killing of the animal was not only applicated in a proper way, but was also done at the appropriate time. In the study of große Beilage (2017), indications for prolonged pain and/or suffering of the animal before its death were detected in 13.2% (n = 64) of the examined fattening pigs and 11.6% (n = 17) of the breeding pigs. In contrast, although very likely less common, post-mortem inspection may possibly show also animals that did not show clear signs of euthanasia (see section 3.1).

5 Minimising welfare problems: improved practices

5.1 Handling

Longer distances or transports of piglets requiring euthanasia within the farm to the place of killing should be avoided. Piglets should be killed close to their home pen in the farrowing unit, out of sight and hearing range of their pen mates if possible. Younger, incurably injured or sick piglets that do not walk independently, should be transported to the killing point by trolley. Animals should not be removed from the pen until the equipment is ready for use and the person performing killing is ready.

5.2 Killing methods

On this topic, see the sections 3.2, 4.2 and 4.3. The duration of the restraint of the animal should be kept to a minimum. Other main points here are to ensure proper equipment settings and maintenance, to use a range of animal-based indicators carefully, and to give the animal the benefit of the doubt when there is any risk of animals regaining consciousness by applying a back-up procedure. For all methods, it should be emphasized that personnel must be adequately and recurrently trained in the assessment of piglets regarding the necessity of euthanasia and the proper application of the methods, in the control of their effectiveness (monitoring procedures using animal-based indicators), in measures to be taken in the event of application failures and on the current state of good professional practice (EFSA, 2020; TVT, 2014). It is strongly recommended to set standards not only for the content of the training for the operators, but also for the necessary qualification of the trainer.

6 Legal requirements

Council Regulation (EC) No 1099/2009 regulates legal requirements for animals at the time of slaughter and killing. Extracts that are particularly relevant to the welfare of piglets for the key areas are listed below. Underlined phrases indicate areas given guidance and improvement measures in the review (for legislation related to handling see also Holmes et al., 2020). Regulations related to handling and moving piglets to killing point and the killing process (including restraint), have been labelled using the terms (handling, killing) between brackets at heading level.

Recital No. 12

It is an ethical duty to kill productive animals which are in severe pain where there is no economically viable way to alleviate such pain. In most cases, animals can be killed respecting proper welfare conditions.

Article 2 Definitions (handling, killing)

(a) 'killing' means any intentionally induced process which causes the death of an animal;
(b) 'related operations' means operations such as handling, lairaging, restraining, stunning and bleeding of animals taking place in the context and at the location where they are to be killed;
(d) 'emergency killing' means the killing of animals which are injured or have a disease associated with severe pain or suffering and where there is no other practical possibility to alleviate this pain or suffering;

(f) 'stunning' means any intentionally induced process which causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death;
(p) 'restraint' means the application to an animal of any procedure designed to restrict its movements sparing any avoidable pain, fear or agitation in order to facilitate effective stunning and killing;
(q) 'competent authority' means the central authority of a Member State competent to ensure compliance with the requirements of this Regulation or any other authority to which that central authority has delegated that competence;
(r) 'pithing' means the laceration of the central nervous tissue and spinal cord by means of an elongated rod-shaped instrument introduced into the cranial cavity.

Article 3 General requirements for killing and related operations (handling, killing)

1. Animals shall be spared any avoidable pain, distress or suffering during their killing and related operations.
2. For the purposes of paragraph 1, business operators shall, in particular, take the necessary measures to ensure that animals:
(a) are provided with physical comfort and protection, in particular by being kept clean in adequate thermal conditions and prevented from falling or slipping;
(b) are protected from injury;
(c) are handled and housed taking into consideration their normal behaviour;
(d) do not show signs of avoidable pain or fear or exhibit abnormal behaviour;
(e) do not suffer from prolonged withdrawal of feed or water;
(f) are prevented from avoidable interaction with other animals that could harm their welfare.

Article 19 Emergency killing (killing)

In the case of emergency killing, the keeper of the animals concerned shall take all the necessary measures to kill the animal as soon as possible.

ANNEX 1 LIST OF STUNNING METHODS AND RELATED SPECIFICATIONS

CHAPTER I

Methods

Table 1 — Mechanical methods (killing)

No	Name	Description	Conditions of use	Key parameters	Specific requirements of Chapter II of this Annex
1	Penetrative captive bolt device	Severe and irreversible damage of the brain provoked by the shock and the penetration of a captive bolt. Simple stunning.	All species. Slaughter, depopulation and other situations.	Position and direction of the shot. Appropriate velocity, exit length and diameter of bolt according to animal size and species. Maximum stun to stick/kill interval(s).	Not applicable.
2	Non-penetrative captive bolt device	Severe damage of the brain by the shock of a captive bolt without penetration. Simple stunning	[…] *This method is currently not allowed for pigs and piglets.*	Position and direction of the shot. Appropriate velocity, diameter and shape of bolt according to animal size and species. Strength of the cartridge used. Maximum stun to stick/kill interval(s).	Point 1.
6	Percussive blow to the head	Firm and accurate blow to the head provoking severe damage to the brain.	Piglets, […] up to 5 kg live weight. Slaughter, depopulation and other situations.	Force and location of the blow.	Point 3.

Table 2 — Electrical methods (killing)

No	Name	Description	Conditions of use	Key parameters	Specific requirements of Chapter II of this Annex
1	Head-only electrical stunning	Exposure of the brain to a current generating a generalised epileptic form on the electro-encephalogram (EEG). Simple stunning.	All species. Slaughter, depopulation and other situations.	Minimum current (A or mA). Minimum voltage (V). Maximum frequency (Hz). Minimum time of exposure. Maximum stun-to-stick/kill interval(s). Frequency of calibration of the equipment. Optimisation of the current flow. Prevention of electrical shocks before stunning. Position and contact surface area of electrodes.	Point 4.
2	Head-to-Body electrical stunning	Exposure of the body to a current generating at the same time a generalized epileptic form on the EEG and the fibrillation or the stopping of the heart. Simple stunning in case of slaughter.	All species. Slaughter, depopulation and other situations.	Minimum current (A or mA). Minimum voltage (V). Maximum frequency (Hz). Minimum time of exposure. Frequency of calibration of the equipment. Optimization of the current flow. Prevention of electrical shocks before stunning. Position and contact surface area of electrodes. Maximum stun-to-stick interval(s), in case of simple stunning(s).	Point 5.

Table 3 — Gas methods (killing)

No	Name	Description	Conditions of use	Key parameters	Specific requirements of Chapter II of this Annex
1	Carbon dioxide at high concentration	Direct or progressive exposure of conscious animals to a gas mixture containing more than 40 % carbon dioxide. The method may be used in pits, tunnels, containers or building previously sealed. Simple stunning in case of slaughter of pigs.	Pigs, [...]. Slaughter only for pigs. Other situations than slaughter for [...] pigs.	Carbon dioxide concentration. Duration of exposure. Maximum stun-to-stick interval(s) in case of simple stunning. Quality of the gas. Temperature of the gas.	Point 7. Point 8.
3	Carbon dioxide associated with inert gases	Direct or progressive exposure of conscious animals to a gas mixture containing up to 40 % of carbon dioxide associated with inert gases leading to anoxia. The method may be used in pits, bags, tunnels, containers or in buildings previously sealed. Simple stunning for pigs if the duration of exposure to at least 30 % of carbon dioxide is of less than 7 minutes. Simple stunning for poultry if the overall duration of exposure to at least 30 % of carbon dioxide is of less than 3 minutes.	Pigs [...]. Slaughter, depopulation and other situations.	Carbon dioxide concentration. Duration of exposure. Maximum stun-to-stick/kill interval(s) in case of simple stunning. Quality of the gas. Temperature of the gas. Oxygen concentration.	Point 8.
4	Inert gases	Direct or progressive exposure of conscious animals to a inert gas mixture such as Argon or Nitrogen leading to anoxia. The method may be used in pits, bags, tunnels, containers or in buildings previously sealed. Simple stunning in case of the slaughter of pigs. [...]	Pigs [...]. Slaughter, depopulation and other situations.	Oxygen concentration. Duration of exposure. Quality of the gas. Maximum stun-to-stick/kill interval(s) in case of simple stunning. Temperature of the gas.	Point 8.

CHAPTER II

Specific requirements for certain methods (killing)

1. Non-penetrative captive bolt device

When using this method business operators shall pay attention to avoid the fracture of the skull[1].

[…]

3. ***[…] percussive blow to the head***

These methods shall not be used as routine methods but only where there are no other methods available for stunning.

[…]

No person shall kill by […] percussive blow to the head more than seventy animals per day.

[…]

4. ***Head-only electrical stunning***

4.1. When using head-only electrical stunning, electrodes shall span the brain of the animal and be adapted to its size.

4.2. Head-only electrical stunning shall be carried out in accordance with the minimum currents set out in Table 1.

Table 1 — Minimum currents for head-only electrical stunning

Category of animals	Animals of porcine species
Minimum current	1.30 A

5. Head-to-body electrical stunning

5.1. Animals of the [...] porcine species.
The minimum currents for head-to-body electrical stunning shall be [...] 1.30 amperes for pigs.

7. Carbon dioxide at high concentration

In the case of pigs, [...] the minimum concentration of 80 % of carbon dioxide shall be used.

[1] Up to now, the use of non-penetrative captive bolt devices on pigs is not permitted according to the Regulation (EC) No. 1099/2009. The regulation requires, when using the non-penetrating bolt shot on other species, that the user shall provide attention to avoid the fracture of the skull. According to the studies of Woods (2012) and Grist (2017, 2018a), which demonstrated good stun quality when the non-penetrative captive bolt was used on piglets, this avoidance of skull fractures in piglets was not possible.

8***. Carbon dioxide, use of inert gases or a combination of those gas mixtures***

Under no circumstances shall gases enter into the chamber or the location where animals are to be stunned and killed in a way that it could create burns or excitement by freezing or lack of humidity.

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Annex. Tables of animal-based welfare indicators

Table A.1. Animal-based indicators (ABI) related to handling and moving pigs to the killing point (handling, H), restraint (R) and gas exposure before loss of consciousness (gas, G) to assess pain and fear and respiratory distress as welfare consequences (modified after EFSA, 2020).

		Assessment of animal-welfare indicators to be used during handling and moving to the point of killing (H), restraint (R) and gas exposure (G)				
	Indicator (ABI)	**Key area to focus on during welfare inspections**			**Short description**	**Welfare consequences**
		H	R	G	.	
3	**Escape attempts**	x	x	x	Attempts to move or run away from the situation (O'Malley et al., 2018)	Pain, fear
4	**High-pitched vocalisations**	x	x	x	Vocalisation (squeal/scream) of a pig during moving and manipulation (Welfare Quality®, 2009; Dalmau et al., 2009)	Pain, fear
7	**Reluctance to move**	x	x	x	Reluctance to move is defined as an animal that (for at least 2 seconds) - stops and does not explore - does not move the body and head (freezing) - does not move the head - refuses to move when coerced by the operator (Welfare Quality®, 2009; Dalmau et al., 2009).	Fear
9	**Head shaking**			x	Rapid shaking of the head, most times accompanied by stretching and/or withdrawal movements of the head (EFSA, 2004; Velarde et al., 2007)	Pain (e.g. aversiveness of CO_2)
10	**Gasping**			x	Deep breath through a gaping-open mouth (Raj and Gregory, 1996; EFSA, 2004)	Respiratory distress
11	**Hyperventilation**			x	Excessive rate and depth of breathing (Raj and Gregory, 1996; EFSA, 2004)	Respiratory distress

Table A.2. Animal-based indicators (ABI) to assess loss of consciousness after resp. during the application of killing methods [physical methods (P) and exposure to gas (G)] (modified after EFSA, 2020).

Loss of consciousness (pain and fear as welfare consequences)				
Indicator (ABI)	**Outcomes**		**Methods**	
	in effectively stunned animals (low risk of pain and fear)	**in ineffectively stunned or re-awakening animals (high risk of pain and fear)**	**P**	**G**
Posture	**Loss of posture, collapse** (EFSA AHAW Panel, 2013)	**Fail to collapse, attempts to regain posture after collapse** (EFSA AHAW Panel, 2013)	x	x
Breathing	**(Immediate onset of) apnea** (absence of breathing)	**Rhythmic breathing** - breathe in a pattern commonly referred to as rhythmic breathing, which may begin as regular gagging and involves respiratory cycle of inspiration and expiration - can be recognised from the regular flank and/or mouth and nostrils movement - if not visible through these movements, recovery of breathing can be checked by holding a small mirror in front of the nostrils or mouth to look for the appearance of condensation due to expiration of moist air (EFSA AHAW Panel, 2013)	x	x
Tonic/clonic seizures	**Onset of tonic-clonic seizures after collapse**	**Fail to show tonic/clonic seizures** - e.g. penetrative captive bolt (mechanical methods): fail to show tonic seizures with paddling and kicking movements immediately after collapse (Van der Wal, 1971) - e.g. electrical methods: showing only tetanus during the flow of the current through the body (EFSA AHAW Panel, 2013)	x	
Corneal and palpebral reflex	**Corneal and palpebral reflex are not present** (can be temporarily positive after effective electrical and penetrative captive bolt stunning; EFSA AHAW Panel, 2013)	**Blinking in response** to the stimulus during testing the palpebral and corneal reflex	x	x
Vocalisation	**No vocalisation**	**Vocalisation is possible** (attention: not all conscious animals may vocalise, EFSA AHAW Panel, 2013)	x	x

Eye movements	**Fixed eyes** (wide open and glassy eyes with clearly visible iris/cornea in the middle); eyeballs rotated in the socket (EFSA AHAW Panel, 2013)	**Eye movements** (EFSA AHAW Panel, 2013): eye tracking to moving objects, spontaneous blinking	x	x

Table A.3. Animal-based indicators (ABI) to assess death after the application of killing methods (modified after EFSA, 2020).

Death	
Indicator (ABI)	**Outcomes**
	in dead animals (no risk of pain and fear)
Body movements	Absence of muscle tone, a complete relaxed body (EFSA AHAW Panel, 2013)
Breathing	absence of breathing, apnea (EFSA AHAW Panel, 2013)
Corneal and palpebral reflex	absence of response to palpebral and corneal stimuli (EFSA AHAW Panel, 2013)
heartbeat	Absence of heartbeat
Pupil size	Dilated pupils (mydriasis) as the onset of brain death (EFSA AHAW Panel, 2013)

European Union Reference Centre for Animal Welfare *Pigs*

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EURCAW-Pigs is the first European Union Reference Centre for Animal Welfare. It focuses on pig welfare and legislation, and covers the entire life cycle of pigs from birth to the end of life. EURCAW-Pigs' main objective is a harmonised compliance with EU legislation regarding welfare in EU Member States. This includes:

- for pig husbandry: Directives 98/58/EC and 2008/120/EC;
- for pig transport: Regulation (EC) No 1/2005;
- for slaughter and killing of pigs: Regulation (EC) No 1099/2009.

EURCAW-Pigs supports:

- inspectors of Competent Authorities (CA's);
- pig welfare policy workers;
- bodies supporting CA's with science, training, and communication.

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