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SCIENTIFIC OPINION

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Welfare of horses during killing for purposes other than slaughter

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

Horses of different ages may have to be killed on-farm for purposes other than slaughter (where slaughter is defined as killing for human consumption) either individually (i.e. on-farm killing of unproductive, injured or terminally ill animals) or on a large-scale (i.e. depopulation for disease control purposes and other situations, such as environmental contamination, disaster management, etc.). The purpose of this opinion is to assess the hazards and welfare consequences associated with the on-farm killing of horses. The killing procedure is divided into Phase 1 (pre-killing), which includes the processes (i) handling and moving the animals to the killing place and (ii) restraint of the animals before application of the killing method; and Phase 2 (stunning and/or killing), which includes stunning and killing of the animals (for methods that require one step for stunning and another for subsequent killing) or killing only (for methods that simultaneously stun and kill the animals). Three stunning and/or killing methods for Phase 2 for horses were identified: (i) penetrative captive bolt followed by killing, (ii) firearms with free projectiles and (iii) lethal injection. Welfare consequences that horses may experience during each process (e.g. handling stress, restriction of movement and injuries during restraint) were identified and potential hazards are listed for all phases, along with preventive and corrective measures. Animal-based measures (ABMs) to assess all identified welfare consequences were proposed. During the application of the stunning and/or killing methods, horses will experience pain and fear if they are ineffectively stunned/killed or if they recover consciousness. A flowchart including ABMs for the assessment of consciousness and death to monitor stunning and killing effectiveness is provided. Additionally, specific practices deemed unacceptable on welfare grounds are listed.

KEYWORDS

animal-based measure, captive bolt, firearm, Horse, killing, lethal injection, stunning

CONTENTS

SUMMARY

In 2009, the European Union (EU) adopted Council Regulation (EC) 1099/2009 'on the protection of animals at the time of killing', which was prepared based on two Scientific Opinions adopted by the European Food Safety Authority (EFSA) issued in 2004 and 2006.

Against this background, the European Commission (EC) requested EFSA to write a Scientific Opinion providing an independent view on the killing of horses for purposes other than slaughter (in which slaughter is defined as killing animals for human consumption).

Specifically, EFSA was asked to: (i) identify the animal welfare hazards and their possible origins in terms of facilities/equipment and staff; (ii) define qualitative or measurable criteria to assess performance on animal welfare (animal-based measures, ABMs); (iii) provide preventive and corrective measures (structural or managerial) to address the identified hazards; and (iv) point out specific hazards related to species or types of animals. In addition, the mandate requested a list of methods or practices considered unacceptable on welfare grounds.

This Scientific Opinion aims to update the above-reported EFSA outputs and to provide the EC with a scientific basis for future discussions at international level on the welfare of animals in the context of killing of horses for purposes other than slaughter. This killing can take place in case of: (a) large-scale depopulation for disease control purposes and other similar situations (such as environmental contamination, disaster management, etc.) and (b) individual on-farm killing of unproductive horses (including injured or terminally ill animals).

The approach used to address the mandate follows a protocol previously developed by EFSA: in summary, for the identification of most elements requested in the mandate (e.g. killing methods, welfare consequences, hazards, ABMs) the protocol is based on expert knowledge elicitation (EKE). For the description of such elements, extensive literature searches were used.

Firstly, the EFSA experts identified the processes related to on-farm killing of horses that should be included in the assessment.

The whole killing is divided into Phase 1 (pre-killing) that includes the processes (i) handling and moving the animals to the killing place and (ii) restraint of the animals before application of the killing methods; and Phase 2, that includes stunning and killing of the animals (for methods that require one step for stunning and another for subsequent killing) or killing only (for methods that simultaneously stun and kill the animals) – hereafter referred to as stunning and/or killing methods.

Secondly, the experts identified the highly relevant WCs that horses may experience during each process of Phase 1 through EKE. In Phase 2, animals might experience highly relevant WCs (e.g. injuries) and underlying negative affective states, i.e. pain and fear, only if they are conscious (e.g. due to mis-stunning or recovery of consciousness). For this reason, the focus for Phase 2 is to prevent the presence of consciousness following stunning (and before the onset of death).

Thirdly, the experts produced a list of possible welfare hazards present during each process and identified corrective measures that can prevent the related WCs. Additionally (or when corrective measures were not considered feasible), measures to mitigate the WCs were proposed. ABMs for the assessment of all welfare consequences were also identified.

Key conclusions that could be considered for risk management decisions (e.g. those on the application of the killing methods) were subjected to a specific analysis to quantify their degree of certainty. This analysis is presented in a separate chapter, with the certainty level indicated in brackets in the relevant conclusions.

Related to Phase 1 (pre-killing), during handling and moving, horses may experience restriction of movement, handling stress and injuries. Rushing horses can cause handling stress and injuries, as well as make animals more difficult to handle subsequently (e.g. during restraint for killing). Moving unhandled horses with their original group and without halters or ropes prevents handling stress. This method is also effective for semi-feral animals that are more at risk of experiencing handling stress, being less accustomed to human interaction. Moving individually handled horses by a halter and rope to the killing area prevents handling stress. Using a companion horse can help encourage reluctant animals to move more willingly.

During restraint, horses experience restriction of movement and may additionally experience handling stress and injuries. Horses should be restrained appropriately avoiding the use of excessive force. The size of the restraint crush should be adjusted to fit the horse size, so that the animal cannot move back and forward or turn around. Animals should only be restrained when the operator is ready to stun them and in any case the duration of restraint should be kept to a minimum.

Related to Phase 2, the stunning and/or killing methods that have been identified are (1) penetrative captive bolt stunning, (2) firearm with free projectile and (3) lethal injection. Ineffective stunning and recovery of consciousness will cause pain and fear.

Penetrative captive bolt stunning is the first step of a two-step killing method, with bleeding, pithing or lethal injection applied as the second step to induce death. When penetrative captive bolt is used, the minimum recommended bolt speed should be 55 m/s, with a bolt diameter of at least 9 mm and a minimum length of 8 cm, ensuring a penetration depth of at least 7 cm. However, further research is needed to validate these parameters for all horse categories.

The use of firearms is a one-step stunning and killing method. After a successful head shot death is immediate. The shooting position for penetrative captive bolts and firearms, (which is about 1 cm above the intersection of a diagonal line extending from the centre of the eye to the centre of the base of the opposite ear), should be used. The bolt should be directed towards the brain stem for maximum effectiveness. Lethal injection should always be done in two steps, i.e. anaesthesia or sedation followed by injection of a lethal substance. After the application of a stunning and/or killing method, the state of unconsciousness and the state of death of the animal should be confirmed using the recommended ABMs

(Figure 4). As a prevention measure, for penetrative captive bolt followed by killing, a stun-to-kill interval of less than 60 s prevents the recovery of consciousness before death. Animals showing signs of recovery of consciousness should be re-stunned/killed immediately in an appropriate way (e.g. adjusting the position or direction of the shot). In case of animals showing signs of life, the killing method should be immediately re-applied correctly (e.g. repeat the pithing to destroy the brain, brain stem and upper spinal cord). The state of death should be confirmed before proceeding to carcass disposal.

Lastly, some practices considered unacceptable on welfare grounds (e.g. severing the spinal cord, use of electric goads to move the animals etc) are listed. These practices must not be used.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

The Union adopted in 2009 Council Regulation (EC) No 1099/2009[1] *on the protection of animals at the time of killing*. This piece of legislation was prepared on the basis of two EFSA opinions respectively adopted in 2004[2] and 2006.[3] The EFSA provided additional opinions related to this subject in 2012,[4] 2013,[5,6,7,8,9,10] 2014[11,12] 2015[13] and 2017.[14,15]

In parallel, since 2005, the World Organisation for Animal Health (WOAH – formerly known as OIE) has developed in its Terrestrial Animal Health Code two chapters covering a similar scope:

- Slaughter of animals (Chapter 7.5).
- Killing of animals for disease control purposes (Chapter 7.6).

The chapter "Slaughter of animals" covers the following species: cattle, buffalo, bison, sheep, goats, camelids, deer, horses, pigs, ratites, rabbits and poultry (domestic birds as defined by the WOAH).

The WOAH has created an ad hoc working group to revise the two chapters.

Against this background, the Commission would like to request the EFSA to review the scientific publications provided and possibly other sources to provide a sound scientific basis for future discussions at the international level on the welfare of animals in the context of slaughter (i.e., killing animals for human consumption) or other types of killing (i.e., killing for other purposes than slaughter).

1.1.2 | Terms of Reference

The Commission therefore considers it opportune to request EFSA to give an independent view on the killing of animals for other purposes than slaughter:

- free moving animals (cattle, buffalo, bison, sheep, goats, horses, pigs);
- animals transported in crates or containers (i.e. rabbits and domestic birds).

The request focuses on the cases of large-scale killing which take place in case of depopulation for disease control purposes and for other similar situations (environmental contamination, disaster management, etc.) outside slaughterhouses.

The request also considers in a separate section the killing of unproductive animals that might be practiced on-farm (day-old chicks, piglets, pullets, etc.)

The request includes the following issues:

- handling,
- restraint,
- stunning/killing,
- unacceptable methods, procedures or practices on welfare grounds.

For each process or issue in each category (i.e., free moving/in crates or containers), EFSA will:

- Identify the animal welfare hazards and their possible origins (facilities/equipment, staff),
- Define qualitative or measurable criteria to assess performance on animal welfare (animal-based measures),

[1]OJ L 303, 18.11.2009, p. 1.

[2]The welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal, 45, 1–29.

[3]The welfare aspects of the main systems of stunning and killing applied to commercially farmed deer, goats, rabbits, ostriches, ducks, geese and quail. EFSA Journal, 326, 1–18.

[4]Electrical requirements for waterbath equipment applicable for poultry. EFSA Journal, 10(6), 2757.

[5]Electrical parameters for the stunning of lambs and kid goats. EFSA Journal, 11(6), 3249.

[6]Monitoring procedures at slaughterhouses for bovines. EFSA Journal, 11(12), 3460.

[7]Monitoring procedures at slaughterhouses for pigs. EFSA Journal, 11(12), 3523.

[8]Monitoring procedures at slaughterhouses for poultry. EFSA Journal, 11(12), 3521.

[9]Monitoring procedures at slaughterhouses for sheep and goats. EFSA Journal, 11(12), 3522.

[10]The use of carbon dioxide for stunning rabbits. EFSA Journal, 11(6), 3250.

[11]The use of low atmosphere pressure system (LAPS) for stunning poultry. EFSA Journal, 12(1), 3488.

[12]Electrical requirements for poultry waterbath stunning equipment. EFSA Journal, 12(7), 3745.

[13]The scientific assessment of studies on electrical parameters for stunning of small ruminants (ovine and caprine species). EFSA Journal, 13(2), 4023.

[14]The low atmospheric pressure system for stunning broiler chickens. EFSA Journal, 15(12), 5056.

[15]The animal welfare aspects in respect of the slaughter or killing of pregnant livestock animals (cattle, pigs, sheep, goats, horses). EFSA Journal, 15(5), 4782.

– Provide preventive and corrective measures to address the hazards identified (through structural or managerial measures),
– Point out specific hazards related to species or types of animals (young, with horns, etc.)

1.2 | Interpretation of the Terms of Reference

This Scientific Opinion concerns the killing of horses for purposes other than slaughter. A separate opinion deals with the welfare of horses at slaughter (EFSA AHAW Panel, 2025) which is referred to in the present document.

The European Commission (EC) asked EFSA to provide an independent view on the welfare of horses during killing for purposes other than slaughter. These killings can take place in case of: (a) large-scale depopulation for disease control purposes and other similar situations (such as environmental contamination, disaster management, etc.) and (b) individual on-farm killing of unproductive animals (the EFSA experts agreed to include animals that are injured or terminally ill in this category). For each of these scenarios, several welfare aspects need to be analysed (including e.g. welfare hazards, hazard origins, animal-based measures and corrective measures).

The term 'on-farm' is used in the present opinion to cover a variety of husbandry conditions in which horses are kept. These can range from fully extensive systems to high-density group housing (Raspa et al., 2020; Raspa et al., 2020; Insausti et al., 2021) and fully stabled horses kept either individually in single boxes (e.g. racehorses) or in groups under shared shelters (Hartmann et al., 2015). In addition, horses may be kept in part stabled/part pastured conditions (Mills & Clarke, 2007). The premises can range from small private yards to large commercial enterprises. In addition, horses can be kept in 'semi-feral' conditions. These animals roam free in large areas, live on pasture or other natural habitats and are only gathered at specific times of the year, if at all (Mendonça et al., 2022; Fletcher et al., accepted for publication). This diversity in husbandry systems significantly impacts the methods required for handling and moving horses, particularly when they are to be killed.

This opinion will use definitions related to the killing of horses provided by Council Regulation (EC) 1099/2009 on the protection of animals at the time of killing, which entered into force in January 2013. In this opinion, killing refers to any intentionally applied procedure that causes the death of the animal.

Also, it is important to note that Council Regulation (EC) 1/2005 defines 'unbroken equidae' as 'equidae that cannot be tied or led by a halter without causing avoidable excitement, pain or suffering'. The term 'broken' refers to horses trained for riding or driving. In this scientific opinion, 'unhandled' describes equines that cannot be tied or led by a halter without avoidable distress, while 'handled' refers to horses previously trained for such activities.

The whole killing is divided into Phase 1 (pre-killing), which includes the processes (i) handling and moving the animals to the killing place and (ii) restraint of the animals before application of the killing methods, and Phase 2 (stunning and/or killing), which includes the methods for stunning and/or killing of the animals.

Some killing methods are defined as two-step methods, since they require a prior step for stunning and another for subsequent killing. In this opinion, the term 'second-step killing method' is used to indicate the killing method applied in two-step methods, while 'backup killing method' is used to indicate the additional killing method to be applied in case of failure of the first one. Other methods simultaneously stun and kill the animals. One-step methods and two-steps methods will be hereafter referred to as stunning and/or killing methods.

Per each phase, the mandate requests the assessment of hazards, related animal-based measures (ABMs), and preventive and corrective actions for each hazard.

The mandate does not require the identification of relevant welfare consequences (WCs) for animals during killing. However, in line with the EFSA guidance on risk assessment in animal welfare (EFSA AHAW Panel, 2012a), the identification of WCs that horses can experience when exposed to hazards is a necessary step for the subsequent identification of hazards required by the mandate. Therefore, in this opinion, WCs were identified per each phase of the killing. The methodology developed for the identification of WCs follows the EFSA guidance on risk assessment in animal welfare in the context of the F2F strategy revision (EFSA AHAW Panel, 2022).

During Phase 1 (pre-killing), the assessment identified highly relevant WCs that could result in negative affective states, particularly pain and/or fear. In the present opinion, the assessment is not focused on the underlying affective states, but on specific WCs, that are considered highly relevant, based on the assumption that measures can be put in place to prevent them.

Phase 2 (stunning and/or killing) involves the application of stunning and/or killing methods intended to render the animal unconscious and to ensure the animal is dead. The analysis of welfare consequences in Phase 2 is based on the assumption that the animal is rendered unconscious at the start of Phase 2, as required by Council Regulation (EC) 1099/2009. Consequently, negative affective states such as pain and fear are not expected to occur unless the animal is mis-stunned/killed or regains consciousness during this phase. If consciousness is present in Phase 2, the animal may experience negative affective states stemming from the welfare consequences identified in Phase 1 (e.g. pain from restriction of movement) as well as from WCs resulting from the stunning and/or killing method (e.g. pain due to neck cutting). For that reason, the negative affective states of pain and fear are directly linked to the animal's conscious state. Preventing consciousness after stunning is therefore critical to avoid these states during Phase 2.

Pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja et al., 2020). Fear is defined as an unpleasant emotional affective state, induced by the perception of a danger or a potential danger that threatens the integrity of the animal (Boissy, 1995). Consciousness

is defined as the capacity to receive, process and respond to information from internal and external environments, and therefore the ability to feel emotions and be sensible to external stimuli (Le Neindre et al., 2017).

According to Council Regulation (EC) 1099/2009, killing should spare animals from unnecessary pain, distress and suffering. However, EFSA experts consider that distress and suffering might include pain and fear (as sensorial and emotional components), and therefore the focus of this opinion is on minimising pain and fear throughout killing.

EFSA has applied the following criteria for the selection of the on-farm killing methods to be included in this assessment:

a) all methods with described technical specifications known by the experts and not only the methods described in Council Regulation (EC) 1099/2009,
b) methods currently used for stunning and killing of horses, and those which are still under development and are likely to become commercially applicable and
c) methods for which the welfare aspects (in terms of welfare hazards, WCs, ABMs, preventive and corrective measures) are described sufficiently in the scientific literature.

The application of these criteria resulted in a list of killing methods and practices assessed in this Scientific Opinion. This may have excluded less described methods.

The main on-farm killing methods identified for horses were: (1) penetrative captive bolt followed by killing, (2) firearms and (3) lethal injection. The assessment will be done separately for each method.

This opinion defines qualitative and/or measurable (quantitative) criteria to assess the effect of hazards on animal welfare (animal-based measures, ABMs) for on-farm killing of horses as requested by the mandate.

This opinion proposes corrective measures for the identified hazards, which will prevent the WCs (e.g. correcting the size of the restraint crush to prevent injuries). Additionally, or when corrective measures for the hazards are not feasible, actions to mitigate the WCs are also discussed (e.g. reducing the duration of restraint to mitigate injuries and handling stress).

Lastly, the mandate requests a list of methods, procedures or practices deemed unacceptable on welfare grounds. These practices are discussed in a specific chapter. However, although scientific risk assessment can support discussions on what practices are unacceptable on welfare grounds, the ultimate decisions on acceptability involve several other considerations (e.g. ethical and socio-economic aspects) that need to be weighted by the risk managers considering the welfare implications.

2 | DATA AND METHODOLOGIES

2.1 | Data

2.1.1 | Data from literature

Information from the scientific publications selected as relevant from the literature search described in Section 2.2.2 and from additional literature identified by the EFSA experts was used for a narrative description and assessment of each phase of the killing event (Phase 1 – pre-killing, Phase 2 – killing).

2.1.2 | Data from expert opinion

Data obtained from the literature were complemented by experts' opinions in order to identify hazards, WCs, ABMs and preventive and corrective measures relevant to the current assessment.

2.2 | Methodologies

2.2.1 | Protocol

This scientific opinion follows the protocol detailed in the methodological guidance developed by the AHAW Panel in the context of the Farm to Fork (F2F) strategy revision (EFSA AHAW Panel, 2022).

The detailed protocol can be found in Annex A. In summary, EFSA experts described the phases and processes involved in the killing of horses on farm. For Phase 1, highly relevant WCs were identified through expert knowledge elicitation and hazards leading to the highly relevant WCs were listed, together with the related preventive and corrective measures and ABMs for assessing the WCs. For Phase 2, hazards leading to presence of consciousness or recovery of consciousness as well as preventive and corrective measures were identified.

2.2.2 | Literature search

A literature search was carried out in peer-reviewed and grey literature to identify hazards related to animal welfare during the killing of horses.

The search focused on the killing process and its hazards. No search was done on indicators of consciousness and death as these were investigated in detail in the EFSA opinion on slaughter of horses (EFSA AHAW Panel, 2025).

Full details of the literature search and results are provided in Appendix A to this opinion.

In total, eight references related to horse killing were retrieved from the search and included in this assessment. In addition, the EFSA experts complemented the information with references from review papers, book chapters and non-peer-reviewed papers they considered relevant for the present assessment.

2.2.3 | Expert knowledge elicitation

As described in Table A1 in Annex A, expert knowledge elicitation (EKE) was used for the sub-questions requiring the identification of WCs, ABMs, hazards, and preventive and corrective (or mitigative) measures. Expert knowledge was mainly elicited via EFSA expert group discussion.

For the identification of highly relevant WCs for the processes of Phase 1, a semi-formal EKE was carried out. The starting point was the list of 33 specific WCs identified by the AHAW Panel in the methodological guidance produced in the context of the F2F strategy revision. Out of these 33 WCs, the experts selected highly relevant WCs based on their individual estimates of duration, intensity and occurrence of the WC in each process (for details on the exercise see Appendix B). Only highly relevant WCs are further reported in this scientific opinion.

2.2.4 | Structure of the opinion

Chapters are organised by phases of the killing. For Phase 1 (pre-killing), WCs are presented in a list and hazards are included within the related WCs. For Phase 2 (killing), subchapters are organised by stunning and/or killing methods and the focus is on pain and fear. Hazards are specifically listed within each stunning and/or killing method.

2.2.5 | Uncertainty analysis

The uncertainty in the assessment performed for this Scientific Opinion was investigated qualitatively following the methods detailed in the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a, 2018b).

The EFSA experts agreed to tackle the uncertainty related to the methodology employed to identify WCs, ABMs and related hazards by describing the sources of uncertainty (Appendix C).

Regarding the overall impact of the identified uncertainties on the conclusions of the opinion, it was agreed to perform an assessment only for a subset of key conclusions that could be considered for risk management decisions (e.g. those on the application of the stunning methods).

Therefore, these conclusions were reformulated into scientifically answerable assessment questions that consider the exposure of animals to a well-defined scenario described in the conclusion. These questions were focused on whether the proportion of animals potentially affected by the scenario (i.e. a given WC or affective state) was above a reference quantity (rQ). The selection of the rQ took into account the impact of the scenario on the animals and the proportion of animals affected that would encourage intervention. The rQ varies depending on the context of the conclusion.

In particular, questions related to stunning and/or killing method application or effectiveness (i.e. in inducing unconsciousness) were formulated so that experts would express their certainty that more than 99% of the animals are properly stunned (i.e. < 1% of the animals being conscious and therefore experiencing pain and fear). This threshold was chosen due to the critical level of pain and fear experienced by animals if not properly stunned before killing. In these conditions, having more than 1% of the animals ineffectively stunned was considered indicative of poor welfare.

Experts were then asked to express their certainty for each question according to three pre-defined agreed certainty ranges (Table 1) derived from the approximate probability scale in the guidance on uncertainty (EFSA Scientific Committee, 2018a, 2018b).

TABLE 1 The three ranges used to express agreed (consensus) certainty around conclusions.

	Certainty range		
Quantitative assessment	**50%–100%**	**66%–100%**	**90%–100%**
Qualitative translation	From more likely than not to almost certain (summarised as 'more likely than not')	From likely to almost certain (summarised as 'likely')	From very likely to almost certain (summarised as 'very likely')

Experts were initially asked to individually select the certainty range that best reflected their degree of confidence when answering each question considered. Then, a group discussion provided an opportunity for experts to present the rationale behind their assessments. A consensus was then sought to identify the range that best reflected overall certainty; if consensus could not be reached, the broader range encompassing all individual judgements was selected.

3 | ASSESSMENT

3.1 | Reasons and methods for on-farm killing of horses

There are several reasons for killing horses on farm other than slaughter. Two main situations can be identified: (i) on-farm killing of individual animals and (ii) large-scale killings for depopulation.

Regarding the first situation, there are different reasons for on-farm killing of one or several animals. One reason is the killing of individuals that are seriously injured or have a disease associated with pain or suffering and where there is no other practical possibility to alleviate this pain or suffering (Binder & Baumgarnter, 2015). Similarly, animals may be killed when they are likely to suffer in the immediate future and when remedial care is not considered possible or appropriate. Secondly, non-viable neonates may also be killed to prevent further suffering.

Large-scale killing on farms or depopulation of horses may be necessary for several reasons: for the control or eradication of certain animal diseases, to deal with a natural disaster situation (e.g. a flood, storm, fire, severe drought or earthquake), as an economic mitigation measure during oversupply or closed marketing channel, in the event of an outbreak of a highly contagious disease among the human population with consequent closure of slaughterhouses or movement restriction (e.g. SARS-CoV2 during COVID-19), or when there is the risk of a zoonotic disease infecting humans (FAWC, 2012).

Indeed, contingency plans exist in most countries to deal with disease outbreaks (e.g. EU Animal Health Law Regulation 2016/42,[16] DEFRA, 2024; AUSVETPLAN, 2015) but they do not describe in detail the killing methods to be used.

On-farm killing conditions differ significantly from slaughterhouses, especially during disease control operations, due to lack of specific handling and restraining facilities. Consequently, some stunning and/or killing methods used in slaughterhouses may not work efficiently or are not feasible for on-farm use. Additionally, in disease control situations, the urgency of action places extra pressure on personnel (Gerritzen & Raj, 2009). Currently, all the available killing methods for horses are designed and applied to individual animals. In some cases, a group of animals is moved to a holding pen and subsequently divided into smaller, more manageable groups, then moved to a designated pen where the killing method is applied individually to the animals.

Regarding the methods that are available for the on-farm killing of horses for other purposes than slaughter, AVMA (2020) recommends the following methods: lethal injection with barbiturates and their derivatives, firearms and penetrative captive bolts. Killing on farm can involve a two-step procedure, where a stunning method is immediately followed by a killing method or a one-step method that simultaneously stuns and kills the animals.

All the methods are applied individually to each animal.

Methods used for on-farm killing of horses are listed in Table 2, together with information on the number and type of steps involved. All methods can be applied to all animal categories, from neonates to adults.

TABLE 2 Methods used for on-farm killing of horses.

Stunning and/or killing method	Number of steps
Lethal injection	Two: (1) anaesthesia or sedation (2) intravenous injection of a lethal substance
Penetrative captive bolt followed by killing.	Two: (1) penetrative captive bolt stunning (2) killing method (e.g. pithing, bleeding or intravenous injection of lethal substance)
Firearms with free projectile	One

3.2 | Types of horses killed on farm

Among domestic animals, horses may have a wide range of functional categories: competition animals, companion animals, therapy animals, working animals (on farm or for transport) or farmed animals for meat or milk production (Fletcher et al., 2022). In addition, horses may live under wild or semi-feral conditions (Minero and Canali, 2009). The present opinion covers all these categories of horses, when they are killed outside the slaughterhouse. The functional category of horses will impact their behaviour and capacity to be handled and manipulated by humans.

[16] https://eur-lex.europa.eu/eli/reg/2016/429/2019-12-14.

Some animals are unhandled and may have a total lack of experience with human contact and be unhabituated to being moved or led (Riva et al., 2022). Others can be fully halter-trained.

Besides the above-mentioned functional categories, horses can also be divided into types, based on:

- Size: ponies are horses whose height at the withers is below 1.48 m without shoes and 1.49 m with shoes. Taller animals are categorised as horses.
- Weight: saddle horses are commonly used for riding and weigh up to 700 kg. Draught horses are used for pulling carriages and usually weigh above 700 kg. Horses weighing less than 700 kg can pull carriages, and horses above 700 kg can be ridden, but for simplicity, horses below and above 700 kg will be termed saddle and draught horses, respectively, in this scientific opinion. In this opinion, an average horse weight is 400 kg.
- Age: a foal is a juvenile horse born the same year; a yearling is a young horse aged 1–2 years. Horses over 2 years of age are considered adults.
- Physiology: a mare is a female horse, a stallion is an intact male and a gelding is a castrated male, all above 2 years of age.

3.3 | Phase 1: Pre-killing

3.3.1 | Handling and moving to the killing areas

In the context of the killing procedures described in this opinion, handling is the process of preparing the animals for the killing, and in most cases involves moving them from the paddock/pasture, home pen or stable to the killing area. Gavinelli et al. (2014) lists animal handling as one of the key stages to be monitored during on-farm killing.

3.3.1.1 | *Highly relevant welfare consequences identified for handling and moving*

As explained in Section 2.2.3, an exercise based on EKE was performed to identify the highly relevant WCs for Phase 1 – handling and moving. Results and related definitions (EFSA AHAW Panel, 2022) are reported in Table 3.

TABLE 3 Welfare consequences identified as highly relevant for Phase 1 – handling and moving.

Phase 1: Handling and moving to the killing area	
Welfare consequence	**Definition**
Handling stress	The animal experiences stress and/or negative affective states such as pain and/or fear resulting from human or mechanical handling.
Injuries	The animal experiences negative affective states such as pain, discomfort or distress due to physical damage to somatic tissue types (bones, joints, skin, muscles).
Restriction of movement	The animal experiences stress and/or negative affective states such as pain, fear, discomfort and/or frustration because it is unable to move freely or walk comfortably (e.g. due to overcrowding, unsuitable floors, being tethered or confined individually in stalls).

A summary of the information retrieved from the literature complemented by expert opinion per each of these WCs is presented in the rest of this section.

Handling stress

Handling stress is mainly due to inappropriate handling by humans and is affected by previous training of the horse (handled vs. non-handled horse).

For handled horses, halters and ropes are the most commonly used devices for handling and moving.

Inappropriate handling may occur when animals are reluctant to move and handlers apply increased pressure on the animal (e.g. with tools or by shouting), often due to time constraints. Another example of inappropriate handling is separating individual horses (e.g. semi-feral horses) from their group, as horses are naturally gregarious animals. Stressful handling can also include shouting and yelling by humans. Horses may not move calmly if distracted by people, noise or objects, and they may stop moving forward or turn back.

The main preventive measure for inappropriate handling is education and training of handlers. Trained and knowledgeable staff, who allocate sufficient time to ensure calm and correct handling, will reduce handling stress.

Because people entering the home pen/paddock is unavoidable and essential to moving animals or performing the killing in the pen or paddock, handlers must be trained to understand the species-specific and individual animal behaviour, as horses can become fearful of humans or turn aggressive to handlers if they are experiencing handling stress.

The previous training of the horse for handling leads to an important distinction between handled and unhandled horses.

For smaller groups or individual horses accustomed to handling, leading them to the killing area using halters or ropes minimises handling stress.

If a horse shows signs of stress or is reluctant to move, using a companion horse first as a demonstrator is a corrective measure.

Separation of mares and unweaned foals can provide considerable stress to both the mare and the foal (Rogers et al., 2012). The stressful impact on foals when separated from their mothers, has been demonstrated using ABMs such as increased injuries and vocalisations (McGee & Smith, 2004). Therefore moving mares and foals together will minimise handling stress.

Unhandled horses, particularly those raised extensively with minimal human contact, that cannot be led by a halter and a rope, will probably experience more handling stress during the pre-killing phase, and are likely to be more fearful and stressed when interacting with handlers.

For unhandled horses, keeping them within their original groups in a calm manner that allows them to walk freely, and moving them without halters or ropes minimises handling stress. This method is especially effective for semi-feral horses, which are less accustomed to human interaction.

Injuries

The main hazards that can lead to injuries during the handling and moving of horses are related to the facilities, the level of training of the horse and the handling by the staff.

In case passageways are used to move horses from the pen to the killing area, hazards relating to the design or maintenance of the facility could lead to injuries due to collisions with solid objects when animals walk in the passageways or try to escape.

As prevention, facilities designed (i) with solid-sided runs that allow several horses to move together and not be distracted during handling and moving; and (ii) without sharp projections, other injurious structures and slippery floors will reduce the occurrence of injuries. Curved passageways work better than right angles, because the leading animals can be seen by the rest of the group.

Inappropriate handling increases the risk of injuries in horses and can exacerbate the severity of pre-existing injuries or conditions (e.g. lameness). Unhandled horses will experience more handling stress, resulting in a panic reaction that may provoke injuries while moving (e.g. due to struggling, bumping into the walls, or falling). The preventive measure for handling stress relates to staff training so that horses are handled quietly and without force (Grandin, 2014).

A mitigation measure for animals that are lame, sick, injured or have difficulties walking by themselves is not to move them and kill them on the spot (i.e. in their home pen or enclosure) to avoid them from experiencing handling stress and pain due to the injuries. The EFSA experts suggest that, if conspecifics are present in the pen, the killing should be done via lethal injection to prevent fear due to auditory stimuli resulting from the noise of captive bolt or firearm shooting.

Restriction of movement

Restriction of movement in horses is mainly due to inadequate facility design, especially related to the passageways connecting the pen area to the killing point. The same hazards and preventive measures presented above for injuries apply, for instance the use of curved passageways so that followers do not lose sight of leading animals, and the avoidance of obstacles and distractions to reduce the risk of animals baulking and/or turning back, impeding the flow. Keeping the passageways clean and dry (e.g. by adding straw or sawdust) can prevent slipping and falling. Additionally, solid boards that are too high and obstruct visual contact with other animals contribute to the risk of animals baulking and impeding the flow. Avoiding the use of too high solid boards prevents this risk.

Lastly, ensuring horses are allowed to move at their natural, unhindered walking pace helps prevent restrictions in movement, facilitating a smoother and safer flow through the facilities.

3.3.2 | Restraint

In the context of the on-farm killing described in this opinion, restraint means the application to an animal of any procedure designed to restrict its movements in order to facilitate the effective application of a stunning and/or killing method. Individual horses may be restrained manually or mechanically to present their head/body to the operator for the correct application of the method. According to the EC factsheet (European Commission, 2017) horses require restraining before being stunned with a penetrative captive bolt gun. Firearms do not always require restraint, but in some cases, animals are restrained by a halter. For lethal injection, horses are restrained via a halter to allow easy access to the jugular vein.

Types of restraints used for captive bolt stunning are:

Headcollar and lead rope, halter.

A halter with a lead rope should be used for handled horses. As a second choice, a head collar and lead rope can be used to secure and restrict the movement of the head. All halters, head collars and other equipment used to restrain or handle horses should be fitted with a method of quick release in case a horse becomes entangled.

If a horse struggles or vocalises while being restrained, it is often an indication that the restraint is causing stress.

Restraint crush

As it is not common to have a restraint box on a farm, temporary restraint structures can be constructed using readily available materials, such as strawbales or wooden boards. These can be used to configure restraint crushes to restrict the movement of horses. Alternatively, restraint devices used for medical care such as echography devices might also be used for restraining horses during on-farm killing.

The size of the restraint crush should not allow horses to go forward or backward or to turn around and must therefore be adjusted to the size of the horses. Adjusting the restraint crush constitutes a good practice, particularly for unhandled animals.

Floor conditions in the restraint and use of force by operators during the movement of horses into the restraint crush are crucial factors affecting both the effective application of the killing method and the stress levels through increased slipping (Fletcher et al., accepted for publication). Operators shouting, as opposed to those speaking calmly or staying quiet, resulted in more force required to move horses into the restraint and more slipping inside the restraint crush (Fletcher et al., accepted for publication).

3.3.2.1 | *Highly relevant WCs identified for restraint*

The highly relevant WCs identified via EKE (see Section 2.2.3) are presented in Table 4.

TABLE 4 Highly relevant welfare consequences for restraint.

Welfare consequence	Definition
Handling stress	See Section 3.3.1.1 (Table 3)
Injuries	See Section 3.3.1.1 (Table 3)
Restriction of movement	See Section 3.3.1.1 (Table 3)

A summary of the information retrieved from literature complemented by expert opinion per each of these WCs is presented in this section.

Restriction of movement

Restriction of movement is inherent to restraint in the restraint crush. It will cause fear in most cases due to the inability of the animal to escape from a novel situation.

An inappropriate size of the restraint crush worsens the situation and is considered the main hazard. A too narrow restraint crush will hamper the entrance of horses, potentially leading to fear and panic response. A restraint crush that is too wide will allow horses to turn around, which will lead to extra handling and time in the box. This might also lead to injuries.

The preventive measure is using restraint equipment designed for horses and adjusted to the size of the animal.

In addition, starting the restraint only when the operator and the equipment are ready to proceed to stunning and killing can shorten the duration of restraint and the associated restriction of movement.

In the case of manual restraint of the head (without a restraint box), a head collar and a lead rope, a halter or a bridle are used to secure and restrict the movement of the horse head. In this case, restriction of movement and injuries are mitigated by calm handling, when the horse is restrained, and by using halters, head collars and other equipment adjusted to the animal size.

Handling stress

Handling stress during restraint occurs during on-farm killing, because in most cases the restraint box is temporarily arranged and might not be adapted to suit the horse size. Gentle handling of horses while restrained and reducing the duration of restraint to the shortest possible can mitigate this stress.

If one person handles and restrains and another stuns/kills the horse there is a higher chance of shooting the animal before it becomes agitated (European Commission, 2017).

Injuries

Injuries during restraint may result from an inappropriate restraint by humans (i.e. too forceful or prolonged). If the operator uses severe force to restrain the animal for the correct application of the stunning or killing method, or if restraint lasts too long, the horse becomes agitated and the risk of injuries is increased.

Injuries may also result from a slippery floor in the restraint box, causing horses to slip and fall during restraint. Slipping causes animals to become agitated.

As prevention, animals must be restrained only when the operator and the equipment are ready to proceed to the stunning and killing procedures. Operators should be trained to handle and restrain horses (e.g. understand the species-specific behaviours) to prevent poor animal welfare outcomes.

A non-slippery floor in the restraint crush is essential to prevent slipping.

3.3.3 | Animal-based measures for all welfare consequences during Phase 1 (pre-killing)

A feasible ABM during the pre-killing phase should be minimally invasive for the animal, be collected quickly, not require any specialised equipment or laboratory test, and lead to no (or only minimal) interference with normal operations, as suggested for animal welfare monitoring during transport (Llonch et al., 2015; Messori et al., 2016).

Tables 5–7 report the ABMs suggested per each WC, along with their description and an indication of the process for which they can be used.

TABLE 5 Animal-based measures (ABMs) for the assessment of handling stress.

ABM	Description	Process
Aggressive behaviour towards the handler	Aggression is defined as threats or harmful actions directed towards the handler and can include threat displays, rearing, kicking, pushing and biting.	Handling and moving Restraint
Avoidance behaviour	Refusal to move forward and/or moving away from the source of an aversive situation (e.g. too much pressure applied by the handler) (EFSA AHAW Panel, 2022)	Handling and moving Restraint
Snorting	Horses make noise with the nose, a forceful quick expiration from the nostrils. Snorting may indicate that horses are curious and examine unknown objects (Stomp et al., 2018). However, it can also be used to warn conspecifics that danger is present if loud and repeated.	Handling and moving Restraint
Flat back ear position	Position of the ears is an indicator (body language) of the horse's behavioural response. For example, ears pricked forward indicate curiosity, ears flat back indicate fear or aggression (McDonnell, 2003).	Handling and moving Restraint

TABLE 6 Animal-based measures (ABMs) for the assessment of injuries.

ABM	Description	Process
Superficial skin lesions	Superficial tissue damage such as bruises and scratches, particularly on the tail, head, back and legs (EFSA AHAW Panel, 2012b; Mansmann & Woodie, 1995).	Handling and moving Restraint
Wounds and sores	Damage to the skin, muscle or bone tissue (EFSA AHAW Panel, 2022)	Restraint
Loud vocalisations	Horses have a large range of auditory signals. Neighs and whinnies are loud, prolonged calls, typically of 1–3 s, beginning high-pitched and ending lower-pitched.	Handling and moving Restraint

TABLE 7 Animal-based measures (ABMs) for the assessment of restriction of movement.

ABM	Description	Process
Unable to walk comfortably/freely	The horse is unable to walk at its own pace, showing short steps, jumping and struggling (EFSA AHAW Panel, 2022).	Handling and moving
Slipping and falling	Falling: Loss of balance in which parts of the body other than hooves and legs are in contact with the floor surface (extrapolated from cattle, Welfare Quality®, 2009). Slipping: Loss of balance in which the animal loses its foothold or the hooves slide on the floor surface. No other body parts except hooves and/or legs are in contact with the floor surface (extrapolated from cattle, Welfare Quality®, 2009).	Handling and moving Restraint
Loud vocalisations	Horses have a large range of auditory signals. Neighs and whinnies are loud, prolonged calls, typically of 1–3 s, beginning high-pitched and ending lower-pitched.	Restraint
Escape attempts	Attempts to go through or over gates and other barriers. Head and neck stretched forward and either held level with the back or slightly raised above or below the back line (extrapolated from cattle, from Lanier et al., 2001).	Restraint

3.4 | Phase 2: Stunning and/or killing

3.4.1 | Introduction

On-farm killing should result in loss of consciousness followed by death without causing avoidable pain or fear.

In the context of killing, the assessment of consciousness is the state that matters, because it is the prerequisite for experiencing any WC (e.g. injuries during neck cutting) and the underlying negative affective states (i.e. pain and fear).

A conscious animal after the application of a stunning and/or killing method is due to either an ineffective stunning and/or killing or to the recovery of consciousness. Recovery of consciousness can occur if the animals are not dead in case of failure of the killing method (in one-step killing methods e.g. firearms) or when the second step is performed with delay or is not properly applied (in two-step killing methods).

Therefore, ABMs can be used to evaluate consciousness before and during killing to detect animals recovering consciousness and to confirm death at the end of the procedure. This prevents the risk of discarding horses that are still alive.

The main on-farm stunning and/or killing methods for horses are penetrative captive bolts, firearms and lethal injection.

For each method the WCs, ABMs, related hazards, and preventive and corrective measures are described in Sections 3.4.2, 3.4.3 and 3.4.4.

3.4.2 | Penetrative captive bolt stunning

3.4.2.1 | *Introduction*

Penetrative captive bolts powered by cartridges are commonly used to stun horses at slaughter. The guns are designed to fire a retractable steel bolt that penetrates the cranium and enters the brain. When correctly used, the impact of the bolt on the skull results in brain concussion and immediate loss of consciousness (EFSA, 2004). The penetration of the bolt into the skull and the subsequent withdrawal causes structural damage to the brain due to cavitation, which results in marked subarachnoid and intraventricular haemorrhages, especially adjacent to the entry wound and at the base of the brain. The bolt diameter, speed and penetration depth are important parameters to ensure the efficacy of the stun. The bolt causes the disruption of brain tissue and helps to prolong the duration of unconsciousness and insensibility (EFSA, 2004).

Some guns have a captive bolt that protrudes from the muzzle when it is in the primed position, while others have a recessed bolt within the muzzle. Normally, when a bolt is fired, it requires a short distance to reach its maximum speed before impacting the skull. Therefore, at the time of firing, guns with protruding bolts should be kept at a short distance (up to 5 mm) from the animal's head, whereas guns with recessed bolts must always be pressed firmly against the head. Various factors, such as anatomical differences due to breed, sex or age of the animal, influence the efficacy of stunning.

Death may occur depending on the degree of injury to the brain, but killing is not always guaranteed (Lambooij & Algers, 2016). Therefore, captive bolt stunning is a reversible stunning method and shall be followed as quickly as possible by bleeding, destruction of the brain and upper spinal cord by pithing, or injection of a lethal substance.

Even though not preferable for biosecurity reasons, bleeding can be performed by severing both carotid arteries or the brachiocephalic trunk (for further description see the Opinion on the slaughter of horses, EFSA AHAW Panel, 2025).

According to the HSA (2016a), pithing involves inserting a flexible wire or polypropylene rod through the hole in the head made by a penetrative captive bolt. The rod is then thrust towards the tail through the brain to the level of the brainstem and into the spinal cord. It is then slid back and forth to cause maximum damage to the brain and upper spinal cord. Adhering to the manufacturer's instructions is vital to use of the pithing rod effectively.

Lethal injection (for details see Section 3.4.4) in unconscious animals using an intravenous (IV) injection of potassium chloride or magnesium sulphate has also been suggested as a second step in two-steps killing methods (AVMA, 2020).

3.4.2.2 | *Hazards for penetrative captive bolt stunning followed by killing and prevention*

An incorrect position of the shot (i.e. point of penetration in the head) or direction may lead to ineffective stunning. For instance, Fletcher et al. (accepted for publication) observed horses slaughtered in an Italian abattoir during four non-consecutive days and reported that 24% (15/62) of horses slaughtered showed signs of ineffective stunning after being shot with a penetrating captive bolt and 10% (6/62) were shot twice. Ineffective stunning was found to occur due to deviation from the correct shooting position recommended by HSA (HSA, 2016a, see below) (range of deviations = −60 to +80 mm sagittal and −55 to +65 mm lateral). In the same study, post-mortem examination of the brains for macroscopic lesions in a subset of 43% (27/62) animals indicated that the majority (85%; 23/27) of effectively stunned horses showed some level of macroscopic damage to the thalamus, midbrain and brainstem structures. In contrast, in ineffectively stunned horses and those that required a second shot, the bolt had either missed the brain completely or inflicted mild damage to temporal or occipital lobes depending upon the extent of deviation from the correct shooting position.

Another example of mis-stunning due to incorrect position or direction of the shot is the study from Caraves and Gallo (2007), who evaluated the use of pneumatically operated (penetrating captive bolt 12 bar airline pressure; 1200 kPa) in 101 horses (frontal shooting position) in Chilean slaughterhouses and reported that 78.2% of the animals were effectively stunned by the first attempt and the rest needed two or more shots. Of the 101 horses stunned, 82% showed no

sign of recovery of consciousness after the shot and during bleeding. When the location of the lesions on the skull was measured with respect to the correct shooting position, only 11.5% of the shots hit the target (within 2 cm). There was a low efficiency in the stunning process of horses, mostly due to inadequate shooting positions, that could be as far as 8 cm from the correct position. Inadequate shooting position can be due to lack of knowledge of staff and/or inefficient restraint (allowing horses to move their head at the moment of the shot).

Another hazard is the operator using a restraining technique that is not adequate to prevent movement of the animal, leading to a wrong application of the method (e.g. wrong shooting position, wrong direction etc.). In a recent study, Fletcher, Benedetti, et al. (n.d.) reported that 28 out 100 horses showed behavioural signs of ineffective stunning in a commercial abattoir in Mexico. In all of them, the shot deviated more than 10 mm from the correct position described by HSA (HSA, 2016a). One possible reason for ineffective stunning with captive bolts is that the horse, although restrained, may have moved the head leading to the wrong shooting position. Incorrect captive bolt parameters (e.g. low cartridge power, low bolt speed, shallow penetration, too narrow bolt diameter and faulty equipment) (EFSA, 2004) are other hazards for ineffective stunning.

Fletcher et al. (accepted for publication) reported that under slaughterhouse conditions, horses were shot with a captive bolt gun with 0.22-inch diameter, 2.5 grains (purple) cartridges, quoted as delivering 238J ± 10% of kinetic energy and recommended for use with small cattle, small pigs, ewes and goats. In this study, 24% (15/62) of the animals showed rhythmic breathing and other signs of consciousness after the first shot. The rates of ineffective stunning were most probably due to the underpowered cartridge, recommended for use on small animals and not on horses.

To prevent failure, according to the Humane Slaughter Association (HSA), the correct site for captive bolt stunning is in the middle of the forehead (Figure 1) and about 1 cm above the intersection point of a diagonal line from the centre of the eye to the centre of the base of the opposite ear (HSA, 2016a).

FIGURE 1 Correct shooting position for captive bolt stunning of horses (©HSA, Humane Slaughter Association): The correct point of entry of the projectile is 1 cm above the intersection of two imaginary lines, each drawn from the centre of the eye to the centre of the base of the opposite ear.

The correct direction of the bolt is when it targets the brain stem (AVMA, 2016) (Figure 2).

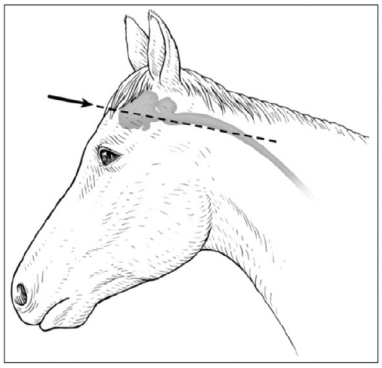

FIGURE 2 Direction of the shot (©AVMA, American Veterinary Medical Association).

The HSA (HSA, 2016a) recommended to use the types of cartridges suggested by the manufacturer for the equipment and type of animal (e.g. foal vs. stallion).

Cartridges vary in strength and are classified according to the amount of propellant (gun powder) they contain, with 3.0 grains and 4.0 grains suggested for larger horses such as draught horses (1 grain = 0.0648 g).

The speed and penetration depth of captive bolts are determined by the captive bolt gun performance. The minimum recommended bolt speed (EC, 2017) is 55 to 70 m/s, with a minimum bolt diameter of 9 mm and a recommended penetration depth of at least 7 cm. The EFSA experts consider that these key parameters lead to an effective stunning of horses, assuming that other factors, such as position and direction of the shot, are applied correctly. However, there are no published data to suggest that these parameters are valid for all horse categories. Furthermore, studies in cattle indicated that even when the recommended captive bolt parameters were applied, not 100% of the cattle were effectively stunned due to reasons related with the thickness of skull (in addition to movement of the animals and lack of skill of the staff) (Atkinson et al., 2023).

Staff training and rotation, use of an appropriate restraint, proper placement and firing of the gun, use of equipment that is fit for the purpose, and regular cleaning and maintenance of the equipment according to the manufacturer's instructions (also see HSA, 2016a) are preventive measures. Routine checking and maintenance of captive bolt guns, particularly when repeat firing occurs in a session, can prevent carbon build-up and ensure performance.

After an ineffective shot, the corrective measures are to re-stun as soon as possible in the correct shooting position, in the correct direction and with the proper captive bolt parameters, or re-stun with a backup method.

When a captive bolt enters the skull, it causes massive damage to the brain and acute swelling around the skull wound. This means that, in case of a second shot, the swelling will absorb much of the impact and therefore the second shock wave will not be effectively transmitted to the brain. As a corrective measure, the repeat position must always avoid the immediate area of the first shot. If the first shot is off target, the second should be placed as close to the correct stunning position as possible. If the first shot is on target, but fails to produce an effective stun, the second shot should be above and to one side. If a third shot is required, this should be above and to the other side of the first shot (HSA, 2016a).

In general, training staff to acquire knowledge and understanding of signs of ineffective stunning and take appropriate measures to mitigate poor welfare outcomes is crucial.

Regarding the second step for this method, the main hazard is the too-long interval between captive bolt stunning and application of a killing procedure (i.e. IV injection of a saturated solution of potassium chloride or overdose of an anaesthetic drug, pithing or bleeding) that may result in recovery of consciousness before the killing is finished.

As a prevention measure, a stun-to-kill interval of less than 60 s prevents the recovery of consciousness before death, as suggested in the EFSA opinion on the slaughter of horses (EFSA AHAW Panel, 2025).

A hazard for pithing applied as a killing procedure is when the rod is not long enough to reach the brain and the spinal cord. If the pithing rod is too short (e.g. intended for sheep), pithing is ineffective because it will not destroy the brain entirely, leading to an increased risk of recovery of consciousness and delaying the onset of death. In commercial practices, pithing rods used for horses have a length ranging from 46 to 80 cm to reach the brain and spinal cord. In addition, an appropriate movement of the rod destroys the brain and brainstem, ensuring death and reducing the reflex (convulsive) kicking, which can occur after stunning.

Hazards for lethal injections applied as a second step in two-step methods are described in Section 3.4.3.

If bleeding is used as a killing method, the main hazard is the failure to completely cut the carotid arteries or the brachiocephalic trunk. Incorrect sectioning will maintain the blood supply to the brain and may lead to recovery of consciousness or delayed onset of death. Lack of skilled operators and use of blunt or short knives are identified as hazard origins.

3.4.2.3 | *Animal-based measures to monitor the effectiveness of penetrative captive bolt stunning followed by killing*

In the killing context, ABMs are the indicators assessed to evaluate if animals are conscious and therefore exposed to negative affective states such as pain and fear.

According to the EC factsheet (EC, 2017), signs of unconsciousness after effective captive bolt stunning are immediate collapse, apnoea, tonic–clonic seizures, absence of eye reflexes (palpebral and corneal), fixed eyes with glazed expression and lack of response to painful stimuli (pinch or prick of the nose or ear). Figure 3 shows these signs and their anatomical localisation.

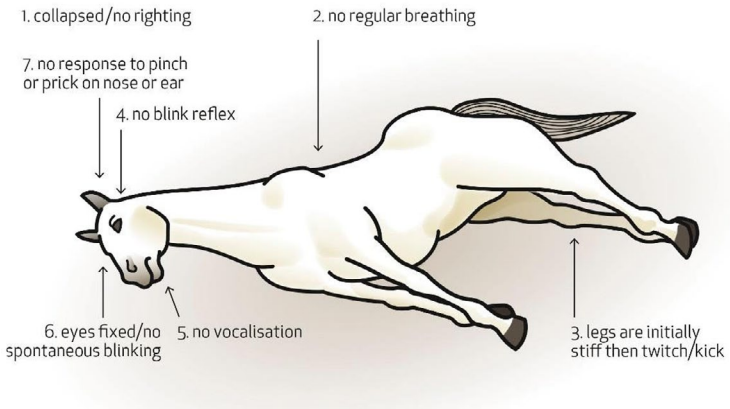

FIGURE 3 Signs of unconsciousness in horses stunned with a penetrative captive bolt (©EC, European Commission).

In line with other scientific opinions on the slaughter of various animal species (e.g. EFSA AHAW Panel, 2013), it is important to assess the overall state of consciousness of the animal, which includes an assessment of both signs of consciousness and unconsciousness. If signs of consciousness appear after stunning, an appropriate backup stunning method applied immediately can mitigate the WCs.

Werner and Gallo (2008) found that after captive bolt stunning, 57% out of the 21 horses studied in one Chilean slaughterhouse showed signs of returning to consciousness during exsanguination, with a most frequent stun-to-stick interval of 1–2 min. The main signs of return to consciousness observed were rhythmic breathing, corneal reflex, ocular movement, head elevation, vocalisation and attempts to regain posture.

In Fletcher et al. (accepted for publication) 15 of the 62 animals (24%) showed signs of ineffective stunning. Three animals failed to collapse and two attempted to get up after collapsing. All 15 animals showed rhythmic breathing, 2 had corneal and palpebral reflexes, and others showed eyeball rotation (5) and nystagmus (6).

Based on these studies and extrapolation of indicators from cattle (EFSA AHAW Panel, 2013), EFSA experts suggest the flowchart in Figure 4 to monitor the effectiveness of penetrative captive bolt stunning of horses followed by a killing method. In the flowchart, ABMs to monitor the state of consciousness during on-farm killing are suggested and included in toolboxes (blue boxes in Figure 4). For each ABM, the corresponding signs of consciousness and unconsciousness as well as corresponding signs of life and death are listed. Following key stage 1 (application of stunning with penetrative captive bolt), if horses show any sign of consciousness, an intervention should be applied (i.e. repeat the stunning). After any reintervention, the state of consciousness should be monitored again. The process can continue to key stage 2 (application of the killing method, e.g. pithing, injection of a lethal substance or bleeding) only when the corresponding signs of unconsciousness are observed. For each step, three or four ABMs that are reliable for monitoring consciousness are suggested (above the dashed line), plus two or three other ABMs, which are less reliable, that can be additionally used (below the dashed line).

Following key stage 2, in case horses show any of the signs of life, an intervention should be applied, namely the killing should be repeated (e.g. repeat the pithing so to destroy the brain, brain stem and upper spinal cord). If signs of death are observed the animals can be processed further (disposal). Signs of death are a relaxed body (complete and irreversible loss of muscle tone, which can be recognised from the limp carcass), cessation of bleeding from the sticking wound and dilated pupils.

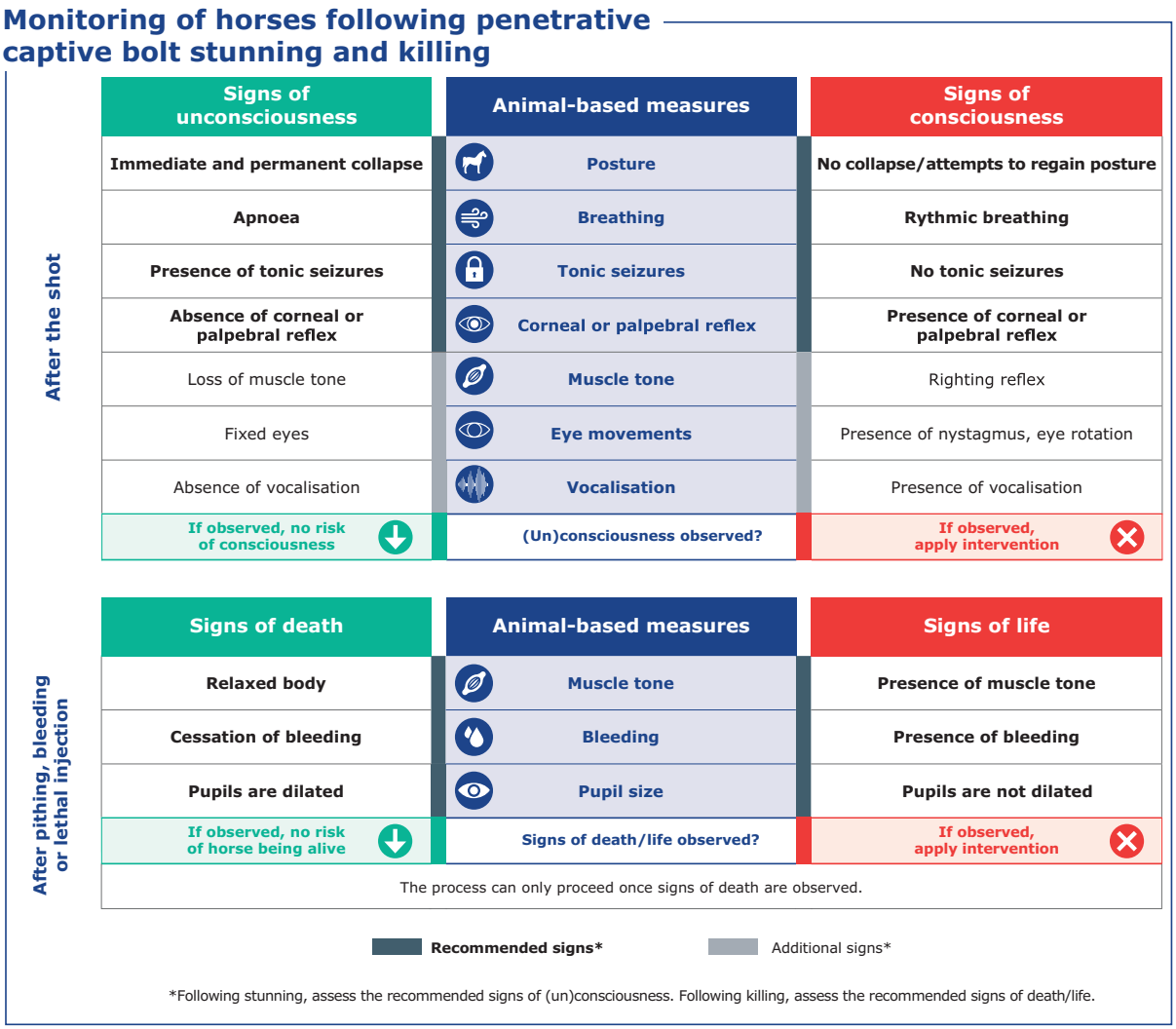

FIGURE 4 Flowchart of ABMs to be used at two steps to monitor the states of consciousness and death. If signs of consciousness or life are observed, intervention should be applied (i.e. re-stunning/killing or application of a backup method).

3.4.3 | Firearms with free projectiles

3.4.3.1 | *Introduction*

Firearms with free projectiles, such as shotguns and rifles, can be used to kill horses. This is a one-step stunning and killing method. After a successful head shot death is immediate. The correct shooting position is the same as for penetrating captive bolts.

The Humane Slaughter Association (HSA, 2016b) lists several firearms for the humane killing of horses, including shotguns (12, 16, 20, 28 and .410 gauges), handguns (.32 to .45 calibre) and rifles (.22, .243, .270 and .308). Therefore, if a .22 is used for humane slaughter, it is best fired from a rifle. In general, when comparing handguns with rifles, the longer the barrel, the higher the muzzle speed. Therefore, if a .22 is used for humane slaughter, it is best fired from a rifle (AVMA, 2016).

Another requirement is that the bullet possesses sufficient energy to penetrate the skull and enter the underlying brain tissue. The impact of the bullet on the skull will send shock waves through the brain. The bullet will tumble while travelling through the brain, creating cavitation and severe structural damage. To improve energy transfer upon impact and prevent exiting the target tissue, bullets are constructed to fragment and/or deform after hitting the target (known as Dum Dum bullets). Deformation projectiles do not lose any material, so that the projectile in the target tissue weighs almost as much as the original projectile. They are mainly used in handguns and have muzzle speeds below 450 m/s. In the case of fragmentation projectiles, the loss of mass accounts for up to half of the original mass.

Millar and Mills (2000) evaluated a .32 free bullet pistol (5.51 g; $n = 12$ horses) and found variation in the degree of intracranial damage by assessing the gross pathology of the brain but did not assess the effectiveness in provoking immediate death or recovery of consciousness. Another study carried out in Brazil ($n = 4$ horses and 4 mules), involving a .22 calibre rifle and warhead projectile, reported that five animals had to be shot two or more times before being rendered unconscious (Machado et al., 2013). In contrast, a study in an English slaughterhouse ($n = 46$ horses) reported no animals showing signs of consciousness following one shot with a free bullet rifle (Gibson et al., 2015). The sample size, firearm/ammunition characteristics, operator competence, animal type and level of restraint may explain the differences in results (Gibson et al., 2015).

Gibson et al. (2015) evaluated the effect of shooting horses and ponies with a .22 long rifle (copper plate hollow-point (≈2.6 g) 40 grains, rounds). All animals ($n = 46$) were immediately rendered unconscious, with only one pony showing an intermittently positive palpebral reflex but no other signs of brainstem function. All animals had some degree of damage to structures of the brainstem or lobes of the cerebrum, while 41 (89%) had damage to the thalamus/hypothalamus. In one pony, the bullet missed the brain but still caused mild damage to the thalamus, midbrain, pons and cerebellum, and this animal showed no signs of consciousness. The findings confirm that free bullet shooting is an effective method of killing horses when using the correct shooting position and the appropriate key parameters.

On farm, handled animals may be shot in close ranges (up to 3 m), whereas unhandled or semi-feral horses might require to be shot from larger distances because, having larger flight zones than handled animals, they may not accept the operator approaching and back off.

3.4.3.2 | *Hazard identification for firearms and prevention*

An incorrect position of the shot, due to lack of skilled operators, operator fatigue, wrong target area or angle of shooting, can lead to ineffective stunning. The correct position has been described by HSA (2016b) as being 2 cm above the crossing of two imaginary lines going from the centre of an eye to the centre of the opposite ear, with the muzzle of the firearm slightly tilted so that the shot is directed through the cerebral cortex towards the brain stem. The EFSA experts consider this shooting position comparable to the one recommended for captive bolt stunning (i.e. 1 cm above the intersection of the two imaginary lines described) and therefore suggest the latter also for firearms.

Sudden movements of the head of the horse could lead to the projectile failing to enter the skull or missing vital parts of the brain.

Ineffective stunning may also occur due to an inappropriate power and calibre of the cartridge, delivering insufficient energy to damage the brain depending on the breed, age, sex and live weight of the horse.

Similarly, the type of projectile might be inappropriate to the type of animal and the shooting distance. Metal-sleeved or jacketed high-speed bullets may exit the skull without sufficiently damaging the brain.

Retz et al. (2014), in a study on cattle, reported that the type of projectile (i.e. deformation or fragmenting bullet), did not have any effect on the effectiveness of destruction of the brain. However, the advantage of using soft-point bullets compared to full metal jackets is that they expand when they hit the target and release more energy into the tissue. This is important to cause sufficient destruction of the brain.

As prevention, the use of appropriate firearms and ammunitions is essential. Staff training can help prevent the incorrect shot position and the choice of inappropriate power, calibre of the cartridge and type of projectile. It is also important to follow the manufacturer's instructions. Training the staff to use adequate practices to monitor (un)consciousness will prevent and correct shooting failures.

Food may be placed in front of the animal to facilitate taking aim and shooting (Longair et al., 1991). The precision of the shot can be ensured by choosing the shortest possible shooting distance and selecting appropriate ammunition for long

shooting distances. For example, rim-fired cartridges may be used for shooting at close range (e.g. up to 3 metres) and centre-fired cartridges for shooting animals at longer distances (e.g. up to 25 metres).

When shooting at short range in stockyards, the use of relatively low-speed ammunition matched to the size of the animals reduces the risk of failure. Hollow-point or soft-point ammunition are preferred to solid-point ammunition, because the latter can penetrate the skull and exit at high velocity, endangering people close by. Hollow-point or soft-point ammunition deform when entering the skull, destroying brain tissue more effectively. If animals are to be shot in their paddocks, high-speed ammunition is adequate for the task (AUSVETPLAN, 2015).

For unhandled animals, the shooter position should respect the flight zone without inducing postural changes in the animal (and thus increasing the risk of a mis-shot). Telescopic devices fitted to rifles increase the accuracy of shooting.

Furthermore, the use of a silencer device can prevent the fear due to loud noises.

A corrective measure in the event of a failed shot is repeat the killing by application of a second shot after correction (e.g. correcting position, direction or bolt key parameters).

3.4.3.3 | *Animal-based measures to monitor the effectiveness of firearms*

Following a firearm shot, animals should be monitored for signs of consciousness and death. The same signs that are suggested for penetrative captive bolt stunning apply also to firearms with free projectiles (see flowchart in Figure 4).

3.4.4 | Lethal injection

3.4.4.1 | *Introduction*

Lethal injection to kill horses of all ages and weight groups involves the injection of first an anaesthetic or sedation drug, followed by the IV administration of an overdose of an anaesthetic drug or a lethal substance (Hinterhofer & Auer, 2015). Lethal injection requires restraint, usually with a halter and a rope. Particular attention should be paid that restraint does not hamper access to the jugular veins.

According to the Federation of Veterinarians of Europe (FVE), the Federation of European Equine Veterinary Association (FEEVA) and AVMA/AAEP (American Veterinary Medical Association /American Association of Equine Practitioners) an IV overdose of barbiturates after sedation is the method of choice to kill horses, as it reliably causes a quick loss of consciousness and death with the minimum amount of pain and distress for the animal. Pentobarbital sodium induces depression of the central nervous system and deep anaesthesia progressing towards respiratory and cardiac arrest. Due to the requirement of a large volume of solution, the procedure must be facilitated by using an IV catheter placed in the jugular vein. To facilitate catheterisation of an excitable equid, a tranquilliser, such as acepromazine, or an α2- agonist can be administered, but these drugs may delay loss of consciousness because of their effect on circulation and may result in varying degrees of muscular activity and agonal gasping. Opioid agonists or agonist-antagonists in conjunction with α2-adrenergic receptor agonists may also be used (AVMA, 2020). Barbiturates, when injected too slowly or in insufficient doses, can cause an initial excitation phase that can result in difficulties for the operator in finishing the injection, therefore leading to an increased risk of administration of a sub-lethal dose. Therefore, sedatives (alpha-2 agonists such as xylazine, detomidine or romifidine) are administered prior to the barbiturate overdose to minimise violent thrashing and provide a more controlled recumbency process (Desjardins & Touzot-Jourde, 2011).

AVMA (2020) considered adjunctive methods for on-farm killing of horses with substances other than barbiturates. These methods are performed on horses anaesthetised with an IV injection of xylazine-ketamine and then killed by one of the following: (1) saturated solution of potassium chloride (KCl) injected IV or intracardially; (2) saturated solution of magnesium sulphate ($MgSO_4$) injected IV; or (3) 60 mL of 2% lidocaine injected intrathecally. A saturated solution of potassium chloride (dosage 75–150 mg/kg IV) or magnesium sulphate (2 mL/kg IV) will induce cardiac arrest. Intrathecal administration of 2% lidocaine hydrochloride to anaesthetised horses resulted in the sequential loss of respiration, cerebrocortical activity, brainstem function and cardiovascular activity. In line with AVMA (2020) the FVE and the FEEVA have stated in a joint statement (FVE/FEEVA, 2021) that an IV injection of a saturated solution of KCl, $MgSO_4$ or any commercially-available euthanasia solution (e.g. T61®) and intrathecal administration of lidocaine are conditionally acceptable methods, i.e. these substances can be administered only to horses under general anaesthesia. The most common commercially-available euthanasia solution is T61® which contains (i) embutramide (a non-barbituric anaesthetic inducing severe cardiovascular depression); (ii) mebezonium iodide (a curarising agent inducing muscular paralysis) and (iii) tetracaine hydrochloride (a local anaesthetic, used because embutramide is irritant). T61® is used at a dose of 4 to 6 mL/50 kg. It is unclear in horses if unconsciousness appears before or after respiratory paralysis, and it is for this reason that anaesthesia must be performed before T61® injection. Unconsciousness must be confirmed before injection of the lethal substance.

3.4.4.2 | *Hazard identification for lethal injection and prevention*

The primary hazard associated with lethal injection is the administration of a dose lower than that recommended by the manufacturer or the use of an incorrect route of administration (i.e. extravascular), resulting in an insufficient dose to

achieve rapid death. The use of any route of administration different from the ones recommended by the manufacturer can be caused by inexperienced operators or sudden movements of the animals during the administration of the drug.

Individual animals may have to be weighed in order to calculate the lethal dose, otherwise some animals may receive less than the dose required to cause rapid death.

Preventive methods to avoid the above-described hazards and their WCs are: adherence to the manufacturer's instructions, training of the staff to use appropriate restraint of the animal to avoid extravasation of the drug and accurate dose calculation using the horses' live weight.

3.4.4.3 | *ABMs for monitoring the effectiveness of lethal injection*

The same signs of consciousness and death that are suggested for penetrative captive bolt stunning apply also to lethal injection (see flowchart in Figure 4).

3.5 | Unacceptable methods, procedures or practices on welfare grounds

The mandate requests to identify methods that are unacceptable on welfare grounds. In response to this ToR, the EFSA experts listed practices and classified them as leading to severe pain and fear, based on expert opinion. However, due to lack of information, the severity of pain and fear associated to these practices is unknown. These practices are considered unacceptable on welfare grounds and should be avoided.

Firstly, the EFSA experts agree with Council Regulation (EC) 1099/2009 on unacceptable methods and practices and the list of prohibited restraining methods. Some of these methods can be related to the killing of horses:

- mechanical clamping or tying of the legs or feet of animals;
- severing the spinal cord, such as using a puntilla or dagger.

Similarly, the EFSA experts agree with the principle in Chapter 7.5.10 of the WOAH Terrestrial code (OIE, 2019), which says that 'methods and practices e.g. restraining methods [...] that cause severe pain and stress in animals, cannot be considered acceptable'.

In addition, the EFSA experts consider unacceptable from the welfare perspective the following practices as they will induce severe pain and fear:

- moving severely injured horses or those unable to move independently;
- using painful procedures to move animals (e.g. electric goads, sticks) or restrain them (e.g. twitches);
- (rope) casting of horse for restraint;
- sticking and bleeding conscious horses;
- painful induction of death in conscious animals (e.g. euthanasia solutions, application of an electric current across the chest to induce cardiac arrest in conscious animals).

Finally, methods, which are likely to be highly painful, and have not been scientifically reported must not be used to kill horses, for instance (but this list is not exhaustive): inflicting injuries and wounds leading to death, burying, drowning, suffocating, adding pesticides or any other toxic substances to feed or water, injection of air, injection of chemical agents or other substances not specifically designed or labelled for killing (i.e. disinfectants, cleaning solutions etc.).

3.6 | Results of the uncertainty analysis

Table 8 shows the key conclusions with potential relevance for risk management. For each conclusion, the table lists the questions formulated to capture the certainty around the corresponding conclusion. Each question refers to the proportion of a population (hypothetically) exposed to the scenario described in the conclusion that would be affected (see Section 2.2.5 for details).

For each question, experts were asked to provide their individual judgement together with the rationale. A consensus range reflecting the collective certainty about the statement was then reached through group discussion or, if consensus could not be achieved, the wider range encompassing all individual judgements was retained.

TABLE 8 Conclusions and questions for uncertainty assessment and final consensus certainty range.

	Conclusion	Question for uncertainty assessment	Certainty range
1	Using adequate captive bolt key parameters (minimum bolt diameter of 9 mm, minimum bolt length of 8 cm and speed of at least 55 m/s) leads to effective stunning when all other factors dependent on the technique (such as position and direction of the shot) are applied correctly.	How certain are you that 99% or more horses stunned with captive bolts with a minimum bolt diameter of 9 mm, a bolt length of 8 cm and a speed of at least 55 m/s are unconscious (assuming stunning is applied correctly)?	66%–100% There is uncertainty because these parameters are not validated on all horse categories and other factors not related to the technique but related to the animal (e.g. conformation and thickness of the skull) will influence the effectiveness of the technique.
2	The correct shooting position for captive bolt stunning is about 1 cm above the intersection of two imaginary lines, each drawn from the centre of the eye to the centre of the base of the opposite ear.	How certain are you that 99% or more horses that have been stunned with captive bolts with a shooting position of about 1 cm above the intersection of two imaginary lines, each drawn from the centre of the eye to the centre of the base of the opposite ear are unconscious (assuming other parameters are applied correctly e.g. bolt power, bullet speed and size etc)?	66%–100% There is uncertainty because animals might be ineffectively stunned even though the shooting position was correct due to other factors linked to animal anatomical variability (e.g. conformation and thickness of the skull).
3	The correct shooting position for firearms is about 1 cm above the intersection of two imaginary lines, each drawn from the centre of the eye to the centre of the base of the opposite ear.	How certain are you that 99% or more horses that have been shot with firearms with a shooting position of about 1 cm above the intersection of two imaginary lines, each drawn from the centre of the eye to the centre of the base of the opposite ear are dead (assuming other parameters are applied correctly e.g. bolt power, bullet speed and size etc)?	90%–100% Despite some uncertainty linked to animal anatomical variability (e.g. conformation and thickness of the skull), shooting with firearms has a very high likelihood of destroying the brain even when the shooting position is slightly deviated.
4	Injection of barbiturates without prior sedation or anaesthesia can lead to pain and fear due to an initial excitation phase resulting in an incorrect injection (e.g. sub-lethal dose or extravascular administration).	How certain are you that 1% or more horses that are injected with barbiturates without prior sedation or anaesthesia experience pain and fear?	90%–100% Very limited uncertainty as almost all horses will show the excitation phase.
5	Injection of lethal substances (e.g. saturated solution of potassium chloride or magnesium sulphate; lidocaine or euthanasia solution) without anaesthesia leads to painful induction of cardiac or respiratory arrest in conscious animals.	How certain are you that 1% or more horses that are injected with lethal substances without prior anaesthesia experience pain and fear?	90%–100% Very limited uncertainty as almost all horses will experience pain.
6	One method of lethal injection is (1) sedation by alpha-2 agonist such as xylazine, detomidine or romifidine followed by (2) overdose of barbiturates. This combination is effective in killing horses.	How certain are you that 99% or more horses that have been injected with this combination are killed?	90%–100% Very limited uncertainty as almost all horses will die with this combination.
7	Another effective method is (1) anaesthesia with xylazine-ketamine and then (2) killing by one of the following substances: saturated solution of potassium chloride (KCl) injected IV or intracardially; saturated solution of magnesium sulphate injected IV; lidocaine injected intrathecally or euthanasia solution injected IV.	How certain are you that 99% or more horses that have been injected with this combination are killed?	90%–100% Very limited uncertainty as most horses will die with this combination.
8	A maximum stun-to-kill interval of 60 s prevents return to consciousness during killing.	How certain are you that 99% or more horses killed within 60 s after effective stunning will not recover consciousness before death?	66%–100% There is uncertainty because this interval is extrapolated from recommendations on cattle and there is no data on horses.

3.7 | Specific hazards related to types of animals or species (ToR 4)

Some horses are at a higher risk of experiencing welfare consequences. Most of these were presented in different sections of the opinion, along with specific preventive measures:

- unhandled and semi-feral horses are more prone to handling stress than handled horses and should be moved in groups and without halters and ropes (see Section 3.3.1.1 under handling stress);
- agitated horses are more susceptible to slips or falls (Fletcher et al., accepted for publication; Fletcher et al., 2023) and should be handled with care;
- horses with specific temperament (e.g. due to previous housing, management and handling, or specific breeds) are more prone to handling stress (Lloyd et al., 2008); to prevent this the operator should adapt to the specific needs of the individual;
- mares panic when separated from their foal and this will lead to stress and agitation; therefore mares and foals should be killed together (see Section 3.3.1.1 under handling stress).

4 | CONCLUSIONS

Preamble: The on-farm killing process is divided into Phase 1, which involves the processes (i) handling and moving the animals to the killing site and (ii) restraint, and Phase 2, which involves the application of stunning and/or killing methods. Certainty assessments are provided in brackets below only for key conclusions that could be considered in risk management decisions.

4.1 | Conclusions for handling and moving

1. The highly relevant welfare consequences that horses may experience during handling and moving are restriction of movement, handling stress and injuries.
2. Rushing horses can cause handling stress and injuries, as well as make animals more difficult to handle subsequently (e.g. during restraint for killing).
3. Moving unhandled horses with their original groups and without halters or ropes prevents handling stress. This method is also effective for semi-feral horses, which are less accustomed to human interaction.
4. Moving individually handled horses by a halter and rope to the killing area prevents handling stress.
5. Using a companion horse can help encourage reluctant animals to move more willingly.

4.2 | Conclusions for restraint

1. Stunning with penetrative captive bolt and killing with lethal injection require restraint of the horse, whereas killing with firearms does not always require restraint.
2. During restraint horses experience restriction of movement and may additionally experience handling stress and injuries.
3. The highly relevant welfare consequences during restraint primarily originate from restraint itself, with hazards including inappropriate restraint (forceful or prolonged), inadequate design of the restraint crush, inappropriate size of the restraint crush and slippery floor in the restraint crush.

4.3 | Conclusions for stunning and/or killing

1. During the application of the killing method, horses can experience pain and fear if they are ineffectively stunned or if they recover consciousness before dying. ABMs related to the state of consciousness can be used to indirectly assess pain and fear during this phase.
2. In two-step killing methods, where stunning is followed by a second step to cause death, delays in performing the second step increase the risk of animals regaining consciousness before death occurs.
3. Penetrative captive bolt stunning is the first step of a two-step killing method. Bleeding, pithing or lethal injection are applied as the second step to induce death.
4. For penetrative captive bolt followed by killing, a maximum stun-to-kill interval of 60 s prevents return to consciousness before death occurs (certainty 66%–100% - likely).
5. Ineffective captive bolt stunning is mostly due to wrong shooting position and/or direction of the shot and/or inappropriate stunning key parameters (bolt length, diameter, speed, penetration depth, etc.).
6. Using adequate captive bolt parameters (minimum bolt diameter of 9 mm, minimum bolt length of 8 cm and speed of at least 55 m/s) leads to effective stunning when all other factors dependent on the technique (such as position and direction of the shot) are applied correctly (certainty 66%–100% - likely).
7. A hazard for pithing applied as a second step following captive bolt stunning is when the rod is not long enough to reach the brain and the spinal cord, leading to the recovery of consciousness and delay in the onset of death.
8. A hazard for bleeding is the failure to completely cut the carotid arteries or the brachiocephalic trunk, leading to recovery of consciousness or delayed onset of death.
9. The use of firearms is a one-step stunning and killing method. After a successful head shot death is immediate.

10. Hazards for firearm killing are the incorrect position of the shot, inappropriate power and calibre of the cartridge or type of projectile.
11. The firearm shooting distance depends on the gun/rifle and ammunition type, operator skill and whether the horse is handled or unhandled.
12. The shooting position described (i.e. about 1 cm above the intersection point of a diagonal line from the centre of the eye to the centre of the base of the opposite ear, with the bolt targeting the brain stem) leads to effective stunning with captive bolt (certainty 66%–100% - likely) and with firearms (certainty 90%–100% – very likely).
13. Lethal injection performed in two steps – first injecting an anaesthetic or sedating drug, followed by an overdose of anaesthetic or a lethal substance – is effective in killing horses of all ages and weights, when properly delivered (i.e. following the manufacturer's instructions about dose, route and rate of administration).
14. For lethal injections, failure to kill the horses is due to incorrect administration routes (e.g. extravascular administration) or sub-lethal doses.
15. One method of lethal injection is (1) sedation by IV injection alpha-2 agonist such as xylazine, detomidine or romifidine followed by (2) an IV overdose of barbiturates. This combination is effective in killing horses (certainty 90%–100% - very likely).
16. Another effective method is (1) anaesthesia with IV injection of xylazine-ketamine and then (2) killing by one of the following substances: saturated solution of potassium chloride (KCl) injected IV or intracardially; saturated solution of magnesium sulphate injected IV; lidocaine injected intrathecally or commercially-available euthanasia solution injected IV (certainty 90%–100% – very likely).
17. Injection of barbiturates without prior sedation or anaesthesia can lead to pain and fear due to an initial excitation phase, resulting in the risk of wrong administration (e.g. sub-lethal dose or extravascular administration) (certainty 90%–100% – very likely).
18. Injection of lethal substances (e.g. lidocaine, commercially-available euthanasia solutions or saturated solutions of potassium chloride or magnesium sulphate) in conscious animals (i.e. without anaesthesia) leads to painful induction of cardiac or respiratory arrest (certainty 90%–100% – very likely).
19. When killing is unsuccessful, horses are subjected to the risk of being disposed of alive and therefore experiencing pain and fear.

4.4 | Conclusions on unacceptable methods and practices on welfare grounds

1. The EFSA experts consider the following practices unacceptable on welfare grounds as they induce severe pain:
    - moving severely injured horses or those unable to move independently;
    - use of painful procedures to move animals (e.g. electric goads, sticks) or restrain them (e.g. twitches);
    - sticking and bleeding of conscious horses;
    - (rope) casting of horse for restraint;
    - painful induction of death in conscious animals (e.g. euthanasia solutions, application of an electric current across the chest to induce cardiac arrest or sticking);
    - any other painful practice such as burying, drowning, suffocating, addition of pesticides or any other toxic substances to feed or water, injection of air, injection of chemical agents or other substances not specifically designed or labelled for the killing of horses (i.e. disinfectants, cleaning solutions etc.). Please note that this list is not exhaustive.

5 | RECOMMENDATIONS

5.1 | General recommendations

1. Design, construction and maintenance of the handling and moving facilities (i.e. passageways from the pen/ paddock/ pasture to the killing area) should account for how horses perceive their environment and meet their welfare requirements to prevent restriction of movement, handling stress and injuries during killing.
2. The whole killing procedure should be carried out by trained and skilled personnel with a good understanding of species-specific behaviour. Staff should know the previous experience and management (e.g. handled vs. unhandled) of the horse(s) to be killed and act accordingly.
3. The welfare of horses should be assessed at each phase of on-farm killing to prevent and correct hazards and mitigate the welfare consequences.
4. Killing practices considered unacceptable on welfare grounds must not be used.

5.2 | Recommendations for handling and moving

1. Horses that are blind, lame, injured, show signs of severe pain or illness, or those unable to move without assistance should be killed in their home pens or pastures, with lethal injection as the preferred method in case conspecifics are present.
2. Horses should be moved gently and never be forced to move faster than their unhindered walking pace.
3. Handled horses should be moved by halter and rope to the killing area.
4. Semi-feral and unhandled animals should be handled with extra care and should be moved with their original group and without halters or ropes.

5.3 | Recommendations for restraint

1. Horses should be restrained appropriately avoiding using excessive force.
2. Animals should only be restrained when the operator is ready to stun them. The duration of restraint should be kept to a minimum.
3. Strawbales or wooden boards can be used to construct a temporary restraint crush.
4. The size of the restraint crush should be adjusted to fit the horse size, so that the animal cannot move back and forward or turn around.
5. The floor in the restraint crush should be clean, dry and non-slippery.

5.4 | Recommendations for stunning and/or killing

1. When penetrative captive bolt is used, the minimum recommended bolt speed should be 55 m/s, with a bolt diameter of at least 9 mm and a minimum length of 8 cm, ensuring a penetration depth of at least 7 cm. However, further research is needed to validate these parameters for all horse categories.
2. The shooting position for penetrative captive bolts and firearms, (which is about 1 cm above the intersection of a diagonal line extending from the centre of the eye to the centre of the base of the opposite ear), should be used. The bolt should be directed towards the brain stem for maximum effectiveness.
3. When pithing is used as a second step after captive bolt stunning, it should be performed with a rod long enough to ensure it reaches the brain, brainstem and upper spinal cord.
4. When bleeding is used as a second step after captive bolt stunning, the severance of the carotid arteries or brachiocephalic trunk should be ensured.
5. For penetrative captive bolt stunning followed by killing, the stun-to-kill interval should be as short as possible and never last more than 60 s.
6. Lethal injection should always be done in two steps, i.e. anaesthesia or sedation followed by injection of a lethal substance.
7. Lethal injection should be performed strictly following the manufacturer's instructions about dose, route and rate of administration.
8. After the application of a stunning and/or killing method, the state of unconsciousness and the state of death should be confirmed, using the recommended ABMs (Figure 4).
9. Animals showing signs of recovery of consciousness should be re-stunned/killed immediately in an appropriate way (e.g. adjusting the position or direction of the shot).
10. In case of animals showing signs of life, the killing method should be immediately re-applied correctly (e.g. repeat the pithing so to destroy the brain, brain stem and upper spinal cord).
11. The state of death should be confirmed before proceeding to carcass disposal using ABMs.

ABBREVIATIONS

A	Ampere
ABM	animal-based measure
EEG	electroencephalogram
EKE	expert knowledge elicitation
Hz	Hertz
IV	intravenous
PCB	penetrative captive bolt
V	volt
WC	welfare consequence

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Literature search outcomes for horses

As described in Section 2.2.1, a literature search was carried out to identify peer-reviewed scientific evidence on the topic of killing of horses that could provide information on the elements requested by the ToRs (i.e. description of the processes, identification of hazards, origins, preventive and corrective measures, welfare consequences and indicators).

To obtain this, firstly a broad literature search was carried out, and the results were then screened and refined as described below.

Sources of information included in the search: Bibliographic database Web of Science.

The search string was designed to retrieve relevant documents to 'animal welfare' during 'killing' of 'horses'. Restrictions applied in the search string related to the processes characterising killing (from handling and moving to killing) of animals and the date of publication (considering only those records published after EFSA, 2004). No language or document type restrictions were applied in the search string.

Web of science search string	
Years 2004–2019[a]	
Search terms	**Category Field searched**
TS = horse OR TS = horses OR TS = equidae OR TS = equine OR TS = equines OR TS = "Equus ferus caballus"	Topic
AND	
TS = kill* OR TS = stun* OR TS = euthanasia	Topic
AND	
TS = handl* OR TS = mov* OR TS = restrain* OR TS = cut* OR TS = bleed* OR TS = conscious* OR TS = pain* OR TS = behav* OR TS = stress*	Topic
AND	
TS = "on farm" OR TS = on-farm	Topic
AND	
TS=Welf* OR TS = "animal welfare"	Topic

[a] Date of the search: 13 July 2024.

Refinement of literature search results

The search yielded a total of 22 records that were exported to an EndNote library together with the relevant metadata (e.g. title, authors, abstract). Titles and abstracts were firstly screened to remove irrelevant publications (e.g. related to species, processes and research purposes that were out of the scope of this opinion) and duplicates, and successively to identify their relevance to the topic.

Full-text publications were screened if the title and abstract did not allow to assess the relevance of a paper. The screening was performed by one reviewer, with support by a second reviewer in cases of doubt; publications that were not considered relevant nor providing any additional value to address the question were also removed. The screening led to eight relevant records:

1. Binder and Baumgarnter (2015)
2. EFSA AHAW Panel (2022)
3. Fletcher et al. (2022)
4. Fletcher et al. (2023)
5. Gibson et al. (2015)
6. Hinterhofer and Auer (2015)
7. Messori et al. (2016)
8. Werner and Gallo (2008)

APPENDIX B

Expert knowledge elicitation

For the identification of highly relevant welfare consequences (sub-question 2 in Annex A) a semi-formal exercise for expert knowledge elicitation (EKE) was carried out. This involved the classification of welfare consequences by the experts first at individual level, followed by group discussion to identify the highly relevant ones by consensus.

The starting point was the list of 33 specific welfare consequences identified by the AHAW Panel in the methodological guidance produced in the context of the F2F strategy revision (for details see section 3.1.1.3, EFSA AHAW Panel, 2022, 2022).

The exercise consists of selecting the highly relevant welfare consequences out of these 33 for each of the following processes of Phase 1: (i) handling and moving and (ii) restraint.

For each process, the EFSA experts classified the 33 welfare consequences into four categories of relevance: (i) non-applicable, e.g. the welfare consequence 'Inability to perform suckling behaviour' was not considered relevant for the animal categories covered by the mandate, (ii) slightly relevant, e.g. the welfare consequence 'inability to perform play behaviour' might apply, but its effect on welfare (in terms of prevalence and/or severity) was considered to be minimal compared to other welfare consequences during on-farm killing, (iii) moderately relevant and (iv) highly relevant.

The relevance was based on an estimate of the severity, duration and occurrence of each WC in each process (EFSA AHAW Panel, 2012a). Severity refers to the intensity of the welfare consequence. Duration refers to the time an animal spends within a specific process. Occurrence refers to the prevalence of animals experiencing the welfare consequence during that specific process.

Owing to the lack of published data, the EFSA experts expressed their qualitative expert opinion on these three parameters.

Expert opinion was elicited in three steps:

- First step: The five EFSA experts individually screened the list of welfare consequences and identified those that would fall in the 'non-applicable' or 'slightly relevant' categories. Their individual judgements were then collated, and those welfare consequences unanimously identified as belonging to these two categories were removed and not considered for further assessment. Those welfare consequences for which there was no consensus on whether they should be considered 'non-applicable' or 'less relevant' remained for further assessment and required an open group discussion to find a consensus.
- Second step: The experts individually screened the list of remaining welfare consequences and identified those that would fall in the category 'highly relevant' and should be kept for further assessment (Sub-question 2). Similarly, as done during the first step in case discrepant opinions emerged, consensus was sought through group discussion.
- Third step: The experts were asked to rank individually all the remaining welfare consequences in the list that were not already identified as highly relevant (and thus kept) or non-applicable or slightly relevant (and thus removed) from the most to the least relevant. Their individual rankings were then discussed again in an open group discussion to assign the remaining welfare consequences to the category 'highly relevant' or 'moderately relevant'.

The scientific opinion only reports, for each of the defined processes, those welfare consequences selected to be highly relevant from this exercise.

APPENDIX C

Sources of uncertainty

Source of uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
Expert group – number & type of experts	A limited number of experts (5) were selected based on the subject (killing of horses), and this may have resulted in a bias in the assessment processes.	The number of highly relevant WCs, ABMs and/or hazards may be incomplete.
Methodology for selection of highly relevant welfare consequences (per each killing phase/process)	The selection of highly relevant WCs per each killing process was achieved by EKE for which a pre-defined scale of relevance was used.	The highly relevant WCs may have been wrongly classified (a welfare consequence selected might have been included in error and similarly, another welfare consequence might have been excluded in error).
Extensive literature search - Language	The search was performed exclusively in English. More studies might have been identified by including references with abstracts in languages other than English.	The ABMs and/or hazards may be incomplete.
Extensive literature search – Publication type	Studies considered included primary research studies identified through the extensive literature search and grey literature (factsheets, guidelines, conference papers, EU reports, book chapters, unpublished papers etc.) known to the EFSA experts, but an extensive search of the grey literature was not conducted, Therefore, there may be reports (grey literature) and other guidance documents on animal welfare of which the Working group is not aware.	The listed ABMs and/or hazards may be incomplete.
Extensive literature search – Search strings	The search criteria were reported, however some synonyms may not have been used in the search strings, and thus less hits might have been retrieved.	The listed ABMs and/or hazards may be incomplete.
Extensive literature search – Source of studies	The search was limited to Web of Science Core Collection and four more specialist/subject-specific databases (AGRICOLA, AGRIS, CABI - Animal Health & Production Compendium and MEDLINE). Although the search was complemented by internet searches and manual searches of the publicly available literature, no data were retrieved from other sources (e.g. industry data). More information could have been retrieved by applying a different methodology (e.g. public call for data).	The listed ABMs and/or hazards may be incomplete.
Extensive literature search – inclusion and exclusion criteria	A conservative approach was applied during the screening phase which could have led to the exclusion of certain studies that could have included relevant information.	Incomplete overview of existing information. The ABMs and/or hazards may be incomplete.
Animal Species and Animal categories considered in the studies retrieved in the literature search	The animal breeds or categories used in the studies retrieved might not be the ones currently used in the EU to study WCs and ABMs, thus requiring an extrapolation exercise from the experts.	Underestimation of the magnitude of the WCs and related ABMs. Overestimation of the magnitude of the WCs in case the retrieved papers are pointing to a specific category of horses.
Killing methods in the studies retrieved in the literature search	The studies retrieved through the literature search could have been performed anywhere in the world, and thus may consider killing conditions different from those currently allowed in the EU, also regarding animal welfare.	Underestimation/overestimation of the level of magnitude of the WCs and related hazards.

ANNEX A

Protocol for the development of the scientific opinion

Annex A is available under the Supporting Information section on the online version of the scientific output.