European Food Safety Authority

EFSA Journal 2013;11(6):3249

SCIENTIFIC OPINION

Scientific Opinion on the electrical parameters for the stunning of lambs and kid goats[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 23 July 2013, replaces the earlier version published on 11 June 2013.*

ABSTRACT

The Panel on Animal Health and Welfare was asked to deliver a scientific opinion on two studies performed by IRTA: "Evaluation of the electrical stunning effectiveness in sheep with a current intensity lower than 1 Ampere" and "Evaluation of the electrical stunning effectiveness with electric currents lower than 1 A in lambs and kid goats". To achieve this, the first step was to define the type of study, critical variables, experimental design, data collection and analysis and reporting needed to supply scientific evidence that a given electrical stunning protocol of small ruminants provides a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A. These criteria were then applied to the two IRTA studies. The submitted studies are not adequate for a full welfare assessment of the alternative method studied because they do not fulfil the eligibility criteria and the reporting quality criteria defined in this opinion. The shortcomings of the studies are identified to make clear where improvements are required. To be considered for a full assessment of the welfare implications of the use of minimum currents lower than 1 A for electrical stunning of small ruminants a study must meet the eligibility standards described herein. A full assessment of the welfare implications of the use of minimum currents lower than 1 A for electrical stunning of small ruminants would need to take into account the restraining methods, the pre-stunning, and the stunning phases of the slaughter process and the correlation of the study findings with the results of other scientific evidence.

KEY WORDS

electrical stunning, lambs, kid goats, reporting criteria

---

[1] On request from the European Commission, Question No EFSA-Q-2013-00237, adopted on 22 May 2013.

[2] Panel members: Edith Authie, Charlotte Berg, Anette Bøtner, Howard Browman, Ilaria Capua, Aline De Koeijer, Klaus Depner, Mariano Domingo, Sandra Edwards, Christine Fourichon, Frank Koenen, Simon More, Mohan Raj, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Ivar Vågsholm, Antonio Velarde, Preben Willeberg and Stéphan Zientara. Correspondence: ahaw@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank Howard Browman and Mohan Raj for the preparatory work on this scientific opinion and the hearing experts: Bert Lambooij, Michael Marahrens and Martin von Wenzlawowicz, and EFSA staff: Andrea Gervelmeyer, Frank Verdonck and Ana Afonso for the support provided to this scientific opinion.

* Minor changes of editorial nature were made. The changes do not affect the overall conclusions of the opinion. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013. Scientific Opinion on the electrical parameters for the stunning of lambs and kid goats. EFSA Journal 2013;11(6):3249, 40 pp. doi:10.2903/j.efsa.2013.3249

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare (AHAW) was asked to deliver a scientific opinion on the use of minimum currents lower than 1 A for electrical stunning of small ruminants. Specifically, the European Food Safety Authority (EFSA) was asked to give its view on the findings of the studies performed by IRTA: "Evaluation of the electrical stunning effectiveness in sheep with a current intensity lower than 1 Ampere" (study 1) and "Evaluation of the electrical stunning effectiveness with electric currents lower than 1 A in lambs and kid goats" (study 2).

As a first step, the type of study and data needed to supply scientific evidence that a given electrical stunning protocol for small ruminants provides a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A were defined (TOR 2). These were then applied to the studies submitted for review to assess the extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A (TOR 1).

EFSA assessed only the stunning procedure itself and did not take into account any pre-stunning phases. The outcome of the assessment in this opinion indicates only whether the submitted study is adequate for a full welfare assessment of the alternative method studied or not, whereas quality and strength of evidence will be assessed at the next stage.

TOR 2: Definition of the type of study and data needed to supply scientific evidence that a given electrical stunning protocol for small ruminants provides a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A

The opinion defines **eligibility criteria** of studies on alternative stunning methods that are based on the legal framework provided in Council Regulation (EC) No 1099/2009 and its Annex I. For consistency with the legislation, the eligibility criteria defined in this opinion specify only the minimum requirements. The minimum criteria that should be reported by studies on stunning methods to fully characterise the stunning intervention were defined to allow assessment of the alternative stunning method. Regarding the outcome measures, the onset and duration of unconsciousness and insensibility should be recorded and reported in all studies. If the onset of unconsciousness/insensibility achieved by the studied stunning intervention is not immediate, then the absence of pain, distress and suffering until loss of consciousness/sensibility also has to be recorded and reported.

Regarding the **intervention**, electrical stunning, the legislation states that the key parameters to be provided are minimum current, minimum voltage, maximum frequency, minimum time of exposure, maximum stun-to-stick/kill interval(s), frequency of calibration of the equipment, optimisation of the current flow, prevention of electrical shocks before stunning, position and contact surface area of electrodes. Studies analysing a modification of a currently permitted method need to describe all of the legal key parameters. In order to ensure a comprehensive description of the applied stunning method, for some parameters additional information on several components of these parameters, which have been defined in this opinion, need to be reported.

Onset of unconsciousness and insensibility is best demonstrated by an electroencephalogram (EEG). Unconsciousness and insensibility after electrical stunning can be ascertained by the induction of a generalised epileptiform activity in the brain, followed by a quiescent EEG. Once the effectiveness of a given stunning method has been shown in controlled environment studies using EEGs, its effectiveness should also be studied in experiments under slaughterhouse conditions. Indicators of recognising a successful stun should be applied in slaughterhouse settings, after their correlation with EEGs has been shown in controlled environment studies. In this opinion, the indicators recognising a successful stun, which need to be ascertained to be sure that the animal is unconscious and insensible

after an electrical stun, have been defined as the presence of presence of tonic seizures, the presence of apnoea and the lack of response to painful stimuli.

If a stunning method does not induce immediate unconsciousness/insensibility, the **absence of pain, distress and suffering until the onset of unconsciousness/insensibility** should be assessed. Pain is a complex phenomenon and is very difficult to measure qualitatively and quantitatively owing to the absence of clear borders among pain, distress and suffering, as these states may not always be distinguishable in animals. At the moment, indirect animal-based measures of pain, distress and suffering have to be used as no direct tool is available to identify pain. Several examples of animal-based measures from the three response types (behavioural changes, physiological changes and neurological changes), which could be applied to observe changes in these responses, are listed in this opinion. It is recommended that the animal-based measures are selected according to their relevance to the respective stunning intervention as shown by the available scientific knowledge of each measure's sensitivity and specificity. It has further been determined that two criteria/rules have to be fulfilled before a stunning method is considered to not induce pain, distress and suffering before the onset of unconsciousness and insensibility, namely that (1) animal-based measures from at least two different response types of the three response types presented above and relevant to the intervention/species must be indicative of absence of pain, distress and suffering before the onset of unconsciousness/insensibility, and that (2) these animal-based measures should be consistent at the level of the individual animal, depending upon the species and the coping strategies.

Studies in a controlled environment should determine the **duration of unconsciousness/insensibility** using EEGs as described for the determination of the onset of unconsciousness/insensibility. The maximal stun-to-stick/kill time interval that guarantees unequivocal loss of consciousness/sensibility until the moment of death can be defined based on these results. The applicability of the stun-to-stick/kill interval should then be analysed in commercial settings using indicators of recognising recovery of consciousness/sensibility that correlate with EEGs as established in controlled environment studies. In this opinion, it has been defined that the indicators recognising a successful stun, which need to be ascertained to be sure that the animal is unconscious and insensible after an electrical stun, are the presence of tonic seizures, the presence of apnoea and the lack of response to painful stimuli.

For the definition of **reporting quality** criteria suitable existing reporting guidelines were identified and their criteria lists slightly modified to allow their use in the context of studies on stunning methods.

The **methodological quality** assessment focuses on the fulfilment of the internal and external validity of the submitted study. Internal validity is reached when the study results reflect reality among the animals under study, whereas external validity is reached when the study results can reasonably be generalised to the broader reference population. It was decided to assess only the main biases affecting internal validity, namely confounding, selection bias and information bias and only in the case that the submitted study fulfils the eligibility criteria.

TOR 1: Assess the extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A based on the submitted studies

The review to assess the extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A, based on the submitted studies was carried out according to the criteria defined under TOR 2. For both study 1 and study 2, the intervention is considered to be insufficiently described. The onset of unconsciousness and insensibility has not been adequately assessed in study 1, while in study 2 it is not possible to assess whether the onset of unconsciousness and insensibility has been adequately assessed. The duration of unconsciousness has not been adequately addressed in study 1; in study 2 it is not possible to assess whether the duration of unconsciousness has been adequately addressed. Neither study 1 nor study 2

fulfils the reporting quality criteria. As the studies did not fulfil the eligibility criteria, the methodological quality of the studies was not assessed. Therefore, the shortcomings of the studies have been highlighted to indicate where improvements are required before the studies can be submitted for a full assessment of the welfare implications of the use of minimum currents lower than 1 A for electrical stunning of small ruminants, which would need to take into account both pre-stunning and stunning phases of the slaughter process and the correlation of the study findings with results of other scientific evidence.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Article 4 (2) of Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing[4] allows the Commission to amend stunning parameters laid down in Annex I to this Regulation as to take into account scientific and technical progress on the basis of an opinion of the EFSA. Any such amendments shall ensure a level of animal welfare at least equivalent to that ensured by the existing methods.

At present, a minimum current of 1 Ampere (A) is required for both head-only[5] and head-to-body[6] electrical stunning of small ruminants. The Commission has received a request from the Spanish authorities and the Catalan meat federation (FECIC) to amend points 4.2 and 5.1 of Chapter II of Annex I to Regulation 1099/2009 as regards the minimum current for small ruminants for respectively head-only and head-to-body electrical stunning. This request is supported by two studies performed by IRTA.

In order to reply to this request, the Commission would like to request the EFSA to review the scientific knowledge on the electrical stunning of small ruminants of these studies.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission therefore considers it opportune to request the EFSA to give an independent view on the use of a lower minimum current than 1 A for electrical stunning of small ruminants.

The scope of this request is limited to the head-only and head-to-body electrical stunning of small ruminants (ovine and caprine species).

The EFSA will give its view on the findings of the study performed by IRTA "Evaluation of the electrical stunning effectiveness in sheep with a current intensity lower than 1 Ampere" and the study performed by IRTA "Evaluation of the electrical stunning effectiveness with electric currents lower than 1 A in lambs and kid goats" with a focus on the following issues:

− The extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A;

− The extent to which the findings of the study are consistent with other sources on electrical stunning of small ruminants (in particular on lowering the current for younger/smaller animals);

− The extent to which the findings of the study can be valid for different breeds of small ruminants;

− Additional requirements possibly linked to the use of minimum currents lower than 1 A for small ruminants, in particular in terms of maximum live weight and possibly of other conditions (minimum voltage, maximum frequency, time of exposure, stun-to-stick interval, etc.).

[4] OJ L 303, 18.11.2009, p. 1.
[5] Point 4.2 of Chapter II of Annex I to Regulation 1099/2009.
[6] Point 5.1 of Chapter II of Annex I to Regulation 1099/2009.

ASSESSMENT

1. Introduction

Electrical stunning consists of the application of a current to the brain that is sufficiently high to induce grand mal epilepsy in the brain, followed by a spreading depression due to hyperpolarisation, rendering the animal unconscious and insensible (EFSA, 2004). Electrical stunning is widely used for stunning small ruminants and can be used as head-only or head-to-body stunning. Annex 1 of Council Regulation (EC) No 1099/2009 specifies the minimum currents for head-only or head-to-body stunning of sheep and goats, but does not differentiate between different subgroups of these, e.g. lambs versus adults. On receipt of the mandate, its terms of reference were discussed with the European Commission service and the following clarifications were made.

EFSA will give its view on the findings of the two studies submitted by the Spanish authorities ("Evaluation of the electrical stunning effectiveness in sheep with a current intensity lower than 1 Ampere", from now on referred to as "study 1" and "Evaluation of the electrical stunning effectiveness with electric currents lower than 1 A in lambs and kid goats", from now on referred to as "study 2"), with a focus on:

TOR 1: The extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A

TOR 2: The type of study and data needed to supply scientific evidence that a given electrical stunning protocol of small ruminants provides a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A

The term "acceptable alternative" in this opinion is defined as an alternative stunning method that is at least as good as those listed in the Council Regulation (EC) No 1099/2009. Specifically, the alternative procedure must induce immediate onset of unconsciousness/insensibility or absence of pain, distress and suffering until the onset of unconsciousness/insensibility and the animal must remain unconscious/insensible until death.

The moment of animal exposure to the electric current is considered as the start of the stunning phase. The pre-stunning handling and restraint methods are not considered in this opinion on account of the terms of reference. However, the implications of the pre-stunning and restraint are very important for animal welfare and should be considered in a full welfare assessment of a stunning method for any given species.

The opinion defines eligibility criteria of studies on alternative stunning methods that are based on the legal framework provided in Council Regulation (EC) No 1099/2009 and its Annex I. For consistency with the legislation, the eligibility criteria defined in this opinion specify only the minimum requirements. The criteria concerning the outcome of the intervention are based on the legal definition of stunning and consequently focus on the onset and duration of unconsciousness and insensibility as well as the absence of pain, distress and suffering in case the onset of unconsciousness and insensibility is not immediate.

EFSA assessed only the stunning procedure itself and did not take into account any pre-stunning phases. A full assessment of the welfare implications of the use of minimum currents lower than 1 A for electrical stunning of lambs and kid goats, which would need to take into account both pre-stunning and stunning phases of the slaughter process and the correlation of the study findings with the results of other scientific studies, is beyond the scope of this mandate as the TORs are restricted to the assessment of the submitted studies. The outcome of the assessment in this opinion indicates only whether the submitted studies are adequate for a full welfare assessment of the alternative method studied, whereas quality and strength of evidence will be assessed at the next stage.

This opinion is just the first step in providing guidance to the AHAW Panel for assessing studies examining alternative stunning methods. A document covering all stunning methods listed in Council Regulation (EC) No 1099/2009 with detailed guidance on assessing alternative stunning methods types will be generated and published in the near future.

2. Approach

The submitted study documents were assessed regarding fulfilment of eligibility criteria, reporting quality and methodological quality criteria. The criteria were first defined (fulfilment of TOR 2) and then applied to assess the submitted studies with the objective of determining the extent to which the use of minimum currents lower than 1 A is an acceptable alternative for the stunning of lambs and kid goats based on the submitted studies (fulfilment of TOR1) (Figure 1). The assessment was first individually carried out by each working group member. The individual assessments were then discussed to reach a consensus on parameters where experts had initially had different opinions.

Eligibility criteria

Council Regulation (EC) No 1099/2009 defines "stunning" in Article 2(f) as "any intentionally induced process which causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death". Furthermore, Article 4 on stunning methods regulates that "animals shall only be killed after stunning in accordance with the methods and specific requirements related to the application of those methods set out in Annex I of the Regulation" and "that the loss of consciousness and sensibility shall be maintained until the death of the animal". The methods referred to in Annex I that do not result in instantaneous death shall be followed as quickly as possible by a procedure ensuring death such as bleeding, pithing, electrocution or prolonged exposure to anoxia. Most of the methods listed in Annex 1 cause immediate onset of unconsciousness, with the exception of controlled atmosphere- or gas-stunning methods. Eligibility criteria that need to be fulfilled by submitted studies were set based on the legislation and focussed on the intervention and the outcome:

For the intervention :

The key parameters described in the legislation and provided by stunning experts

For the outcome :

A. Immediate onset of unconsciousness and insensibility **OR**
B. Absence of avoidable pain, distress and suffering until the loss of consciousness and sensibility **AND**
C. Duration of the unconsciousness and insensibility (until death)

The minimum criteria that should be reported by studies on stunning methods to fully characterise the stunning intervention were defined to allow assessment of the alternative stunning method. Regarding the outcome measures, the onset and duration of unconsciousness and insensibility should be recorded and reported in all studies. If the onset of unconsciousness/insensibility achieved by the studied stunning intervention is not immediate, then the absence of pain, distress and suffering until the loss of consciousness/sensibility also has to be recorded and reported.

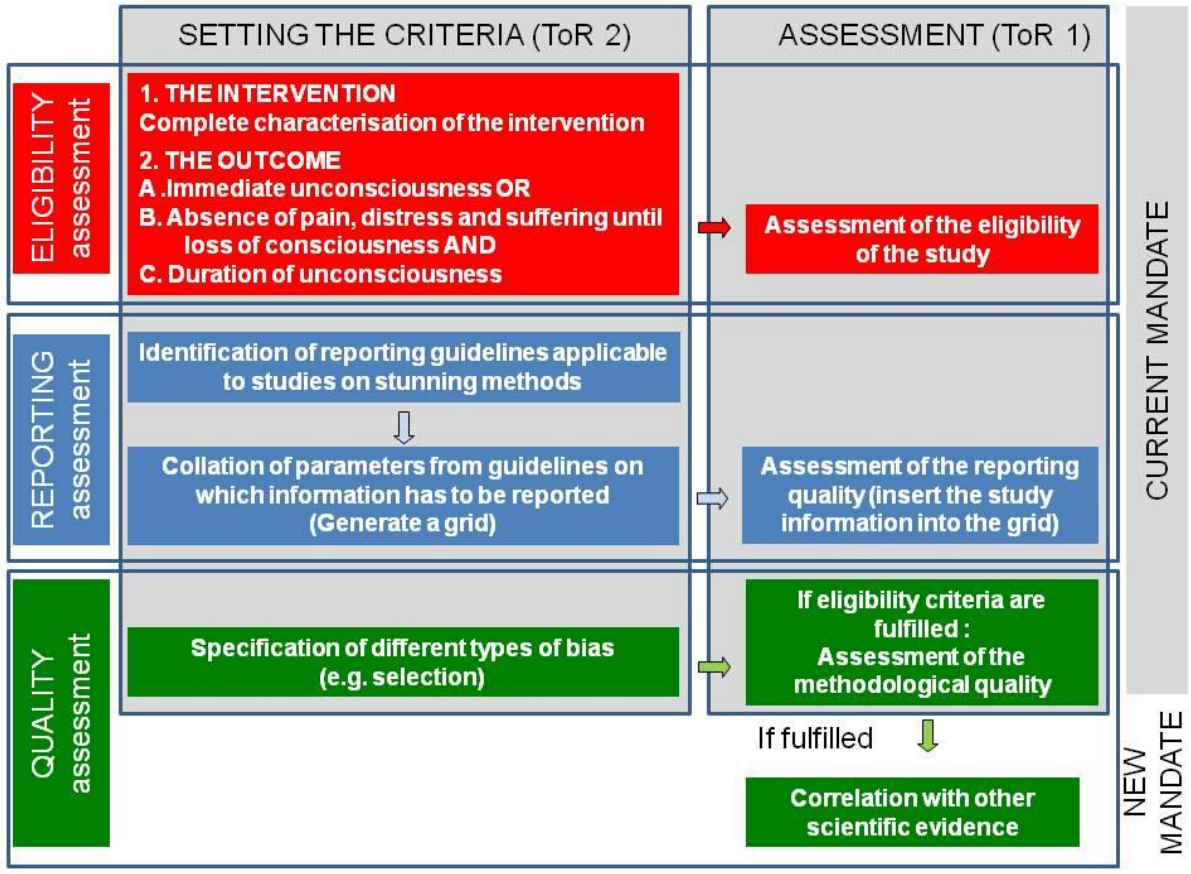

Figure 1: The approach of the mandate.

Reporting quality

Inconsistencies in the reporting of scientific studies have been identified in the fields of both human and veterinary medicine. Therefore, reporting guidelines designed to increase the transparency of conducting and reporting such scientific studies have been developed by various groups in the past. As these guidelines were not developed to be applied specifically to studies on stunning methods, the two most relevant guidelines were identified. Both guidelines were screened and the relevant parameters in relation to studies on stunning methods were listed and later used as the basis for assessing the reporting quality of the submitted studies.

Quality assessment

The methodological quality of the submitted studies is assessed only if the eligibility criteria are fulfilled. At this stage, the presence of biases affecting internal validity is assessed: confounding, selection and information bias.

An analysis of the external validity of the results of the submitted studies, including comparing them with other available scientific evidence will only be performed if all requirements of the previous steps of the assessment (assessment of eligibility criteria, reporting quality criteria and methodological quality criteria) have been met by the submitted study. However, this analysis is beyond the time frame of the current mandate and will be performed only if the European Commission provides a new mandate for this task.

Furthermore, results obtained under controlled laboratory conditions need to be confirmed in a range of slaughterhouse conditions. Therefore, the analysis of alternative stunning methods requires a first phase of the study under controlled (laboratory) conditions to analyse the animals' responses (unconsciousness, absence of pain) using the most sensitive and specific methods and to find a

correlation with non-invasive parameters that can be applied during the second phase of the study in slaughterhouses. The eligibility criteria should be applied to both phases of the study. Information obtained in other species can be used as an indication, but should be confirmed in the species under investigation because coping strategies, pain thresholds and tolerances are species and individual specific.

Possible conclusions
When all criteria regarding eligibility, reporting quality and methodological quality have been assessed individually, an overall conclusion is provided. There are two possible overall conclusions of the assessment made in this opinion:

- All the criteria regarding eligibility, reporting quality and methodological quality are fulfilled and the results are conclusive.

  This means that the study on the alternative method provides sufficient detail regarding the intervention and the outcome with conclusive results allowing to conclude that it does induce immediate onset of unconsciousness/insensibility and that unconsciousness/insensibility lasts sufficiently long to cover the stun-to-stick interval and onset of brain death through loss of blood.

  In consequence, the study could be further assessed in the context of additional scientific evidence, and taking account of both pre-stunning and stunning phases and restraint methods of the slaughter process, under a new mandate.

- Not all the criteria regarding eligibility, reporting quality and methodological quality are fulfilled or the results of the submitted study are inconclusive.

  This means that the study does not provide sufficient detail regarding the intervention and the outcome and/or the results are inconclusive as to whether it does induce immediate onset of unconsciousness/insensibility and whether unconsciousness/insensibility lasts sufficiently long to cover the stun-to-stick interval and onset of brain death through loss of blood.

  In consequence, the assessment would highlight the shortcomings to indicate where improvements are required before the study can be further assessed in the context of additional scientific evidence and taking account of both the pre-stunning and stunning phases and restraint methods of the slaughter process.

3. Eligibility criteria

As described in section 2, the requirements specified in this section are based on the definition of stunning laid down by Council Regulation (EC) No 1099/2009[7] on the protection of animals at the time of killing and are applied as eligibility criteria for assessing studies in this opinion.

3.1. Specification of eligibility criteria

3.1.1. Intervention

At the moment, head-only and head-to-body electrical stunning is permitted in all species when the technical criteria described in Annex I of Council Regulation (EC) No 1099/2009 are fulfilled. When using head-only electrical stunning, the legislative requirements prescribe that the brain should be exposed to a current generating a generalised epileptiform activity in the electroencephalogram (EEG) and the electrodes should span the brain of the animal and be adapted to its size. In addition, the stunning intervention should be carried out in accordance with the minimum current of 1 A for animals of ovine and caprine species, regardless of their age. For head-to-body electrical stunning, the

[7] COUNCIL REGULATION (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. OJ L 303, 18.11.2009, p. 1-30.

electrodes should span the brain and heart leading to a generalised epileptiform activity in the EEG and the fibrillation or stopping of the heart. The minimum currents should be 1 A for sheep and goats. The legislation states that the key parameters to be provided are: minimum current, minimum voltage, maximum frequency, minimum time of exposure, maximum stun-to-stick/kill interval(s), frequency of calibration of the equipment, optimisation of the current flow, prevention of electrical shocks before stunning, position and contact surface area of electrodes. Studies analysing a modification of a currently permitted method need to describe all of the legal key parameters. Some parameters are divided into several detailed components to ensure a comprehensive description of the applied stunning method (Table 1).

For studies researching a new or modified simple stunning method, animals should be stunned without sticking to establish the duration of unconsciousness achieved by the stunning itself in proof-of-concept studies under controlled laboratory conditions. The experimental protocol should consider humane endpoints and therefore, in the case of the long-term adverse effects of the stun experienced, the animal should be re-stunned and bled as soon as it regains consciousness.

Table 1: Parameters to be provided when applying a stunning method based on head-only and head-to-body electrical stunning, based on Annex I of Council Regulation (EC) No 1099/2009 and further specifications of components of the parameters

Parameter	Component	Description
Minimum current (A or mA)	Current type	The electrical current used to stun animals can be either sine or square wave alternating current (bipolar or biphasic) or pulsed direct current (monopolar or monophasic). Define the current type used
	Waveform	The waveform of current used for stunning animal varies widely and includes clipped or rectified sine or square waves. The proportion of clipping also varies widely. Define the waveform used including the proportion of clippings; report the marks-spaced ratio, when pulsed direct current is used
	Minimum current[a]	Specify the minimum current (A or mA) to which animals are exposed. Explain how this value was obtained. Normally, when using sine wave alternating current the minimum current will be expressed as root mean square current. When a pulsed direct current is used, the minimum will be expressed as average current. Describe how the minimum current was calculated
	Latency[a]	Specify how soon the minimum current was reached after the intervention was applied to the animal
Minimum voltage (V)	Exposed minimum voltage (V)[a]	Specify the minimum voltage (V) to which animals are exposed. Explain how this value was measured (e.g. peak voltage, peak-peak voltage, root mean square voltage or average voltage). Root mean square voltage is the recommended description of the exposed minimum voltage
	Delivered minimum voltage (V)[a]	According to the Ohm's law, the amount of voltage required to deliver 1 A will depend upon the electrical resistance in the pathways, which in turn is determined by several factors. Describe how the stunning equipment was set up to deliver the minimum current level to the animal
Maximum frequency (Hz)	Maximum frequency (Hz)	If applicable, define the maximum frequency (Hz) applied to the animal
	Minimum frequency (Hz)	If applicable, define the minimum frequency (Hz) applied to the animal
Minimum time exposure[a]		Define the minimum duration of electrical exposure applied to the animals
Maximum stun-to-stick-/kill		Describe the maximum stun-to-stick/kill interval that has been applied

Parameter	Component	Description
interval(s) [a,b]		to guarantee unconsciousness/insensibility of the stunned animal until the moment of death (except for proof-of-concept studies where the duration of unconsciousness must be determined without sticking)
Frequency of calibration of the equipment		Provide information on the method used for and the time intervals between consecutive calibrations of the equipment
Optimisation of the current flow	Electrode characteristics	The form of the stunning tongs or electrodes and the material are important to overcome the resistance in the pathway. Provide a description of the electrode (form/shape, presence and description of spikes (depth of penetration), wetting)
	Electrode appearance	The condition (e.g. corroded) and cleanliness (fat and wool cover, carbonisation of dirt) of stunning electrodes contribute to the electrical resistance. Electrodes should be cleaned regularly using a wire brush to prevent build-up of materials. Describe the appearance of the electrodes as well as the method used to clean them between use on individual animals
	Animal restraining	Describe how animals are restrained
Prevention of electrical shocks before stunning		Explain how the animals are protected from inadvertent, unintentional electrical shocks immediately before the stunning intervention is initiated. For instance, the stunning electrodes could be placed firmly without slipping and held with uniform pressure throughout the duration of stunning to ensure that the current flows uninterruptedly
Position and contact surface area of electrodes	Position of the electrodes	Specify the anatomical position where the electrodes are attached to the animal and the method to hold electrodes in place during the intervention. Placement and application of electrodes should be described and validated
	Type of electrode	Provide information on the type of electrodes used (e.g. tong, wand, …)
	Animal skin condition	The amount of wool/hair covering the head at the site of stunning electrode position is critical as the electrical resistance increases with the increasing amount of wool. The wool should be wetted to reduce electrical resistance and improve current flow. Provide a description of the study population in relation to the wool/hair cover, cleanliness of the coat (e.g. clipped or not, breed, wet/dry head)

[a]Provide information on mean or median and range and standard deviation or interquartile range.
[b]In case of simple stunning.

3.1.2. Outcome

3.1.2.1. Onset of unconsciousness and insensibility

The EFSA Scientific Report of the Scientific Panel for Animal Health and Welfare on a request from the Commission related to welfare aspects of animal stunning and killing methods concludes that stunning and stunning/killing methods should ideally induce an immediate (e.g. in less than one second) and unequivocal loss of consciousness and sensibility. Electrical stunning methods are considered to lead to immediate onset of unconsciousness and insensibility (EFSA, 2004).

The neuronal basis of consciousness with regard to stunning is presented in detail in the EFSA 2004 report. The normal functioning of neurons in the thalamus and cerebral cortex is accepted as a necessary condition for perceptual processes and consciousness. Therefore, stunning methods should disrupt the depolarised state of neurons in the brain and thereby render animals unconscious and insensible. The extent of disruption caused by a stunning method and the induction of unconsciousness and insensibility are best demonstrated using EEGs (EFSA, 2004). EEGs or electrocorticograms (ECoGs) are widely used to record the spontaneous and evoked electrical activity in the brain to ascertain the state of consciousness and sensibility following stunning. EEG signatures correlated with loss of consciousness are reported in humans (e.g. Gandelman-Marton and Neufeld, 2012; Purdon et al., 2013) and different animals, but can depend on how unconsciousness is induced, e.g. on whether

electrical, mechanical or modified atmosphere stunning is used (e.g. Raj et al., 1992 and 1998; Cook et al., 1995 and 1996, EFSA, 2004; Gerritzen et al., 2004 and 2006; Benson et al., 2012a, 2012b).

Two kinds of electrical stunning methods are used at present for small ruminants, head-only and head-to-body application of an electrical current. The electrical stunning of animals with a current of sufficient magnitude and duration leads to long-lasting strong depolarisation of the cell membrane leading to grand mal epilepsy. The grand mal epilepsy is followed by a period of quiescence in the EEG, which is referred to as spreading depression and occurs as a result of hyperpolarisation. When these two EEG manifestations occur after electrical stunning, the animals are considered to be unconscious and insensible (EFSA, 2004). Therefore, unconsciousness and insensibility can be ascertained by the following EEG patterns:

- After head-only electrical stunning:
    - induction of a generalised epileptiform activity in the brain, which can be recognised from the predominance of 8–13 Hz high-amplitude EEG activity **AND**
    - the epileptiform activity is followed by a quiescent EEG after head-only electrical stunning.
- After head-to-body electrical stunning:
    - induction of a generalised epileptiform activity in the brain, which can be recognised from the predominance of 8–13 Hz high-amplitude EEG activity **AND**
    - the epileptiform activity is followed by a quiescent EEG when cardiac ventricular fibrillation leading to cardiac arrest is induced during head-to-body stunning.

The occurrence of the epileptiform activity ensures immediate onset of unconsciousness during head-to-body stunning and the onset of quiescent EEG confirms the successful induction of cardiac arrest.

It is important to note that once the effectiveness of a given stunning method has been shown in controlled environment studies using EEGs, its effectiveness should also be studied in experiments under slaughterhouse conditions. Indicators of recognising a successful stun (see paragraph below) should be applied in slaughterhouse settings, after their correlation with EEGs has been shown in controlled environment studies.

Indicators of recognition of a successful electrical stun:

Generalised epileptiform activity induced by head-only or head-to-body stunning results in immediate collapse of the animal and occurrence of tonic seizures, which can be used as behavioural indicators (depending on the slaughter process). Head-only electrical stunning induced tonic seizure leads to clonic seizure. On the other hand, head-to-body stunning induced tonic seizure may be very short and the clonic seizure will be absent, due to cardiac fibrillation in animals. The occurrence of tonic seizure after the application of the electric current followed by apnoea, or lack of response to painful stimuli, can be used together to recognise effective electrical stunning (as monitoring points) under slaughterhouse conditions. However, under the head-only stunning situation, the animal has the capacity to recover consciousness straight after the tonic seizure, i.e. to resume breathing during clonic seizures. Seizures can also be induced by currents below the level needed to induce epileptiform activity in the brain/unconsciousness. Electro-immobilisation, which may occur during electrical head-to-body stunning, can prevent the animal from showing reactions to painful stimuli although it is sensible. For these reasons, it is necessary that all three indicators (presence of tonic seizures, apnoea, lack of response to painful stimuli) need to be ascertained to be sure that the animal is unconscious and insensible.

Indicators of failed stunning are escape behaviour, often with prolonged purposeful vocalisation, absence of the typical tonic or clonic muscle activity, resumption of rhythmic breathing, during and after the current application or righting attempts after current application. If the eyeball is able to focus and follow stimuli from the surroundings, the animal is conscious (EFSA, 2004).

Studies on alternative stunning methods should explain in detail how and when the onset of unconsciousness and insensibility is measured. It is recommended that the methods used have previously been published in peer-reviewed journals, that data are provided at the individual animal level and that actions are taken to prevent the possibility of bias (see section 5) as much as possible. In the case of EEG measurements, all parameters crucial for assessment of the EEG data should be specified (e.g. the electrode position at the skull or on the brain itself, the configuration of the electrode (transhemispheric or from the same hemisphere of the brain)). In order to estimate quantitative changes occurring in the EEG, the method used to derive the transformations of EEG signals must be described. In addition, the indicators used to assess recognition of a successful stun should be relevant to the respective stunning intervention, based on the available scientific knowledge of each indicator's sensitivity and specificity. Furthermore, the scoring system applied to categorise/classify the indicators should be clearly defined. It is essential that the observers making the measurements of the indicators have been carefully trained and that scoring systems are adapted to the species and the stunning conditions. Information on all these aspects should be provided and will be assessed by the AHAW Panel based on the scientific knowledge available at that time.

3.1.2.2. Absence of pain, distress and suffering until the loss of unconsciousness and sensibility

Effective electrical stunning, with currents of sufficient magnitude, is considered to lead to immediate onset of unconsciousness and insensibility; therefore the absence of pain, distress and suffering between the application of the stun and the onset of unconsciousness and insensibility does not need to be assessed.

However, any attempt to stun an animal with a current less than that required for achieving immediate loss of consciousness and sensibility will be painful and it is also known that the amount of current necessary to induce seizures is less than that required to induce epileptiform activity in the brain, indicative of unconsciousness and insensibility. Therefore, the assessment of the onset of unconsciousness and insensibility by EEG is required to eliminate any uncertainties.

If a stunning method does not induce immediate unconsciousness/insensibility, the absence of pain, distress and suffering until the onset of unconsciousness/insensibility should be assessed. Pain is a complex phenomenon and is very difficult to measure qualitatively and quantitatively owing to the absence of clear borders among pain, distress and suffering, as these states may not always be distinguishable in animals. At the moment, indirect animal-based measures of pain, distress and suffering have to be used as no direct tool is available to identify pain. In addition, thresholds for pain, distress and suffering can be different between animals within and between species. Inherent concealing of pain in animals has been reported (Underwood, 2002). Several definitions of pain are frequently reported in the scientific literature (e.g. Zimmermann, 1986; IASP, 1994; Molony, 1997; Broom, 2001; OIE, 2012). Kavaliers (1988), based on the International Association for the Study of Pain 1979 definition, suggested that for non-humans, pain is an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species-specific behaviour, including social behaviour. Although there are more recent definitions, this one is considered to be appropriate for this opinion. Previous EFSA opinions and scientific papers focus on assessing three "response types" for the evaluation of pain: behavioural changes, physiological changes and neurological changes.

Table 2: Overview of response types and animal-based measures associated with pain, distress and suffering during the induction of unconsciousness and insensibility

Response type	Groups of animal-based measures	Example	References
Behaviour	Vocalisations	e.g. number and duration, intensity, spectral components	EFSA, 2005; Le Neindre et al., 2009; Atkinson et al., 2012; Landa, 2012; Llonch et al., 2012a, 2012b, 2013
	Postures and movements	e.g. kicking, tail flicking, avoidance	Jongman et al., 2000; EFSA, 2005; McKeegan et al., 2006; Gerritzen et al., 2007; Velarde et al., 2007; Kirkden et al., 2008; Svendsen et al., 2008; Dalmau et al., 2010; Atkinson et al., 2012; Landa, 2012; Llonch et al., 2012a, 2012b, 2013
	General behaviour	e.g. agitation, freezing	EFSA 2005; Landa, 2012
Physiological response	Hormone concentrations	e.g. HPA[a] axis: cortisol, ACTH[b]; sympathetic system: adrenalin, noradrenaline	Mellor et al., 2000; EFSA, 2005; Le Neindre et al., 2009; Coetzee et al., 2010; Landa, 2012
	Blood metabolites	e.g. glucose, lactate, free fatty acids	EFSA, 2005; Vogel et al., 2011; Landa 2012; Mota-Rojas et al., 2012
	Autonomic responses	e.g. heart rate, blood pressure, respiratory rate, body temperature, dilatation of the pupil, sweating	Martoft et al., 2001; EFSA ,2005; Gerritzen et al., 2007; Rodriguez et al., 2008; Svendsen et al., 2008; Dalmau et al., 2010; Le Neindre et al., 2009; McKeegan et al., 2011; Atkinson et al., 2012; Landa, 2012; Llonch et al., 2012a, 2012b, 2013
Neurological response	Brain activity	e.g. EEG, ECoG	Raj et al., 1998; Martoft et al., 2001; Murrell et al., 2003; EFSA, 2005; Gibson et al., 2009; Johnson et al., 2012; Llonch et al., 2012a, 2012b, 2013

[a]HPA, hypothalamic–pituitary–adrenal.
[b]ACTH, adrenocorticotrophic hormone.

Studies on alternative stunning methods should assess at least animal-based measures from behavioural, physiological and neurological response types (see Table 2) using methods previously published in peer-reviewed journals, and data should be provided at the individual animal level. In the methods section of the studies, it should be explained how and when the animal-based measures were performed and analysed. It is recommended that the animal-based measures are examined under experimental conditions - for each animal undergoing the stunning procedure - (1) during exposure of the animal to the procedure/apparatus without the actual stunning (providing a baseline result) and again (2) during exposure of the animal to the full procedure/apparatus including the stunning act. Comparison of the two observations differentiates between pain, distress and suffering due to the handling process vs pain, distress and suffering due to the stunning itself. Animals may be acclimatised or sensitised to the new procedure apparatus in the second operation, depending upon the species, the circumstances and the severity of pain, distress and suffering. In the event of a high pre-stun response, additional experiments with an adjusted experimental design should be sought to enable a more critical evaluation of the stunning itself. Making pre- and post-stunning observations on the same animal reduces the risk of selection bias. The scoring system of the measure should be clearly defined. The uniformity of high scores among the animals exposed to the stunning intervention (as evidenced by a low standard deviation of the response) is an indication of the presence of pain, distress and suffering. The greater the variance, the more plausible is the argument that it is a matter of the individual animal's response (EFSA, 2005). On the other hand, highly variable animal responses could also indicate inconsistent effects of the alternative stunning method. The various animal-based measures should be examined independently of each other and in all animals in the study population.

It is recommended that the animal-based measures are selected according to their relevance to the respective stunning intervention as shown by the available scientific knowledge of each measure's sensitivity and specificity. Detailed experimental protocols should be provided to allow assessment of the limitations of the selected animal-based measures. For instance, animals connected to measuring equipment may behave differently, the effect of the sampling procedure or the latency of a physiological response could influence the results obtained with physiological parameters, and exposure of an animal to a new environment could change its autonomic responses. Therefore, the combination of indicators to be used depends on the design of the study and the animal species.

Animal-based measures to identify pain, distress and suffering are often subjective and have a relatively low specificity and/or sensitivity (EFSA, 2005; Le Neindre et al., 2009). Therefore, two criteria/rules have to be fulfilled before a stunning method is considered not to induce pain, distress and suffering before the onset of unconsciousness and insensibility:

- Animal-based measures from at least two different response types out of the three response types presented in Table 2 relevant to the intervention/species (e.g. behavioural and physiological) must be indicative of the absence of pain, distress and suffering before the onset of unconsciousness/insensibility. This means that these animal-based measures should not be significantly different when the response of the animals exposed to the procedure/apparatus without the stunning act is compared with their response following exposure to the procedure/apparatus including the stunning act, provided that the pain and distress responses are not already maximum before the actual stunning.
- In general, these animal-based measures should be consistent at the level of the individual animal, depending upon the species and the coping strategies (that is, consistent with respect to their interpretation).

Finally, it is essential that the observers making the measurements have been carefully trained and that scoring systems are adapted to the species and the stunning conditions. Information on all these aspects should be provided and will be assessed by the AHAW Panel based on the scientific knowledge available at that time.

3.1.2.3. Duration of the unconsciousness and insensibility

Council Regulation (EC) No 1099/2009 states that unconsciousness/insensibility induced by stunning should last until the moment of death. Studies in a controlled environment should determine the duration of unconsciousness/insensibility using EEG as described in section 3.1.2.1. Based upon the obtained results (e.g. the shortest time to recovery of consciousness observed minus 2 SD), the maximal stun-to-stick/-kill time interval can be defined that guarantees unequivocal loss of consciousness/sensibility until the moment of death (EFSA, 2004). The applicability of the stun-to-stick/-kill interval should then be analysed in commercial settings using indicators that recognise recovery of consciousness/sensibility that correlate with EEGs as established in controlled environment studies. The selection of useful indicators will also depend upon the stunning method and the species involved. It is acceptable that studies on alternative stunning methods assess only the duration of unconsciousness as this will always precede the recovery of sensibility.

The duration of unconsciousness induced by head-only stunning is equal to the duration of epileptiform activity plus the duration of the quiescent phase and depends upon factors such as the position of the electrodes, the current level and the stun duration (Velarde et al., 2000; Beyssen et al., 2004; Berg et al., 2012). On the other hand, head-to-body electrical stunning induces epileptiform activity followed by a quiescent EEG when cardiac ventricular fibrillation is induced during head-to-body stunning leading to cardiac arrest. These EEG patterns can be used to ascertain the duration of unconsciousness and insensibility in controlled environment studies.

Indicators of recovery of consciousness after stunning are listed in EFSA's 2004 scientific opinion, but their sequence depends on the stunning method. Recovery of spontaneous breathing is considered to be the earliest indicator of recovery of consciousness, which may begin as regular gagging (a brainstem reflex of forced/laboured breathing through the mouth) in a recumbent animal. These gagging movements gradually lead to resumption of rhythmic breathing. There is a lack of information on the correlation of EEG and the sequence or the time to recovery of other indicators of consciousness, such as pupillary, palpebral or corneal reflex. However, return of corneal reflex has been used to recognise recovery of consciousness in pigs under slaughterhouse conditions (EFSA, 2004). In conclusion, it is recommended that the indicator that is most sensitive in detecting recovery be used. Studies on alternative stunning methods should explain in detail how and when the onset of unconsciousness and insensibility is measured. It is recommended that the methods used have previously been published in peer-reviewed journals, that data are provided at the individual animal level and that actions are taken to prevent the possibility of bias (see section 5) as much as possible. In the case of EEGs, all parameters crucial for assessment of the EEG data should be specified (e.g. the electrode position on the skull or on the brain itself, the configuration of the electrode (transhemispheric or from the same hemisphere of the brain)). In order to estimate quantitative changes occurring in the EEG, the method used to derive the transformation of electroencephalography signals must be described. In addition, the indicators used to assess recognition of a successful stun should be relevant to the respective stunning intervention based on the available scientific knowledge of each indicator's sensitivity and specificity. Furthermore, the scoring system applied to categorise/classify the indicators should be clearly defined. It is essential that the observers making the measurements of the indicators have been carefully trained and that scoring systems are adapted to the species and the stunning conditions. Information on all these aspects should be provided and will be assessed by the AHAW Panel based on the scientific knowledge available at that time.

3.2. Assessment of the eligibility of the submitted study

An assessment of all the eligibility criteria, defined in section 3.1, was performed and detailed information is provided in Appendix A.

3.2.1. Intervention

In study 1, the reporting of the intervention lacks detailed information regarding several key components of the parameters listed in Annex I of Council Regulation (EC) No 1099/2009, and some essential components of the parameters are not reported at all. It is not specified whether the reported amperage values are root mean square, average or peak current values and whether the reported frequency is the minimum or maximum frequency. No information is provided on the type, the waveform and the voltage of the applied current. The reporting of the optimisation of the current flow used in the study lacks detail regarding the electrode characteristics and appearance as well as the restraining of animals. The description of the position and contact surface area of the electrodes fulfils the requirements only partially, as it is not described how the application of electrodes was validated. For these reasons, the intervention is considered to be insufficiently described.

In study 2, the reporting of the intervention lacks detailed information regarding several key components of the parameters listed in Annex I of Council Regulation (EC) No 1099/2009, and some essential components of the parameters are not reported at all. It is not specified whether the reported amperage and voltage values are root mean square, average or peak current values and whether the reported frequency is the minimum or maximum frequency. No information is provided on the type and the waveform of the applied current. Information on the electrode characteristics and appearance is not reported; only photographs are provided. The description of the position and contact surface area of the electrodes fulfils the requirements only partially, as it is not described how the correct electrode position was verified during stunning. Therefore, the intervention is considered to be insufficiently described.

3.2.2. Outcome

3.2.2.1. Onset of unconsciousness and insensibility

In study 1, the onset of unconsciousness and insensibility has not been ascertained by EEG. It is considered that the onset of unconsciousness has been adequately addressed using indicators; however, several shortcomings have been noted in the report and are pointed out here. The indicators that were assessed are reported (presence and intensity of the tonic phase, presence and intensity of the clonic phase, absence of corneal reflex, absence of respiratory movements, absence of blinking), but no detailed description of how the indicators were measured is provided other than that after stunning, the animals were placed in lateral recumbency, and assessments were carried out at 10-second intervals for 150 seconds. No scoring system for assessing the intensity of the tonic and clonic phase is described. Owing to the absence of EEGs, it is considered that the onset of unconsciousness has not been adequately assessed in the study.

In study 2, the onset of unconsciousness and insensibility has been ascertained by EEG, but neither the position of the electrodes nor the electrode configuration, the method used to derive the transformations of the EEGs or the results are of these measurements are reported. It is considered that the onset of unconsciousness has been adequately addressed using indicators; however, several shortcomings have been noted in the report and are pointed out here. The indicators to detect onset of unconsciousness/insensibility and how they were measured, as well as the beginning and end of the measurement, are reported, but no description of the scoring system for assessing the intensity of the clonic phase is provided. Owing to the absence of information regarding the EEGs, it is not possible to assess whether the duration of unconsciousness and insensibility has been adequately assessed in the study.

3.2.2.2. Absence of pain, distress and suffering

As effective electrical stunning, with currents of sufficient magnitude, leads to immediate onset of unconsciousness and loss of sensibility, the eligibility criteria for absence of pain, distress and suffering do not need be applied. However, as the submitted studies do not report any EEG data, or any of the indicators associated with the absence of pain, distress and suffering, it is impossible to

conclude that the onset of unconsciousness and insensibility was immediate or that pain, distress and suffering were absent.

3.2.2.3. Duration of unconsciousness and insensibility

In study 1, the duration of unconsciousness and insensibility was not measured by electroencephalography. It is considered that the duration of unconsciousness has been adequately addressed using indicators; however, several shortcomings have been noted in the report and are pointed out here. The indicators that were used to detect onset of unconsciousness/insensibility are reported, but no detailed description of how the indicators were measured is provided other than that after stunning, the animals were placed in lateral recumbency, and assessments were carried out at 10-second intervals for 150 seconds. Owing to the absence of EEGs, it is considered that the duration of unconsciousness was not adequately addressed in the study.

In study 2, the duration of unconsciousness and insensibility was ascertained by electroencephalography, but neither the position of the electrodes nor the electrode configuration, the method used to derive the transformations of the EEG or the results of these measurements are reported. It is considered that the duration of unconsciousness has been adequately addressed using indicators, as the indicators that were used to detect duration of unconsciousness/insensibility, how they were measured and the beginning and end of measurement are reported. However, owing to the absence of information regarding the EEGs, it is not possible to assess whether the duration of unconsciousness was adequately addressed in the study.

4. Reporting assessment

4.1. Identification of reporting guidelines applicable to studies on stunning methods

Studies on alternative stunning methods should analyse the equivalence to the requirements prescribed in Council Regulation (EC) No 1099/2009: induction of immediate onset of unconsciousness/insensibility or absence of pain, distress and suffering until the onset of unconsciousness/insensibility and the duration of unconsciousness/insensibility until death. Several study designs could be applied. At the moment, several guidelines are available on reporting of randomised controlled and observational studies[8], but none of these guidelines can be applied directly to studies on stunning methods. The REFLECT[9] statement and the STROBE[10] statement were identified as the most suitable guidelines that could be applied to studies on stunning methods. The REFLECT statement is a reporting guideline for randomised controlled trials in animals. The STROBE statement is a reporting guideline for observational studies on humans but can be readily adapted to animals.

Collation of parameters from guidelines on which information has to be reported:

A checklist that could be applied to studies on stunning methods should be generated, taking into account the specificities related to the design of randomised controlled trials or observational studies. However, this could not be done within the time frame of this mandate. As preparatory work before generating such a checklist, all of the parameters from the checklist of the REFLECT and the STROBE statements were listed and reviewed. The parameters dealing with information that could be valuable to assess the reporting quality of studies on stunning methods are briefly described in Table 3**Error! Reference source not found.**. The description of the parameters was modified in some cases to allow their use in the context of studies on stunning methods.

[8] http://www.equator-network.org/
[9] http://www.reflect-statement.org/statement/
[10] http://www.strobe-statement.org/

Table 3: Parameters used to assess the reporting quality of studies on stunning methods, per section of the study report

Parameter	Description
Introduction	
Background and rationale	Explain the scientific background and rationale for the investigation being reported
Objective	Describe the specific objectives and hypotheses. Clearly state primary and secondary objectives (if applicable)
Materials and methods	
Study population	Give characteristics of the study population (species, breed, animal type (e.g. dairy or beef cattle), and weight) and potential confounders (health status, fasting, water deprivation, husbandry system); indicate the number of animals with missing data for each variable of interest
Number of animals (sample size)	How was the sample size determined and, when applicable, explanation of any interim analyses and stopping rules. Experimental/intervention units must be described and information on whether true replication was done is needed
Intervention	Precise details of the interventions intended for each group, how and when interventions were actually administered. In addition, specifications of the requirements for the stunning method are provided in section 3.1.1
Outcome	Clearly define all primary outcomes (onset of unconsciousness/insensibility, absence of pain, distress and suffering and duration of unconsciousness/insensibility) and ancillary outcomes (e.g. heart beat, tail flicking). Report category boundaries when continuous variables were categorised. Specifications of the requirements for the assessment of unconsciousness and insensibility as well as absence of pain, distress and suffering are provided in sections 3.1.2.1–3.1.2.3
Bias and confounding	Describe any efforts to address potential sources of bias that are relevant to the study design and could affect internal and external validity of the study. Concerning external validity, report methods to control for sampling bias. Was any comparison made between the reference population and animals under study? Concerning internal validity, report methods to control for selection bias, information bias and confounding. These may include random allocation, matching, blocking stratification for randomised controlled trials, and multivariable analytical methods
Blinding (masking)	Specify if blinding was performed or not. If done, describe who was blinded (e.g. the data collector, the data analyst) as well as how and when it was done. If the process was different for outcomes, clarify per outcome (e.g. behaviour data was blinded but electrocardiography data were not)
Statistical methods	Describe all statistical methods used to summarise the data and test the hypotheses, including those used to control for confounding; include information about data transformations. Describe any methods used to examine subgroups and interactions; explain how missing data were addressed. Guidance can be found in Lang (2013)
Results	
Numbers analysed	Basic information about the distribution of important confounders and effect modifiers in the each study group (age, weight, sex). If variables are continuous provide means (SD) if normally distributed, if not provide medians and interpercentile ranges, ranges, or both. Report the upper and lower boundaries of interpercentile ranges and the minimum and maximum values of ranges, numbers of study units (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (e.g. 10/20, not 50 %)
Outcomes and estimations	For each outcome, report a summary of results for each group (although it is recommended that data are made available at individual animal level, at least in studies performed in a controlled environment); give unadjusted estimates

Parameter	Description
	and their precision (e.g. 95 % confidence interval) and, if applicable, confounder-adjusted estimates and number. If the design includes non-independent observations ensure variance components are reported. Make clear which confounders were adjusted for
Adverse events	Describe all important adverse events or side effects in each intervention group and report the number of adverse events in each group and indicate if they appear prior to or after unconsciousness is reached. For example, in the case of electrical stunning, high electrical resistance could cause overheating of the stunning electrodes, leading to poor stunning as well as burn marks on the skin
Ancillary analyses	Report the outcome of any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory
Discussion	
Key results and interpretation	Summarise key results with reference to study objectives; provide a well-founded interpretation of results considering objectives, limitations, taking into account sources of potential bias or imprecision, multiplicity of analyses, results from similar studies, and other relevant evidence
External validation	Discuss the potential for external validation of the study results (e.g. applicability of the stunning method in slaughterhouses in different Member States)
Other	
Funding	Give the source of funding and the role of the funders for the submitted study

4.2. Assessment of the reporting quality based on the selected parameters

An assessment of all the reporting quality criteria, defined in section 4.1, was performed, and detailed information is provided in Appendix B.

Study 1 has several shortcomings in the description of materials and methods as well as in the reporting and discussion of the results. The experimental unit and the way the sample size was determined are not specified. Information on potential confounders and the number of animals with missing data is not provided. No information on whether and how blinding was carried out in the study is reported. There are no true replicates in experiments as all of the animals were from the same source population and were not allocated to the controls and treatments in a truly random manner (meaning that they are not statistically independent units).No efforts to address potential sources of bias relevant to the study design or to control for confounding are reported and the results of the statistical analyses used are incompletely reported. The number of animals included in each analysis is not specified and data are not presented in absolute numbers. Information on confounders is lacking. No information regarding adverse events has been reported and the potential for external validation of the study results is not discussed. The role of the funders is not reported. Therefore, the study does not fulfil the reporting quality criteria.

Study 2 has several shortcomings in the description of materials and methods as well as in the reporting and discussion of the results. Information on potential confounders and the number of animals with missing data is not provided. It is not explained how the sample size was determined, nor is it specified what the experimental/intervention unit is. No information on whether and how blinding was carried out in the study is reported. The number of animals is reported, but it is not explained how the sample size was determined. There are no true replicates in experiments as 15 animals were tested for each treatment (five animals of each commercial category on each of the three days of the experiment), but all of the animals were from the same source population and were not allocated to the controls and treatments in a truly random manner (meaning that they are not statistically independent units). No efforts to address potential sources of bias relevant to the study design, including confounding, are reported. The statistical analyses used are described, yet their results are incompletely reported. The number of animals included in each analysis is not specified and data are not presented in absolute numbers. Information on confounders is lacking. No information regarding adverse events is reported and the potential for external validation of the study results is not discussed.

The role of the funders is not reported. Therefore, the study does not fulfil the reporting quality criteria.

5. Quality assessment

The methodological quality criteria focus on elements in the report that allow the assessment of the internal and external validity of the submitted study. Internal validity is reached when the study results reflect reality among the animals under study, whereas external validity is reached when the study results are reasonably generalised to the broader reference population. The main biases affecting internal validity are confounding, selection bias and information bias (Rothman, 2002). The most relevant bias affecting external validity is sampling bias. It is assumed that a high-quality study is conducted in such a way that these biases are minimised. Assessment of other parameters that might be related to the methodological quality of a study could not be considered owing to the short deadline of the mandate.

5.1. Specification of different types of potential biases impacting on internal validity

5.1.1. Confounding

Confounding can be described as the mixing together of the effects of two or more factors. It is present when the observed measure of association between a given exposure/intervention factor and an outcome becomes biased owing to the effects of one or more extraneous factors. Confounding can be controlled in the study design, for example by matching, or during the data analysis by stratification or adjusting (Dohoo et al., 2010).

5.1.2. Selection bias

Selection bias arises in studies that compare two or more groups, such as an intervention versus a control. If the way in which study subjects are selected to go into the different groups creates groups that differ in other characteristics, then the estimate of the effect of the intervention made will be potentially confounded. For instance, in experimental conditions, it is recommended that, for methods not inducing immediate unconsciousness, the animal-based measure for pain, distress and suffering is analysed for each animal undergoing the stunning procedure twice: first without the stunning act (giving the baseline result per animal) and afterwards with the stunning act.

5.1.3. Information bias

Information bias is a collective term for misclassification bias and measurement bias and arises from incorrectly classifying or measuring the study subject's exposure, extraneous factors and/or outcome status. It can alter the magnitude and the direction of estimates of association and can affect different measures of association differently. Misclassification bias results from assigning study individuals into incorrect categories because of errors in classifying exposure, outcome or both, while measurement bias results from errors in measuring quantitative factors, e.g. owing to lack of accuracy or a lack of precision (Dohoo et al., 2010).

5.2. Specification of different types of potential biases impacting on external validity

5.2.1. Sampling bias

Where study subjects systematically differ from those to whom the results are likely to be applied, a study is described as having a sampling bias (e.g. a study may have used only heavy animals but the method is intended to be used later on animals with a broad weight range). It essentially relates to definitions of and relationships between the reference population (to which one wishes to generalise), the target population (from which one is sampling) and the eligible or study population (those eventually enrolled).

Assessment of this criterion is beyond of the scope of this mandate.

5.3. Quality assessment of the internal validity of the submitted study

As the studies did not fulfil the eligibility criteria, the methodological quality of the studies was not assessed.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Conclusions on TOR 1

- Regarding fulfilment of the eligibility criteria it is concluded that:
    - For both study 1 and study 2, the intervention is considered to be insufficiently described.
    - The onset of unconsciousness and insensibility has not been adequately assessed in study 1.
    - It is not possible to assess whether the onset of unconsciousness and insensibility has been adequately assessed in study 2.
    - The duration of unconsciousness has not been adequately addressed in study 1.
    - It is not possible to assess whether the duration of unconsciousness has been adequately addressed in study 2.
- Regarding fulfilment of the reporting criteria it is concluded that:
    - Neither study 1 nor study 2 fulfils the reporting criteria.
- Regarding fulfilment of the quality criteria it is concluded that:
    - As the studies did not fulfil the eligibility criteria, the methodological quality of the studies was not assessed.

RECOMMENDATIONS

Recommendations on TOR 1

- Further studies on the use of minimum currents lower than 1 A for electrical stunning of small ruminants are needed, which should include the eligibility criteria set out in this opinion.

Recommendations on TOR 2

- As a follow-up action, a document covering all stunning methods listed in Council Regulation (EC) No 1099/2009 with detailed guidance on assessing alternative stunning methods is proposed.
- Alternative stunning methods should be first studied under controlled (laboratory) conditions to analyse the animals' responses (unconsciousness, absence of pain, distress and suffering) using the most sensitive and specific methods and to find a correlation with non-invasive parameters that can be applied during the second phase of the study in slaughterhouses. In a second step, the results obtained under controlled laboratory conditions need to be confirmed under a range of slaughterhouse conditions.
- The criteria for eligibility, reporting quality and study quality defined in this document should be applied to studies carried out under controlled (laboratory) conditions as well as to studies carried out under slaughterhouse conditions.

- Information obtained in other species can be used as an indication, but should be confirmed in the species under investigation because coping strategies, pain thresholds and tolerances are species and individual specific.
- For studies researching a new or modified stunning method, animals should be stunned without sticking to establish the duration of unconsciousness achieved by the stunning itself in proof-of-concept studies under controlled laboratory conditions.
- The onset and the duration of unconsciousness and insensibility should be ascertained using EEGs or ECoGs in studies carried out under controlled (laboratory) conditions.
- The onset and the duration of unconsciousness and insensibility should be ascertained assessing the presence of tonic seizures, the presence of apnoea and the lack of response to painful stimuli in studies carried out under slaughterhouse conditions.
- Data reported in studies on alternative stunning methods should be provided at the individual animal level.

REFERENCES

Atkinson S, Velarde A, Llonch P and Algers B, 2012. Assessing pig welfare at stunning in Swedish commercial abattoirs using CO2 group-stun methods. Animal Welfare, 21, 487 495.

Benson ER, Alphin RL, Rankin MK, Caputo MP and Johnson AL, 2012a. Electroencephalogram-based methodology for determining unconsciousness during depopulation. Avian Diseases, 56, 884 890.

Benson ER, Alphin RL, Rankin MK, Caputo MP, Kinney CA and Johnson AL, 2012b. Evaluation of EEG based determination of unconsciousness vs. loss of posture in broilers. Research in Veterinary Science, 93, 960 964.

Berg C, Nordensten C, Hultgren J and Algers B, 2012. The effect of stun duration and level of applied current on stun and meat quality of electrically stunned lambs under commercial conditions. Animal Welfare, 21, 131–138.

Beyssen C, Babile R and Fernandez X, 2004. The effect of current intensity during 'head-only' electrical stunning on brain function in force-fed ducks. Animal Research, 53, 155–161.

Broom DM, 2001. The evolution of pain. Vlaams Diergeneeskundig Tijdschrift, 70, 17–21.

Coetzee JF, Gehring R, Tarus-Sang J and Anderson DE, 2010. Effect of sub-anesthetic xylazine and ketamine ('ketamine stun') administered to calves immediately prior to castration. Veterinary Anaesthesia and Analgesia, 37, 566–578.

Cook CJ, Devine CE, Gilbert KV, Smith DD and Maasland SA, 1995. The effect of electrical head-only stun duration on electroencephalographic-measured seizure and brain amino acid neurotransmitter release. Meat Science, 40, 137–147.

Cook CJ, Maasland SA, Devine CE and Gilbert KV, 1996. Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. Research in Veterinary Science, 60, 255–261.

Dalmau A, Rodriguez P, Llonch P and Velarde A, 2010. Stunning pigs with different gas mixtures: aversion in pigs. Animal Welfare, 19, 325–333.

Dohoo I, Martin W and Stryn H, 2010. Veterinary Epidemiologic Research. VER Inc., Charlottetown, PE, 865 pp.

EFSA (European Food Safety Authority), 2004. Welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal, 45, 1–29.

EFSA (European Food Safety Authority), 2005. Aspects of the biology and welfare of animals used for experimental and other scientific purposes. The EFSA Journal, 292, 1–46.

Gandelman-Marton R and Neufeld MY, 2012. State of consciousness and interictal epileptiform discharges predict seizure occurrence during routine EEG. Neurologist, 18, 125–127.

Gerritzen MA, Lambooij B, Reimert H, Stegeman A and Spruijt B, 2004. On-farm euthanasia of broiler chickens: effects of different gas mixtures on behavior and brain activity. Poultry Science, 83, 1294–1301.

Gerritzen M, Lambooij B, Reimert H, Stegeman A and Spruijt B, 2007. A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. Applied Animal Behaviour Science, 108, 179–185.

Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, Johnstone AC and Mellor DJ, 2009. Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. New Zealand Veterinary Journal, 57, 77–83.

IASP (International Association for the Study of Pain), 1994. Part III: Pain Terms, A Current List with Definitions and Notes on Usage (209-214). In: Classification of Chronic Pain, Second Edition, IASP Task Force on Taxonomy. Eds Merskey H and Bogduk N. IASP Press, Seattle.

Johnson CB, Gibson TJ, Stafford KJ and Mellor DJ, 2012. Pain perception at slaughter. Animal Welfare, 21, 113–122.

Jongman EC, Barnett JL and Hemsworth PH, 2000. The aversiveness of carbon dioxide stunning in pigs and a comparison of the CO2 stunner crate vs. the V-restrainer. Applied Animal Behaviour Science, 67, 67–76.

Kavaliers M, 1998. Evolutionary and comparative aspects of nociception. Brain Research Bulletin, 21, 923–931.

Kirkden RD, Niel L, Lee G, Makowska IJ, Pfaffinger MJ and Weary DM, 2008. The validity of using an approach-avoidance test to measure the strength of aversion to carbon dioxide in rats. Applied Animal Behaviour Science, 114, 216–234.

Landa L, 2012. Pain in domestic animals and how to assess it: a review. Veterinarni Medicina, 57, 185–192.

Lang T and Altman DG, 2013. Basic statistical reporting for articles published in biomedical journals: the statistical analyses and methods in the published literature or the SAMPL guidelines.. In: Smart P, Maisonneuve H, Polderman A (eds). Science Editors' Handbook. European Association of Science Editors.

Le Neindre PGR, Guémené D, Guichet J-L, Latouche K, Leterrier C, Levionnois OMP, Prunier A, Serrie A and Servière J, 2009. Animal pain: identifying, understanding and minimising pain in farm animals. Multidisciplinary scientific assessment, Summary of the expert report. INRA, Paris, 98 pp.

Llonch P, Dalmau A, Rodriguez P, Manteca X and Velarde A, 2012a. Aversion to nitrogen and carbon dioxide mixtures for stunning pigs. Animal Welfare, 21, 33–39.

Llonch P, Rodriguez P, Velarde A, de Lima VA and Dalmau A, 2012b. Aversion to the inhalation of nitrogen and carbon dioxide mixtures compared to high concentrations of carbon dioxide for stunning rabbits. Animal Welfare, 21, 123–129.

Llonch P, Rodriguez P, Jospin M, Dalmau A, Manteca X and Velarde A, 2013. Assessment of unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures. Animal, 7, 492–498.

McKeegan DEF, McIntyre J, Demmers TGM, Wathes CM and Jones RB, 2006. Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. Applied Animal Behaviour Science, 99, 271–286.

McKeegan DEF, Sparks NHC, Sandilands V, Demmers TGM, Boulcott P and Wathes CM, 2011. Physiological responses of laying hens during whole-house killing with carbon dioxide. British Poultry Science, 52, 645–657.

McPherson A, Rojas L, Bauerschmidt A, Ezeani CC, Yang L, Motelow JE, Farooque P, Detyniecki K, Giacino JT and Blumenfeld H, 2012. Testing for minimal consciousness in complex partial and generalized tonic-clonic seizures. Epilepsia, 53, e180–e183.

Martoft L, Lomholt L, Kolthoff C, Rodriguez BE, Jensen EW, Jorgensen PF, Pedersen HD and Forslid A, 2002. Effects of CO_2 anaesthesia on central nervous system activity in swine. Laboratory Animals, 36, 115–126.

Mellor DJ, Cook CJ and Stafford KJ, 2000. Quantifying some responses to pain as a stressor. Biology of Animal Stress, 171–198.

Molony V and Kent JE, 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. Journal of Animal Science, 75, 266–272.

Mota-Rojas D, Bolanos-Lopez D, Concepcion-Mendez M, Ramirez-Telles J, Roldan-Santiago P, Flores-Peinado S and Mora-Medina P, 2012. Stunning swine with CO_2 gas: controversies related to animal welfare. International Journal of Pharmacology, 8, 141–151.

Murrell JC, Johnson CB, White KL, Taylor PM, Haberham ZL and Waterman-Pearson AE, 2003. Changes in the EEG during castration in horses and ponies anaesthetized with halothane. Veterinary Anaesthesia and Analgesia, 30, 138–146.

OIE (World Organisation for Animal Health), 2012. Use of animals in research and education. In: Terrestrial Animal Health Code, OIE, Paris, 2012.

Purdon PL, Pierce ET, Mukamel EA, Prerau MJ, Walsh JL, Wong KFK, Salazar-Gomez AF, Harrell PG, Sampson AL, Cimenser A, Ching S, Kopell NJ, Tavares-Stoeckel C, Habeeb K, Merhar R and Brown EN, 2013. Electroencephalogram signatures of loss and recovery of consciousness from propofol. Proceedings of the National Academy of Sciences of the United States of America, 110, E1142–E1151.

Raj ABM, Gregory NG and Wilkins LJ, 1992. Survival rate and carcass downgrading after the stunning of broilers with carbon dioxide-argon mixtures. Veterinary Record, 130, 325–328.

Raj ABM, Wotton SB, McKinstry JL, Hillebrand SJW and Pieterse C, 1998. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of broiler chickens during exposure to gas mixtures. British Poultry Science, 39, 686–695.

Rodriguez P, Dalmau A, Ruiz-de-la-Torre JL, Manteca X, Jensen EW, Rodriguez B, Litvan H and Velarde A, 2008. Assessment of unconsciousness during carbon dioxide stunning in pigs. Animal Welfare, 17, 341–349.

Rothman KJ, 2002. Epidemiology: An Introduction. Oxford University Press, Cambridge, MA, 240 pp.

Svendsen O, Jensen SK, Karlsen LV, Svalastoga E and Jensen HE, 2008. Observations on newborn calves rendered unconscious with a captive bolt gun. Veterinary Record, 162, 90–92.

Underwood WJ, 2002. Pain and distress in agricultural animals. Journal of the American Veterinary Medical Association, 221, 208–211.

Velarde A, Ruiz-de-la-Torre JL, Stub C, Diestre A and Manteca X, 2000. Factors affecting the effectiveness of head-only electrical stunning in sheep. Veterinary Record, 147, 40–43.

Velarde A, Cruz J, Gispert M, Carrion D, de la Torre JLR, Diestre A and Manteca X, 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. Animal Welfare, 16, 513–522.

Vogel KD, Badtram G, Claus JR, Grandin T, Turpin S, Weyker RE and Voogd E, 2011. Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. Journal of Animal Science, 89, 1412–1418.

Zimmermann M, 1986. Behavioral investigations of pain in animals. Archiv für Geflügelkunde, 50, 115–115.

APPENDIX A. Assessment of the eligibility criteria

Table 4: Information provided by the submitted studies in relation to the intervention

Parameter	Component	Information provided in the submitted study 1	Fulfilment criterion (yes or no)
Minimum current (A or mA)	Current type	Not reported	No
	Waveform	Not reported	No
	Minimum current[a]	It is reported that 0.7 A and 1.0 A were used, but it is not clear whether the reported value is root mean square, average or peak current	No
	Latency[a]	Not reported	No
Minimum voltage (V)	Exposed minimum voltage (V)[a]	Not reported	No
	Delivered minimum voltage (V)[a]	Not reported	No
Maximum frequency (Hz)	Maximum frequency (Hz)	50 Hz is mentioned, but it is not clear whether it is minimum or maximum frequency	No
	Minimum frequency (Hz)	Not reported	No
Minimum time exposure[a]		Stun was applied for four seconds but method of control and average and range are not reported	No
Maximum stun-to-stick/kill interval(s)[a,b]		Not relevant in trial 1 as animals were allowed to recover in this study, in trial 2 the reported time between stunning and slaughter by neck-cutting was 7.3 ± 1.46 seconds. However, no EEG data assessing the duration of unconsciousness/insensibility after the stunning intervention are reported. Therefore, the maximum stun-to-stick/kill interval could not be defined adequately	No
Frequency of calibration of the equipment		Not reported	No
Optimisation of the current flow	Electrode characteristics	Not reported	No
	Electrode appearance	A picture is presented without full description	No
	Animal restraining	Some aspects are reported (animals were moved through a 6-metre long restraining system that transported the animals individually to the stunning and bleeding area)	Yes
Prevention of electrical shocks before		Not reported	No

Parameter	Component	Information provided in the submitted study 1	Fulfilment criterion (yes or no)
stunning			
Position and contact surface area of electrodes	Position of the electrodes	The targeted electrode position is reported (between the eyes and the ears on either side of the head (trial 1) and above the spinal cord, behind the position of the heart in the case of head-to-body (trial 2), but validation of correct position during stunning is not described	No
	Type of electrode	A photo and the manufacturer's name are presented, but no description is provided.	No
	Animal skin condition	The skin condition is not reported in sufficient detail, it is only stated that 2 (trial 1) or none (trial 2) of the study animals presented high amounts of wool on their heads	No

[a]Provide information on mean or median and range and standard deviation or interquartile range.
[b]In case of simple stunning.

Table 5: Information provided by the submitted studies in relation to the intervention

Parameter	Component	Information provided in the submitted study 2	Fulfilment criterion (yes or no)
Minimum current (A or mA)	Current type	It is reported that the stunning system used in the study provides constant current, but the maximum voltage available to the stunner is not reported and no records of the constant current delivered are provided.	No
	Waveform	Not reported	No
	Minimum current[a]	The values of 1.0 A, 0.7 A, 0.5 A, or 0.3 A have been applied in the study, but it is not clear whether the reported values are root mean square, average or peak current	No
	Latency [a]	Not reported	No
Minimum voltage (V)	Exposed minimum voltage (V) [a]	Average voltage is presented in Table 1 for each treatment group but it is not clear whether it is root mean square, average or peak voltage.	No
	Delivered minimum voltage (V) [a]	It is reported that the voltage was modulated by the stunning system according to the changes in the resistance between the electrodes and that voltage was recorded during experiments, but it is not reported	No
Maximum frequency (Hz)	Maximum frequency (Hz)	50 Hz is mentioned, but it is not clear whether it is minimum or maximum frequency	No
	Minimum frequency (Hz)	Not reported	No
Minimum time exposure [a]		It is reported that the stun was applied for three seconds but the range and the method of control are not reported	No

Parameter	Component	Information provided in the submitted study 2	Fulfilment criterion (yes or no)
Maximum stun-to-stick/kill interval(s) [a,b]		Animals were neck-cut (two carotid artery/two jugular vein) after stunning, the average ± SD of the stun-to-stick/-kill interval are reported. However, there are no EEG data assessing the duration of unconsciousness/insensibility after the stunning intervention	Yes
Frequency of calibration of the equipment		Not reported	No
Optimisation of the current flow	Electrode characteristics	Not reported, only photographs provided	No
	Electrode appearance	Not reported, only photographs provided	No
	Animal restraining	It is reported that animals were individually restrained in sternal recumbency in a V-restrainer with manual restraining of the head	Yes
Prevention of electrical shocks before stunning		Not reported	No
Position and contact surface area of electrodes	Position of the electrodes	The targeted electrode position (between the eyes and the ears on either side of the head for HO[c]/between the eyes and the ears on either side of the head and the body electrode was placed above the spinal cord, behind the position of the heart for HB[d]) is reported, but no verification of correct position during stunning is described	No
	Type of electrode	Not reported, only photograph and manufacturer (two electrodes tongs (PZ004, Gozlin, Modena, Italy) for HO, three electrodes tong (Jarvis, Auckland, New Zealand) for HB) of electrodes are provided	No
	Animal skin condition	Not reported	No

[a]Provide information on mean or median and range and standard deviation or interquartile range.
[b]In case of simple stunning.
[c]HO: head only
[d]HB: head to body

Table 6: Information provided by the submitted studies in relation to the onset of unconsciousness and insensibility

	Information provided in the submitted study 1	**Is the induction of unconsciousness/insensibility addressed adequately? (yes, no or not possible to assess)**
EEG	Not studied	NO
Indicator(s) to detect onset of unconsciousness/insensibility	The indicators that were assessed are reported (presence and intensity of the tonic phase, presence and intensity of the clonic phase, absence of corneal reflex, absence of respiratory movements, absence of blinking). No detailed description of how the indicators were measured is provided other than that after stunning, the animals were placed in lateral recumbency, and assessments were carried out at 10-second intervals for 150 seconds. No scoring system for assessing the intensity of the tonic and clonic phase is described	YES

Table 7: Information provided by the submitted studies in relation to the **onset of unconsciousness and insensibility**

	Information provided in the submitted study 2	**Is the induction of unconsciousness/insensibility addressed adequately? (yes, no or not possible to assess)**
EEG	Measured, but neither the position of the electrodes, the electrode configuration, the method used to derive the transformations of the EEG nor the results are of the measurements are reported.	NO
Indicator(s) to detect onset of unconsciousness/insensibility	The indicators and how they were measured are reported: onset and duration of tonic and clonic phases; intensity of clonic phase; absence of rhythmic breathing (as indicated by the movements of the flanks); absence of corneal reflex (through physical stimulation of the cornea); absence of response to painful stimuli (by means of a prick in the upper lip); absence of spontaneous blinking (by direct observation). The beginning and end of the measurement is reported (started after electrical stunning application, lasted until brain death due to exsanguination). No description of the scoring system is provided than that the intensity of clonic phase was assessed subjectively (0 = moderate movement; 1 = severe movement)	YES

Table 8: Information provided by the submitted study in relation to animal-based measures (ABMs) associated with pain, distress and suffering during the induction of unconsciousness and insensibility

Response type	Groups of ABMs	Information provided in the submitted study 1	Do the ABMs suggest pain, distress and suffering? (yes, no or not possible to assess)
Behaviour	Vocalisations	No information provided	No assessment possible
	Postures and movements	No information provided	No assessment possible
	General behaviour	No information provided	No assessment possible
Physiological response	Hormone concentrations	No information provided	No assessment possible
	Blood metabolites	No information provided	No assessment possible
	Autonomic responses	No information provided	No assessment possible
Neurological response	Brain activity	No information provided	No assessment possible

Table 9: Information provided by the submitted study in relation to animal-based measures (ABMs) associated with pain, distress and suffering during the induction of unconsciousness and insensibility

Response type	Groups of ABMs	Information provided in the submitted study 2	Do the ABMs suggest pain, distress and suffering (yes, no or not possible to assess)
Behaviour	Vocalisations	No information provided.	No assessment possible
	Postures and movements	No information provided.	No assessment possible
	General behaviour	No information provided.	No assessment possible
Physiological response	Hormone concentrations	No information provided.	No assessment possible
	Blood metabolites	No information provided.	No assessment possible
	Autonomic responses	No information provided.	No assessment possible
Neurological response	Brain activity	No information provided.	No assessment possible

Table 10: Information provided by the submitted studies in relation to the duration of unconsciousness and insensibility

	Information provided in the submitted study 1	**Is the duration of unconsciousness/insensibility addressed adequately? (yes, no or not possible to assess)**
EEG	Not studied	No
Indicator(s) to detect onset of unconsciousness/insensibility	The indicators that were assessed are reported (presence and intensity of the tonic phase, presence and intensity of the clonic phase, absence of corneal reflex, absence of respiratory movements, absence of blinking). No detailed description of how the indicators were measured is provided other than that after stunning, the animals were placed in lateral recumbency, and assessments were carried out at 10-second intervals for 150 seconds. No scoring system for assessing the intensity of the tonic and clonic phase is described	Yes

Table 11: Table 11: Information provided by the submitted studies in relation to the duration of unconsciousness and insensibility

	Information provided in the submitted study 2	**Is the duration of unconsciousness/insensibility addressed adequately? (yes, no or not possible to assess)**
EEG	Measured, but the position of the electrodes, the electrode configuration, the method used to derive the transformations of the EEG and the results are not reported	No
Indicator(s) to detect onset of unconsciousness/insensibility	The indicators and how they were measured are reported: time to return of rhythmic breathing; time to return of corneal reflex; time to return of response to painful stimuli; time to return of spontaneous blinking. The beginning and end of the measurement is reported (started after electrical stunning application, lasted until brain death due to exsanguination)	Yes

Appendix B. Reporting assessment

Table 12: Assessment of the reporting quality parameters by the submitted studies

Parameter	Information provided in the submitted study 1	Fulfilment criterion (yes or no)
Introduction		
Background and rationale	The study describes that Regulation 1099/2009 (EC) sets out that the minimum electric current for head-only and head-to-body electrical stunning for sheep is 1.0 A regardless the weight and age of the animals or the presence of wool and that with the application of the new Regulation, the higher current may cause an increase of the haemorrhages in the carcass and consequently a downgrade of the final product quality	Yes
Objective	The objective was to study the effectiveness of head-only and head-to-body electrical stunning in lambs with an electric current of 0.7 A and its effect on the final product quality, specifically to (trial 1) assess the effectiveness of the head-only and head-to-body electrical stunning with an electric current of 0.7 A to induce unconsciousness, and in the case of head-to body stunning, to induce cardiac fibrillation and to (trial 2) compare the blood loss and carcass quality during the head-to-body electrical stunning between 0.7 A and 1 A	Yes
Materials and methods		
Study population	The species, the breeds, the average weight and range of weight are reported. In addition, information on the duration of the lairage period and on provision of water and feed during lairage is reported. Information on potential confounders and the number of animals with missing data is not provided	No
Number of animals (sample size)	The sample size is reported (80 animals). It is not explained how the sample size was determined, neither specified what is the experimental/intervention unit. There are no true replicates in experiments as all of the animals were from the same source population and were not allocated to the controls and treatments in a truly random manner (meaning that they are not statistically independent units)	No
Intervention	See Table 4	See Table 4
Outcome	See Tables 6, 8 and 10	See Tables 6, 8 and 10
Bias and randomisation	It is reported that animals were homogeneously divided into study group 1 and 2 according to live weight. No efforts to address potential sources of bias relevant to the study design or to control for confounding are reported	No
Blinding (masking)	Not reported	No
Statistical methods	The statistical methods used are described (mixed model analysis of variance for continuous outcome measures, "stunning system" was included as fixed effect, "live weight" as a covariate, general linear model analysis of variance for binomial variables, significance level was set at $p < 0.05$ (trial 1); mixed model analysis of variance for continuous outcome measures, the fixed effect was "current intensity", "live weight" and "stun-to-stick interval" were considered covariates, general linear model analysis of variance for binomial variables, significance level was set at $p < 0.05$ (trial 2)), but are incompletely reported	No
Results		
Numbers analysed	The number of animals included in each analysis was not specified and data were not presented in absolute numbers.	No

Parameter	Information provided in the submitted study 1	Fulfilment criterion (yes or no)
	There is no information on important confounders	
Outcomes and estimations	The following animal-based indicators were measured, but not thoroughly assessed because of the limits of the study design: onset of unconsciousness: trial 1: presence of tonic phase, presence of clonic phase, presence of mild clonic phase, absence of respiratory movements, absence of corneal reflex, absence of spontaneous blinking: %; trial 2: presence of tonic phase, presence of clonic phase, presence of severe clonic phase, absence of respiratory movements, absence of corneal reflex: %; duration of unconsciousness: trial 1: time to return of corneal reflex, time to return of respiratory movements, time to return of spontaneous blinking: mean ± s for different current values and electrode applications (HO[a], HB[b]); trial 2: return of corneal reflex, return of respiratory movements: %, no time to return reported; average stun-to-stick interval: mean ± s	No
Adverse events	Not reported	No
Ancillary analyses	In trial 2 blood loss, presence of petechiae, ecchymoses were measured and reported	Yes
Discussion		
Key results and interpretation	The statistics are incompletely reported.	No
Validation	Not reported	No
Other		
Funding	The source of funding was reported (Mercabarna, Barcelona), but the role of the funders was not detailed	No

NA, not applicable
[a]HO: head only
[b]HB: head to body

Table 13: **Table 13:** Assessment of the **reporting quality parameters** by the submitted studies

Parameter	**Information provided in the submitted study 2**	**Fulfilment criterion (yes or no)**
Introduction		
Background and rationale	The study describes that the strength of electric current flowing through the brain is the factor that determines the loss of consciousness of the animal. It states, that the new Regulation sets out the minimum current of 1.0 A for sheep and goats, regardless the weight and age of the animal and that the Humane Slaughter Association recommend for larger sheep 1.0 A and 0.6 A for lambs. It reports that currents below 1 A for lambs is also applied in other countries such as Australia and New Zealand (AS 4696-2007 and Animal Welfare Act, 1999, respectively) and that the use of a higher current level to stun the animals provokes a higher intensity of the tonic and clonic phase and an increase in blood pressure with rupture of vessels inducing the presence of ecchymoses in the muscles and fractures)	Yes
Objective	The objective is to assess the effectiveness of "head-only" electrical stunning with electric currents of 0.3, 0.5, 0.7 and 1 A in inducing unconsciousness in lambs and kid goats until brain death due to bleeding, and to study its effects on carcass quality and stun marks on the skin	Yes
Materials and methods		
Study population	The species, the breed, the average weight and range of weight are reported. In addition, information on the duration of the lairage period and on provision of water and feed during lairage is reported. No information on potential confounders and the number of animals with missing data is provided	No
Number of animals (sample size)	The number of animals is reported (360 lambs, 120 kid goats), but it is not explained how the sample size has been determined. The number of animals is reported (360 lambs, 120 kid goats), but it is not explained how the sample size has been determined. There are no true replicates in experiments as 15 animals were tested for each treatment (five animals of each commercial category on each of the three days of the experiment), but all of the animals were from the same source population and were not allocated to the controls and treatments in a truly random manner (meaning that they are not statistically independent units)	No
Intervention	See Table 5	See Table 5
Outcome	See Tables 7, 9 and 11	See Tables 7, 9 and 11
Bias and randomisation	It is reported only that the allocation of animals to study groups depended on the species and live weight categories and that animals were randomly selected for each treatment (no further specification reported). No efforts to address potential sources of bias relevant to the study design or any design features used to control for confounding are reported	No
Blinding (masking)	Not reported	No
Statistical methods	The statistical methods used are described (latency measures analysed with mixed model ANOVA (PROC MIXED), fixed effects "stunning system", the "electric current", "live body weight", "stun to stick interval" covariates, "day of slaughter" random effect, if significant differences ($p < 0.05$) least square means comparison test (LSMEANS) adjusted to multiple comparisons test of Tukey, if any of the covariates significant effect on the model ($p < 0.05$), linear	No

Parameter	Information provided in the submitted study 2	Fulfilment criterion (yes or no)
	correlation between the covariate and the dependent variable was analysed; binomial variables analysed using a generalised linear model ANOVA (PROC GENMOD) following a binomial distribution, fixed effects "stunning system", "electric current", covariates "live body weight", "stun to stick" interval) but are incompletely reported	
Results		
Numbers analysed	The numbers of animals included in the analysis are not specifically reported, results are stated as average with a range or percentages without denominator for each commercial category and intervention, not in absolute numbers	No
Outcomes and estimations	The following measures were reported, but not thoroughly assessed because of the limits of the study design: average voltage (± SD) reported for each current level; average live body weight for each commercial category; average stun to stick interval for each commercial category; for each commercial category presence of tonic phase: all; presence of clonic phase: percentage; severity of clonic phase: percentage for HO[a] and HB[b]; absence of respiratory movements: percentage; absence of corneal reflex: percentage; absence of blinking: percentage; absence of response to pain stimuli = percentage; % of return of rhythmic breathing; % of return of corneal reflex; return of response to painful stimuli = %; return of spontaneous blinking = %; time to return of rhythmic breathing=mean ± s; time to return of corneal reflex = mean ± s; time to final loss of rhythmic breathing = mean ± s; time to final loss of corneal reflex=mean ± s	No
Adverse events	Not reported	No
Ancillary analyses	Additional analyses reported were blood loss, skin quality, carcass quality (haemorrhages, ecchymoses)	Yes
Discussion		
Key results and interpretation	The conclusions are reported without mentioning the limitations, potential bias or other relevant evidence and the statistics are incompletely reported	No
Validation	Not reported	No
Other		
Funding	The source of funding was reported (Interovic), but the role of the funders was not detailed	No

ANOVA, analysis of variance; NA, not applicable.
[a]HO: head only
[b]HB: head to body

APPENDIX C. QUALITY ASSESSMENT

The quality assessment was not carried out, as neither study fulfilled the eligibility quality criteria.

GLOSSARY

Adverse event	Any observation in animals that is unfavourable and unintended and occurs after the intervention
Immediate unconsciousness	Induce immediate (e.g. in less than one second) and unequivocal loss of consciousness and sensibility
Insensibility	An animal can be presumed to be insensible when it does not show any reflexes or reactions to stimulus such as sound, odour, light or physical contact
Maximum stun-to-stick/kill interval(s)	This is the legal parameter describing the time interval between the end of the stunning and the moment of killing by any method (e.g. sticking, neck cutting)
Simple stunning	Stunning methods that do not result in instantaneous death
Stunning	Means any intentionally induced process that causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death.
True replicate	This means that more than one (statistically independent) experimental or observational unit was subjected to the same treatment. Each unit with the same treatment is called a replicate. True replication permits the estimation of variability within a treatment. Without estimating variability within treatments, it is impossible to do statistical inference, hence most models for statistical inference require true replication
Unconsciousness	This is a state of unawareness (loss of consciousness) in which there is temporary or permanent impairment of brain function and the individual is unable to respond to normal stimuli, including pain

ABBREVIATIONS

CO_2	Carbon dioxide
ECG	Electrocardiogram
ECoG	Electrocorticogram
EEG	Electroencephalogram
TOR	Term of reference