Assessment of studies evaluating alternative stunning methods

Andrea Gervelmeyer

SCFCAH meeting
Brussels, 14 June 2013
Overview

Legal background
Request from Member State and Commission
TORs addressed by EFSA
Opinions:
  Approach
  Assessment
Further steps
Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing
• Lays down rules for killing of animals
• Lists stunning methods, related specifications and specific requirements for certain methods in Annex 1

**Article 4 (2) on stunning methods:**
Annex I may be amended to take account of scientific and technical progress on the basis of an opinion of EFSA.

Any such amendments shall ensure a level of animal welfare at least equivalent to that ensured by the existing methods.
Head-only and head-to-body electrical stunning of small ruminants - current situation:

• minimum current of 1 Ampere (A) for both
• no differentiation according to weight/size of animal

Request from Spanish authorities and Catalan meat federation (FECIC):

• amend points 4.2 and 5.1 of Chapter II of Annex I to Regulation 1099/2009 as regards the minimum current for small ruminants for respectively head-only and head-to-body electrical stunning
• reduce currents for smaller animals to avoid meat quality and hide problems
• request supported by two studies performed by IRTA
Carbon dioxide stunning of rabbits - current situation: use of carbon dioxide is not allowed for stunning rabbits

Request from Spanish authorities:
• allow the use of carbon dioxide as method for stunning rabbits
• transitional period in order to enable collection of additional scientific data
• supported by one study
view on findings of studies performed by IRTA with focus on:
- extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A
- extent to which findings of the studies are consistent with other sources on electrical stunning of small ruminants (in particular on lowering the current for younger/smaller animals)
- extent to which findings of the studies can be valid for different breeds of small ruminants;
- additional requirements possibly linked to use of minimum currents lower than 1 A for small ruminants (maximum live weight, possibly other conditions (min. V, max. Hz, time of exposure, stun-to-stick interval, etc.))

Deadline May 2013
view on findings of study performed by Polytechnic University of Valencia (Spain) and Animal Technology Centre CITA-ITAVIA:

− extent to which use of CO$_2$ is, in principle, an acceptable alternative for stunning of rabbits compared to the welfare advantages/disadvantages related to other stunning methods used for rabbits under commercial conditions;
− extent to which findings of study are consistent with other sources of information;
− requirements possibly attached to use of carbon dioxide for stunning rabbits, (minimum or maximum gas concentration, duration of exposure, stun-to-stick interval, quality of the gas, temperature of the gas, type of recording and maintenance etc.);
− extent to which findings of study can be valid in the context of other rabbit slaughterhouses in the EU.

Deadline Sep 2013
EFSA approach

Previous experience:

*Inconsistencies* with reporting of intervention studies in animal health area, *lack of harmonization* of designing and reporting intervention studies studying stunning interventions’ efficacy

Clear guidance to researchers on how studies will be assessed by EFSA:

Minimum criteria need to be fulfilled for a given study so that it can be considered for assessment as a potential alternative to stunning methods and related specifications listed in Council Regulation (EC) No 1099/2009
Mandate on lamb stunning

Electrical stunning of lambs (2 studies submitted)

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

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

Deadline May 2013
**Mandate on rabbit stunning**

**CO₂ stunning of rabbits** (1 study submitted)

**TOR1**: the extent to which the use of CO₂ is an acceptable alternative for the stunning of rabbits based on the submitted study

**TOR2**: the **type of study and data needed** to supply scientific evidence that the use of CO₂ is an acceptable alternative for the stunning of rabbits

Deadline Sep 2013
TOR 2

Eligibility criteria:

For the intervention:
- Key parameters described in legislation and provided by stunning experts

For the outcome:
- Immediate onset of unconsciousness and insensibility OR
- Absence of avoidable pain, distress and suffering until the loss of consciousness and sensibility
  AND
- Duration of the unconsciousness and insensibility (until death)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum current (A or mA)</td>
<td>Current type</td>
<td>The electrical current used to stun animals can be either sine or square wave alternating current (bipolar or biphasic) or pulsed direct current (monopolar or monophasic). Define the current type used</td>
</tr>
<tr>
<td>Waveform</td>
<td></td>
<td>The waveform of current used for stunning animal varies widely and includes clipped or rectified sine or square waves. The proportion of clipping also varies widely. Define the waveform used including the proportion of clippings; report the marks-spaced ratio, when pulsed direct current is used</td>
</tr>
<tr>
<td>Minimum current</td>
<td></td>
<td>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</td>
</tr>
<tr>
<td>Latency</td>
<td></td>
<td>Specify how soon the minimum current was reached after the intervention was applied to the animal</td>
</tr>
<tr>
<td>Minimum voltage (V)</td>
<td>Exposed minimum voltage (V)</td>
<td>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.</td>
</tr>
</tbody>
</table>
Eligibility criteria – unconsciousness

• unconsciousness and insensibility should be ascertained by EEG in controlled environment settings
• once the effectiveness of a given stunning method has been shown in controlled environment studies using EEGs, its effectiveness should also be studied in experiments under slaughterhouse conditions
• Indicators of recognising a successful electrical stun (presence of tonic seizures, apnoea, lack of response to painful stimuli) should be applied in slaughterhouse settings, after their correlation with EEGs has been shown in controlled environment studies
### Eligibility criteria – absence of pain, distress and suffering

<table>
<thead>
<tr>
<th>Response type</th>
<th>Groups of animal-based measures</th>
<th>Example</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behaviour</td>
<td>Vocalisations</td>
<td>e.g. number and duration, intensity, spectral components</td>
<td>EFSA, 2005; Le Neindre et al., 2002; 2012a, 2012b, 2013</td>
</tr>
<tr>
<td></td>
<td>Postures and movements</td>
<td>e.g. kicking, tail flicking, avoidance</td>
<td>Jongman et al., 2000; EFSA, 2005; Le Neindre et al., 2007; Kirkden et al., 2008; Sy et al., 2012; Landa, 2012; Llonch et al., 2012</td>
</tr>
<tr>
<td></td>
<td>General behaviour</td>
<td>e.g. agitation, freezing</td>
<td>EFSA 2005; Landa, 2012</td>
</tr>
<tr>
<td>Physiological</td>
<td>Hormone concentrations</td>
<td>e.g. HPA(^a) axis: cortisol, ACTH(^b): sympathetic system: adrenalin, noradrenaline</td>
<td>Mellor et al., 2000; EFSA, 2005; Le Neindre et al., 2002; 2012a, 2012b, 2013</td>
</tr>
<tr>
<td>response</td>
<td>Blood metabolites</td>
<td>e.g. glucose, lactate, free fatty acids</td>
<td>EFSA, 2005; Vogel et al., 2011; Landa, 2012</td>
</tr>
<tr>
<td></td>
<td>Autonomic responses</td>
<td>e.g. heart rate, blood pressure, respiratory rate, body temperature, dilatation of the pupil, sweating</td>
<td>Martoft et al., 2001; EFSA, 2005; G et al., 2008; Dalmau et al., 2010; Le Neindre et al., 2012; Landa, 2012; Llonch et al., 2012</td>
</tr>
<tr>
<td>Neurological</td>
<td>Brain activity</td>
<td>e.g. EEG, ECoG</td>
<td>Raj et al., 1998; Martoft et al., 2001; Landa, 2012</td>
</tr>
</tbody>
</table>
Reporting quality criteria:

• reporting guidelines designed to increase the transparency of conducting and reporting scientific studies have been developed by various groups in the past, adopted by many scientific journals

• REFLECT\textsuperscript{1} statement and STROBE\textsuperscript{2} statement were identified as most suitable guidelines that could be applied to studies on stunning methods
  – REFLECT : reporting guideline for randomised controlled trials in animals
  – STROBE : reporting guideline for observational studies on humans but can be readily adapted to animals

\textsuperscript{1}http://www.reflect-statement.org/statement/
\textsuperscript{2}http://www.strobe-statement.org/
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>Background and rationale</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
</tr>
<tr>
<td>Objective</td>
<td>Describe the specific objectives and hypotheses. Clearly state primary and secondary objectives (if applicable)</td>
</tr>
<tr>
<td><strong>Materials and methods</strong></td>
<td></td>
</tr>
<tr>
<td>Study population</td>
<td>Give characteristics of the study population (species, breed, animal type (e.g. dairy or beef cattle), and weight) and potential confounders (health status, fasting, water deprivation, husbandry system); indicate the number of animals with missing data for each variable of interest</td>
</tr>
<tr>
<td>Number of animals (sample size)</td>
<td>How was the sample size determined and, when applicable, explanation of any interim analyses and stopping rules. Experimental/intervention units must be described and information on whether true replication was done is needed</td>
</tr>
<tr>
<td>Intervention</td>
<td>Precise details of the interventions intended for each group, how and when interventions were actually administered. In addition, specifications of the requirements for the stunning method are provided in section 3.1.1</td>
</tr>
<tr>
<td>Outcome</td>
<td>Clearly define all primary outcomes (onset of unconsciousness/insensibility, absence of pain, distress and suffering and duration of unconsciousness/insensibility) and ancillary outcomes (e.g. heart beat, tail flicking). Report category boundaries when continuous variables were categorised. Specifications of the requirements for the assessment of unconsciousness and insensibility as well as absence of pain, distress and</td>
</tr>
</tbody>
</table>
Study quality criteria:

- methodological quality of the submitted studies assessed only if the eligibility criteria are fulfilled
- presence of biases affecting internal validity assessed:
  - confounding bias
  - selection bias
  - information bias
- analysis of external validity of the results of the submitted studies only if all requirements of the previous steps of assessment have been met by submitted study
- beyond time frame of current mandate
Approach - lambs and rabbits

1. THE INTERVENTION
   Complete characterisation of the intervention

2. THE OUTCOME
   A. Immediate unconsciousness OR
   B. Absence of pain, distress and suffering until loss of consciousness AND
   C. Duration of unconsciousness

Assessment of the eligibility of the study

Identification of reporting guidelines applicable to studies on stunning methods

Collation of parameters from guidelines on which information has to be reported (Generate a grid)

Assessment of the reporting quality (insert the study information into the grid)

Specification of different types of bias (e.g. selection)

If eligibility criteria are fulfilled:
   Assessment of the methodological quality

If fulfilled:
   Correlation with other scientific evidence
## Assessment of studies (TOR 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Component</th>
<th>Information provided in the submitted study 1</th>
<th>Fulfilment criterion (yes or no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum current (A or mA)</td>
<td>Current type</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Waveform</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Minimum current(^1)</td>
<td>It is reported that 0.7 A and 1.0 A were used, but it is not clear whether the reported value is root mean square, average or peak current</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Latency(^2)</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Minimum voltage (V)</td>
<td>Exposed minimum voltage (V)(^2)</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Delivered minimum voltage (V)(^2)</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Maximum frequency (Hz)</td>
<td>Maximum frequency (Hz)</td>
<td>50 Hz is mentioned, but it is not clear whether it is minimum or maximum frequency</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Minimum frequency (Hz)</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Minimum time exposure(^3)</td>
<td>Stun was applied for four seconds but method of control and average and range are not reported</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Maximum interval (s)(^4)</td>
<td>stun-to-stick (kill)</td>
<td>Not relevant in trial 1 as animals were allowed to recover in this study, in trial 2 the reported time between stunning and slaughter by neck-cutting was 7.3 ± 1.46 seconds. However, no EEG data assessing the duration of unconsciousness/insensibility after the stunning intervention are reported. Therefore, the maximum stun-to-stick (kill) interval could not be defined adequately</td>
<td>No</td>
</tr>
<tr>
<td>Frequency of calibration of the equipment</td>
<td>Not reported</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Optimisation of the current flow</td>
<td>Electrode characteristics</td>
<td>Not reported</td>
<td>No</td>
</tr>
</tbody>
</table>
Potential assessment outcome A

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

– sufficient detail regarding intervention and outcome with conclusive results

– conclusion: method induces immediate onset of unconsciousness/insensibility and that unconsciousness/insensibility lasts sufficiently long to cover stun-to-stick interval and onset of brain death through loss of blood

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

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

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

In consequence: assessment would highlight shortcomings, indicate where improvements are required before the study can be further assessed
Assessment of studies (Lambs)

• Regarding fulfilment of the eligibility criteria it is concluded that:
  – For both study 1 and study 2, the intervention is considered to be insufficiently described.
  – The onset of unconsciousness and insensibility has not been adequately assessed in study 1. It is not possible to assess whether the onset of unconsciousness and insensibility has been adequately assessed in study 2.
  – The duration of unconsciousness has not been adequately addressed in study 1. It is not possible to assess whether the duration of unconsciousness has been adequately addressed in study 2.

• Regarding fulfilment of the reporting criteria it is concluded that:
  – Neither study 1 nor study 2 fulfils the reporting criteria.

• Regarding fulfilment of the quality criteria it is concluded that:
  – As the studies did not fulfil the eligibility criteria, the methodological quality of the studies was not assessed.
Regarding fulfilment of the **eligibility criteria** it is concluded that:

- The intervention is considered to be insufficiently described.
- The onset and the duration of unconsciousness were not assessed in the study.
- An assessment of whether pain, distress and suffering were present during the induction phase was not done.

Regarding fulfilment of the **reporting criteria** it is concluded that:

- The study does not fulfil the reporting criteria.

Regarding fulfilment of the **quality criteria** it is concluded that:

- As the study did not fulfil the eligibility criteria, the methodological quality of the study was not assessed.
**Recommendations on TOR 1**

*Electrical stunning of lambs*

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

*CO₂ stunning of rabbits*

- Further studies on the use of CO₂ as an acceptable alternative for the stunning of rabbits are needed, which should include the eligibility criteria set out in this opinion.
Recommendations on TOR 2

• Alternative stunning methods should be first studied under **controlled (laboratory) conditions** to analyse the animals’ responses (unconsciousness, absence of pain, distress and suffering) using the **most sensitive and specific methods** and to **find a correlation with non-invasive parameters** that can be applied during the second phase of the study in slaughterhouses.

• In a second step, the results obtained under controlled laboratory conditions need to be **confirmed under a range of slaughterhouse conditions**.

• The **criteria for eligibility, reporting quality and study quality** defined in this document should be applied to studies carried out under controlled (laboratory) conditions as well as to studies carried out under slaughterhouse conditions.
• For studies researching a new or modified stunning method, animals should be **stunned without sticking** to establish the **duration of unconsciousness** achieved by the stunning itself in proof-of-concept studies under controlled laboratory conditions.

• The onset and the duration of **unconsciousness** and insensibility should be ascertained using **EEGs** or **ECoGs** in studies carried out under **controlled (laboratory) conditions**.
Recommendations on TOR 2

Under slaughterhouse conditions, the onset and the duration of unconsciousness and insensitivity should be ascertained using the **indicator** that **best** detects unconsciousness and that has been shown to be **correlated** with EEGs in laboratory experiments. If different indicators are not in agreement, following on from the precautionary principle and to benefit animal welfare, the one that **indicates the longest time interval between application of the stunning intervention and onset of unconsciousness** should be used.

**Data** reported in studies on alternative stunning methods should be provided at the **individual animal level**.
• As no specific indicator is available for pain, combinations of animal-based measures for pain, distress and suffering should be used as a proxy, selected according to their relevance to the respective stunning intervention as shown by the available scientific knowledge of each measure’s sensitivity and specificity.

• Studies on alternative stunning methods should assess at least animal-based measures from behavioural, physiological and neurological response types, using methods previously published in peer-reviewed journals.
Further work: Guidance

Guidance of the EFSA Scientific Committee or Scientific Panel:

• Explains principles behind EFSA’s procedures and approaches to scientific risk assessments to risk assessors (including Scientific Committee or Scientific Panels), risk managers and/or applicants of dossiers submitted for evaluation.
TOR of guidance

• define the criteria against which studies evaluating the efficacy of stunning interventions regarding animal protection during stunning will be assessed
  • checklist of reporting quality criteria, eligibility criteria and further study quality criteria
  • scientific reasoning for each checklist item
  • description of the guidance development process
  • explain how studies will be evaluated
  • scope: mechanical, electrical and gas methods for the main livestock species (bovines, sheep, goats, pigs, poultry, and rabbits)

• public consultation of draft guidance 15 Jul – 8 Sep

• adoption of guidance in Nov 2013
Further work: Guidance

• Intervention: mechanical stunning methods
• Intervention: electrical waterbath stunning
• Intervention: modified atmosphere stunning methods

• add species-specific aspects where needed

• further development of study quality criteria

• criteria for evaluating studies
The 2 opinions „Electrical stunning of lambs and kid goats“ and „CO₂ stunning of rabbits“ are available on EFSA‘s website at
