GUIDANCE OF THE AHAW PANEL

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Guidance on the assessment criteria for applications for new or modified stunning methods regarding animal protection at the time of killing

EFSA Panel name on AHAW
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Abstract

This guidance defines the process for handling applications on new or modified stunning methods and the parameters that will be assessed by the EFSA Animal Health and Welfare (AHAW) Panel. The applications, received through the European Commission, should contain administrative information, a checklist of data to be submitted and a technical dossier. The dossier should include two or more studies (in laboratory and slaughterhouse conditions) reporting all parameters and methodological aspects that are indicated in the guidance. The applications will first be scrutinized by the EFSA's APDESK Unit for verification of the completeness of the data submitted for the risk assessment of the stunning method. If the application is considered incomplete, additional information may be requested from the applicant. If considered complete, it will be subjected to assessment phase 1 where the suitability of the data related to parameters for the scientific evaluation of the stunning method will be examined by the AHAW Panel. Such parameters focus on the stunning method and the outcomes of interest, i.e. immediate onset of unconsciousness or absence of avoidable pain, distress and suffering until the loss of consciousness, and duration of the unconsciousness (until death). The applicant should also propose methodologies and results to assess the equivalence with existing stunning methods in terms of welfare outcomes. Applications passing assessment phase 1 will be subjected to the following phase 2 which will be carried out by the AHAW Panel and focuses on the animal welfare risk assessment. In this phase, the Panel will assess the outcomes, conclusions and discussion proposed by the applicant. The results of the assessment will be published in a scientific opinion.

Keywords: Stunning, slaughter, animal welfare, reporting guidance

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Panel [or Scientific Committee] members: [add names in the format Name Surname, Name Surname and Name Surname].

Minority opinion: In case of a minority opinion, please add: [Part of this/This] scientific output is not shared by the following member(s) of the Panel: [add names in the format Name Surname, Name Surname and Name Surname].

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Summary

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1. Introduction

1.1. Background and Terms of Reference as provided by EFSA

Council Regulation (EC) No 1099/2009[1] on the protection of animals at the time of killing 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". Annex I of the Regulation lists the stunning methods and related specifications. Article 4 of the Regulation allows the Commission to amend Annex I to this Regulation after taking account of 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.

Several studies assessing the efficacy of modified protocols of stunning methods listed in Annex I or novel stunning methods have been submitted to the Commission who has requested EFSA's assessment on the studies (M-2013-0114, M-2013-0077 and M-2013-0076).

In order to respond to the mandates, the AHAW Panel of EFSA in 2013 has issued a guidance document (EFSA-Q-2013-00532) that establishes the criteria for evaluating such studies. In particular, the process set up by the guidance foresees two phases of assessment: i) assessment phase 1: the submitted studies in support of the new method or modified protocol are first checked against criteria related to eligibility, reporting and methodological quality; ii) assessment phase 2: the submitted studies are fully assessed in terms of welfare implications, i.e. pain, distress and suffering, and evaluated to assess if the proposed stunning method is able to provide a level of animal welfare at least equivalent to that ensured by the existing methods.

In 2013, studies submitted for the above mentioned mandates did not pass assessment phase 1, i.e. the studies submitted by the applicants did not provide complete information related to eligibility, reporting and methodological quality. Subsequently, in 2016, the EU Commission requested EFSA to review a series of scientific studies to assess a new stunning system for poultry based on low atmospheric pressure (M-2016-0109). In this case, the submitted studies passed assessment phase 1 as described in the guidance. It was therefore required to proceed to the assessment phase 2, i.e. the full assessment of the new stunning method, to evaluate whether it provides a level of animal welfare at least equivalent to that ensured by the currently allowed methods.

On the basis of the experience acquired during the latter assessment of the low atmospheric pressure stunning method, the AHAW Panel noted that some aspects of the guidance needed to be reviewed

[1] 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.

and refined for assessment phase 1 as well as further steps that needed to be completed for assessment phase 2 to ascertain the equivalence to the existing stunning methods.

The experience acquired also has shown that guidance and requirements have to be proportionate to the issue at stake. Indeed stunning methods are rarely subject to fundamental research due to limited budget for such activities.

It is likely that further studies in support of modified protocols of existing stunning methods or new stunning methods for animals at slaughter will be carried out and submitted to EFSA for assessment. Therefore, a revision and completion of the EFSA guidance is required.

1.2. Interpretation of the Terms of Reference

This guidance defines the process and the criteria that will be applied to the scientific assessment of applications related to new or modified legal stunning methods. The scope of this guidance is limited to new stunning methods, or modified legal stunning methods used at slaughter. It does not cover methods that are exclusively used for depopulation nor other forms of on-farm slaughter or killing (e.g. emergency killing methods).

2. Guidance for handling applications on stunning methods for animals

2.1. Procedure

In accordance with Regulation (EC) No 1099/2009 Article 4 (2), the Commission can amend Annex I to the Regulation, which includes approved stunning methods and their specifications, on the basis of a scientific assessment provided by EFSA. Any amendment shall ensure a level of animal welfare at least equivalent to that ensured by the existing stunning methods by taking into account the magnitude of pain, distress and suffering. In addition, Article 14(3)(b) of the same Regulation provides that its Annex II concerning layout, construction and equipment of slaughterhouses may be amended to take account of scientific and technical progress.

EFSA will assess the application for a new or modified stunning method through a procedure that foresees the following sequence (also summarised in Figure 1):

1) the applicant prepares a dossier and submits it to the EC;

2) the EC decides on sending a mandate to EFSA requesting scientific assessment of the dossier;

3) EFSA (APDESK Unit) performs a **completeness check of the application**: the submitted application on the new or modified method is checked against the completeness of the information and data submitted by the applicant (see chapter 2.4.);

If the application is considered incomplete by APDESK, EFSA may ask for a revision of the dossier (which after resubmission will be submitted to 3)) or it may fully reject the application.

5) Upon agreement from EC about the timeline for execution of the tasks, EFSA will proceed to the **suitability check of the data in preparation of the risk assessment (assessment phase 1)**: EFSA (AHAW Panel) will verify if the information used to describe and scientifically evaluate the method – e.g. statistical methods, welfare measures – is adequate (see chapter 3). In case the suitability check is negative, EFSA may ask for a revision of the dossier (which after resubmission will be submitted to 3)) or it may fully reject the application.

6) Stunning methods passing assessment phase 1 will be subjected to a **risk assessment of the stunning method (assessment phase 2)**: the submitted application is fully assessed by the AHAW Panel for (see chapter 4):

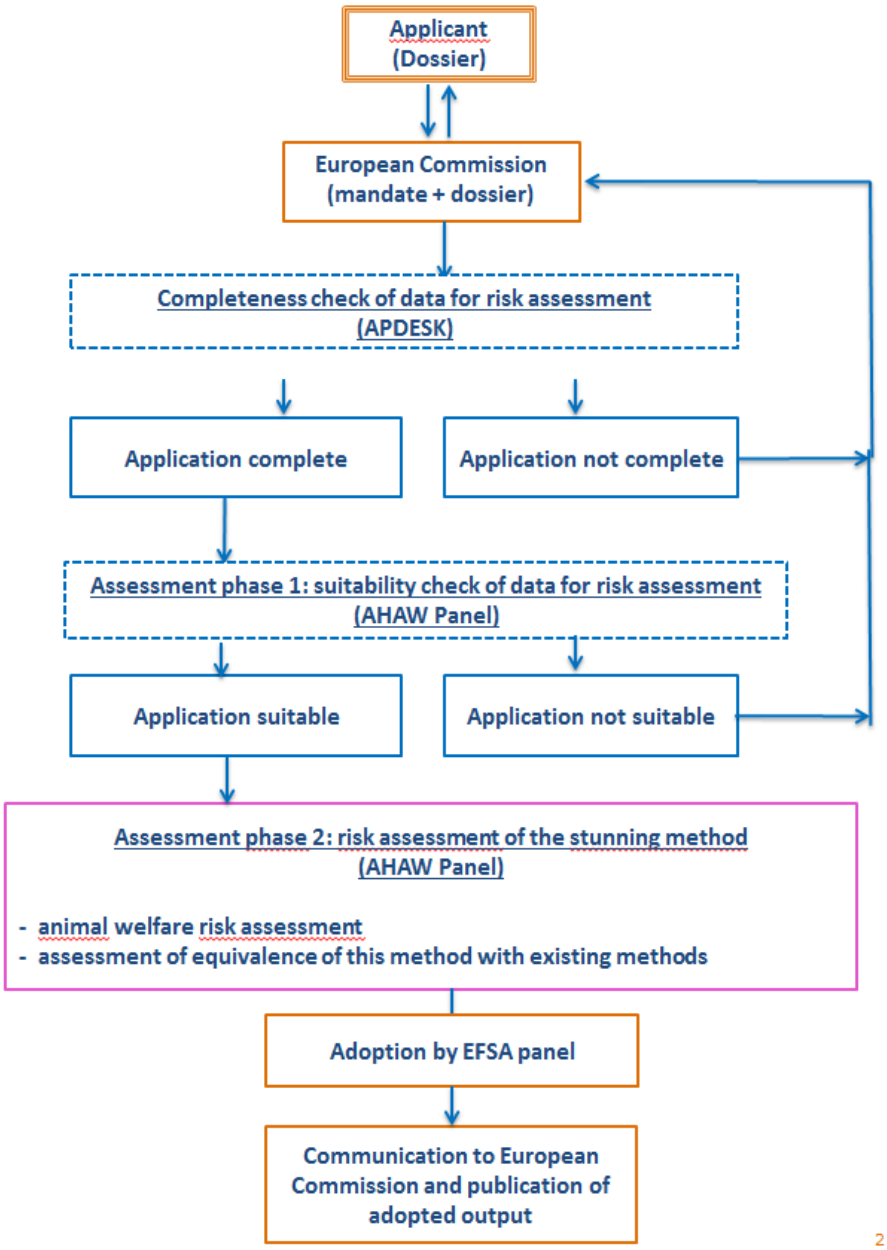
- animal welfare risk assessment (i.e. assessment of the outcomes of the method in terms of welfare implications, i.e. pain, distress and suffering), and

- the assessment of the equivalence with at least one of the existing methods (i.e. to assess if the proposed stunning method is able to provide a level of animal welfare at least equivalent to that ensured by the existing methods listed in Annex 1 of EC Regulation 1099/2009).

7) the EFSA AHAW panel provides the EC with a scientific opinion on the animal welfare outcome assessment and publishes it in the EFSA Journal, in accordance with Article 29(1) of Regulation (EC) No 178/2002[2].

The EC will decide about the authorisation of the new method.

Figure 1. Flowchart showing the procedure for handling applications on animal stunning methods



[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 031, 1.2.2002, p.1, as last amended.

2.2. Submission of an application for stunning methods for animals

Any applicant or any business operator seeking an authorisation for a new or modified stunning method shall submit an application to the EC, which will possibly make the application available to EFSA. From reception of an application, EFSA will issue an acknowledgement of receipt letter to the EC, with the applicant in copy of the correspondence. At that moment, the application is registered in the EFSA Register of Questions and receives a unique identification number (e.g. EFSA-Q-YYYY-XXXX referred to as "EFSA Question number"). The status of the application is regularly updated in the Register of Questions database and can be monitored by the applicant.

2.2.1. Documentation

When submitting an application, the following documents and particulars shall be provided to EC:

- **Administrative part**, containing all the administrative information related to the application using the format provided in Annex A1– Administrative information.
- **Technical dossier**: includes detailed reports of all studies performed in support of the application (see below in 2.3.1.). When preparing the technical dossier, applicants should follow the scientific requirements described in this guidance. Audio-video material demonstrating the method and other material considered relevant for the understanding of the method by the applicant (e.g. histological images, thermographic material) and bibliographic references should be provided in separate files.
- **Completeness checklist**: the applicant should compile the checklist provided in Annex B – Completeness checklist, in WORD format.
- **Justification for confidential information**, consisting in a statement justifying why the confidential information included in the dossier might significantly harm the applicant's competitive position. Applicants should submit the justification using the format provided in Annex C – Justification for confidential information.

EFSA will receive the above documentation directly from the EC. Applicants shall not submit their applications directly to EFSA.

2.3. Preparation of the dossier

2.3.1. Submission format

The above listed documentation should be submitted using standard electronic data carriers (i.e. USB key, CD-ROM). It should be accompanied by the original of a signed cover letter listing the annexes of the application.

A USB key or a CD-ROM shall be provided with the **complete and full information**. This copy shall therefore include:

- Administrative part (Annex A);
- Technical dossier and annexes as separate pdf documents (one pdf document for each annex) with **confidential information highlighted**;
- Completeness checklist (Annex B)
- Justification for confidential information (Annex C);
- When applicable, the agreement on data sharing (see chapter xx).

A USB key or a CD-ROM **without confidential information** should also be provided. This copy shall therefore ONLY include:

- Administrative part (Annex A);
- Technical dossier and annexes as separate pdf documents (one pdf document for each annex) **without confidential information** or with **confidential information blanked out**;
- When applicable and if it is not requested to be considered as confidential, the agreement on data sharing (see chapter xx).

2.3.2. Studies provided in the dossier

The technical dossier should include detailed reports of all studies performed in support of the application, i.e. scientific reports and/or papers fully documenting the performed experiments, analytical methods and outcomes.

The number of studies submitted in the dossier depends on the number of experiments that the applicant considers necessary for demonstrating the efficacy of the proposed method. Overall, studies provided in the dossier should include experiments carried out: 1) at laboratory level and 2) at commercial (slaughterhouse) level. This is due to the fact that research evaluating stunning methods requires well controlled studies under laboratory conditions to characterize the animals' responses to the stunning method (onset of unconsciousness, magnitude of pain, distress and suffering). The most valid measures available (e.g. electroencephalograms (EEG)) should be used and the correlations between these measurements and non-invasive animal based measures that can be applied in commercial slaughterhouse conditions should be established. Secondly, studies performed under slaughterhouse conditions are intended to assess the feasibility of the method and to assess whether the results obtained in the laboratory studies can also be achieved in a commercial context. Consequently the submitted dossier should contain two or more studies.

2.3.3. Language

In order to facilitate the evaluation of the applications, scientific and technical documentation should be submitted in English. EFSA may ask the applicant to translate the parts of the dossier that would not be submitted in English.

2.3.4. File format, size and name

The technical dossier and annexes and all references cited should be provided preferably as portable document format (PDF). The electronic files should not be password-protected. Each PDF document should be accessible to allow reading, printing, word searching and copying of text from the file using Adobe Acrobat® Standard (version 7.0 or later) software. Text and figures of all parts of the application should be fully legible.

The size of single documents should be limited to 30 MB.

When no standard name is recommended, the file name should be concise and informative of its content and contain no more than 40 characters including spaces.

2.3.4.1. Standard Units and abbreviations

The International System of Units (SI)[3] must be used. Explanation for acronyms and abbreviations should be provided in the text when they are used for the first time.

2.3.5. Bibliographical references

[3] http://www.bipm.org/utils/common/pdf/si_brochure_8_en.pdf

The applicant should include in the relevant section of the technical dossier references to all published and unpublished studies. These references should be provided as full text in separate pdf documents.

2.4. Completeness check of data for risk assessment and validation of the application

After reception, the Applications Desk Unit (APDESK) checks the completeness of the application against the requirements detailed in this guidance. To do that, EFSA will check the completeness of the data submitted for the risk assessment following the checklist (Annex B) provided by the applicant and verifying that the information is effectively provided in the technical dossier. The completeness check relates to the description of the stunning method (see chapter 3.1.), the description of the individual studies submitted (see chapter 3.2.) and the overall integration of findings from all studies (see chapter 3.3.). The applicant should follow the same structure of the checklist (i.e. chapter headings of the guidance) when building dossiers in relation to studies on new or modified stunning interventions.

EFSA endeavours to have the first outcome of the completeness check available and communicated to the applicant within 30 working days after the reception date.

The completeness check process might require further exchange of information between the applicant and EFSA. In such case, EFSA informs the applicant, in writing, if certain parts of the application need modification or completion, in order to proceed to validation. This may also prolong the time required for the completeness check. After receiving a request for additional information, the applicant should submit the response within 30 days. When this is not possible, the applicant should indicate to EFSA the date by which the response is expected. EFSA will notify the acceptance of the new submission date via e-mail. When responding to EFSA questions, the applicant should submit an updated version of the entire application. EFSA advises to accompany the submission of an updated application with a cover letter wherein the applicant precisely describes how each EFSA question was addressed. Missing information should be incorporated in all relevant parts of the application.

EFSA endeavours to inform the applicant within 30 working days if the updated application is complete or if further revision is required.

2.5. Interaction with EFSA staff during preparation, submission and completeness check

EFSA has implemented some initiatives to support applicants in understanding the evaluation process of applications for stunning methods and to engage with them during the life-cycle of applications.

If an applicant is seeking information during the preparation of an application on aspects related to data for risk assessment, EFSA encourages the use of the APDESK web form (link) to submit any queries to EFSA. EFSA endeavours to reply within 15 working days of reception of the query.

If an applicant is seeking information on the status of an application already submitted the applicant may check this information in the EFSA Register of questions database.

During the completeness check, applicants have the possibility to contact the staff in the APDESK Unit. A telephone conference may be organised to further clarify the outcome of the completeness check.

2.6. Confidentiality of the submitted studies

EFSA has obligations in terms of independence of its scientific risk assessment and transparency deriving from its Founding Regulation (EC) No 178/2002, specifically Articles 37 and 38 of Regulation (EC) No 178/2002. In particular, according to Article 38(1)(b) and (c) of Regulation (EC) No 178/2002 EFSA shall publish "the opinions of the Scientific Committee and the Scientific Panels immediately after adoption, minority opinions always being included" and "without prejudice to Articles 39 and 41, the information on which its opinions are based".

EFSA shall ensure confidentiality of information "for which confidential treatment has been requested and justified, except for information which must be made public if circumstances so require, in order to protect public health", in accordance with Article 39 of the same Regulation. For the purpose of assessing the confidentiality claims for information contained in applications, particularly in studies, EFSA has developed an internal procedure for evaluating those claims and their justification.

The assessment of confidentiality claims and their justification is done according to objective criteria which were settled by EFSA taking inspiration from sectoral food and feed legislation were confidentiality criteria are defined. Applicants are invited to provide additional elements to substantiate their confidentiality claims, allowing EFSA to assess whether the publication or release of this information may undermine the protection of:

- The privacy and integrity of individuals, for example names or personal data (information allowing the identification of persons) of persons working in laboratories, in the sense of Regulation (EC) No 45/2001,
- The company commercial interests,
- The Intellectual property (in case a patent or copyrights exist).

For example:

- information, documents or data, which should normally be deemed to undermine the protection of the commercial interests or of privacy and integrity of the individuals concerned:
    - Information on the method of manufacture and manufacturing process,
    - Information on the complete composition data of the product,
    - Personal data, such as names, addresses, telephone and fax numbers, e-mail addresses, letterheads of persons involved in building the method,
    - the names of authors of unpublished studies,
    - Links between a producer or importer and the applicant/requestor or the authorization holder,
    - Proprietary data, or data for which copyrights are claimed,
    - Analytical test data
    - Commercial and industrial related information outlining strategies, programs or plans of concerned business operators, etc.
- Information likely not to be considered confidential:
    - Name of the method, product, substance, organism, health claim,
    - Name and address of the applicant/requestor or authorization holder,
    - The list of references, title, study and publication dates of published and unpublished studies,
    - Publicly available/published studies, the names of the authors,
    - Information of direct relevance to the assessment of safety of humans, animals or of the environment,
    - The indication of the purity of the active substance, neither as minimum purity as manufactured nor as purity used in studies,
    - Details of representative uses or registered uses.
    - The method(s) of analysis.

3. Assessment phase 1: suitability check of data for risk assessment

Once the completeness of the submitted data is confirmed, the AHAW Panel will check for the suitability of the data needed for the scientific evaluation of the stunning method; for instance it will check if experimental materials and analytical methods are adequate.

In this phase, the AHAW Panel may request further analysis by the applicant or may request the applicant to provide raw data in order to perform additional analyses. In this case, EFSA might need to readjust the deadline proposed to the EC.

3.1. Description of the stunning method

The following information and parameters have to be reported in the technical dossier.

3.1.1. Name of the method

A name and acronym (if appropriate) for the method is to be provided.

3.1.2. Description of the method including potential sources of pain, distress and suffering

The applicant is expected to provide a comprehensive technical description of the method and the biological mechanism associated with the induction of unconsciousness. The level of detail should be sufficient to reproduce the method. Any handling and restraining of live animals that are integral parts of the method should be described (e.g. restraining of animal and presentation of head to the operator). The potential sources of pain, distress and suffering associated with handling, restraint and application of the method should be identified and described.

The applicant must also specify under what commercial conditions the new or modified stunning method should be applied, namely detailed information on animal characteristics (e.g. species, size and weight of the animal) and any other factor that may be relevant for effective use of the method (e.g. throughput rate in slaughterhouse).

3.1.3. Key parameters of the effective use of the method

According to (EC) Regulation 1099/2009, key parameters are defined as the critical factors for ensuring proper stunning of all animals subjected to the stunning process and listed in Annex 1 of (EC) Reg. 1099/2009. The Appendix A of this guidance provides details on parameters to be provided for the description of the stunning methods related to various existing methods. Some key parameters are divided into several detailed components to ensure a comprehensive description of the applied stunning method.

For modified stunning methods, the applicant should provide all relevant information concerning key parameters associated with the modification. In case of a new stunning method, the applicant should propose a list of key parameters (e.g. minimum current for electrical stunning) following the rationale for key parameters listed in Annex 1 of (EC) Reg. 1099/2009 for existing methods and provide the relevant information associated (value of the key parameter e.g. amperage of the current).

3.1.4. Scientific basis of induction and maintenance of unconsciousness for this method

The applicant should take into consideration that 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 neuronal function 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 by recording electrical activity of the brain using EEGs (EFSA, 2004).

The applicant should describe the neurological mechanism underlying the induction and maintenance of unconsciousness. Describe if onset of unconsciousness is immediate or not. Information should be reported on whether the induced unconsciousness is reversible or not.

3.1.5. Potential causes of system failure and chances of occurrence

Chances and the potential causes of system failure need to be characterised. The system may fail because of the physical features of the system (e.g. electricity breakdown in case of electrical

stunning, poor maintenance of the gun in case of mechanical stunning) or because of animal factors (e.g. different size and weight of the animals, presence of horns).

3.2. Description of the individual studies submitted

3.2.1. Introduction

3.2.1.1. Background and rationale

Explain the scientific background and rationale for the method / investigation being reported.

3.2.1.2. Objective

Describe the specific objectives and hypotheses. Clearly state primary and secondary objectives (if applicable).

3.2.2. Materials and methods (for each single study)

The applicant should consider the EFSA guidance on statistical reporting (EFSA, 2014) for the full description of materials and methods. Basic information needed in the dossier is reported in the next paragraphs (from 3.2.2.1 to 3.2.5.2).

3.2.2.1. Method

Specify technical details of the methods applied to each different study, how and when methods were actually administered.

Study population

Give characteristics of the study population (species, breed/genotype, animal type (e.g. dairy or beef cattle), age and weight) and potential confounders (e.g. health status, transport, fasting, water deprivation, husbandry system).

Sampling strategy

Sample size determination and sampling techniques should be described and justified. Where applicable, explanation of any interim analyses should be provided. Experimental units (e.g. individual animal vs group of animals) must be described such that the level of true replication (independent observations) can be determined.

Experimental design

The experimental treatment, the number of animals in an experimental unit as well as the number of experimental units/treatments have to be described and justified.

Ethical considerations

For studies conducted at laboratory level, the experimental protocol must apply humane endpoints as specified in various international (e.g. http://www.animalethics.org.au/legislation/international) or European guidelines on the ethical use of animals in research (e.g. Directive 2010/63/EU). The research reported should cite the granting body, date and reference number for animal ethics approvals associated with the work within the methods of the document.

Randomisation and blinding

Describe any efforts to address potential sources of bias that are relevant to the study design and could affect the validity of the results of the study. Report methods used to control for sampling bias, selection bias, information bias, observer bias and confounding; for example, random allocation, matching, blocking stratification for randomised controlled trials, and multivariable analytical methods. 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 it was done (e.g. when it started and when it ceased). If the process was different for different outcomes, clarify per outcome (e.g. behaviour data were blinded but electroencephalography data were not).

Reporting data quality (if the applicant uses external data)

The applicant should provide details of quality assurance regarding what is detailed in the guidance on statistical reporting (EFSA, 2014).

Reporting the methods of analysis

Describe and justify 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.

3.2.2.2. Measurement of the outcomes

The (EC) Regulation 1099/2009 stipulates reversible stunning as 'simple stunning' and irreversible stunning as 'stunning'. It is also stated in the Regulation that animals shall be spared any avoidable pain, distress or suffering during their killing and related operations, and more importantly, animals subjected to simple stunning should remain unconscious until death occurs through exsanguination. In case of simple stunning, the two carotid arteries or the vessels from which they arise shall be systematically severed. To assess the onset of unconsciousness and death, and the magnitude of pain, distress and suffering, animal based measures (ABMs) should be used. These measures can be i) neurological (e.g. EEG records), ii) physiological (e.g. heart rate variability), iii) behavioural (e.g. escape attempts) or iv) physical reflexes (e.g. tonic-clonic seizures).

Onset and duration of unconsciousness and time to death

If the method does not induce immediate unconsciousness, the time from the start of the method to onset of unconsciousness should be recorded. When the method induces reversible loss of consciousness, animals should be stunned without exsanguination to establish the duration of unconsciousness achieved by the stunning itself in proof-of-concept studies under controlled laboratory conditions. There may be circumstances in which a method intended, designed or described as a simple stunning method would lead to irreversible stunning (death) in some animals. Under this situation, the proportion of animals in each of these two categories should be reported for studies carried out under laboratory and slaughterhouse conditions. In animals subjected to reversible stunning, the duration of unconsciousness should be sufficient to prevent recovery following the method, until death occurs through bleeding. The ABMs used to determine the time to death should be described. The maximum permissible stun-to-stick interval can be calculated by the shortest duration of unconsciousness of any individual induced by the stunning method, minus the longest time death after exsanguination. If the method is applied to animals in groups (group stunning) then the duration of unconsciousness induced with the method should outlast the time to time to death in the last animal in a group to be shackled and bled-out.

The time to onset of death should be reported for the proportion of animals that died by the stunning method. It is also important to report the time to time to death due to exsanguination in animals subjected to simple stunning and which blood vessels severed at exsanguination should also be reported.

As explained earlier, studies should be conducted in laboratory conditions and repeated under slaughterhouse conditions. In laboratory conditions, neurological measures of spontaneous or evoked electrical activity of the brain recorded using EEG or ECOG should be used to assess the onset and duration of unconsciousness and time to death, in combination with other ABMs. The correlation between neurological measures and other ABMs such as behavioural or physical measures will also be used to allow interpretation of behavioural and physical measures where neurological measures cannot be obtained (i.e. in slaughterhouse conditions).

466

467 Use of neurological measures

468 The applicant should define and provide evidence for validity of criteria used to unequivocally assess
469 unconsciousness and recovery of consciousness (if method leads to simple stunning) or time to death.

470 Electroencephalograms (EEGs) or electrocorticograms (ECoGs) are widely used to record the
471 spontaneous and evoked (somatosensory, visual and auditory evoked potentials or responses)
472 electrical activity in the brain to ascertain the state of consciousness following stunning and time to
473 death. Established stunning methods induce unique brain states that are incompatible with the
474 persistence of consciousness (cf appendix B).

475 Studies on stunning methods should report in detail the EEG criteria and the methodology used to
476 determine the onset and duration of unconsciousness and time to death. It is required that the
477 methodology used in the determination of the onset and the duration of unconsciousness and time to
478 death has previously been accepted in appropriate internationally recognised and peer-reviewed
479 journals and that actions are taken to prevent the possibility of any kind of bias.

480 In the case of EEGs (or ECoGs), all parameters crucial to the assessment of the data should be
481 specified (e.g. the EEG recording electrode position on the skull or on the brain itself, the
482 configuration of the electrode (transhemispheric or from the same hemisphere of the brain), the
483 background noise filtration method employed in the data acquisition and analysis, calibration and
484 certification of equipment). In order to estimate quantitative changes occurring in the EEG (or ECoGs),
485 the method used to acquire data (analogue or digital, data sampling rate) and to derive the
486 transformations of EEG data must be described. In addition, the measures used to assess recognition
487 of unconsciousness should be relevant to the respective stunning method, based on the available
488 scientific knowledge of each measure's sensitivity and specificity.

489

490 Use of animal behavioural measures, physiological measures and physical reflexes

491 The applicant should define and provide evidence for validity of criteria to assess unconsciousness and
492 recovery of consciousness (if method leads to simple stunning) or time to death

493 Altered electrophysiological brain states are associated with certain behavioural patterns and physical
494 reflexes. The correlation between EEG/ECoG evidence of unconsciousness and ABM has been
495 characterized for established stunning methods, permitting the use of those ABM as proxies for
496 unconsciousness in slaughterhouse conditions (see appendix B). Therefore, such ABM for monitoring
497 the effective use of a stunning method in slaughterhouses should be included, as required in the (EC)
498 Regulation 1099/2009. It is also important to describe the earliest ABMs representing the induction to
499 unconsciousness and the recovery of consciousness such that effective monitoring can be performed
500 in slaughterhouses and an appropriate back-up stunning method applied if necessary.

501 Description of these ABMs should be provided and the validated methodology used in assessment and
502 timing of recording and analysis should also be described. The biological relevance of the measures in
503 relation to the method and the state of (un)consciousness or death (e.g. motor incoordination, early
504 unconsciousness, death) should be provided. Detailed experimental protocols should be provided to
505 allow assessment of the limitations of the selected measures. The selection of a suitable combination
506 of measures to be used depends upon the design of the study, whether behaviours are specific to the
507 type of stimulus and, are inhibited or hindered from manifestation, and the test species. The scoring
508 system applied to categorise/classify the ABM should be defined. It is essential that the observers
509 making the measurements are carefully trained and that scoring systems are adapted to the species
510 and the stunning conditions.

511 Correlation of neurological and other ABMs

512 The applicant should establish and report correlations between neurological criteria and other ABMs
513 for determining onset of unconsciousness and the recovery of consciousness or time to death, using
514 data from controlled laboratory studies. These correlations can also be substantiated using previously
515 validated criteria from the scientific literature.

In studies carried out under slaughterhouse conditions, the onset and the duration of unconsciousness and insensibility should be ascertained using the ABM that best detects unconsciousness / recovery of consciousness and that has been shown to be correlated with EEGs in laboratory experiments. This will allow the use of behavioural measures as proxies.

Magnitude of pain, distress and suffering

The applicant should first describe potential sources of pain, distress and suffering. Any restraint that is an integral part of the stunning method should be included in the overall assessment.

Secondly, the applicant should measure the magnitude of pain, distress and suffering. 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 them. In addition, thresholds for pain, distress and suffering can be different between animals within and between species.

The validity of criteria used to assess pain, distress and suffering should be provided. **The duration** of pain, distress and suffering can be assessed from the time to loss of consciousness at individual animal and group/treatment levels. **The severity** of these poor animal welfare states should be qualitatively assessed using validated measures. Previous EFSA opinions and scientific papers focus on assessing three categories of measures for the evaluation of pain: behavioural changes, physiological changes and neurological changes. Groups of animal-based measures that could be applied to observe changes in these responses were identified, based on previous EFSA opinions, an expert report and a scientific review of the field of pain assessment in animals (EFSA, 2005; Le Neindre et al., 2009; Landa, 2012). As no specific measure is available for pain, combinations of categories of measures for pain, distress and suffering should be used as a proxy for pain (see a non-exhaustive list in Table 1).

If the severity of these states of poor welfare increases or decreases progressively during application of the method, then clear description of the time to onset and duration for different intensities should be provided.

Magnitude (duration x severity) of pain, distress and suffering can be derived from the above mentioned neurological, physiological and behavioural responses. This should be done in laboratory study(ies) using appropriate experimental protocols, including sham controls. Such protocols should also facilitate evaluation of individual animal responses consecutive to restraining procedures, if any, and to the method. It is essential that side operation effect, like during restraint, is assessed separately from the stunning operation by itself. Indeed, the risk that a peak response induced by e.g. restraining is masking the response from the stunning should be avoided. In study(ies) carried out under slaughterhouse conditions, previously validated behavioural measures can be measured alone as proxies for pain, distress and suffering. Where feasible, physiological and neurological parameters should also be investigated.

It is also important to describe whether the entire animal population subjected to the method would experience these poor welfare states, and whether the magnitude would vary according to other factors (e.g. genotype, production system).

Poor animal welfare outcomes can also occur due to mis-stunning or recovery of consciousness either prior to neck cutting or during exsanguination. Therefore, the proportion of animals recovering consciousness prior to neck cutting or during exsanguination, if any, should be reported.

559 **Table 1:** Overview of categories animal-based measures associated with pain, distress and suffering during the induction of unconsciousness
560

1. **Category of ABMs**	2. **ABMs**	3. **Example**	4. **References**
Behavioural measures	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, retreat attempts, escape attempts	EFSA 2005; Velarde et al., 2007; Dalmau et al., 2010; Landa, 2012
Physiological measures	Hormone concentrations	e.g. HPA[a] axis: corticosteroids, ACTH[b]; sympathetic system: adrenaline, noradrenaline	Mellor et al., 2000; EFSA, 2005; Le Neindre et al., 2009; Coetzee et al., 2010; Landa, 2012
	Blood metabolites	e.g. glucose, lactate, LDH[c]	EFSA, 2005; Vogel et al., 2011; Landa 2012; Mota-Rojas et al., 2012
	Autonomic responses	e.g. heart rate and heart rate variability, blood pressure, respiratory rate, body temperature	Martoft et al., 2001; EFSA ,2005; Borell et al. 2007; 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 measures	Brain activity	e.g. EEG, ECoG	Gibson et al., 2009

561 [a]HPA, hypothalamic–pituitary–adrenal, [b]ACTH, adrenocorticotrophic hormone, [c]LDH, Lactate dehydrogenase

3.2.3. Reporting the results

3.2.3.1. Reporting outcomes and estimations

Reporting of the studies should conform with appropriate international reporting guidelines, for example CONSORT, STROBE, ARRIVE and others (see http://www.equator-network.org).

For each single study, the applicant should report the complete results for each group of animals (for both laboratory and commercial condition) concerning:

- data at both the individual animal and group levels including the level of variation between animals
- any missing data for each variable of interest
- unadjusted estimates 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.

This applies to the following categories of variables:

- Proportion of animals mis-stunned: Report the proportion of mis-stunned animals and consequences of the mis-stunning in terms of animal welfare.
- Time to onset of unconsciousness: In the case of a method not inducing immediate onset of unconsciousness, appropriate analyses to demonstrate the exact temporal sequence of the onset of the different welfare measures and the variations between animals should be applied (e.g. survival curve, boxplots describing the dispersion of the data around the median time to onset of the different welfare measures, graphical representation of the event sequence).
- Duration of pain, distress and suffering: Determine and report the time for which the animals will be conscious and able to feel pain distress and suffering. In this objective, the timing about the appearance of the different behavioural, physiological and neurological events should be presented so that the exact sequence could be determined for an animal and for each group of animals.
- Magnitude of pain, distress and suffering: Quantitative and qualitative results related to the magnitude of pain, distress and suffering should be provided at the individual and group level (e.g. necropsy lesions, behaviour intensity or frequency).
- Duration of unconsciousness: In the case of a method inducing reversible stunning (simple stunning), appropriate analyses to demonstrate the exact temporal sequence of the onset of the different welfare measures regarding the recovery of consciousness and the variations between animals should be applied.
- Frequency of animals recovering consciousness before death
- Time to death
- Proportion of dead animals: The proportion of dead animals after the stunning process and before the sticking
- Stun-to-stick interval: the applicant should calculate and report stun-to-stick interval which will prevent recovery of consciousness prior to or during bleeding (in case of simple stunning).
- Adverse events: Additionally, the applicant should describe all important adverse events or side effects in each method group. Describe the event, reporting 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 head-only electrical stunning, it should be reported that high electrical resistance could cause overheating of the stunning electrodes, leading to poor

stunning as well as burn marks on the skin that could be related to pain if animals are still conscious.

3.2.3.2. Reporting uncertainty

Uncertainty analysis is the process of identifying limitations in scientific knowledge and evaluating their implications for scientific conclusions. The applicant should list and describe potential sources of uncertainty and methodologies to analyse the uncertainty.

3.2.4. Discussion and conclusions

3.2.4.1. Reporting interpretation of results

Summarise key results with reference to study objectives; provide a well-founded interpretation of results considering the purpose, the objectives and the limitations, taking into account sources of potential bias or imprecision, multiplicity of analyses, results from similar studies, and other relevant evidence.

Give conclusion about the efficiency of the stunning process and the consequences in terms of animal welfare.

3.2.5. Conflicts of interest

Report the sources of funding and the role of the funders for the submitted study. State any potential conflicts of interest.

3.3. Overall integration of findings from all studies

3.3.1. Demonstration of equivalence with existing methods

Article 4 (2) of Regulation 1099/2009 requires that the new or modified stunning method ensures a level of animal welfare which is at least equivalent to that ensured by the existing methods. Therefore, the applicant should compare the proposed new or modified method with existing methods in terms of animal welfare. Various methodologies can be employed to do this and they should preferably be based on the comparison of welfare outcome measures indicative of the animals' response to the method, or e.g. a ranking of the welfare hazards involved (EFSA, 2017). If the applicant proposes a different methodology, the bibliographic reference justifying the choice should be reported.

For the comparison based on welfare outcome measures as the preferred option, a quantitative and/or qualitative approach should be adopted using:

- Quantitative approach: In case valid ABMs can be identified and applied to both new and existing methods, equivalence assessment should be achieved through data obtained from literature review and/or through an experiment. For the correct procedure to identify relevant literature please refer to the EFSA Guidance on the "Application of systematic review methodology to food and feed safety assessments to support decision making" (EFSA, 2010) or other relevant guidance documents.

- Qualitative approach: In case no valid ABMs can be found which apply to both the new and existing methods OR the quantitative approach reveals inconclusive results across several measures, the equivalence assessment should be achieved through expert knowledge elicitation on the welfare outcome measures. A guidance document that can be used for reference when eliciting expert knowledge was produced by EFSA (EFSA, 2014b).

3.3.1.1. Quantitative approaches

The preferred way of demonstrating equivalence is through a quantitative approach, which is only possible if the measures are equally applicable to the new/modified and existing methods. Once data have been obtained, either from experiments or literature review, the difference between methods can be quantified by pair wise comparison of the measure. For example, if both methods rely on inhalation of a noxious gas, the time to loss of posture may be measurable in both and can be used for comparative purposes. Assuming the magnitude of pain, distress and suffering is similar in the compared methods, a faster loss of posture will indicate a quicker unconsciousness and therefore relatively better welfare.

It is preferable to use multiple measures to compare pair-wise between methods, through a quantitative comparison of all available measures. The analysis will look at the outcomes of each measure comparison, across all welfare outcome measures that were included in the study. In the example above between systems using a noxious gas, in addition to loss of posture there could be a second outcome measure called 'escape attempts' which can also be compared quantitatively between the different methods. If both welfare outcome measures suggest less suffering in one of the two methods, the conclusion is straightforward.

Welfare outcome measures which are common to existing stunning methods and readily available in literature are listed in section 2.1.1.2.

3.3.1.2. Qualitative approaches

When multiple measures that are comparable across methods are used, it is possible that they bring inconclusive results about animal welfare. For example, in the comparison described above, the new method may result in a faster loss of posture, but the animals show a higher level of escape attempts. In that case a qualitative step is needed to evaluate the different measures in combination with each other: a 'weighting' of both measures is required to be able to compare their relative importance for animal welfare (Spoolder et al, 2003).

Similarly, if the welfare outcome measures are not the same for the existing and new stunning method, a qualitative approach is needed. This may be the case when comparing e.g. gas stunning and electrical stunning methods. For example, poor welfare outcomes such as 'gasping' during gas stunning can be compared qualitatively with 'wing flapping' during shackling associated with electrical stunning.

Spoolder et al. (2003) discussed different techniques for qualitative comparisons. Most commonly, the measure scores are linked to a range or step indicating 'severity', which can then be compared quantitatively. The minimum and maximum of each measure are determined *a priori* by the experts, and represent the weighting process. For example, the experts consider that the maximum number of wing flaps in a given time period is 70, representing the highest level of discomfort ("score 10"). To the observed value of wing flaps, a proportional score is then assigned. This can be done across all measures, thus transforming them to the same comparable metrics of 0 - 10. These scores can be added to calculate an overall score for each stunning method.

Far more complex approaches (Spoolder et al., 2003) exist using e.g. non-linear equations calculated on the basis of multiple comparisons between measurements of the relevant measures with a 'gold standard'.

Once the applicant has decided for one of these techniques, they have to set up an expert knowledge elicitation process to do the comparison of the measures among the methods (see for example EFSA guidance on expert knowledge elicitation).

Depending on the approach, the applicant should provide information on the methodology used for the literature search (e.g. the search string), the experimental protocol, qualitative and quantitative data obtained and used, the approach used in conducting the EKE, and the background and expertise of the EKE experts (Chatham House Rules should be applied: the list of participants and a summary of discussion and judgements of an expert judgment can be recorded and included in an expert judgement report but the statements and judgements will not be attributed to specific experts).

699

700 ### 3.3.2. Overall discussion and conclusions

701 #### 3.3.2.1. Results regarding welfare impact

702 The overall results from all single studies should be discussed with a view to integrating the efficacy of
703 the method in terms of the animal welfare impact.

704 #### 3.3.2.2. External validity

705 Discuss the potential for external validity of the study results (e.g. whether study results can be
706 extrapolated beyond the study population and experimental conditions).

707 In addition, the throughput rate should be specified where appropriate (e.g. studies under
708 slaughterhouse conditions).

709 #### 3.3.2.3. Discussion on equivalence with existing methods

710 Discuss how the new method compares with existing methods based on literature review or
711 experimental comparative studies demonstrating that the novel method is at least equivalent to the
712 existing ones regarding the animal welfare outcomes (at all stages of the process) or expert
713 judgement.

714 In the situation where direct quantitative comparisons are not possible, qualitative critical appraisal
715 can be performed. Different methods to elicit expert knowledge on various subjects are specified in
716 the "EFSA guidance on expert knowledge elicitation"
717 (https://www.efsa.europa.eu/it/efsajournal/pub/3734).

718

719 # 4. Assessment phase 2: risk assessment of the stunning method

720 In this phase the AHAW Panel will proceed to fully assess the new or modified stunning method
721 proposed by the applicant. In particular, two main aspects will be characterised: i) the animal welfare
722 risk assessment i.e. the analysis of the animal welfare outcomes resulting from the stunning method
723 and ii) the validation of the equivalence of the proposed stunning methods with existing approved
724 methods.

725 ## 4.1. Animal welfare risk assessment

726

727 For the assessment of pain, distress and suffering and the onset and duration of unconsciousness or
728 death the measures chosen by the applicant will be scrutinized in terms of validity. This will be done
729 based on the justification provided by the applicant concerning the choice of the measures. The
730 measures will be compared with the scientific state-of-the-art, taking as far as possible e.g. species,
731 animal category, breed/genetic lines into account.

732

733 ### 4.1.1. Assessment of onset and duration of unconsciousness

734 The EFSA assessment of stunning methods will involve evaluation of the methodology and criteria
735 used for determining unconsciousness. Similarly results of the welfare outcomes will be scrutinised.

736 #### 4.1.1.1. Methodological aspects

737 The methodologies used in the evaluation of the stunning method will be assessed for validity and
738 reliability, including the criteria and the thresholds used for the determination of unconsciousness. In
739 particular the brain mechanisms associated with the induction of unconsciousness and the scientific

rationale used in the selection of the neurological measures will be evaluated. The choice of the behavioural and physical reflexes measures selected for assessment of unconsciousness will be reviewed. The methodology to establish the correlation between neurological and other ABMs will be evaluated.

4.1.1.2. Results regarding onset and duration of unconsciousness and death

The assessment of the effectiveness of the submitted method as regards unconsciousness considers, including validity of criteria and methodology:

- frequency of correctly stunned animals
- time to onset unconsciousness during exposure
- time to recovery of consciousness in case of reversible stunning
- duration of unconsciousness in case of reversible stunning
- time to death during exposure to stunning method in the case of irreversible stunning
- maximum permissible time between the end of exposure and exsanguination
- time to death due to exsanguination in the case of reversible stunning

4.1.2. Assessment of pain, distress and suffering associated with the pre-stunning process, during induction of unconsciousness and due to mis-stunning

4.1.2.1. Methodological aspects

The measures chosen by the applicant will be scrutinized to assess the extent to which they are likely to provide valid and reliable information on the experience of pain, distress and suffering by the animals in question. This will be done based on the justification provided by the applicant which will be contrasted with the scientific state-of-the-art, taking as far as possible e.g. species, animal category, breed/genetic lines into account. For example, if the incidence of vocalizations is used in the CAS (controlled atmosphere stunning) stunning of pigs, the available scientific evidence for its significance as a measure of pain, distress and suffering will be checked. Additionally, in the case of less specific measures such as blood metabolites, the use of complementary measures which allow a combined interpretation will be checked.

4.1.2.2. Evidence of pain, distress and suffering

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:

- The ABM 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 a stunning method, the response of animals exposed to the procedure/apparatus without the application of stunning (control or sham operation) should not be significantly different from the response of the animals exposed to the procedure/apparatus with stunning (treatment).

- In general, these ABM 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).

If there is evidence that the method leads to pain, distress and suffering, the evaluation will be based on the proportion of animals affected as well as, where possible, the magnitude/severity of the infliction and the duration of the negative experience. For this purpose, the existing literature and/or expert opinion will be used to aid in data interpretation.

Table 1 report an overview of categories and examples of ABM associated with pain, distress and suffering during the induction of unconsciousness and insensibility that can be used to verify that the

784 stunning method does not induce pain, distress and suffering before the onset of unconsciousness
785 and insensibility. The examples are not exclusive and other measures may be appropriate.

786

4.1.3. Assessment of external validity

This part of the assessment considers whether, if available, the results from studies carried out in different research groups are consistent and lead to similar conclusions. Furthermore, it is taken into account to which degree the findings from laboratory studies are consistent with those from pilot-plant scale or studies carried out under commercial conditions. Finally, the applicability to different commercial slaughter conditions and the potential impact of environmental conditions in a wider sense (such as climatic conditions, transport conditions of animals, slaughter speed) will be reviewed.

4.2. Assessment of equivalence of the method with existing stunning methods

EFSA will assess the approach proposed by the applicant based on the comparability of the welfare outcome measures between the different methods, the quality of the literature search (e.g. scientific relevance of the search string, comprehensiveness, state of the art), the quality of the experimental protocol, qualitative and quantitative data provided, the background and expertise of the experts contributing to the EKE, and the approach used in conducting the EKE.

The evaluation of the results will be based on whether the results follow logically from the methodology applied, and whether the conclusions follow from the results obtained.

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.

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.

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

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 (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. The EFSA Journal 2010; 8(6):1637 90 pp. doi:10.2903/j.efsa.2010.1637

European Food Safety Authority, 2014. Guidance on Statistical Reporting. EFSA Journal 2014;12(12):3908, 18 pp., doi:10.2903/j.efsa.2014.3908.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2017. Scientific Opinion on the low atmospheric pressure system for stunning broiler chickens. EFSA Journal2017;15(12):5056, 86 pp.

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.

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

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

837 Landa L, 2012. Pain in domestic animals and how to assess it: a review. Veterinarni Medicina, 57,
838 185–192.

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

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

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

847 Llonch P, Rodriguez P, Jospin M, Dalmau A, Manteca X and Velarde A, 2013. Assessment of
848 unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures. Animal, 7, 492–
849 498.

850 McKeegan DEF, McIntyre J, Demmers TGM, Wathes CM and Jones RB, 2006. Behavioural responses of
851 broiler chickens during acute exposure to gaseous stimulation. Applied Animal Behaviour Science,
852 99, 271–286.

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

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

858 Mota-Rojas D, Bolanos-Lopez D, Concepcion-Mendez M, Ramirez-Telles J, Roldan-Santiago P, Flores-
859 Peinado S and Mora-Medina P, 2012. Stunning swine with CO2 gas: controversies related to animal
860 welfare. International Journal of Pharmacology, 8, 141–151.

861 Spoolder, H.A.M., De Rosa, G., Hörning, B., Waiblinger, S. and Wemelsfelder, F., 2003. Integrating
862 parameters to assess on-farm welfare. Animal Welfare, 12: 529-534

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

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

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

871

872 # Glossary

873

Adverse events	A poor animal welfare outcome recorded in a study of a stunning method.
Bias	Systematic deviation of a measurement from the 'true' value leading to either an over- or underestimation of the treatment effect. Bias can originate from many different sources, such as allocation of subjects, measurement, interpretation, publication and review of data.
Blinding (masking)	Blinding or masking is the process used in epidemiological studies and clinical trials in which the observers and the subjects have no knowledge as to which treatments subjects are assigned to. This is done in order to minimise bias occurring in the subject response and outcome measurement. In single-blind studies only the subjects are blind to their allocations, whilst in double-blind studies both observers and subjects are ignorant of the treatment allocations.
Confounding	The bias arising from the co-occurrence or mixing of the effects of extraneous factors - referred to as confounders - with the main effect(s) of interest in a study.
External validity	Refers to the extent to which a study's results provide a correct basis for generalisation beyond the setting of the study and the particular subjects studied. It implies the applicability of the results of a study to another group or population.
Information bias	A bias that occurs during data collection. The most frequent information bias is misclassification bias, which is present when the detection of the exposure status (exposure identification bias) and/or the outcome assessment (disease identification bias) is biased, i.e. exposed/diseased individuals are classified as non-exposed/non-diseased and vice versa. A common source of misclassification is the inaccuracy of diagnostic tests.
Internal validity	Refers to the extent to which a causal conclusion from a study is warranted, which is determined by the degree to which a study minimises bias or systematic error. Biases of concern include sampling bias, selection bias, information bias and confounding.
Objective	Describes the scope of the study and the specific hypotheses to be verified. Depending on the study primary and secondary objectives could be defined.
Outcome	An outcome is an indicator/variable measured in an animal to assess the safety, efficacy or other objective of a study.
Sample size	Number of units selected to enter the trial.
Sampling bias	A bias in which a sample is collected in such a way that some members of the target population are less likely to be included than others.
Selection bias	Systematic differences between comparison groups in prognosis or responsiveness to treatment.
Stunning method	A method that is applied to an animal to render it unconscious.
Randomization	A process of allocating participants to treatment or control groups within a controlled trial by using a random mechanism, such as coin toss, random number table, or computer-generated random numbers.
Unconsciousness	A state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to respond to normal stimuli, including pain.

Uncertainty	All types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question.

874 # Annex A – Administrative data of the applicant

875

Applicant[4] (Company name):
Telephone:
E-mail:
Address (street, number):
Post code:
City/Town:
Country:
Name in full of contact person responsible for the application[5]:
Company:
Telephone:
E-mail:
Address (street, number):
Post code:
City/Town:
Country:

This request is for evaluation of the stunning method for the inclusion in Annex 1 of EC Regulation 1099/2009

Name of the stunning method:
Animal species/categories

◉ New intervention	○ Modification of intervention (Article 4 Reg (EC) 1099/2009)

[4] In case of more than one company submitting an application, their names and addresses should be provided.
[5] To facilitate communication, only one contact person per application should be indicated.

876

877

Annex B – Completeness checklist

The completeness checklist should be submitted using a common word processing format (e.g. MS Word).[6]

The completeness checklist is meant to support applicants in the building up of applications for the authorisation of a new or modified stunning method. The completeness checklist follows the sections, headings and numbering detailed in the guidance (chapter 3.1. for the description of the stunning method, chapter 3.2. for the description of the individual studies submitted and chapter 3.3. for the overall integration of findings from all studies.

For each section, applicants can identify which information has been provided or not provided and if not provided, a justification should be included. The definitions of the different options are detailed below:

- **Information provided:** the parameters is required and the information is provided by the applicant in the technical dossier.
- **Not provided (to be justified):** the parameter is required but the information is not provided by the applicant of the technical dossier. A proper justification for the omission of that data needs to be provided in the technical dossier.

At the end of each section, applicant can add comments in the designated "Comments" box.

All the fields in blue are reserved for EFSA's use.

Please note that in case of more studies to be submitted as part of the overall application for the new or modified stunning method, the checklist section 3.2. is to be duplicated and made specific for each single study.

COMPLETENESS CHECKLIST

Name of the stunning method:

Application number:

	PROVIDED	NOT PROVIDED	EFSA AGREES	EFSA COMMENTS
3.1. Description of the stunning method				
▪ 3.1.1. Name of the method	☒	☒	☒	
▪ 3.1.2.Description of the method including potential sources of pain, distress and suffering	☐	☐	☐	
▪ 3.1.3.Key parameters of the effective use of the method	☐	☐	☐	
▪ 3.1.4.Scientific basis of induction and maintenance of unconsciousness for this method	☐	☐	☐	
▪ 3.1.5.Potential causes of system failure and chances of occurrence	☐	☐	☐	
Comments				
3.2. Description of the individual studies submitted				
3.2.1. Introduction				

[6] The word document Appendix A can be downloaded from the section 'Supporting information'

▪ 3.2.1.1. Background and rationale ▪	☐	☐	☐	
▪ 3.2.1.2. Objective	☐	☐	☐	
Comments				
3.2.2. Materials and methods				
3.2.2.1. Method				
▪ Study population	☐	☐	☐	
▪ Sampling strategy	☐	☐	☐	
▪ Ethical considerations	☐	☐	☐	
▪ Experimental design	☐	☐	☐	
▪ Randomisation and blinding	☐	☐	☐	
▪ Reporting data quality (if the applicant uses external data)	☐	☐	☐	
▪ Reporting the methods of analysis	☐	☐	☐	
Comments				
3.2.2.2. Measurement of the outcomes				
▪ Onset and duration of unconsciousness and time to death	☐	☐	☐	
▪ Magnitude of pain, distress and suffering	☐	☐	☐	
Comments				
3.2.3. Reporting the results				
▪ 3.2.3.1. Reporting outcomes and estimations	☐	☐	☐	
▪ 3.2.3.2. Reporting uncertainty	☐	☐	☐	
Comments				
3.2.4. Discussion and conclusions				
▪ 3.2.4.1. Reporting interpretation of results	☐	☐	☐	
3.2.5. Conflicts of interest	☐	☐	☐	
Comments				
3.3. Overall integration of findings from all studies				
3.3.1. Demonstration of equivalence with existing methods	☐	☐	☐	
Comments				
3.3.2. Overall discussion and conclusions				
▪ 3.3.2.1. Results regarding welfare impact	☐	☐	☐	
▪ 3.3.2.2. External validity	☐	☐	☐	
▪ 3.3.2.3. Discussion on equivalence with existing methods	☐	☐	☐	
Comments				

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Annex C – Justification for confidential information

The applicant may indicate which information submitted is to be treated as confidential on the ground that its disclosure might significantly harm its competitive position. Verifiable justification must be given in such cases.

Annex C shall be updated during the life-cycle of the application each time a request for treating a piece of information as confidential is claimed by the applicant (original submission, missing information, additional information).

Information requested to be considered as confidential	Justification
Section x.y (submitted on YYYY/MM/DD)	
Annex X (submitted on YYYY/MM/DD)	
Section x.y.z (submitted on YYYY/MM/DD)	
Annex X (submitted on YYYY/MM/DD)	

Appendix A – Details for key parameters to be provided for method

Annex I of the (EC) Regulation 1099/2009 requires key parameters for each stunning method to ensuring proper stunning of all animals subjected to the process, as the efficiency of each stunning method is based on the control of key parameters and its regular evaluation. The key parameters related to various existing methods are provided below. Some parameters are divided into several detailed components to ensure a comprehensive description of the applied stunning method.

A.1. Mechanical stunning methods

A.1.1. Penetrative captive bolt

Penetrative captive bolt stunning is permitted in all species and the key parameters are described in Annex I of Council Regulation (EC) No 1099/2009.

Table 1: Parameters to be provided when applying a mechanical stunning method based on penetrative captive bolt, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA ad-hoc expert working group

Parameter	**Component**	**Description** (all specifications should be in internationally recognised units)
Position and direction of the shot	Restraining system	Describe how the animal and its head are restrained during the stunning procedure to facilitate accurate shooting.
	Position of captive bolt gun	Specify the topographical / anatomical position of the gun on the head, direction and angle of firing. Provide the distance between the muzzle of the gun and the skull surface at the intended bolt penetration site.
	Bolt penetration site	Specify the anatomical position of the penetration site - indicating the presence of any topographical features of the study population, such as the presence of horns or thick ridges on the skull, which may influence the selection of the shooting position, including the deviation from the intended penetration site.
Appropriate velocity, bolt length and diameter of bolt according to animal size and species	Captive bolt gun characteristics	Provide details of the device including whether it is pneumatic or cartridge driven or spring operated, trigger operated or contact firing, and recessed bolt or non-recessed bolt. Provide details of the calibration method used for the assessment of the impact of captive bolt.
	Cartridge or compressed air specifications	Specify the cartridge calibre / grain / explosive content or the air pressure.
	Bolt dimensions, mass and velocity	Specify the bolt length) and its exit length (i.e. the length protruding from the barrel after firing), the bolt diameter, bolt mass and bolt velocity at the time of impacting the skull. Describe the shape of the tip of the bolt (e.g. mushroom shaped, flat, curved with sharp edges).
	Animals	Provide details on the species, breed, type (e.g. beef or dairy cattle), age and weight of the animals in the study population.
	Equipment maintenance, cleaning and storage conditions	Provide details on the storage conditions, and the frequency and time intervals between consecutive maintenance and cleaning of the equipment. Where manufacturer maintenance instructions are available, provide the details and how they were implemented.

Maximum stun to stick/kill interval(s)[a]		Describe the maximum stun-to-stick/kill interval and the exsanguination method (blood vessels cut) that have been applied to guarantee non-recovery of consciousness and sensibility of the stunned animal until the time to death (except for proof-of-concept studies where the duration of unconsciousness must be determined without sticking, or if the stunning method is proven to be irreversible).

927 [a]provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter
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929 A.1.2. Non-penetrative captive bolt

930 The non-penetrative captive bolt method of stunning is permitted for use in ruminants (of less than 10
931 kg of live weight), poultry, rabbits and hares.

932 **Table 2:** Parameters to be provided when applying a mechanical stunning method based on non-
933 penetrative captive bolt stunning, based on Annex I of Council Regulation (EC) No 1099/2009 and on
934 further details of requirements as determined by the EFSA ad-hoc expert working group
935

Parameter	**Component**	**Description** (all specifications should be in internationally recognised units)
Position and direction of the shot	Restraining system	Describe how the animal and its head are restrained. Indicate how the head is restrained during the stunning procedure. Provide all information relevant to describing the restraining system used to facilitate accurate shooting.
	Position of captive bolt gun	Specify the topographical / anatomical position of the gun on the head (e.g. on the frontal bone), direction (directed towards the mouth or throat) and angle of firing (e.g. perpendicular to the frontal bone). Provide the distance between the muzzle of the gun and the skull surface at the intended bolt penetration site.
	Bolt impact site	Specify the anatomical position of the impact site - indicating the presence of any topographical features of the study population, such as the presence of horns or thick ridges on the skull, which may influence the selection of the shooting position.
Appropriate velocity, diameter and shape of bolt according to animal size and species	Captive bolt gun characteristics	Provide details of the device including whether it is pneumatic, cartridge driven, spring or trigger operated, or contact firing, and recessed bolt or non-recessed bolt (i.e, bolt level with end of gun muzzle). Provide details of the calibration method used for the assessment of the impact of the captive bolt.
	Cartridge or compressed air specifications	Specify the strength of cartridge (see below) or the air pressure.
	Bolt dimensions, mass and velocity	Specify bolt diameter (including the diameter of the bolt head), size and shape, bolt mass and bolt velocity at the time of impacting the skull.
	animal	Provide details on the species, breed, type (e.g. beef or dairy cattle) age and weight of the animals in the study population.
	Equipment maintenance, cleaning and storage conditions	Provide details on the storage conditions, and the frequency and time intervals between consecutive maintenance and cleaning of the equipment. Where manufacturer maintenance instructions are available, provide the details and how they were implemented.
Strength of the cartridge used		. Specify the cartridge strength described by calibre/ grain/ explosive content, using internationally recognised units.

Maximum stun to stick/kill interval(s)[a]		Describe the maximum stun-to-stick/kill interval and the exsanguination method (blood vessels cut) that have been applied to guarantee non-recovery of consciousness and sensibility 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).

[a]provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter

A.2. Electrical stunning methods

A.2.1. Head-only and head-to-body stunning

Head-only and head-to-body electrical stunning are permitted in all species.

Table 3: 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 on further details of requirements as determined by the EFSA ad-hoc expert working group

Parameter	5. **Component**	6. **Description** (all specifications should be in internationally recognised units)
Minimum current (A or mA)	Current type	Define the current type used (i.e. sine or square wave alternating current (bipolar or biphasic) or pulsed direct current (monopolar or monophasic).
	Waveform	Define the waveform used including the proportion of clippings; report the mark: space ratio, when pulsed direct current is used. If multiple frequencies and waveforms are used, describe them.
	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. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle.
	Latency[a]	Specify how soon the minimum current was reached after the method was applied to the animal. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle.
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. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle.
	Delivered minimum voltage (V)[a]	Describe how the stunning equipment was set up to deliver the minimum current level to the animal. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle. Describe how the present constant current was applied (e.g. variable voltage/constant current stunner).
Maximum frequency (Hz)	Maximum frequency (Hz)	If applicable, define the maximum frequency (Hz) applied to the animal. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle.
	Minimum frequency (Hz)	If applicable, define the minimum frequency (Hz) applied to the animal. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle.
Minimum time exposure[a]		Define the minimum duration of electrical exposure applied to the animals. In a multiple-cycle method of head-to-body stunning system, details should be provided for each cycle.
Maximum stun-to-stick-/kill interval(s)[a,b]		Describe the maximum stun-to-stick/kill interval and the exsanguination method (blood vessels cut) that have been applied to guarantee

Parameter	5. Component	6. **Description** (all specifications should be in internationally recognised units)
		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).
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	Provide a description of the electrode (form/shape, presence and description of spikes (depth of penetration), wetting).
	Electrode appearance	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 method is initiated.
Position and contact surface area of electrodes	Position of the electrodes	Specify the topographical anatomical position where the electrodes are attached to the animal and the method to hold electrodes in place during the method.
	Type of electrode	Provide information on the type of electrodes used (e.g. tong, wand, …)
	Animal skin condition	Provide a description of the study population in relation to the wool/hair/feather 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.

A.2.2. Electrical waterbath stunning

Electrical waterbath stunning is permitted for use in poultry.

Table 4: Parameters to be provided when applying a stunning method based on electrical waterbath stunning, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA ad-hoc expert working group

Parameter	**Component**	**Description** (all specifications should be in internationally recognised units)
Minimum current (A or mA)	Current type	Define the used current type (i.e. bipolar or biphasic) or pulsed direct current (monopolar or monophasic).
	Waveform	Define the used waveform including the proportion of clippings; report the mark: space ratio, when pulsed DC is used.
	Minimum current[b]	Specify the minimum current (A or mA) to which birds 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.

Minimum voltage (V)	Exposed minimum voltage (V)[b]		Specify the minimum voltage (V) to which birds 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 when using sine wave alternating current. When a pulsed direct current is used, the minimum will be expressed as average voltage. Describe how the minimum voltage was calculated.
	Delivered minimum voltage (V)[b]		Describe how the stunning equipment was setup to deliver the minimum current level to each bird.
Maximum frequency (Hz)	Maximum frequency (Hz)		Define the maximum frequency (Hz) applied to the birds when a combination(s) of different frequencies are used.
	Minimum frequency (Hz)		Define the minimum frequency (Hz) applied to the birds when a combination(s) of different frequencies are used.
Frequency of calibration of the equipment			Provide information on the method used for and the time intervals between consecutive calibrations of the equipment.
Prevention of electrical shocks before stunning			Explain how the birds are protected from inadvertent, unintentional electrical shocks immediately before the stunning method is initiated.
Minimising pain at shackling			Describe the measures taken to minimise pain during shackling of the birds.
Optimisation of the current flow	Shackles	Wetting the leg-shackle contact area	Specify if shackles are wet prior to hanging live birds.
		Contact with earth bar	Explain how contact between the shackle and the earth bar was ensured during the stunning procedure.
	Waterbath and electrode characteristics		Provide a description of the dimensions of the waterbath and electrode.
	Water conductivity		Specify the concentration of food-grade salt added to the fresh water bath to improve electrical conductivity.
	Electricity source characteristics		Specify whether the waterbath stunners are supplied with a constant current or a constant voltage source.
	Electrical resistance/impedance		Provide details on the species, breed, age, sex and weight and on the cleanliness of the birds.
Maximum shackle duration before the waterbath[b]			Specify the time interval between shackling of the bird and stunning.
Minimum time of exposure for each bird[b]			State the number of birds in the waterbath at any one time and the minimum duration of exposure to the electrical current applied to each bird.
Immersion of the birds up to the base of the wings			Specify the immersion depth and describe measures taken to minimise variation in depth of immersion.
Maximum stun-to-stick/kill interval(s) for frequency over 50 Hz[a, b]			Describe the maximum stun-to-stick/kill interval and the exsanguination method (blood vessels cut) that have

	been applied to guarantee unconsciousness and insensibility of the stunned bird until the moment of death (except for proof-of-concept studies where the duration of unconsciousness must be determined without sticking).

[a]in case of simple stunning; [b]provide information on mean or median and range and standard deviation or interquartile range

A.3. Modified atmosphere stunning methods

A.3.1. Carbon dioxide (CO_2) at high concentrations and carbon dioxide in two phases

Exposure to high CO_2 concentrations is permitted in pigs, mustelids, chinchillas and poultry, except for ducks and geese..

Table 5: Parameters to be provided when applying a stunning method based on high CO_2 concentrations or CO_2 in two/multiple phases, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA ad-hoc expert working group

Parameter	**Component**	**Description** (all specifications should be in internationally recognised units)
CO_2 concentration	Initial CO_2 concentration[a]	Specify the initial CO_2 concentration to which animals are exposed at the initiation of the stunning (at first contact with the modified atmosphere).
	Targeted CO_2 concentration(s)[a]	Specify the targeted CO_2 concentration used to stun the animals. If animals are exposed to CO_2 in a step-wise manner in a pre-filled chamber system, several CO_2 target concentrations could be applied.
	Final CO_2 concentration[a]	Specify the final/highest CO_2 concentration to which animals are exposed.
	CO_2 concentration gradient	If animals are exposed to CO_2 in a step-wise manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
	Animal stocking density and type	Specify the animal density (number and kg/m^2) during the CO_2 exposure phase and report the species, breed and age of animals.
	Monitoring	Describe how, where and when the CO_2 concentration was monitored. The calibration methods applied should be reported
Duration of method[7]	Time to reach exposure of animal to targeted CO_2 concentration [a]	Report the time elapsing until animals are exposed to the targeted CO_2 concentration. If animals are exposed to CO_2 in a step-wise manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
	Total duration of targeted CO_2 exposure [a]	Report the total duration of exposure of animals to the targeted CO_2. If animals are exposed to CO_2 in a step-wise manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
Maximum stun-to-stick/-kill interval(s) [a,b]		Describe the maximum stun-to-stick/-kill interval and exsanguination method (blood vessels cut) that have been

[7] Referring to the legal parameter 'duration of exposure'

Parameter	Component	Description (all specifications should be in internationally recognised units)
		applied to guarantee unconsciousness and insensibility of the stunned animal until the moment of death (except for proof-of-concept studies in which the duration of unconsciousness must be determined without sticking).
Quality of the gas	CO_2 source	Specify the source of the CO_2.
	Gas composition of the atmosphere	Clarify if CO_2 was applied in an air atmosphere or if other gases (e.g. O_2) were added. If other gases were added in addition to CO_2, provide information on their concentration (in accordance with the key parameter "CO_2 concentration").
	Humidity and temperature	Report how and when humidity of the gas and temperature inside the chamber were monitored, and, if needed, adjusted.
Temperature of the gas		Specify the temperature of the gas used at the point of entry in the chamber and the average temperature of the gas mixture (after the gas has been mixed with air atmosphere) inside the chamber.

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

A.3.2. Carbon dioxide associated with inert gases

Table 6: Parameters to be provided when applying a stunning method based on CO_2 associated with inert gases, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA ad-hoc expert working group

Parameter	Component	Description (all specifications should be in internationally recognised units)
Inert gases	Type of inert gases used to create the atmosphere	Specify the gases that were used to create the atmosphere.
CO_2 and O_2 concentration	Initial CO_2 and O_2 concentration[a]	Specify the initial CO_2 and O_2 concentration in the gas mixture to which animals are exposed at the initiation of the stunning (at first contact with the modified atmosphere).
	Targeted CO_2 and O_2 concentration(s)[a]	Specify the targeted CO_2 and O_2 concentration in the gas mixture used to stun the animals.
	Final CO_2 and O_2 concentration[a]	Specify the final/highest CO_2 and final O_2 concentration in the gas mixture to which animals are exposed.
	CO_2 and O_2 concentration gradient	The CO_2 and O_2 concentration in the atmosphere should be maintained uniformly; if there are any variations in the composition of the atmosphere, these should be described. If a multi-stage system with a different gas composition in each stage is used, these should be clearly described for each stage. Conditions described for two- or multistage CO_2 stunning apply here.
	Animal stocking density	Specify the animal density (number and kg/m^2) during the gas mixture exposure phase and report the species, breed and age of animals.
	Monitoring	Describe how, where and when the CO_2 and O_2 concentration were monitored.

		The calibration methods applied should be reported
Duration of method[8]	Time to reach exposure of animal to targeted CO_2 and O_2 concentration[a]	Report the time elapsing until animals are exposed to the targeted CO_2 and O_2 concentration. If animals are exposed to the gas mixture in a step-wise manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
	Total duration of targeted CO_2 and O_2 exposure[a]	Report the total duration of exposure of animals to the targeted gas mixture. If animals are exposed to the gas mixture in a multi-stage manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
Maximum stun-to-stick/kill interval(s)[b]		Describe the maximum stun-to-stick/kill interval and the exsanguination method (blood vessels 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).
Quality of the gas	CO_2 and inert gases source	Specify the source of the CO_2 and inert gases.
	Humidity and temperature	Report how and when humidity and temperature were monitored and, if needed, adjusted.
Temperature of the gases		Specify the temperature of the gas used at the point of entry in the chamber and the average temperature of the gas mixture (after the gas has been mixed with air atmosphere) inside the chamber.

[a]provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter; [b]In case of simple stunning

A.3.3. Inert gases

Exposure to inert gases is allowed for stunning / killing pigs and poultry for slaughter. The key parameters and the components to ensure effective use are listed in Table 7.

Table 7: Parameters to be provided when applying a stunning method based on inert gases, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA ad-hoc expert working group

Parameter	**Component**	**Description** (all specifications should be in internationally recognised units)
Inert gases	Type of inert gases (Nitrogen, Argon, Helium)	Specify the gas or gases that are part of the modified atmosphere.
	Concentration of inert gases	Specify their concentration expressed by

[8] Referring to the legal parameter 'duration of exposure'

		volume of residual oxygen.
Oxygen concentration	Initial inert gases or oxygen concentration[a]	Specify the initial inert gases or oxygen concentration to which animals are exposed at the initiation of the stunning (at first contact with the modified atmosphere).
	Targeted inert gases or oxygen concentration(s)[a]	Specify the targeted oxygen concentration used to stun the animals. If animals are exposed to the gas mixture in a multi-stage manner in a pre-filled chamber system, several oxygen target concentrations could be applied.
	Final inert gases or oxygen concentration[a]	Specify the final/highest inert gases or oxygen concentration to which animals are exposed.
	Inert gases or oxygen concentration gradient	The inert gases or oxygen concentration in the atmosphere should be maintained uniformly; if there are any variations in the composition of the atmosphere, these should be described. If a multi-stage system with a different gas composition in each stage is used, the compositions at each stage should be clearly described. Conditions described for two- or multistage CO_2 stunning apply here.
	Animal stocking density	Specify the animal density (number and kg/m^2) during the phase of exposure to the modified atmosphere and report the species, breed and age of animals.
	Monitoring	Describe how, where and when the inert gases concentration was monitored. The calibration methods applied should be reported
Duration of method[9]	Time to reach exposure of animal to targeted inert gases or residual oxygen concentration[a]	Report the time elapsing until animals are exposed to the targeted inert gases or oxygen concentration. If animals are exposed to the modified atmosphere in a multi-stage manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
	Total duration of targeted inert gases or residual oxygen exposure[a]	Report the total duration of exposure of animals to the targeted gas mixture. If animals are exposed to the modified atmosphere in a multi-stage manner in a pre-filled chamber system, the concentrations at each step and the duration of the exposure to each concentration and the transition time between each step must be reported.
Maximum stun-to-stick/kill interval(s)[b]		Describe the maximum stun-to-stick/kill interval and exsanguination method (blood vessels cut) that have been applied to

[9] Referring to the legal parameter 'duration of exposure'

		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).
Quality of the inert gas	Source	Specify the source of the inert gases.
	Humidity and temperature	Report how and when humidity and temperature were monitored and, if needed, adjusted.
Temperature of the gases		Specify the temperature of the gas used at the point of entry in the chamber and the average temperature of the gas mixture (after the gas has been mixed with air atmosphere) inside the chamber.

[a]provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter; [b]In case of simple stunning

A.3.4. Low atmosphere pressure

The low atmosphere pressure stunning (LAPS) is a stunning system where animals are rendered unconscious in a decompression chamber by exposing them to a gradual reduction in partial pressure of oxygen. This stunning method is currently not approved for use in the EU. Therefore, no parameters are defined by Council Regulation (EC) No 1099/2009. The parameters and components listed in table 8 have been derived by the EFSA AHAW panel.

Table 8: Parameters considered relevant by the EFSA AHAW panel for stunning methods based on low atmosphere pressure

Parameter	**Component**	**Description** (all specifications should be in internationally recognised units)
Animal species and density	Animal species/ age/ type and stocking density (number per m^2 and kg of body weight/ m^2)	Specify the animal density in the crate or containers during the decompression. Provide details on the species, breed, type, age and weight of the animals in the study population.
Duration of method intervention [10]	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multi-phase system[a]	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	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

[10] Referring to the legal parameter 'duration of exposure' of other stunning methods

		incidence of birds surviving the treatment should be reported
Rate of changes in partial pressure of oxygen	Time/partial pressure of oxygen treatment	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. Specification of the light source if present.
Maximum stun-to-stick/kill interval(s)[b]		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]provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter;
[b]In case of simple stunning