European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the use of low atmosphere pressure system (LAPS) for stunning poultry[1]

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

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ABSTRACT

The EFSA's Panel on Animal Health and Welfare (AHAW Panel) was asked to deliver a scientific opinion on the use of a low atmosphere pressure system (LAPS) for stunning poultry. Four documents were provided by the European Commission (EC) as the basis for an assessment of the extent to which the LAPS is able to provide a level of animal welfare at least equivalent to that ensured by the current allowed methods for stunning poultry. The LAPS is described as rendering poultry unconscious by gradually reducing oxygen tension in the atmosphere leading to progressive hypoxia in the birds. In order to be allowed in the EU, new stunning methods must ensure 1) absence of pain, distress and suffering until the onset of unconsciousness, and 2) that the animal remains unconscious until death. The submitted studies were peer-reviewed by the AHAW Panel as outlined in its "Guidance on the assessment criteria for studies evaluating the effectiveness of stunning intervention regarding animal protection at the time of killing". It is unclear from the submitted documents whether the rate of decompression used in LAPS induces unconsciousness and death without causing avoidable pain and suffering in poultry. The assessed studies did not pass the eligibility assessment and, therefore, no further assessment was undertaken.

KEY WORDS

low atmosphere pressure system, LAPS, stunning, poultry, slaughter

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Animal Health and Welfare (AHAW) was asked to deliver a scientific opinion on the use of a low atmosphere pressure system (LAPS) for stunning poultry prior to slaughter. Four documents were provided by the EC as the basis for an assessment of the extent to which the LAPS is able to provide a level of animal welfare at least equivalent to that ensured by the current allowed methods for stunning poultry. In the case that the outcome of the assessment was positive, the EC requested an assessment of the conditions under which the LAPS could be used in a commercial context.

The LAPS is described as rendering poultry unconscious by gradually reducing oxygen tension in the atmosphere leading to progressive hypoxia in the birds. This intervention is not permitted in the EU. In order to be allowed in the EU, new stunning methods must ensure a level of welfare at least equivalent to that of the methods already provided in Council Regulation 1099/2009.

In this opinion, the term 'acceptable alternative' is defined as an alternative stunning intervention that is at least as good as those listed in the Council Regulation (EC) 1099/2009. In particular, for interventions that do not induce immediate unconsciousness, the alternative procedure should ensure 1) absence of pain, distress and suffering until the onset of unconsciousness and, 2) that the animal remains unconscious and insensible until death.

Following the adoption of two Scientific Opinions on the stunning of rabbits by CO_2 and the electrical stunning of lambs (EFSA, 2013a, b), the EFSA Panel on Animal Health and Welfare developed a guidance on the process and criteria applied by EFSA to assess studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA, 2013c). The approach and criteria defined in that guidance were applied here.

The eligibility criteria that must be fulfilled by submitted studies are set in the EFSA guidance (EFSA, 2013c) and focus on the intervention and the animal welfare outcome. For the LAPS intervention, the key parameters about which information must be provided are: animal density, duration of intervention, rate of decompression, rate of changes in partial pressure of oxygen, temperature/humidity/illumination of the chamber, maximum stun-to-stick/kill interval(s) and calibration of the LAPS equipment and monitoring system. For the outcome of the LAPS intervention, a description of the onset and duration (until death) of unconsciousness and insensibility and a demonstration of the absence of pain, distress and suffering until the loss of consciousness and sensibility must be provided. As requested by the Commission, the assessment focussed on the animal welfare aspects of the LAPS. None of the four studies met the eligibility criteria and, therefore, neither reporting nor methodological quality were assessed. It is unclear from the submitted studies whether the rate of decompression used in the LAPS induces unconsciousness and death without causing avoidable pain and suffering in poultry. Since the rate of decompression was not fully described in the submitted studies, it was not possible to evaluate consistency with other sources of information.

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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 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, the use of low atmosphere pressure system is not allowed for stunning poultry.

The Commission has received a request from a private business operator to allow the use of low atmosphere pressure system as a method for stunning poultry. This request is supported by four scientific publications (see attachment).

In order to reply to this request, the Commission would like to request the EFSA to review the scientific publications provided and possibly other sources if available and assess to which extent the system proposed for stunning poultry is able to provide a level of animal welfare at least equivalent to that ensured by the current allowed methods and, in case of favourable reply, under which conditions.

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 low atmosphere pressure system for stunning poultry.

- The scope of this request is limited to the stunning of broiler chicken for slaughter (i.e. killing for human consumption).
- The EFSA will give its view on the four scientific publications attached to this request with a focus on the following issues:
  - The extent to which the use of low atmosphere pressure system is, in principle, an acceptable alternative for the stunning of broiler chicken compared to the welfare advantages/disadvantages related to other stunning methods allowed in the EU for broiler chicken;
  - The extent to which the findings are consistent with other sources of information;
  - Requirements attached to the use of low atmosphere pressure system;
  - The extent to which the findings may be valid under commercial conditions in the EU.

[4] 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.

ASSESSMENT

1. Introduction

The low atmospheric pressure system (LAPS) is a new intervention that has been claimed to render poultry unconscious by gradually reducing oxygen tension in the atmosphere leading to progressive hypoxia in animals. This intervention is not permitted in the EU. In order to be allowed in the EU, new stunning methods must ensure a level of welfare at least equivalent to that ensured by the methods already provided in Council Regulation 1099/2009.

Following the adoption of two Scientific Opinions on the stunning of rabbits by CO_2 and the electrical stunning of lambs (EFSA, 2013a, b), the EFSA Panel on Animal Health and Welfare (AHAW Panel) developed a guidance on the process and criteria applied by EFSA to assess studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA, 2013c). On the receipt of this mandate, its terms of reference were discussed with the European Commission services and the following clarifications were made.

EFSA will give its independent view on the findings of the four studies submitted to the Commission:

- STUDY 1 - "Physiological responses to low atmospheric pressure stunning (LAPS) and implications for welfare" - McKeegan et al., in press[5], from now referred as "study 1";
- STUDY 2 - " A new humane method of stunning broilers using low atmospheric pressure" - Vizzier-Thaxton et al. (2010), from now on referred to as "study 2";
- STUDY 3 - "The effects of low atmosphere stunning and deboning time on broiler breast meat quality" - Schilling et al. (2012), from now on referred to as "study 3";
- STUDY 4 - "The Effects of Low-Atmosphere Stunning and Deboning Time on Broiler Breast Meat Quality" - Battula et al. (2008), from now on referred to as "study 4".

The assessment of the submitted studies was carried out in a manner analogous to the approach followed in previously adopted opinions and as outline in the EFSA AHAW Panel guidance (EFSA, 2013c). The assessment focuses on:

- TOR 1: the extent to which the use of low atmosphere pressure system is, in principle, an acceptable alternative for the stunning of broiler chicken compared to the welfare advantages/disadvantages related to other stunning methods allowed in the EU for broiler chicken;
- TOR 2: the extent to which the findings are consistent with other sources of information.

An assessment of the evidence of validity under commercial conditions as well as the definitions of requirements attached to the use of LAPS would be performed only if the outcome of the assessment of suitability was positive.

The term "acceptable alternative" is defined as an alternative stunning intervention that is at least as good as those listed in the Council Regulation (EC) 1099/2009. In particular, for interventions that do not induce immediate unconsciousness, the alternative procedure should ensure 1) absence of pain, distress and suffering until the onset of unconsciousness, and 2) that the animal remains unconscious and insensible until death.

[5] Published later as McKeegan et al, 2013.

2. Approach

The submitted studies were assessed following the approach and specific criteria outlined in the guidance document (EFSA, 2013c), summarized below. The assessment was first conducted independently by each working group member. The individual assessments were then discussed to reach a consensus on parameters for which the experts had expressed different opinions.

As requested by the Commission, the assessment focussed on the animal welfare aspects of the LAPS. Further, the assessment only refers to the stunning procedure itself; it does not take into account pre-stunning phases. The outcome of the assessment applies only to whether the assessed study is eligible to be passed on to the next phase in the process: a full assessment of the animal welfare implications of the proposed alternative stunning intervention, including both pre-stunning and stunning phases, and an evaluation of the quality, strength and external validity of the evidence presented (Figure 1).

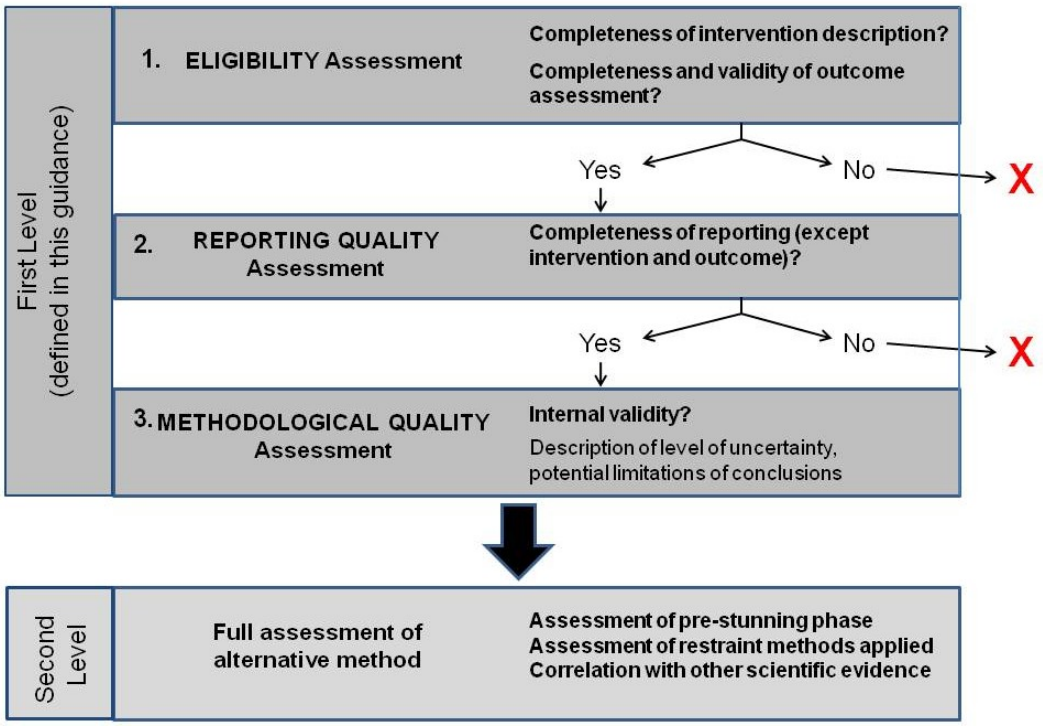

Figure 1: The approach of the assessment of studies evaluating alternative stunning methods (X=exclusion of study from further assessment; in this case a description of the shortcomings and indications where improvements are required before the study can be assessed further, will be provided)

Where a study meets the necessary eligibility criteria regarding the description of the intervention and outcome, then a reporting quality assessment is carried out.

Research studies evaluating stunning methods require well controlled studies under laboratory conditions (Figure 2, I) as a first step, to characterise the animals' responses (unconsciousness, absence of pain) using the most sensitive and specific methods available (e.g. EEG, blood samples) and to establish the correlations between these measurements and non-invasive parameters that can be applied in slaughterhouses (Figure 2). The second step, studies under slaughterhouse conditions (Figure 2, II) is intended to assess whether the results obtained in the laboratory can also be achieved in a slaughterhouse context. The eligibility criteria will be applied to both steps of the research on stunning methods. Information obtained on 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.

Type	Conditions	Elements of research recommended
I. Proof of concept	Study under controlled laboratory conditions	A. Comprehensive record of stunning intervention and key parameters B. Assessment of onset and duration of unconsciousness by EEG or ECoG C. Assessment of absence of pain, distress and suffering using behavioural **and** either physiological **or** neurological animal-based measures D. Comprehensive record of outcome assessment E. Stunning without sticking to establish duration of unconsciousness achievable with simple stunning intervention
II. Ground truthing	Study under slaughterhouse conditons	F. Comprehensive record of stunning intervention and key parameters G. Assessment of onset and duration of unconsciousness using animal-based measures H. Assessment of absence of pain, distress and suffering using behavioural **and** either physiological **or** neurological animal-based measures I. Comprehensive record of outcome assessment J. Assessment of absence of pain, distress and sufferring during restraint/pre-stunning if it deviates from conventional methods and/or is potentially painful

Figure 2: Recommended approach for research on stunning methods

It is important to note that in controlled environment studies electroencephalograms (EEGs) or electrocorticograms (ECoGs) should be used to demonstrate the effectiveness of a given stunning intervention (Figure 2, B). Indicators for recognising a successful stun should be applied in slaughterhouse settings, after their correlation with EEGs has been demonstrated in controlled environment studies (Figure 2, G). Further details can be found in the guidance document (EFSA. 2013c).

Studies on stunning methods should explain in detail how and when the onset of unconsciousness and insensibility is measured (Figure 2, B, C, G, H). In the case of EEGs (or ECoGs), all parameters crucial to the assessment of the electroencephalogram data should be specified (e.g. the EEG recording electrode position on the skull or on the brain itself, the configuration of the electrode (transhemispheric or from the same hemisphere of the brain), the background noise filtration method employed in the data acquisition and analysis). In order to estimate quantitative changes occurring in the EEG (or ECoGs), the method used to derive the transformations of electrical brain signals must be described (Figure 2, B).

Moreover, it should be explained how and when the animal-based measures were recorded and analysed (Figure 2, G, H, I). Furthermore, data should be provided at the individual animal level.

For any intervention that does not lead to an immediate onset of unconsciousness and insensibility, the time to loss of consciousness after the application of the stunning intervention and signs of pain, distress and suffering until the onset of unconsciousness should be recorded in all animals and reported as individual animal level data or mean or median and range and standard deviation or interquartile range (Figure 2, B, C, G, H).

As described in detail in the EFSA guidance and as presented in Figure 1, the following steps are to be followed:

1) 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.

The eligibility criteria that must be fulfilled by submitted studies related to LAPS were set in the EFSA guidance (EFSA, 2013c) and focus on the intervention and the outcome:

a) For the intervention:

This intervention is not currently approved for use in the EU, and therefore, no parameters are defined by Council Regulation (EC) No 1099/2009. The parameters and components were defined by the experts on stunning methods consulted during the preparation of this opinion.

b) For the outcome:

A. Onset of unconsciousness and insensibility OR

B. Absence of pain, distress and suffering until the loss of consciousness and sensibility

AND

C. Duration of the unconsciousness and insensibility (until death).

To allow assessment of new or modified legal stunning methods, the minimum criteria that fully define and characterise the stunning intervention were defined using previously published scientific data. Regarding measures of the outcome, the onset and duration of unconsciousness and insensibility should be recorded and reported. If the onset of unconsciousness and insensibility achieved by the stunning intervention is not immediate, then the absence of pain, distress and suffering until the loss of consciousness and sensibility must also be recorded and reported.

2) Reporting quality criteria

Reporting quality will only be assessed when the scientific study has passed the eligibility assessment (Figure 1). Inconsistencies in the reporting of scientific studies – which make it difficult to assess and compare them - have been identified in human and veterinary medicine. Therefore, the guidance document identified the relevant parameters that will be used as the basis for assessing the reporting quality of submitted studies on stunning methods.

3) Methodological quality criteria

The methodological quality of the submitted study will be assessed only if the eligibility and reporting quality criteria are met (Figure 1). In that case, the information provided in the study will be used to identify and assess possible biases (e.g. selection, attrition and performance bias) that might affect the study's internal validity.

4) Possible outcomes of the assessment

As described in the guidance document, two outcomes of the assessment are possible:

a) The criteria regarding eligibility, reporting and methodological quality are fulfilled.

This means that the study on the new or modified legal intervention provides sufficient detail regarding the intervention and the outcome to allow for a conclusion to be reached about the suitability (or lack thereof) of the intervention. In that case, a full assessment of the animal welfare implications of the proposed alternative stunning intervention, including both pre-stunning and stunning phases, and an evaluation of the quality, strength and external validity of the evidence presented would be carried out at the next level of the assessment (Figure 1).

b) The criteria regarding eligibility, reporting and methodological quality are not fulfilled.

This means that the study does not provide sufficient detail regarding the intervention and the outcome to allow for a conclusion to be reached about its suitability (or lack thereof). In that case, the assessment report would highlight the shortcomings and indicate where improvements are required before the study could be assessed further.

3. Eligibility criteria

3.1. Specification of eligibility criteria

3.1.1. Intervention

LAPS is a stunning system where animals are rendered unconscious prior to slaughter by gradually reducing oxygen tension in the atmosphere to achieve a progressive hypoxia. The induction of unconsciousness with LAPS is not instantaneous. This intervention is not currently approved for use in the EU, and therefore, no parameters are defined by Council Regulation (EC) No 1099/2009. The parameters and components listed in Table 1 were defined by the experts on stunning methods consulted during the preparation of this opinion.

Table 1: Suggested parameters to be provided when applying an intervention based on low atmosphere pressure system for stunning poultry as determined by the EFSA ad-hoc expert working group (EFSA, 2013c)

Parameter	Component	Description (all specifications should be in internationally recognised units)
Animal density	Animal species/ age/ type and stocking density (number/m^2 and kg of body weight/ m^2)	Specify the animal density in the crate or containers during the decompression.
Duration of intervention[a]	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multi-phase system[b]	Report the time elapsing until animals are exposed to the targeted pressure and corresponding partial pressure of oxygen. Report the duration of exposure to the target pressure and corresponding partial pressure of oxygen; If animals are exposed to a multi-stage system, report the target pressure in each stage and the duration of the exposure to each step as well as the transition time between each step.
Rate of decompression	Time/pressure treatment graphic representation	Describe the rate at which pressure changes are achieved in the chamber through a time/pressure curve. If decompression is achieved in more than one step, the profile for each step should be described. Re-pressurisation of the chamber prior to opening of door should be described and any incidence of birds

Parameter	Component	Description (all specifications should be in internationally recognised units)
		surviving the treatment should be reported.
Rate of changes in partial pressure of oxygen	Time/partial pressure of oxygen treatment graphic representation	Describe the rate at which partial pressure of oxygen changes in the chamber in relation to the rate of decompression. If decompression is achieved in more than one step, the profile for each step should be described.
Temperature/ humidity/ illumination of the chamber		Specify the temperature and humidity profile inside the chamber. Specify the light source if present.
Maximum stun-to-stick/kill interval(s)[c]		Describe the maximum stun-to-stick/kill interval and the exsanguination method (blood vessel cut) that have been applied to guarantee unconsciousness and 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). Report the stun- to-stick/kill interval(s) for the last animal stuck that did not recover consciousness in a group stunning situation
Calibration of the LAP equipment and monitoring system		Describe how the decompression procedure was controlled and how and with which frequency the equipment was calibrated. The monitoring equipment should be regularly calibrated. The calibration methods applied should be reported.

[a] Referring to the legal parameter 'duration of exposure' of other stunning methods;
[b] Provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter;
[c] In case of simple stunning.

3.1.2. Outcome

3.1.2.1. Onset of unconsciousness and insensibility

Alternative stunning methods should disrupt the neuronal function and thereby render animals unconscious and insensible. The extent of disruption caused by a stunning intervention and the induction of unconsciousness and insensibility are best demonstrated using EEGs and ECoGs (EFSA, 2004, 2013d). As described in the EFSA guidance (2013c), it is acceptable that studies reporting interventions assess the onset of unconsciousness as this state is always accompanied by the onset of insensibility.

Animals are rendered gradually unconscious and insensible during exposure to gas mixtures, and the animals may show signs of different stages of anaesthesia as seen in clinical veterinary practice. In general, the different stages of anaesthesia include (1) muscle jerk (voluntary and involuntary excitation), (2) anaesthesia (light, medium and deep), (3) respiratory and cardiovascular depression, and finally (4) death. The stage of voluntary excitement may not be seen in animals when the induction of unconsciousness is smooth and non-aversive. However, the rate of induction of unconsciousness, hence the duration of different stages of anaesthesia, during exposure of animals to a low atmosphere pressure may vary and depends mainly upon the level of oxygen tension in the atmosphere.

In physiological terms, exposure of animals to LAPS is analogous to simulated exposure to high altitudes and, if the partial pressure is low enough is expected to produce loss of consciousness and sensibility via hypoxia. Hypoxia inhibits brain function, as evidenced from the gradual depression leading to the abolition of spontaneous and evoked electrical activity. The physiological brain mechanisms associated with the induction of unconsciousness and insensibility and the EEG manifestations appear to be common to all terrestrial vertebrate animals. The survival time of different regions of the brain and the spinal cord to the effects of hypoxia may vary. When animals are exposed

to low atmosphere pressure, there is a transition period during which conscious EEG patterns change to unconscious EEG patterns, but EEG pattern interpretation is subjective. Loss of consciousness through hypoxia results in hyper synchronisation of the brain electrical activity as evidenced from the appearance of slow waves (high amplitude, low frequency activity) in the EEGs of mammals, leading to quiescent EEGs. In poultry, however, only quiescent EEGs occurred without the manifestation of slow waves. Nevertheless, brain evoked potentials are abolished during the appearance of slow waves in the EEGs or during the occurrence of a profoundly suppressed or quiescent EEGs. Therefore, it is recommended that abolition of evoked electrical activity in the brain should be used as an indicator of unconsciousness when EEG manifestations are ambiguous.

Therefore, the reliable criteria to be employed to assess LAPS in broilers during controlled laboratory studies are (as previously reported for pigs and poultry during exposure to gas mixtures):

- Profoundly suppressed or quiescent EEGs. This is indicative of a complete loss of spontaneous brain activity or a reduction of EEG total power content to less than 10 % of the pre-stun EEG power content, and occurs after exposure to anoxia (e.g. Raj et al., 1998; Rodríguez et al., 2008; Llonch, 2013).
- Abolition of evoked electrical activity in the brain (somatosensory evoked potentials, auditory evoked potentials or flash visual evoked potentials), which is indicative of the brain's incapacity to receive and process external stimuli (e.g. Raj et. al., 1997; Martoft, 2001; Rodríguez et al., 2008).

Established stunning methods induce unique brain states that are incompatible with the persistence of consciousness. These altered brain states are associated with certain behavioural patterns and physical reflexes which can be used as animal-based indicators. The correlation between EEG evidence of unconsciousness and animal-based indicators is characterized for established stunning methods, permitting the use of animal-based indicators as proxies for unconsciousness. A list with indicators for recognition of a successful stun in different species after exposure to hypoxic atmospheres using gas mixtures is provided in previous EFSA opinions (EFSA, 2004, 2013d). Studies in poultry and pigs concerning welfare suggest that loss of posture is the earliest behavioural sign of the onset of unconsciousness. However, it may not always be possible to determine the time to loss of posture as animals start with muscle jerks before or simultaneously they lose posture depending upon the rate of induction of hypoxia/anoxia (Raj et al., 1997; Rodríguez et al., 2008). Other indicators of effective stunning include dilated pupils, absence of palpebral, corneal and pupillary reflexes, apnoea, relaxed body/lack of muscle tone and absence of response to painful stimuli such as nose pricking. In conclusion, in studies carried out under slaughterhouse conditions, the onset and the duration of unconsciousness and insensibility should be ascertained using the indicator that best detects unconsciousness and that has been shown to be correlated with EEGs in laboratory experiments. If different indicators are not in agreement, following on from the precautionary principle and to benefit animal welfare, the one that indicates the longest time interval between application of the stunning intervention and onset of unconsciousness should be used.

In addition to EEG evidence, partial pressure of oxygen in arterial blood and/or pulsoximetry could be used as a direct measure of hypoxia in animals. Evidence should be provided in support of the conclusion that the values reported are incompatible with the persistence of consciousness.

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

If a stunning intervention does not induce immediate unconsciousness and insensibility, the absence of pain, distress and suffering until the onset of unconsciousness and insensibility should be assessed. Loss of consciousness during LAPS is not immediate and animals may experience pain, distress and suffering. At the moment, indirect animal-based measures of pain, distress and suffering have to be used as no direct tool is available to identify them.

Seven "groups of animal-based measures" associated with pain, distress and suffering during the induction of unconsciousness and insensibility are presented in the guidance document (Table 9; EFSA, 2013c): vocalisations, posture and movements, general behaviour; hormone concentrations, blood metabolites, automatic responses and brain activity. Some research papers that describe the use of a particular animal-based measure to assess pain, distress and suffering are included as examples, but the list is not exhaustive. Behavioural, physiological and neurological responses to pain, distress and suffering can be different between animals within and between 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 the LAPS is considered not to induce pain, distress and suffering before the onset of unconsciousness and insensibility:

- This means that these animal-based measures should not be significantly different between the appropriate control and treatment groups. In this regard, in the absence of pain, distress and suffering due to the application of the intervention, the response of animals exposed to LAPS without decompression (control or sham operation) should not be significantly different from the response of the animals exposed to LAPS with decompression (treatment). The possibility that the control itself has not produced a maximum response - such that no further increases in response could occur due to the additional pain and distress caused by the intervention - should be demonstrated.

- In general, these animal-based outcomes 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).

3.1.2.3. Duration of unconsciousness and insensibility (until death)

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

In general, animals are considered to be unconscious as long as the altered brain states, as recognised from the profound changes in EEGs that are unique to the intervention and are established to be incompatible with the persistence of consciousness are demonstrated immediately after intervention. When changes occurring in the spontaneous EEGs are ambiguous, abolition of evoked electrical activity in the brain (somatosensory, visual or auditory evoked potentials) can be used as an indicator of unconsciousness. Recovery of spontaneous or evoked electrical activity in the brain can also be used to ascertain the time to recovery of consciousness in animals following the application of reversible stunning. In this regard, the time to return of total EEG power content (voltage squared) to 10 % or more of the pre-stun level has been used as an indicator of recovery of consciousness (e.g. Raj et al., 2006). The time to recovery of spontaneous activity has been reported to coincide with the time to recovery of evoked activity in the brain (Raj and O'Callaghan, 2004).

Indicators of recovery of consciousness after stunning are listed in EFSA scientific opinion (EFSA, 2004, 2013d), but their sequence depends on the stunning intervention. 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. It is recommended that the indicator that is most sensitive in detecting recovery be used. Indicators that can be measured at different stages during slaughter can be found in EFSA (2013d).

3.2. Assessment of the eligibility criteria of the submitted studies

3.2.1. Intervention

"Physiological responses to low atmospheric pressure (LAPS) and their implications for welfare" - McKeegan et al. (2013) (henceforth referred to as "study 1")

The intervention is insufficiently described, making it impossible to obtain details of the variables that are critical to an assessment of the health and welfare of the chickens during the intervention. For example, neither the rate of decompression represented graphically (it is described as "a gradual curve"), nor oxygen partial pressure is provided.

A continuous EEG trace, from pre-exposure until death during the LAPS intervention, is not presented. Therefore, it is not possible to assess the changes in brain activity that occur in association with hypoxia nor the rate of induction of unconsciousness. Neither the range nor standard deviation corresponding to the time to onset of unconsciousness, as determined using the EEG criteria (total power and median frequency), is reported in Study 1. This makes it impossible to characterize the variability between birds. It is not clear how much of the changes occurring in the EEG criteria were due to the period of darkness in the chamber as no control (i.e. sham operation) data are presented in this study.

The conservative estimate of time to loss of consciousness is reported to be approximately 40 s, but it is not clear whether this is the minimum, maximum or average. Nevertheless, this interpretation is not supported by the behaviour data presented in Study 1. For example, the range of time to loss of posture is reported to be 20 to 69 s. Loss of posture has been reported as the earliest behavioural indicator of onset of unconsciousness during exposure to argon-induced hypoxia. Therefore, the time to onset of unconsciousness during exposure to LAPS could be as long as 69 s. The range of time to onset of wing flapping is reported to be 96 to 159 s. It has been suggested in previous studies on stunning of poultry with carbon dioxide or argon-induced anoxia that wing flapping occurs as a consequence of the loss of brain control over the spinal cord, and hence, the time to onset of wing flapping could be used as a behavioural indicator of the time to onset of unconsciousness (Raj and Gregory, 1990; Raj et al., 1991). Therefore, the time to loss of consciousness could be as long as 159 s after exposure to LAPS.

The absence of a clear description of the sequence of other behavioural events that might be indicative of the reaction of chickens to the LAPS intervention, such as vocalisation, gasping, head shacking, makes assessing the welfare consequence of the intervention impossible. Further, changes in temperature and humidity in the chamber during the intervention were not reported.

The resting heart rate in bantam chickens was found to be 100 beats per minute and, after walking on a treadmill at a sub-maximal speed, increased to 200 beats per minute (Green et al., 2009). The heart rates of 400 beats per minute reported in Study 1 seem abnormally high, and probably near-maximal. This indicates that the birds were already stressed before the intervention. The fact that the heart rate of the birds was already near maximal before the intervention severely compromises interpretation of the heart rate data collected during the intervention.

"A new humane method of stunning broilers using low atmospheric pressure" - Vizzier-Thaxton et al. (2010) (henceforth referred to as "study 2")

The intervention is insufficiently described, making it impossible to obtain details of the variables that are critical to an assessment of the health and welfare of the chickens during the intervention. For

example, neither the rate of decompression represented graphically (it is described as "a gradual curve"), nor oxygen partial pressure is provided.

It is stated that behavioural observations were made for 280 s (pg. 346 of the submitted study), which was described as the duration of the intervention in Study 1. Therefore, we must assume that the intervention in Study 2 was the same as in Study 1 and, therefore, that the results are comparable.

- It is reported that, "The first movement is associated with an awareness of a change in atmosphere that occurs approximately 60 s after pressure is reduced. A period of head movement begins approximately 70 s later. This is followed by wing flapping." (pg. 346, line 7)

It is not clear whether the 'awareness of the rate of change' or 'head movement' could be considered as aversive reactions in birds, and the welfare implications of these behaviours are not adequately described.

- It is reported in Table 3 that the average time to first movement was 58.7 s and the average time from first movement to loss of posture was 64.9 s.

In this study, the average time to loss of posture was reported to be 123.6 s (58.7 + 64.9 s), which is considerably longer than the time to loss of posture reported in Study 1.

As detailed above, there are unexplained inconsistencies between Studies 1 and 2 in terms of the estimated times to loss of consciousness.

"The effects of low atmospheric stunning and deboning time on broiler breast meat quality" - Schilling et al. (2012) (henceforth referred to as "study 3")

The intervention is insufficiently described, making it impossible to obtain details of the variables that are critical to an assessment of the health and welfare of the chickens during the intervention. For example, the only information provided about the intervention is that the atmospheric pressure to which the chickens were exposed was equivalent to an elevation of approximately 10 000 m and that the chickens were inside the LAPS for 2 minutes after loss of posture and for a total of 2.5 minutes. It is stated that additional details of the intervention are available in Cheek and Cattarazzi (2010), but that document was not one of those submitted for assessment. No observations relating to the health or welfare of the chickens before or during the intervention are reported.

"The Effects of Low-Atmosphere Stunning and Deboning Time on Broiler Breast Meat Quality - Battula et al. 2008 (henceforth referred to as "study 4")

The intervention is insufficiently described, making it impossible to obtain details of the variables that are critical to an assessment of the health and welfare of the chickens during the intervention. For example, the only information provided about the intervention is that the atmospheric pressure to which the chickens were exposed was 597-632 mmHg and that they were inside the LAPS for 2 minutes. No observations relating to the health or welfare of the chickens before or during the intervention are reported.

3.2.2. Outcome

Study 1

Based on electrocardiogram (EKG) data, cardiac arrest, and on isoelectric EEGs registered in all experimental birds, the chickens were dead when they were removed from the LAPS. No information on animal-based measures associated with pain, distress and suffering during the induction of unconsciousness and insensibility were provided.

Study 2

It is unclear from the blood analyses presented in Table 4 of Study 2 whether the cause of death was hypoxia. This is because the authors do not state whether the blood is from the arterial or venous blood supply, but only that it was taken from the heart. If the blood sampled was arterial, then the only measured variables that were not in the normal range for birds are the values for PO_2 (the partial pressure of oxygen, which we assume is in units of Torr, but the units are not stated in Study 2), Hct (haematocrit), Hgb (haemoglobin concentration), and pH. This PO_2 value in the arterial blood is definitely low enough to cause death in most bird species, particularly considering that hypoxia is induced relatively quickly with the proposed intervention. The slightly low pH is also consistent with the explanation that the birds died from hypoxia (it indicates a metabolic acidosis). The Hct and Hgb values are relatively low, and indicate that the birds were anaemic. However, since the values are similar between LAPS and electrical stunning, this is probably a characteristic of the birds themselves, and not the stunning treatment (although it is hard to tell in the absence of a pre-stunning measurement). The low Hct and Hgb levels would accentuate the effect of a low PO_2, possibly hastening death. It is possible that a low arterial PO_2 would have been better tolerated if the birds hadn't had such low Hct and Hgb values. This observation indicates that caution should be applied when generalizing these findings to other poultry. Nevertheless, arterial PO_2 values this low are sufficient to kill most birds with normal Hct and Hgb values. In contrast, if the blood sampled was venous, then it was probably not sufficiently low to cause death. This leaves the cause of death equivocal since, if by chance venous rather than arterial blood was sampled (i.e. from the right ventricle, which is blood that has returned from the peripheral tissues and has not yet been re-oxygenated in the lungs), then the PO_2 and pH values would reflect the deoxygenated blood supply.

The histopathological evidence reported of haemorrhagic lesions in the lungs and other organs could be interpreted as evidence of compromised welfare in these birds during the application of the intervention. However, overall, the information provided does not allow any conclusions to be taken about the outcome in relation to the impact of the intervention on the welfare of the chickens.

Study 3

No information on outcome was provided beyond stating that all of the chickens were dead when they were removed from the LAPS.

Study 4

No information on outcome was provided beyond stating that all of the chickens were dead when they were removed from the LAPS.

4. Reporting quality

The reporting quality of a study submitted for assessment is evaluated against each of the criteria in Table 10 (Section 4) of the guidance document (EFSA, 2013c). The decision over whether the overall reporting quality is sufficient will be based upon the judgment of the panel experts engaged to assess the submitted studies.

4.1. Assessment of the reporting quality of the submitted studies based on the selected parameters

The assessed studies did not pass the eligibility assessment and, therefore, reporting quality was not assessed (as per Figure 1).

5. Methodological quality

The methodological quality of a research study can be determined by assessing its precision and its internal and external validity. These elements are related to the extent to which the study's design, implementation, data acquisition, analysis and interpretation of results 1) minimise systematic errors

(biases) that compromise the study's internal validity; 2) minimise random errors that reduce the precision of the measurements made in the study; 3) allow broad applicability of the results beyond any single study (= external validity). Details of the methodological quality criteria assessment are presented in chapter 5 of the guidance document.

5.1. Quality assessment of the internal validity of the submitted studies

The assessed studies did not pass the eligibility assessment and, therefore, methodological quality was not assessed (as per Figure 1).

5.2. The extent to which the findings are consistent with other sources of information;

A simple literature search uncovered articles published mainly by the same research groups. However, since the rate of decompression is not fully described in the submitted studies, it was not possible to evaluate their consistency with these other studies. For this reason, the assessment was based exclusively on the documents submitted.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- None of the four studies meet the eligibility criteria and, therefore, were not assessed further.
- The manner in which the LAPS procedure is described in the submitted studies is inconsistent and, therefore, it was not possible to ascertain the specifics of the key parameters of the intervention nor their impact on the welfare of the chickens. The animal welfare outcome of the intervention is incompletely described in the submitted studies, leaving it unclear whether the rate of decompression used in LAPS induces unconsciousness and death without causing avoidable pain and suffering in poultry.

RECOMMENDATIONS

- Researchers are advised to consult the guidance document on the process and criteria applied by EFSA to assess studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing.

REFERENCES

Battula V, Schilling MW, Vizzier-Thaxton Y, Behrends JM, Williams JB and Schmidt TB, 2008. The Effects of Low-Atmosphere Stunning and Deboning Time on Broiler Breast Meat Quality. Poultry Science, 87, 1202-1210.

Cheek H and Cattarazzi B, inventors and assignees, 2010. United States Process Patent 7662030. Method for humanely stunning and slaughtering poultry using controlled low atmospheric pressure. Feb. 16, 2010.

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 2004, 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 2005, 292, 1–46.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013a. 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

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013b. Scientific Opinion on the use of carbon dioxide for stunning rabbits. EFSA Journal 2013;11(6):3250, 33 pp. doi:10.2903/j.efsa.2013.3250

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013c. Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing. EFSA Journal 2013;11(12):3486, 41 pp. doi: 10.2903/j.efsa.2013.3486

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013d. Scientific Opinion on monitoring procedures at slaughterhouses for poultry. EFSA Journal 2013;11(12):3521, 65 pp. doi:10.2903/j.efsa.2013.3521

Green JA , Halsey LG, Wilson RP and Frappell PB, 2009 Estimating energy expenditure of animals using the accelerometry technique: activity, inactivity and comparison with the heart-rate technique. Journal of Experimental Biology, 212, 471-482.

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, 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.

Martoft L, Jensen EW, Rodriguez BE, Jorgensen PF, Forslid A and Pedersen HD, 2001. Middle-latency auditory evoked potentials during induction of thiopentone anaesthesia in pigs. Laboratory Animals, 35, 353–363.

McKeegan DE, Sandercock DA and Gerritzen MA, 2013. Physiological responses to low atmospheric pressure stunning and the implications for welfare. Poultry Science, 92, 858-868.

Raj ABM and Gregory NG, 1990. Effect of rate of induction of carbon dioxide anaesthesia on the time to onset of unconsciousness and convulsions. Research in Veterinary Science, 49, 360-363.

Raj ABM, and O'Callaghan M, 2004. Effects of electrical water bath stunning current frequencies on the spontaneous electroencephalograms and somatosensory evoked potentials in hens. British Poultry Science, 45: 230-236.

Raj ABM, Gregory NG and Wotton SB, 1991. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning in argon-induced hypoxia. British Veterinary Journal, 147, 322-330.

Raj ABM, Johnson SP, Wotton SB and McKinstry JL, 1997. Welfare implications of gas stunning pigs: 3. The time to loss of somatosensory evoked potentials and spontaneous electroencephalogram of pigs during exposure to gases. British Veterinary Journal, 153, 329-340.

Raj ABM, O'Callaghan M and Knowles T, 2006. The effect of amount and frequency of alternating current used in water bath stunning and neck cutting methods on spontaneous electroencephalograms in broilers. Animal Welfare, 15, 7-18.

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.

Schilling MW, Radhakrishnan V, Vizzier-Thaxton Y, Christensen K, Joseph P, Williams JB and Schmidt TB, 2012. The effects of low atmosphere stunning and deboning time on broiler breast meat quality. Poultry Science, 91, 3214-3222.

Vizzier-Thaxton Y, Christensen KD, Schilling MW, Buhr RJ and Thaxton JP, 2010. A new humane method of stunning broilers using low atmospheric pressure. Journal of Applied Poultry Research, 19, 341-348.

APPENDIX. ASSESSMENT OF THE ELIGIBILITY CRITERIA

Study 1: "Physiological responses to low atmospheric pressure stunning (LAPS) and implications for welfare" (McKeegan et al., 2013)

Table 2: Information provided by the submitted study in relation to the intervention

Parameter	Component	Comment	Fulfilment criterion (yes or no)
Animal density	Animal species/ age/ type and stocking density (number/m^2 and kg of body weight/ m^2)	Broilers chicken, 28-30 days of age, For the rest no information was provided.	No/not provided
Duration of intervention	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multi-phase system	The information reported regards only the entire LAPS cycle from doors closed to doors opening, lasting 280 sec. The process is not sufficiently described. There is no information provided about the variability and duration of the intervention.	No
Rate of decompression	Time/pressure treatment graphic representation	The study reports that the decompression consists of a gradual curve of reducing pressure over 75% of the cycle and that the final pressure is maintained over the remaining 25% but no information is provided if air removing grade is gradual or not. A pressure / time profile curve is needed to assess the rate of decompression. The pressure achieved in this study was reported to be 20% of ambient pressure but no supporting measurements were provided. No information in regards to re-pressurisation.	No
Rate of changes in partial pressure of oxygen	Time/partial pressure of oxygen treatment graphic representation	The study refers to a final pressure maintained over the remaining 25% of the cycle and being not greater than an 80% reduction in ambient pressure. The pressure achieved in this study should therefore be 20% of ambient pressure. The data are not expressed in absolute values and no information is provided on the corresponding partial pressure of oxygen. The corresponding partial pressure of oxygen levels indicative of hypoxia is not provided to assess the magnitude of hypoxia.	No
Temperature/ humidity/ illumination of the chamber		Information not provided.	No
Maximum stun-to-stick/kill interval(s)		Not applicable in this case. The intervention reported in the study is irreversible.	Not applicable
Calibration of the LAP equipment and monitoring system		No information is provided.	No

Table 3: Information provided by the submitted study in relation to the onset of unconsciousness and insensibility

Parameter	Comment	Is the induction of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	EEG was measured but incomplete data was provided. The procedures to install the electrodes and their positions are described. The method used to derive the transformations of the EEG is reported. The sampling of the EEG signal for analysis seems to be different for the different birds and therefore is not comparable. The information reported therefore does not allow a full assessment of the EEG data.	Not possible to assess
Animal-based indicators to detect onset of unconsciousness and insensibility	The evaluation of the EEG data does not allow precise determination of the time to unconsciousness and does not associate unconsciousness with animal-based indicators.	No

Table 4: Information provided by the submitted study in relation to animal-based measures associated with pain, distress and suffering during the induction of unconsciousness and insensibility

Response type	Groups of animal-based measures (ABMs)	Comments	Do the ABMs suggest pain, distress and suffering (yes, no or not possible to assess)
Behaviour	Vocalisations	No information provided.	Not possible to assess
	Postures and movements	No information provided in relation to assessment of pain, distress and suffering.	Not possible to assess
	General behaviour	No information provided in relation to assessment of pain, distress and suffering.	Not possible to assess
Physiological response	Hormone concentrations	No information provided.	No
	Blood metabolites	No information provided.	No
	Autonomic responses	Information is provided on the changes in heart rate responses but not in relation to assessment of pain, distress and suffering.	No
Neurological response	Brain activity	EEG was measured but not in relation to assessment of pain, distress and suffering.	No

Table 5: Information provided by the submitted study in relation to the duration of unconsciousness and insensibility or evidence of death in the case of irreversible intervention

Parameter	Comments	Is the duration of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	In case of irreversible stunning, it is necessary to demonstrate that the intervention kills the animal. In this case, we need indicators of death rather than indicator of unconsciousness. It is reported isoelectric EEG occurred in all the experimental birds before exiting the LAPS.	Yes
Animal-based indicators to detect duration of unconsciousness and insensibility or death	In case of irreversible stunning, it is necessary to demonstrate that the intervention kills the animal. It is reported that, based on EKG data, cardiac arrest occurred in all the experimental birds before exiting the LAPS.	Yes

Study 2: "A new humane method of stunning broilers using low atmospheric pressure" (Vizzier-Thaxton et al., 2010)[6]

Table 6: Information provided by the submitted study in relation to the intervention

Parameter	Component	Comment	Fulfilment criterion (yes or no)
Animal density	Animal species/ type and stocking density (number/ m^2 and kg of body weight/ m^2).	Broiler chickens For the rest no information was provided	No
Duration of intervention	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multi-phase system.	No information is provided	No
Rate of decompression	Time/pressure treatment graphic representation.	No information is provided	No
Rate of changes in partial pressure of oxygen	Time/partial pressure of oxygen treatment graphic representation.	No information is provided	No
Temperature humidity/ illumination of the chamber		No information is provided	No
Maximum stun-to-stick/kill interval(s)		Not applicable in this case The intervention reported in the study is irreversible	Not applicable
Calibration of the LAP equipment and monitoring system		No detailed information is provided	No

[6] (The assessment of this study only focuses on the intervention of low atmospheric pressure and not on the electrical stunning intervention)

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

Parameter	Comment	Is the induction of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	Not measured.	No
Animal-based indicators to detect onset of unconsciousness and insensibility	Some indicators are used but the purpose is not clear. In particular, the use of loss of posture as indicator to assess onset of unconsciousness is not properly described. Onset of wing flapping could be used as indicators of onset of unconsciousness, but wing flapping were not interpreted in that context.	No

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

Response type	Groups of animal-based measures (ABMs)	Comment	Do the ABMs suggest pain, distress and suffering (yes, no or not possible to assess)
Behaviour	Vocalisations	No information provided.	No
	Postures and movements	Information provided is not sufficient to interpret the use of the indicators in relation to assessment of pain, distress and suffering.	Not possible to assess
	General behaviour	Information provided is not sufficient to interpret the use of the indicators in relation to assessment of pain, distress and suffering.	Not possible to assess
Physiological response	Hormone concentrations	Corticosterone concentration was not used to assess the welfare of birds during decompression. It was reported only in comparison to electrically stunned broilers and it was no compared to a baseline control.	No
	Blood metabolites	No metabolites indicators of stress were measured.	No
	Autonomic responses	No information provided.	No
Neurological response	Brain activity	Not measured.	No

Table 9: Information provided by the submitted study in relation to the duration of unconsciousness and insensibility or evidence of death in the case of irreversible intervention

Parameter	Comment	Is the duration of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	Not measured	No
Animal-based indicators to detect duration of unconsciousness and insensibility	In case of irreversible stunning, it is necessary to demonstrate that the intervention kills the animal. No indicator of death is used.	No

Study 3: The effects of low atmosphere stunning and deboning time on broiler breast meat quality (Schilling et al., 2012)

Table 10: Information provided by the submitted study in relation to the intervention

Parameter	Component	Comment	Fulfilment criterion (yes or no)
Animal density	Animal species/ age/ type and stocking density (number/m^2 and kg of body weight/ m^2)	In the described study, 2 full live haul cages with 250 commercial broilers each, were inserted in the chamber. No information is provided on the stocking density in cages/chamber.	No
Duration of intervention	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multi-phase system	Information is reported only on the total time during which broilers were kept in the container (2.5 min). Corresponding partial pressure of oxygen is not provided. All broilers were maintained in the LAPS chamber for 2 min after loss of posture for a total time of 2.5 min in the container where the pressure was reduced to that at an approximate elevation of 10,000 m.	No
Rate of decompression	Time/pressure treatment graphic representation	The study refers to another publication illustrating the low atmospheric pressures created in LAPS (in the range of 150 to 230 mmHg) and rate of change from sea level. It also refers to the commercial prototype that was used (Technocatch LLC, Kosciusko, MS). Information is given on the type of vacuum pumps rated at 14 $m^{3/}$ min.	No
Rate of changes in partial pressure of oxygen	Time/partial pressure of oxygen treatment graphic representation	No information is provided.	No
Temperature/ humidity/ illumination of the chamber		No information is provided.	No
Maximum stun-to-stick/kill interval(s)		No information is provided.	No
Calibration of the LAP equipment and monitoring system		A computer-based data acquisition and control system was used to monitor tank pressure and control pump action but data are not provided. No information is provided on the calibration of the system.	No

Table 11: Information provided by the submitted study in relation to the onset of unconsciousness and insensibility

Parameter	Comment This study is not relevant to the assessment of impact on health and welfare	Is the induction of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	No information is provided.	no
Animal-based indicators to detect onset of unconsciousness and insensibility	Time to onset of unconsciousness is not provided.	no

Table 12: Information provided by the submitted study in relation to animal-based measures associated with pain, distress and suffering during the induction of unconsciousness and insensibility

Response type	Groups of animal-based measures (ABMs)	Comments	Do the ABMs suggest pain, distress and suffering (yes, no or not possible to assess)
Behaviour	Vocalisations	No information is provided.	Not possible to assess
	Postures and movements	It is reported that broilers are maintained in the LAPS chamber for 2 min after loss of posture but no data are provided.	Not possible to assess
	General behaviour	No information is provided.	Not possible to assess
Physiological response	Hormone concentrations	No information is provided.	Not possible to assess
	Blood metabolites	No information is provided.	Not possible to assess
	Autonomic responses	No information is provided.	Not possible to assess
Neurological response	Brain activity	No information is provided.	Not possible to assess

Table 13: Information provided by the submitted study in relation to the duration of unconsciousness and insensibility or evidence of death in the case of irreversible intervention

Parameter	Comments This study is not relevant to the assessment of impact on health and welfare	Is the duration of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	No information is provided.	Not possible to assess
Animal-based indicators to detect duration of unconsciousness and insensibility and death	No information is provided.	Not possible to assess

Study 4: The Effects of Low-Atmosphere Stunning and Deboning Time on Broiler Breast Meat Quality (Battula et al., 2008)

Table 14: Information provided by the submitted study in relation to the intervention

Parameter	Component	Comments	Fulfilment criterion (yes or no)
Animal density	Animal species/ age/ type and stocking density (number/m^2 and kg of body weight/ m^2)	The study reports that cages with 24 broilers were placed into the chamber but the stocking density in cages/chamber is not reported.	No
Duration of intervention	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multi-phase system	Information is provided on the time during which broilers were kept in decompression chamber (2 min) but corresponding partial pressure of oxygen is not provided. It is not specified if decompression starts as soon as door closes.	No
Rate of decompression	Time/pressure treatment graphic representation	The study refers to the commercial prototype that was used (Technocatch LLC, Kosciusko, MS). Information is given on the type of vacuum pumps rated at 14 m^3/min. The low atmospheric pressure achieved in the chamber was described as being 597 to 632 mmHg.	No
Rate of changes in partial pressure of oxygen	Time/partial pressure of oxygen treatment graphic representation	No information is provided.	No
Temperature/ humidity/ illumination of the chamber		No information provided.	No
Maximum stun-to-stick/kill interval(s)		Information not provided; the study only reports that after ataxia, broilers were decapitated manually.	No
Calibration of the LAP equipment and monitoring		A computer-based data acquisition and control system was used to monitor tank pressure and control pump action. No information is provided on the calibration of the system.	No

Table 15: Information provided by the submitted study in relation to the onset of unconsciousness and insensibility

Parameter	Comments This study is not relevant to the assessment of impact on health and welfare	Is the induction of unconsciousness and insensibility addressed adequately? (yes, no or not possible to assess)
EEG	No information provided.	no
Animal-based indicators to detect onset of unconsciousness and insensibility	Time to onset of unconsciousness is not provided.	no

Table 16: Information provided by the submitted study in relation to animal-based measures associated with pain, distress and suffering during the induction of unconsciousness and insensibility

Response type	Groups of animal-based measures (ABMs)	Comments	Do the ABMs suggest pain, distress and suffering (yes, no or not possible to assess)
Behaviour	Vocalisations	No information is provided.	Not possible to assess
	Postures and movements	It is reported that after ataxia (loss of posture, resulting in the inability to maintain a standing position and no neck tension at the onset of unconsciousness), broilers were decapitated manually but no information that would permit an assessment of pain, distress and suffering is provided.	Not possible to assess
	General behaviour	No information is provided.	Not possible to assess
Physiological response	Hormone concentrations	No information is provided.	Not possible to assess
	Blood metabolites	No information is provided.	Not possible to assess
	Autonomic responses	No information is provided.	Not possible to assess
Neurological response	Brain activity	No information is provided.	Not possible to assess

Table 17: Information provided by the submitted study in relation to the duration of unconsciousness and insensibility or evidence of death in the case of irreversible intervention

Parameter	Comment	Is the duration of unconsciousness/insensibility addressed adequately? (yes, no or not possible to assess)
EEG	No information is provided.	Not possible to assess
Animal-based indicators to detect duration of unconsciousness/ insensibility or death	No information is provided.	Not possible to assess

GLOSSARY AND ABBREVIATIONS

ABBREVIATIONS

ABM	Animal-based measure
AHAW Panel	EFSA Panel on Animal Health and Welfare
EC	European Commission
EFSA	European Food Safety Authority
ECoG	Electrocorticogram
EEG	Electroencephalogram
EKG	Electrocardiogram
Hct	Hematocrit
Hgb	Haemoglobin concentration
LAPS	Low atmosphere pressure system