



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

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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

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Christian Gortázar Schmidt, Miguel Angel Miranda, Søren Saxmose Nielsen, Antonio Velarde,
Hans-Hermann Thulke, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Mohan Raj,
Preben Willeberg, Christoph Winckler, Remigio Marano, Frank Verdonck, Denise Candiani and
Virginie Michel

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 scrutinised by the EFSA's Applications Desk (APDESK) Unit for verification of the completeness of the data submitted for the risk assessment of the stunning method. If the application is considered not valid, additional information may be requested from the applicant. If considered valid, it will be subjected to assessment phase 1 where 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 the 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.

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Summary

This guidance defines the process for handling applications on new or modified stunning methods and the parameters that will be assessed by the European Food Safety Authority (EFSA) Animal Health and Welfare (AHAW) Panel. The guidance includes four sections.

Section 1 introduces the aim of the guidance and presents the terms of reference of the mandate. It clarifies the animal species of concern and it explains that a 'modified stunning method' as used in the guidance document refers to any method that does not correspond to the definition of 'approved methods' in Regulation 1099/2009.

Section 2 includes the guidance for the applicants about the overall process for handling the applications for a new or modified stunning method. 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 scrutinised by the EFSA's Applications Desk (APDESK) Unit for verification of the completeness of the data submitted for the risk assessment of the stunning method. If the application is considered not valid, additional information may be requested from the applicant. If considered valid, it will be subjected to assessment phase 1.

Section 3 describes 'assessment phase 1'. In this phase, the AHAW Panel will examine the data, submitted by the applicant, related to the parameters for the scientific evaluation of the stunning method. Such parameters focus on the stunning method and the outcomes of interest, i.e. immediate onset of unconsciousness or the 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.

Section 4 describes 'assessment phase 2'. This phase will be carried out by the AHAW Panel and focuses on the animal welfare risk assessment. The Panel will assess the outcomes, conclusions and discussion proposed by the applicant. The aim of 'assessment phase 2' is to finally evaluate if the proposed new or modified stunning method is equivalent to the approved methods from the welfare outcome perspective. The results of the assessment will be published in a scientific opinion.

Annexes A, B and C include templates for the applicant to submit administrative data, completeness checklists and justification for confidential information, respectively. In Appendix A, the details for the key parameters to be provided for describing the stunning methods are presented, as derived from Annex I of (EC) Regulation 1099/2009.

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1. Introduction

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

Council Regulation (EC) No 1099/2009¹ 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 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. European Food Safety Authority (EFSA) carried out a public consultation of the draft version of this guidance in order to receive input from the scientific community and all interested parties on the assessment criteria established for applications for new or modified stunning methods. The report from the public consultation is published on EFSA's website (EFSA, 2018). Following the comments received through the public consultation, the following clarifications need to be made. In the context of this guidance, the term 'data' refers to quantitative measurements of characteristics including summary statistics, analysis and its results (raw data refers to data not yet summarised) and the term 'information' is a more general term related to data and knowledge, where data represents values attributed to parameters, and knowledge signifies understanding of real facts or abstract concepts.

'Modified stunning method' as used in the guidance document refers to any method that does not correspond to the definition of 'approved methods' in Regulation 1099/2009.

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.

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).

Regarding the animal species of concern, Article 2 of Regulation 1099/2009 defines 'animal' as any vertebrate animal, excluding reptiles and amphibians. This guidance therefore refers to the same.

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 process applications for a new or modified stunning method similar to applications for regulated products (as described in the Administrative guidance²) through a procedure that foresees the following sequence (also summarised in Figure 1):

- submission: the applicant prepares a dossier and submits it to the European Commission and the European Commission decides on sending a mandate to EFSA requesting scientific assessment of the dossier;
- completeness check phase: EFSA's Applications Desk Unit (APDESK) checks the submitted application on the new or modified method against the completeness of the information and data submitted by the applicant (see Section 2.4);

If the application is considered 'valid', it will proceed to the following step;

- check of the data in preparation of the risk assessment (assessment phase 1): Upon agreement from European Commission about the timeline for execution of the tasks, EFSA (Animal Health and Welfare (AHAW) Panel) will verify if the information and data used to describe and scientifically evaluate the method – e.g. statistical methods, welfare measures – are adequate (see Section 3). In case data provided are considered not sufficient or inadequate, EFSA may ask for additional data and if considered necessary further analyses to be carried out by the applicant.

In case provision of additional data or performance of further analyses is not possible for the applicant, EFSA may proceed to the adoption of a scientific output based on the originally submitted dossier (including results from assessment phase 1 and reporting the reasons why the application was considered inadequate). The output will be delivered to European Commission and published on EFSA's website.

If data are considered sufficient and adequate for the assessment, the application will proceed to the next step of the process:

- risk assessment of the stunning method (assessment phase 2): an application considered valid from assessment phase 1 will be fully assessed by the AHAW Panel for (see Section 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).
- the EFSA AHAW panel provides the European Commission 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 178/2002³. The European Commission will decide about the authorisation of the new method.

² <http://www.efsa.europa.eu/it/supporting/pub/1362e>

³ 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.

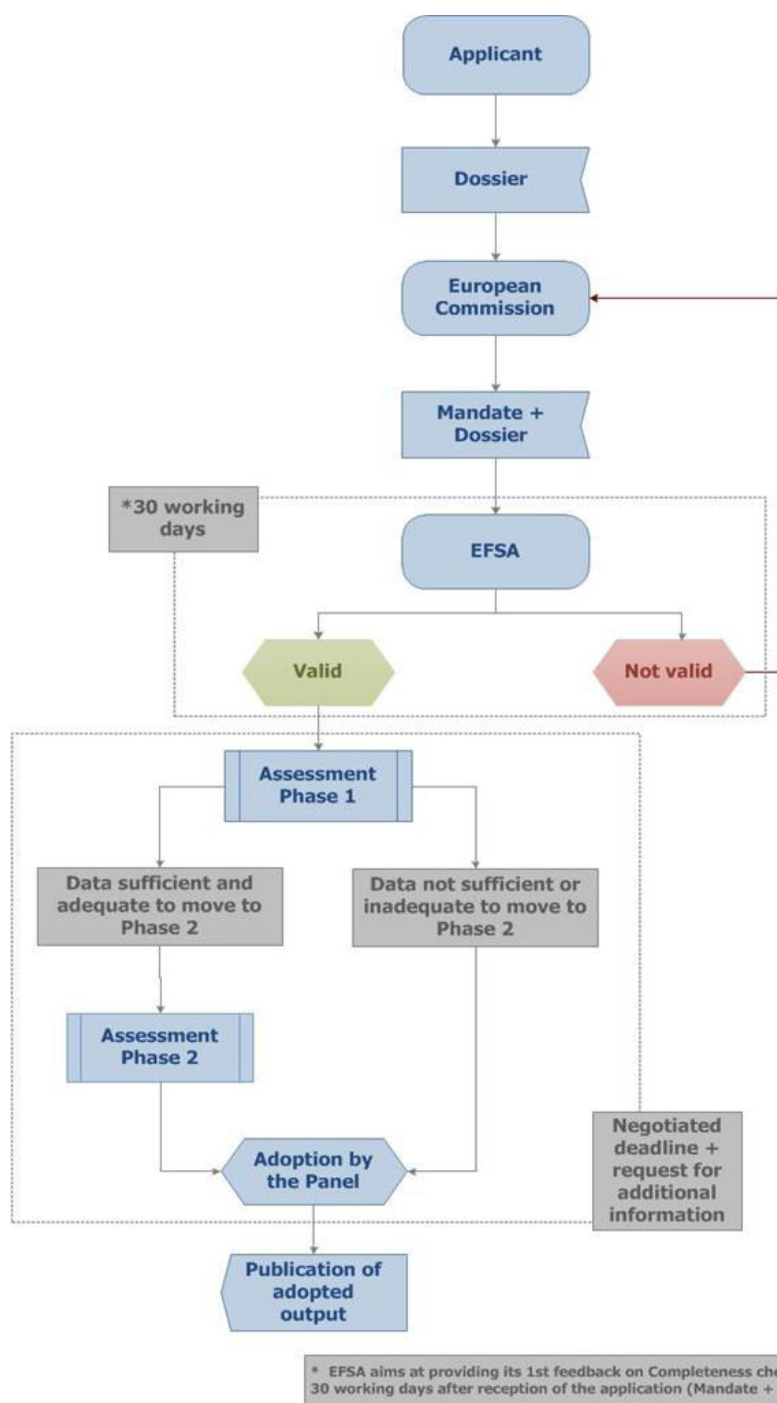


Figure 1: Flowchart showing the procedure for handling applications on animal stunning methods

2.2. Submission of an application for stunning methods for animals

Any person seeking an authorisation for a new or modified stunning method shall submit an application to the European Commission, which will possibly make the application available to EFSA. From reception of an application, EFSA will issue an acknowledgement of receipt letter to the European Commission, 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 should be provided to European Commission:

- 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 Section 2.3.1). When preparing the technical dossier, applicants should follow the scientific requirements described in this guidance. Audio–video material demonstrating the method, other relevant material (e.g. histological images, thermographic material) and bibliographic references should be provided in separate annexes.
- 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 only from the European Commission. Applicants should 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 (e.g. 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 and data. 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 or data highlighted;
- Completeness checklist (Annex B);
- Justification for confidential information or data (Annex C);
- When applicable, the agreement on data sharing (see Section 2.6).

A USB key or a CD-ROM without confidential information or data should also be provided, in line with EC Regulation 178/2002. 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 data or with confidential information or data blanked out;
- When applicable and if it is not requested to be considered as confidential, the agreement on data sharing (see Section 2.6).

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 require well-controlled studies under laboratory conditions to characterise 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 (ABMs) 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 normally be password protected. In case of password-protected files, then the applicant should send the passwords on separated couriers to EFSA. The applicant should keep in mind that EFSA complies with applicable provisions on regulatory data protection or confidentiality as outlined in EC Regulation 178/2002, including on the obligations to make publicly available the data and information on which its opinions are based. EFSA staff and external experts have the understanding that the information contained in the system should be treated on a professional secrecy basis and should not be made public.

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)⁴ 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

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, APDESK checks the completeness of the application and validates if it fulfils the legal requirements outlined in Regulation (EC) No 1099/2009 and 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 and data are effectively provided in the technical dossier. The completeness check relates to the description of the stunning method (see Section 3.1), the description of the individual studies submitted (see Section 3.2) and the overall integration of findings from all studies (see Section 3.3). The applicant should follow the same structure of the checklist (i.e. section 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 and/or data 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 and/or data, the applicant should submit the response within 30 working 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

⁴ http://www.bipm.org/utils/common/pdf/si_brochure_8_en.pdf

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 and/or data should be incorporated in all relevant parts of the application.

EFSA endeavours to inform the applicant within 15 working days if the updated application is valid or if further revision is required.

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

EFSA has implemented several initiatives to support applicants in understanding the evaluation process of applications for regulated products and to engage with them during the life cycle of applications.

Figure 2 below shows the different services that applicants can take advantage of in the different phases of the life cycle of the application. The complete list of support initiatives in place and a full description of each service currently implemented can be found in the 'EFSA's Catalogue of support initiatives during the life cycle of applications for regulated products' (EFSA, 2016).

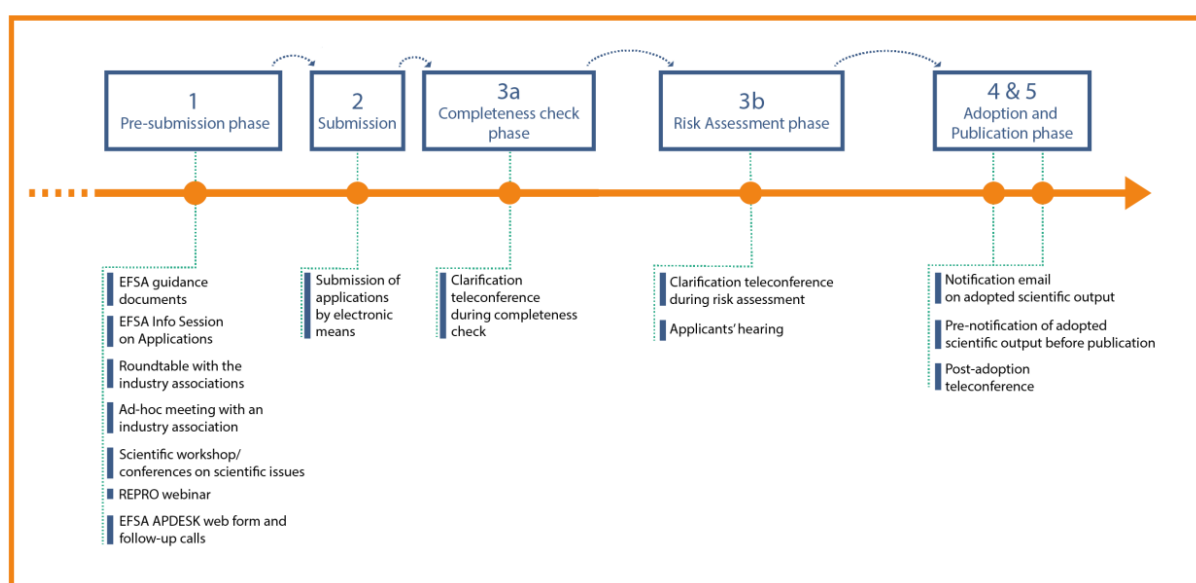


Figure 2: Overview of EFSA support initiatives available during the life cycle of an application for a new or modified stunning method

If an applicant is seeking information during the preparation of an application on aspects related to data for the scientific assessment, EFSA encourages the use of the APDESK web form 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 to EFSA, 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. In each correspondence related to an application, the contact details of the EFSA staff following the specific application within the APDESK unit are clearly mentioned to allow direct interaction between EFSA staff and the applicant. Applicants can contact EFSA staff to request further clarifications following a request for missing information letter or to clarify any outstanding issues during the completeness check phase. A telephone conference may be organised to further clarify the outcome of the completeness check.

During the scientific assessment phase, applicants have the possibility to contact the staff of the AHAW team. In each correspondence related to an application, the contact details of the EFSA staff within the AHAW team are mentioned. A telephone conference may take place to further clarify the questions.

In addition, upon request from EFSA, applicants might be invited to attend a specific agenda item of a working group or Panel meeting – either in person or via teleconference – to answer questions

about the submitted data and to clarify any outstanding issues on the application. EFSA will decide if this is necessary after examining the written response from the applicant to the EFSA's initial request for information or in case the experts of the Working group and/or Panel need to clarify any outstanding issues on the application.

Following the publication of an EFSA scientific opinion, applicants have the possibility to request post-adoption teleconference. The EFSA staff may organise the teleconference to explain the scientific rationale of the final opinion from the Panel.

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 where 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 authorisation holder,
 - Proprietary data or data for which copyrights are claimed,
 - Analytical test data,
 - Commercial- and industrial-related information outlining strategies, programmes 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 authorisation 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: check of data for risk assessment

Once the application is declared valid, the AHAW Panel will check the data needed for the scientific evaluation of the stunning method; for instance, it will check if experimental materials and analytical methods are sufficient and adequate.

In this phase, the AHAW Panel may request further data 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 European Commission.

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 are 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 or analogous structures 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, 2014a) for the full description of materials and methods. Basic information and data needed in the dossier is reported in the next paragraphs (from Section 3.2.2.1 to Section 3.3.2.3).

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. Expected effect size of the treatment outcomes and adequate power calculation should be considered.

Ethical considerations

For studies conducted at laboratory level, the experimental protocol must apply humane endpoints as specified in various international (e.g. <http://www.animaethics.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, 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 estimated by the shortest duration of unconsciousness of any stunned animal treated with the stunning method, minus the longest time till death after exsanguination. If it is impossible to measure the ABMs individually for each animal of a group experiment (group stunning), then population ranges and population estimates should be used to derive appropriate intervals. When ABMs can be measured individually, then means and confidence intervals can be calculated. In this case, the duration of unconsciousness induced with the method should outlast the 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 death due to exsanguination in animals subjected to simple stunning and which blood vessels were severed at exsanguination should also be reported.

As explained earlier, studies should be conducted in laboratory conditions and performed under slaughterhouse conditions. In laboratory conditions, neurological measures of spontaneous or evoked electrical activity of the brain recorded using EEGs or electrocorticograms (ECoGs) 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).

Use of neurological measures

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

EEG or ECOG are widely used to record the spontaneous and evoked (somatosensory, visual and auditory evoked potentials or responses) electrical activity in the brain to ascertain the state of consciousness following stunning and time to death. Established stunning methods induce specific brain states that are incompatible with the persistence of consciousness.

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

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

Use of animal behavioural measures, physiological measures and physical reflexes

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

Altered electrophysiological brain states are associated with certain behavioural patterns and physical reflexes. The correlation between EEG/ECoG evidence of unconsciousness and ABM has been characterised for established stunning methods, permitting the use of those ABM as proxies for unconsciousness in slaughterhouse conditions. It is worth noting that environmental conditions, such as darkness in a stunning chamber, may cause occurrence of EEG changes suggestive of unconsciousness before the occurrence of loss of posture (Martin et al., 2016b,c). Therefore, EEG criteria must be validated in controlled conditions and their correlation with ABMs well established for monitoring the effective use of a stunning method in slaughterhouses should be included, as required in the (EC) Regulation 1099/2009. It is also important to describe the earliest ABMs representing the induction to unconsciousness and the recovery of consciousness such that effective monitoring can be performed in slaughterhouses and an appropriate backup stunning method applied if necessary.

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

Correlation of neurological and other ABMs

The applicant should establish and report correlations between neurological criteria and other ABMs for determining onset of unconsciousness and the recovery of consciousness or time to death, using data from controlled laboratory studies. These correlations can also be substantiated using previously 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 make measurements to assess 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 ABMs 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 ABMs 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 ABMs for pain, distress and suffering should be used as a proxy for pain (see a non-exhaustive list in Table 1). Studies comparing the responses of animals to interventions with and without analgesic treatment may be useful for assessing the occurrence of pain, and potentially indicate the magnitude of pain. Care needs to be taken in the choice of analgesic and its dose to minimise the side effects of the analgesic on the animals' responses. In particular, it is important to ensure the chosen analgesic does not have sedative effect (Martin et al., 2016a).

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 stunning 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 mis-stunned animals and of those recovering consciousness prior to neck cutting or during exsanguination, if any, should be reported.

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

Category of ABMs	ABMs	Example	Reference
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,b, 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), Verhoeven et al. (2015), Llonch et al. (2012a,b, 2013)
	General behaviour	e.g. agitation, freezing, retreat attempts, escape attempts	EFSA (2005), Velarde et al. (2007), Dalmau et al. (2010), Landa (2012)

Category of ABMs	ABMs	Example	Reference
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), von 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,b, 2013)
Neurological measures	Brain activity	e.g. EEG, ECoG	Gibson et al. (2009)

(a): Hypothalamic–pituitary–adrenal.

(b): Adrenocorticotrophic hormone.

(c): 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, 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, including exclusion criteria applied;
- 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, AHAW Panel, 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. This 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 consciousness will indicate relatively better welfare.

It is preferable to use multiple pair-wise comparisons between methods, i.e. considering all measures which are equally applicable to the new/modified and existing methods. The final analysis will comprise all welfare outcome measures for which such comparisons can be made. 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.

When (animal-based) measures are individually compared between the methods in order to statistically establish an overall result, appropriate corrections should be applied to control for false-positive results.

Welfare outcome measures which are common to existing stunning methods and readily available in literature are listed in Section 3.2.2.2.

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).

3.3.2. Overall discussion and conclusions

3.3.2.1. Results regarding welfare impact

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

3.3.2.2. External validity

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

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

3.3.2.3. Discussion on equivalence with existing methods

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

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

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

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

4.1. Animal welfare risk assessment

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

4.1.1. Assessment of onset and duration of unconsciousness

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

4.1.1.1. Methodological aspects

The methodologies used in the evaluation of the stunning method will be assessed for validity and reliability, including the criteria and the thresholds used for the determination of unconsciousness. In 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 prestunning process, during induction of unconsciousness and due to mis-stunning

4.1.2.1. Methodological aspects

The measures chosen by the applicant will be scrutinised 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 vocalisations 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 prior to the onset of unconsciousness and insensibility:

- The ABMs chosen by the applicant should not indicate a greater magnitude of pain, distress and suffering in the treatment group compared to the appropriate control group. The response of animals exposed to the procedure/apparatus without the application of stunning (control or sham operation) should not be different from the response of the animals exposed to the procedure/apparatus with stunning (treatment).
- In general, the outcomes of the different ABMs on an individual animal should point into the same direction allowing for a clear interpretation about the consequence on animal welfare.

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

4.1.3. Assessment of external validity

This part of the assessment considers to which degree the findings from laboratory studies are consistent with those from pilot-plant scale, studies carried out under commercial conditions or studies carried out under different settings. 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.

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Glossary and abbreviations

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 personnel 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
Data	quantitative measurements of characteristics including summary statistics, analysis and its results (raw data refers to data not yet summarised)
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
Randomisation	a process of allocating units of replication 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
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
Uncertainty	all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question
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
ABMs	animal-based measures
AHAW	Animal Health and Welfare
APDESK	Applications Desk Unit
CAS	controlled atmosphere stunning
ECoGs	electrocorticograms
EEG	electroencephalograms
LAPS	low atmosphere pressure stunning
PDF	portable document format

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 A.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)

(a): Provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter.

A.1.2. Non-penetrative captive bolt

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

Table A.2: Parameters to be provided when applying a mechanical stunning method based on non-penetrative captive bolt 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)
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 A.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	1. Component	2. 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 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

Parameter	1. Component	2. Description (all specifications should be in internationally recognised units)
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 A.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

Parameter	Component		Description (all specifications should be in internationally recognised units)
Prevention of electrical shocks before stunning			Explain how the birds are protected from inadvertent, unintentional electrical shocks immediately before the stunning method are 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)		
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 (CO₂) at high concentrations and carbon dioxide in two phases

Exposure to high CO₂ concentrations is permitted in pigs, mustelids, chinchillas and poultry, except for ducks and geese.

Table A.5: Parameters to be provided when applying a stunning method based on high CO₂ concentrations or CO₂ 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 prefilled 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 prefilled 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 were monitored. The calibration methods applied should be reported
Duration of method ^(c)	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 prefilled 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 prefilled 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 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
Illumination of the chamber		Specify the light source if present
Calibration of the equipment and monitoring system		Describe 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 the case of simple stunning.

(c): Referring to the legal parameter 'duration of exposure'.

A.3.2. Carbon dioxide associated with inert gases

Table A.6: Parameters to be provided when applying a stunning method based on CO₂ 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 multistage 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 ^(c)	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 prefilled 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 multistage manner in a prefilled 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
Illumination of the chamber		Specify the light source if present
Calibration of the equipment and monitoring system		Describe 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.

(c): Referring to the legal parameter 'duration of exposure'.

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 A.7.

Table A.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 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 multistage manner in a prefilled 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 multistage 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 ^(c)	Time to reach exposure of animal to targeted inert gases or residual oxygen concentration ^(a)
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 multistage manner in a prefilled 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 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

Parameter	Component	Description (all specifications should be in internationally recognised units)
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
Illumination of the chamber		Specify the light source if present
Calibration of the equipment and monitoring system		Describe 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.

(c): Referring to the legal parameter 'duration of exposure'.

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 European Union (EU). Therefore, no parameters are defined by Council Regulation (EC) No 1099/2009. The parameters and components listed in Table A.8 have been derived by the EFSA AHAW panel.

Table A.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 ^(c)	Time to achieve the target pressures and corresponding partial pressure of oxygen in a single-phase system or multiphase 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 multistage 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 Repressurisation of the chamber prior to opening of door should be described and any 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

Parameter	Component	Description (all specifications should be in internationally recognised units)
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.

(c): Referring to the legal parameter 'duration of exposure' of other stunning methods.