SCIENTIFIC OPINION

Scientific Opinion on monitoring procedures at slaughterhouses for pigs[1]

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

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ABSTRACT

This scientific opinion proposes toolboxes of welfare indicators for developing monitoring procedures at slaughterhouses for pigs stunned with the head-only electrical method or carbon dioxide at high concentration. In particular, the opinion proposes welfare indicators together with their corresponding outcomes of consciousness, unconsciousness or death. The opinion proposes a toolbox of indicators and the outcomes to be used to assess consciousness in pigs at three key stages of monitoring: (a) after stunning and during shackling and hoisting, (b) during sticking and (c) during bleeding. Various activities—including a systematic literature review, an online survey and stakeholders' and hearing experts' meetings—were conducted to gather information about specificity, sensitivity and feasibility of the indicators that are to be included in the toolboxes for monitoring welfare. On the basis of information gathered during these activities, a methodology was developed to select the most appropriate indicators that could be used in the monitoring procedures. The frequency of checking differs according to the role of each person with responsibility for ensuring animal welfare at slaughter. The personnel performing stunning, shackling, hoisting and/or bleeding will have to check all the animals and confirm that they are not conscious following stunning. For the animal welfare officer, who has the overall responsibility for animal welfare, a mathematical model for the sampling protocols is proposed, giving some allowance to set the sample size of animals that he/she needs to check at a given throughput rate (total number of animals slaughtered in the slaughterhouses) and tolerance level (number of potential failures—animals that are conscious after stunning; animals that are not unconscious or not dead after slaughter without stunning). The model can also be applied to estimate threshold failure rate at a chosen throughput rate and sample size. Finally, different risk factors and scenarios are proposed to define a 'normal' or a 'reinforced' monitoring protocol, according to the needs of the slaughterhouse.

KEY WORDS

stunning, slaughter, consciousness, welfare indicators, monitoring procedures

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SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver scientific opinions on monitoring procedures at slaughterhouses for different animal species and stunning methods. In particular, the opinions will (i) provide indicators assessing signs of (a) consciousness, in the case of slaughter with stunning, and (b) unconsciousness and (c) death of the animals, in the case of slaughter without stunning, which have been selected based on their performance (i.e. sensitivity, specificity and feasibility of the indicator); (ii) indicate the most common risk factors and their welfare consequences to determine the circumstances of the monitoring procedures; and (iii) provide examples of sampling protocols, based on different possible scenarios.

The current opinion deals with the assessment of consciousness in pigs stunned with the head-only electrical method or carbon dioxide at high concentration. The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in pigs following stunning, the risk of poor welfare can be detected better if pig welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the indicators were phrased neutrally (e.g. corneal reflex) and the outcomes were phrased either suggesting unconsciousness (e.g. absence of corneal reflex) or suggesting consciousness (e.g. presence of corneal reflex). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses. Two different toolboxes of selected indicators are proposed to check for signs of consciousness in pigs after head-only and carbon dioxide stunning. Various activities (two stakeholder consultations, a systematic literature review, an online survey addressed to experts involved with monitoring welfare at slaughter) were carried out in order to obtain information on the sensitivity, specificity and feasibility of the indicators. Based on such information, the most appropriate indicators were selected and a toolbox of indicators to be used in monitoring procedures was proposed. The use of animal-based indicators is similar to the use of a diagnostic or statistical ‘test’ with either a positive or negative outcome. In the case of stunning of the animals, the major interest is to detect the undesired outcome, namely the presence of consciousness in animals. For this reason, the toolboxes propose indicators and their outcomes.

Each of the toolboxes provides a set of recommended indicators and another set of additional indicators. The people responsible for monitoring have to choose the most appropriate set of indicators (at least two indicators) from these toolboxes according to their expertise and the available infrastructure in a slaughterhouse.

Toolboxes for head-only electrical stunning:

After stunning of the animals prior to slaughter the indicators should be repeatedly checked to detect consciousness through the three key stages of monitoring during the slaughter process: after stunning (between the end of stunning and shackling), during sticking (cutting brachiocephalic trunk) and during bleeding.

The recommended indicators in Toolbox 1 (for monitoring after head-only electrical stunning) are tonic/clonic seizures, breathing and the corneal or palpebral reflex. Additionally, spontaneous blinking, posture and vocalisations may be used.

For Toolbox 2 (for monitoring at sticking after head-only electrical stunning), the recommended indicators to be used are breathing, tonic/clonic seizures and muscle tone. Additionally, the corneal or palpebral reflex, spontaneous blinking and vocalisations may be used.

For Toolbox 3 (for monitoring during bleeding after head-only electrical stunning) the recommended indicators are breathing and muscle tone. Additionally, vocalisations, the corneal or palpebral reflex and spontaneous blinking may be used.

Toolboxes for carbon dioxide stunning:

The recommended indicators in Toolbox 4 (for monitoring after stunning with carbon dioxide) are muscle tone, breathing and the corneal or palpebral reflexes. Additionally, response to nose prick or ear pinch and vocalisations may be used.

For Toolbox 5 (for monitoring at sticking after carbon dioxide stunning), the recommended indicators to be used are muscle tone, breathing and vocalisations. Additionally, the corneal or palpebral reflex and response to nose prick or ear pinch may be used.

For Toolbox 6 (for monitoring during bleeding after carbon dioxide stunning), the recommended indicators are muscle tone and breathing. Additionally, the corneal or palpebral reflex and vocalisations may be used.

The personnel performing stunning, shackling, hoisting and/or bleeding will have to check all the animals to rule out the presence of consciousness following head-only electrical or carbon dioxide stunning. The person in charge of monitoring the overall animal welfare at slaughter (i.e. animal welfare officer) has to check a certain sample of slaughtered animals for approval. A mathematical model is proposed which can be used to calculate the sample size that he/she needs to check at a given throughput rate (total number of animals slaughtered in the slaughter plant) and tolerance level (number of potential failures—animals that are conscious after electrical or carbon dioxide stunning). Finally, different risk factors and scenarios are proposed to define, in addition to a ‘normal’ sampling procedure, a ‘reinforced’ protocol to be used if required by the particular circumstances and needs of the slaughterhouse.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Article 16 of Council Regulation (EC) No 1099/2009[4] on the protection of animals at the time of killing[5] requires slaughterhouse operators to put in place and implement monitoring procedures in order to check that their stunning processes deliver the expected results in a reliable way.

Article 16 refers to Article 5 which requires operators to carry out regular checks to ensure that animals do not present any signs of consciousness or sensibility in the period between the end of the stunning process and death.

Those checks shall be carried out on a sufficiently representative sample of animals and their frequency shall be established taking into account the outcomes of previous checks and any factors which may affect the efficiency of the stunning process.

According to Article 16(2), a monitoring procedure shall include in particular the following:

(a) indicators designed to detect signs of unconsciousness and consciousness or sensibility in the animals (before death or release from restraint, in case of slaughter without stunning, = indicators A); or indicators designed to detect the absence of signs of life in the animals slaughtered without stunning (before undergoing dressing or scalding = indicators B);

(b) criteria for determining whether the results shown by the indicators previously mentioned are satisfactory;

(c) the circumstances and/or the time when the monitoring must take place

(d) the number of animals in each sample to be checked during the monitoring.

Furthermore, Article 16 (4) specifies that: "The frequency of the checks shall take into account the main risk factors, such as changes regarding the types or the size of animals slaughtered or personnel working patterns and shall be established so as to ensure results with a high level of confidence."

The Commission plans to establish EU guidelines concerning monitoring procedures at slaughterhouses.

The purpose of the Commission is to provide a sort of "toolbox" for establishing monitoring procedures so that slaughterhouse operators can use scientifically based procedures which will provide them proper information on their stunning processes. The guidelines will also be used by the competent authorities in order to check that slaughterhouse operators are not using unreliable monitoring procedures.

In order to prepare these guidelines, a sound basis for checks on stunning as laid down in Articles 5 and 16 of the above-mentioned regulation is needed.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

The Commission therefore considers it opportune to request the EFSA to provide an independent view on the indicators and elements for putting in place monitoring procedures at slaughterhouses for the following methods and scope, in light of the most recent scientific developments.

- The scope of this request includes the following groups of methods/species[6]:

  (1) penetrative captive bolt for bovine animals,

4 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = OJ:L:2009:303:0001:0030:EN:PDF
5 OJ L 303, 18.11.2009, p. 1.
6 Wording used for the stunning methods refers to Annex I to Regulation (EC) No 1099/2009.

(2) head-only electrical stunning for pigs,

(3) head-only electrical stunning for sheep and goats,

(4) electrical waterbath for poultry (chickens and turkeys),

(5) carbon dioxide at high concentration for pigs,

(6) all authorised gas methods to slaughter chickens and turkeys (carbon dioxide in two phases, carbon dioxide associated with inert gases and inert gases alone).

(7) Slaughter without stunning for bovine animals,

(8) Slaughter without stunning for sheep and goats,

(9) Slaughter without stunning for chickens and turkeys.

- For each group the EFSA, based on the relevant scientific basis and on indicators' performances, will provide indicators A (loss of consciousness or sensibility for all groups) or indicators B (absence of signs of life for groups 7 to 9 only) as well as the other elements of the monitoring procedure (criteria for satisfactory results in terms of animal welfare, circumstances and sampling procedure, including minimum sampling and frequency) (sampling procedures are needed only for groups 1 to 6 since checks must be systematic for groups 7 to 9).

- For that purpose, the EFSA will take into account that:

  – Indicators should be able to detect, with high level of confidence, unsatisfactory stunning/slaughtering practices within the sample observed. Hence, the EFSA should specify the criteria for selecting indicators, based on the level of sensitivity and specificity for each indicator.

  – At least two indicators are required for each process but more could be recommended.

  – Indicators will be used at slaughterhouses, which imply human (work safety, accessibility), physical (line speed, difficulties to observation, etc.) and economic (time, costs) constraints. Hence, the EFSA could indicate the possible limitations related to the measurement of each indicator.

  – Circumstances to determine the monitoring procedure have to address the risk factors most commonly associated with each group of methods/species (for example the penetrative captive bolt is likely to be more sensitive to the competence of the staff than a highly mechanised method). Hence, for each groups of methods/species, the EFSA should indicate the most common risk factors and their welfare consequences to determining the circumstances of the monitoring procedure (e.g. when the staff shifts change if staff is an important risk factor).

  – Monitoring procedures can be dynamic instruments and different indicators and sampling procedures could be used on the same slaughter line depending on the previous results and other risk factors. Hence, based on different possible scenarios, the EFSA should provide examples of different sampling protocols (like "new line/reinforced", "regular", "light") and the minimum sampling needed for indicators 'A' (even when results appear to be fully satisfactory).

ASSESSMENT

1. Introduction

1.1. General introduction

According to Council Regulation (EC) No 1099/2009, on the protection of the animals at the time of killing, animals must be rendered unconscious and insensible by the stunning method and they must remain so until death occurs through bleeding. One way of achieving this animal welfare requirement would be to monitor the state of consciousness and unconsciousness in animals at three key stages: (1) immediately after stunning, (2) at the time of neck cutting or sticking and (3) during bleeding until death occurs.

Within the scope of this Regulation, it is the responsibility of the food business operator (FBO) to ensure that the welfare of the animals is not compromised from the time of their arrival until they are slaughtered.

The ‘personnel’ performing stunning, shackling, hoisting and/or bleeding (hereafter referred to as the ‘personnel’) must hold a certificate of competence, awarded after training and assessment by independent organisations, attesting that they have the knowledge and skills required to recognise the signs of both effective and ineffective stunning and, in the event of a failure, to re-stun the animal. In addition, the ‘personnel’ performing slaughter (sticking, i.e. severing brachiocephalic trunk) in pigs should have a certificate of competence attesting that they are aware of the need, and have the skills required, not only to perform prompt and accurate slaughter but also to perform checks for the signs of recovery of consciousness prior to sticking to ensure that every animal is unconscious at the time of sticking. The personnel should also be able to ascertain the possibility or potential for recovery of consciousness in animals during bleeding and take action, if necessary (e.g. use a back-up stunner).

Finally, the person in charge of the overall animal welfare at slaughter (i.e. animal welfare officer) should be able to monitor the animals during the entire process, from stunning to bleeding, and ascertain that they do not show any signs of consciousness and also that death occurs before further carcase dressing operations or scalding begin. Under laboratory conditions, the induction and maintenance of unconsciousness and insensibility following stunning can be ascertained by recording the brain activity using electroencephalography (EEG) or electrocorticography (ECoG). The effectiveness of stunning and the duration of unconsciousness induced by the stunning method can be recognised from the unique brain state and associated EEG manifestations. When stunning-induced EEG or ECoG are changes are ambiguous, abolition of auditory, somatosensory or visual evoked potentials in the brain has been used to ascertain the brain responsiveness to these external stimuli. The effectiveness of stunning and sticking can also be recognised under the field conditions from the characteristic changes in the behaviour of animals (e.g. loss of posture), physical signs (e.g. onset of seizures, cessation of breathing, fixed eye) and from the presence or absence of response to physiological reflexes (e.g. response to an external stimulus such as blinking response to touching the cornea (corneal reflex), response to pain stimulus such as nose prick or toe pinching). In the scientific literature, these physical signs and reflexes have been referred to as indicators of unconsciousness or consciousness and used to monitor welfare at slaughter of animals (see, for example, EFSA, 2004, 2006).

At all of the key stages, monitoring is carried out to identify animals that are improperly stunned, or recover from stunning and therefore the attention is focused on the indicator of consciousness. Effectively stunned animals are expected to remain unconscious during key stages 2 and 3 until death occurs. It is thought that, for this monitoring system to be effective, it is important to define indicators (see sections 3.4 and 3.5 and the glossary), identify their pathophysiological basis of the stunning method and its relevance or appropriateness to key stages of monitoring, and also to describe how the indicator may be manifested or can be used to recognise consciousness at a particular key stage of monitoring.

1.2. Definitions

Consciousness is a state of awareness which requires the function of the brain stem and projections in the relevant cortical regions. Following everyday neurological practice (Zeman, 2001) consciousness is generally equated with the waking state and the abilities to perceive, interact and communicate with the environment and with others, which is referred to as sensibility. Consciousness is a matter of degree, and a range of conscious states extends from waking through sleep until unconsciousness is reached. For the purpose of this opinion, an animal is considered 'conscious' as long as a degree of consciousness is detected.

Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to perceive external stimuli (which is referred to as insensibility) and control its voluntary mobility and, therefore, respond to normal stimuli, including pain (EFSA, 2004).

For the Dialrel project (von Holleben, 2010) 'unconsciousness' is defined in a similar way to that used by anaesthesiologists: "*Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence the individual is unable to respond to normal stimuli, including pain*."

According to the Regulation 1099/2009, the **sensibility** of an animal is essentially its ability to feel pain. In general, an animal can be presumed to be insensible when it does not show any reflexes or reactions to stimuli such as sound, odour, light or physical contact.

In the context of this scientific opinion, consciousness includes sensibility and unconsciousness includes insensibility.

Death is a physiological state of an animal, in which respiration and blood circulation have ceased as the respiratory and circulatory centres in the medulla oblongata are irreversibly inactive. Owing to the permanent absence of nutrients and oxygen in the brain, consciousness is irreversibly lost. In the context of application of stunning and stun/kill methods, the main clinical signs of death are absence of respiration (and no gagging), absence of pulse and dilated pupils (EFSA, 2004, 2006).

1.3. Physiology of head-only electrical stunning

Head-only electrical stunning with a current of sufficient magnitude induces immediate loss of consciousness in pigs through the induction of a generalised epileptiform activity in the brain (Simmons, 1995; Berghaus and Troeger, 1998). The neurophysiological basis of a generalised epileptiform activity and associated loss of consciousness is well documented in the scientific literature (see EFSA, 2004, report for details).

Successful induction of epileptiform activity is manifested as immediate collapse of the animal owing to the onset of tonic seizure. During the tonic phase, the animals show tetanus (rigidly extended legs), breathing is absent and the eyeballs may be fixed or obscured (cornea not visible due to eyeball rotation into the socket). The tonic phase is followed by two clonic phases, which can manifest as a galloping, cantering or erratic kicking action (Simmons, 1995; Berghaus and Troeger, 1998; Gregory, 1998).

The duration of tonic seizure is influenced by several factors (e.g. type of animal, electrical parameters), but is usually of the order of seconds (Anil and McKinstry, 1992, 1998; Simmons, 1995). Tonic seizure is followed by clonic seizures lasting for seconds and terminating in loss of muscle tone, which can be recognised from drooping ears and limp legs, especially when the animals have been shackled and hoisted on to the overhead bleeding rail. Additionally, reflexes that would require brain control are also abolished. For example, the palpebral (elicited by touching eyelashes or inner or outer canthus of the eye), corneal (elicited by touching the cornea) and pupillary (elicited by focusing bright light into the pupil) reflexes and response to external stimuli including pain (e.g. nose prick) are also abolished during the period of unconsciousness (Anil, 1991).

Ineffective head-only electrical stunning can occur for various reasons (e.g. low current, poor tong position, poor electrical contact between the stunning tongs and head of the animal, dirty or corroded stunning tongs, lack of pressure applied during stunning, slipping of tongs during stun application, incorrect area of application) and, as a consequence, the animal may not experience the generalised epileptiform activity required to achieve unconsciousness, leading to different behavioural manifestations and retention of reflexes (Anil *et al*., 1997). This situation can be recognised from the failure to collapse, the absence of tonic–clonic seizures and the presence of breathing (including laboured breathing), and, in extreme cases, animals may also vocalise. Ineffectively stunned animals and those recovering consciousness will show also spontaneous blinking or positive eye reflexes (palpebral, corneal and pupillary). Head righting (attempt to raise head) after stunning and body arching during bleeding are also signs of consciousness.

Effectively stunned animals, i.e. unconscious animals, are bled out by inserting a knife through the thoracic inlet and cutting the brachiocephalic trunk, causing rapid onset of brain death (Wotton and Gregory, 1986; Anil *et al*., 2000). Prompt and accurate sticking of effectively stunned animals results in rapid onset of brain death, and therefore animals do not show signs of recovery of consciousness at any key stages of monitoring. In addition, depending upon the stun-to-stick interval, the duration of tonic–clonic seizures may be reduced as a result of onset of death. This means that, if stunning has been effective, and the duration of unconsciousness induced by the stunning method is longer than the sum of time between the end of stunning and sticking (stun-to-stick interval) plus the time it takes for animal to die through blood loss, the animal will remain unconscious until death occurs. On the other hand, ineffective stunning or prolonged stun-to-stick interval and/or inappropriate/inadequate sticking will lead to animals showing signs of recovery of consciousness during bleeding. These signs include recovery of breathing manifested as regular gagging, recovery of muscle tone manifested as righting reflex, vocalisation, response to nose prick or ear pinch and positive corneal reflex.

1.4. Physiology of carbon dioxide stunning

Exposure of pigs to high concentrations of carbon dioxide (> 80 % by volume in air) leads to metabolic acidosis (reduction in blood pH) and, as a consequence, significant reduction in the pH of the cerebrospinal fluid bathing the brain, which, in turn, induces gradual loss of consciousness and sensibility through inhibition of the spontaneous brain activity (Rodriguez *et al*., 2008). The neurophysiological basis and the consequences of brain inhibition are well documented in the scientific literature (see EFSA, 2004, report for details). The survival time of different regions of the brain and the spinal cord following exposure to carbon dioxide varies and, as a consequence, the clinical signs observed at the exit of the gas chamber are also expected to vary according to the concentration of carbon dioxide and the duration of exposure to the target concentration. It is worth noting that a prolonged exposure to high concentration of carbon dioxide (>80 % by volume in air) would be necessary to prevent recovery of consciousness and sensibility during shackling, hoisting, sticking and bleeding (Rodriguez *et al*., 2008; Llonch *et al*., 2012). Under batch or group stunning situations, the duration of unconsciousness and insensibility becomes more critical because the time interval between the end of exposure to gas mixture and sticking (stun-to-stick interval) would be considerably longer for the last animal in a group (Raj, 1999).

The earliest sign of onset of unconsciousness and insensibility during exposure of pigs to high concentrations of carbon dioxide is the loss of posture followed by onset of convulsions. Field observations of gas stunning suggest that it may not always be possible to determine the exact time to loss of posture as pigs start to convulse prior to loss of posture (frequently described as the excitation phase). However, as exposure to a gas mixture continues, these convulsions stop, leading to a complete loss of muscle tone. There is also a suppression of respiration, which can be evidenced from progressively declining rate and depth of breathing, resulting in complete cessation of any respiratory activity, including gagging at the exit of the chamber (Raj, 1999). Other signs of unconsciousness induced by exposure to high concentrations of carbon dioxide include fixed eyes, dilated pupil, absence of the palpebral (elicited by touching eyelashes or inner or outer canthus of the eye), corneal (elicited by touching the cornea) and pupillary (elicited by focusing bright light into the pupil) reflexes and absence of response to painful stimuli such as nose prick or ear pinch (Raj, 1999; Rodriguez *et al*., 2008). In addition, Rodriguez *et al.* (2008) reported that 82 % of the animals exposed to a high concentration of carbon dioxide did not show a corneal reflex at the exit of the stunner. However, the remaining 18 % of the animals lost corneal reflex only 18 seconds after the exit from the stunner.

Ineffective or unsuccessful stunning can occur for various reasons, for example low carbon dioxide concentration or short exposure time. As a consequence, the animal may not suffer inhibition of the brain to the magnitude required to achieve unconsciousness and insensibility, leading to different behavioural manifestations and retention of reflexes. This situation can be recognised in extreme cases from vocalisation and retention of some muscle tone and, as a consequence, failure to collapse or attempt to regain posture. Ineffectively stunned animals and those recovering consciousness will show breathing, spontaneous blinking or positive eye reflexes (palpebral, corneal and pupillary) and vigorous kicking, especially of the hind legs. Head righting (attempt to raise head) after stunning and body arching during bleeding are also signs of consciousness.

Effectively stunned animals, i.e. unconscious and insensible pigs, are bled out by inserting a knife through their thoracic inlet and cutting the brachiocephalic trunk. Prompt and accurate sticking of effectively stunned animals results in rapid onset of death, and therefore animals do not show signs of recovery of consciousness/sensibility at any key stages of monitoring. This means that when stunning has been effective, and the duration of unconsciousness induced by the stunning method is longer than the sum of time between the end of stunning and sticking (stun-to-stick interval) plus the time it takes for animal to die through blood loss, the animal will remain unconscious until death occurs. On the other hand, ineffective stunning or prolonged stun-to-stick interval and/or inappropriate/inadequate sticking will lead to animals showing signs of recovery of consciousness during bleeding (Raj, 1999). These signs include recovery of breathing manifested as regular gagging, recovery of muscle tone manifested as the righting reflex, positive corneal reflex, vocalisation and response to nose prick or ear pinch.

2. Materials and methods

2.1. Indicators and criteria for selection of the indicators

The mandate requests EFSA to select **indicators A**, designed to detect signs of consciousness in the animals after stunning.

For the sake of clarity and consistency, indicators checking the state of consciousness and unconsciousness will be used in this opinion instead of indicators A, as shown in Table 1.

The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in animals following stunning, the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the indicators were phrased neutrally (e.g. posture) and the outcomes were phrased either suggesting unconsciousness (e.g. immediate collapse) or suggesting consciousness (e.g. no collapse/attempts to regain posture). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses.

Table 1: Correspondence between indicators suggested in the ToR of the mandate and indicators proposed in this scientific opinion

Species	Method	Key stage	Indicators		
			Indicators from mandate's ToRs	**Checking state of**	**Outcome related to animal welfare**
Pigs	Stunning with head-only electrical method or carbon dioxide at high concentration	Key stage 1 = A immediately after stunning until shackling		Consciousness and unconsciousness	Consciousness
		Key stage 2 = A during sticking		Consciousness and unconsciousness	Consciousness
		Key stage 3 = A during bleeding		Consciousness and unconsciousness	Consciousness

The indicators investigated in this opinion were selected based on previous EFSA opinions (2004, 2006) and amended in Working Group discussion on the basis of feedback from (i) a stakeholder meeting at which interested parties were consulted by means of a questionnaire (referred to in this opinion as questionnaire 1), (ii) a systematic literature review, (iii) an online survey of experts involved in monitoring of welfare at slaughter or neck cutting in the form of a questionnaire (questionnaire 2), (iv) public consultation on the scientific opinion on bovines and (v) a technical meeting with selected experts. Their suitability for inclusion in a monitoring system was determined during Working Group discussions on the basis of their sensitivity and specificity, and their feasibility for use at different key stages of the slaughter process.

2.1.1. Feasibility

The feasibility of an indicator is considered in relation to physical aspects of its assessment. These include, for example, the position of the animal relative to the assessor, the assessor's access to the animal and the line speed. Feasibility for the purpose of this opinion does not include economic aspects. It is very likely that the feasibility of assessing an indicator is influenced by the key stage of the slaughter process, i.e. after stunning, at sticking and during bleeding animals can be in different positions and proximity relative to the assessor, which may affect how easily the indicator can be used.

2.1.2. Sensitivity and specificity

The use of animal-based indicators is similar to the use of a diagnostic or statistical test with either a positive or negative outcome. The performance of a test (i.e. the indicator) is usually described by its sensitivity and specificity. The estimation of sensitivity and specificity requires a definition of what can be considered a positive or negative outcome of checking for an indicator. The definitions of sensitivity and specificity of indicators differ depending on whether they are used in situations where animals are slaughtered with stunning or without stunning.

2.1.2.1. Sensitivity and specificity during slaughter with stunning

When monitoring the effectiveness of the stunning, in order to safeguard animal welfare, it is of major interest to detect those animals that are not properly stunned or recover consciousness after stunning. A positive outcome of the checked indicator is that based on which the animal is considered conscious. A negative test outcome of the indicator is that based on which the animal is considered not conscious (i.e. animal is considered unconscious).

Sensitivity is thus calculated as the number of truly conscious animals considered conscious based on the outcome of the indicator (A in Table 2) divided by the number of all conscious animals (A + C), multiplied by 100 (in short, sensitivity is the percentage of truly conscious animals that the indicator tests as conscious).

Specificity is calculated as the percentage of truly unconscious animals (B + D) that the indicator does not test as conscious (D).

Table 2: Sensitivity and specificity of indicators during slaughter with stunning

Slaughter with stunning		Truth: the animal is conscious?	
		Yes	No
Is the animal considered conscious, based on the outcome of the indicator?	Yes	**A**	B
	No	C	**D**

An indicator for slaughter with prior stunning is considered to be 100 % sensitive if it detects all the conscious animals as conscious; an indicator is considered to be 100 % specific if it detects all the unconscious animals as unconscious.

2.2. Establishing the ability of the indicators to detect welfare problems at slaughter

2.2.1. Stakeholder meeting and questionnaire 1

A stakeholder meeting was held on 30 January 2013 in order to inform all interested parties about this mandate. The meeting was opened to participants from all EU Member States representing research groups, FBOs licensed to own premises to slaughter animals, animal welfare officers employed by the FBO, auditing companies, the European Commission, Member State Competent Authorities, members of EFSA's Stakeholders Consultative Platform and non-governmental organisations (NGOs) with proven experience in the field of humane slaughter. The meeting was an opportunity for the experts to exchange experience and information on the animal-based indicators most commonly used to check unconsciousness in pigs, during slaughter with stunning. More than 100 experts or persons claiming to be experts associated with the slaughter of animals participated in the meeting. Traditionally, animal welfare monitoring in slaughterhouses involves checking for unconsciousness or death, following the application of a stunning method. However, a questionnaire on the use of animal-based indicators to check for the state of consciousness and unconsciousness at slaughter was distributed to all participants. The questionnaire asked about (i) the indicators that are mostly used and their use in combinations; (ii) the timing of the assessment of unconsciousness and death based on such indicators; (iii) the problems encountered during the assessment (feasibility of the indicators); and (iv) the respondent's opinion of the reliability of the indicators. The participants were also asked to suggest names of experts with practical knowledge in the field of slaughter to be contacted for the subsequent online survey (section 2.2.3).

2.2.2. Systematic literature review

A systematic literature review was conducted in order to summarise the currently available data describing the sensitivity and specificity of indicators checking the state of consciousness and unconsciousness or life and death for all stun-kill methods and species combinations (O'Connor *et al.*, 2013). Traditional animal welfare monitoring in slaughterhouses involves checking for outcomes of unconsciousness, following the application of a stunning method. Therefore, in order to obtain information on sensitivity and specificity, a systematic review was conducted of studies in which outcomes of unconsciousness and outcomes of death were measured using EEG. In such studies, the indicators of interest (e.g. no corneal reflex, no breathing, loss of posture) were tested against the results of EEG (e.g. a stunned animal does not show a corneal reflex and its unconsciousness is confirmed by EEG).

2.2.3. Questionnaire 2 (online survey)

In addition, an online survey was launched using a questionnaire to gather subjective opinion from experts with knowledge and experience in stunning and slaughtering of animals. The conduct of the survey was outsourced to an external communication company and its final technical report can be found on EFSA's website (Sellke, 2013). The survey was structured on the basis of the results from the questionnaire distributed at the stakeholder meeting held on 30 January 2013 and was addressed to approximately 160 participants. In order to avoid confusion, the assessments of feasibility, sensitivity and specificity of the indicators were presented in separate sections of the questionnaire. The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in animals following stunning, the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the selected indicators were phrased neutrally (e.g. posture) and the outcomes were phrased positively, suggesting unconsciousness (e.g. immediate collapse), or negatively, suggesting consciousness (e.g. no collapse/attempts to regain posture). This approach is commonly used in animal health studies (e.g. testing for the presence of a disease) but very new to animal welfare monitoring in slaughterhouses.

Regarding feasibility, for each species and method, questions were asked on how easily the indicators are applied and checked at each key stage of the stunning and slaughter process. For each key stage the feasibility ratings were computed into a feasibility score across all respondents that weighed the proportion of ratings easy against the proportion of ratings difficult as presented in the equation below:

Feasibility score = (No of 'easy' respondents – No of 'difficult' respondents)/No of all respondents

For example, in the case of data distribution of easy = 3, normal = 6 and difficult = 1, the score would be +0.2, i.e. (3 – 1)/10.

The resulting score was between +1 and –1 and covers the median rating as well as the tendency across all ratings, thus providing an overview of the distribution of the data and associated variability.

In addition, the survey asked respondents to assess the sensitivity and specificity of the indicators. This information was elicited by asking respondents to estimate, for each indicator, the proportion of truly conscious and the proportion of truly unconscious animals that would be considered conscious, based on the outcome of the indicator (i.e. A and B in Table 2). Sensitivity and specificity were estimated across all respondents using either the direct or weighted average of individual data values. The weights are provided by the uncertainty rating assigned by each respondent to every answer, which ranged between 1 and 3 (1 = 'not sure', 2 = 'rather sure', 3 = 'very sure'). Prior to calculations, the data were closely examined for consistency and corrected according to the following rules: answers associated with the uncertainty rating 'do not know' were excluded (e.g. 8/212 for electrical stunning); if the uncertainty rating was omitted, answers were re-set to the lowest uncertainty weight (i.e. 1 = 'not sure'; 12/213); if a respondent's answer to a sequence of questions reversed the logic (i.e. "2 % of truly unconscious animals will not show eye movements") then the corresponding values in the data record were reversed as '100 % minus rating' (35/213). Ratings were not reversed if variability across the respondents was too large to conclude logical inconsistency. In total, 213 and 185 individual ratings were evaluated for electrical and gas stunning respectively.

2.2.4. Working Group discussions

The outcomes of all previous activities were assessed and discussed within the Working Group of experts developing this scientific opinion. In addition, a technical meeting with a group of external experts (five academics, two from NGOs, one representative from the poultry industry, one representative from the red meat industry and two representatives from European Commission (EC)) was held on 3 September 2013. During the meeting the results obtained during the preceding activities of the Working Group were discussed, with the aim of advising the Working Group on the content of the toolboxes for head-only electrical stunning and carbon dioxide stunning. The experts invited to this meeting had previous access to the draft opinion, including the toolbox of indicators, and were asked to give their comments. During the meeting various presentations were given to stimulate discussion. A public consultation on the draft scientific opinion was also held during August–September 2013 (EFSA AHAW Panel, in press).

2.3. Developing the sampling protocol

In order to develop a monitoring procedure for slaughter with stunning, the mandate from the Commission requests EFSA to estimate the optimal frequency with which animals should be checked for signs of consciousness following stunning. This sampling frequency should take into account risk factors associated with the stunning procedure. For the optimal sampling fraction (or sampling frequency) to be calculated, at least two components need to be quantified: first, the highest proportion of insufficiently stunned animals that may be considered acceptable; and, second, the quantitative effects of the risk factors (individually or in combination) on the frequency of ineffective stunning.

Both components are problematic. Regarding the level of acceptability, the legislation specifies that no animals should show signs of consciousness following stunning. All animals should be stunned properly, and therefore the threshold level for the acceptability of ineffective stunning is zero. The second component requires a large number of data on the interactive effects of risk factors on stunning effectiveness, given a wide range of circumstances under which animals are stunned in European abattoirs. These data are not available.

However, it is possible to model the relationship between the fraction of slaughtered animals sampled and the minimum proportion of ineffectively stunned animals that will be detectable using a certain sampling protocol. Understanding this relationship allows the risk manager (and others concerned) to relate the economic and other costs associated with a particular sample size to the benefits associated with improved detection levels (i.e. improved animal welfare).

2.3.1. The statistical background of the model

The relationship can be modelled using existing approaches for process monitoring (e.g. continuous quality assurance regarding threshold failure rate in computer chip production). Although the statistical relationship is identical to those applied in planning disease surveillance, the related terminology (e.g. design prevalence) was considered less appropriate for addressing the issue of mis-stunned animals and therefore this text adheres to the terminology of failure management. For the statistical model, we used the following parameters:

1. Threshold failure rate for proportion of mis-stunned animals. This specifies the minimum proportion of animals that are ineffectively stunned which will still be detected by the sampling protocol.

2. Sensitivity of the indicators. As defined previously, this is the percentage of truly conscious animals detected as conscious by the indicator.

3. Slaughter population. This is the total number of animals slaughtered under the same circumstances as determined by risk factors (see Table 7). Note that the slaughter population is independent of the line speed, and can cover a period of minutes, hours or even days.

4. Sampling fraction. This is the proportion of the slaughter population which is assessed in the sampling protocol.

5. Accuracy of the sampling protocol. This is the percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if the number of ineffectively stunned animals was higher than the prescribed threshold failure rate would allow.

Please note that for the electrical or carbon dioxide stunning situations, specificity is not considered for the purposes of this model, as the specificity of an indicator is not related to the risks associated with reduced welfare.[7]

[7] It should be noted that a low specificity of the indicator, although not representing an animal welfare issue, definitely represents an issue from a FBO perspective. An indicator with low specificity would more often misclassify unconscious

Given these parameters, the details of the monitoring protocol can be calculated from Equation 1 (Cannon, 2001).

$$SF = \frac{n}{SP} \cong \frac{\left(1-(1-A)^{1/(SP \cdot FR)}\right) \cdot (SP - 0.5(A(SP \cdot FR) - 1)}{ISe}$$

Where:

A = requested accuracy of the sampling protocol
FR = standard threshold failure rate
ISe = indicator sensitivity
n = number of animals tested
SF = sample size or sampling fraction
SP = slaughter population

The objective was to use Equation 1 to estimate the threshold failure rate (FR) associated with a given sampling fraction. However, Equation 1 cannot be solved for the FR in an algebraic way. For this reason, it was necessary to solve the equation numerically. For the purpose, the R[8] function 'uniroot' was used.

Solving Equation 1 numerically, it was then possible to determine the minimum detectable FR associated with each SF value. The results could then be plotted in a diagram (see Figure 1). Once the relationship is formalised, it is also possible to read the results the other way round, i.e. to estimate what is the minimum SF needed to detect a given threshold FR, with a given accuracy, accounting for the indicator sensitivity and the slaughter population.

animals as conscious. Obviously, this represents a problem from a FBO perspective as an unnecessary corrective action must be taken, entailing a waste of money and time.

8 R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

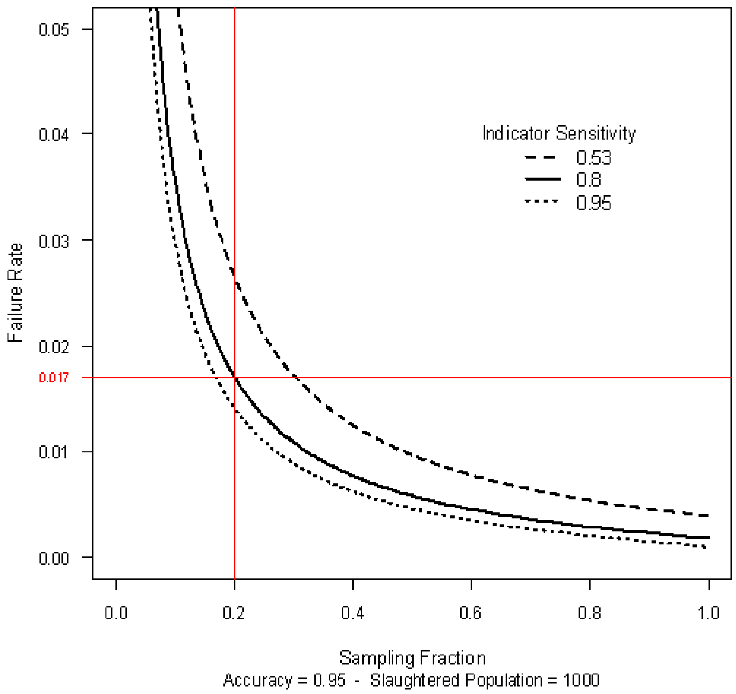


Figure 1: Example graph of the relationship between the parameters defining a sampling protocol (SF and detectable threshold FR for fixed values of accuracy (here 95 %) and slaughter population (here 1000 animals) and various scenarios for indicator sensitivity)

In Figure 1, a slaughter population of 1 000 animals and a required accuracy of 95 % are assumed. The red horizontal and vertical lines on the diagram form the basis for the following illustration: using an indicator with a sensitivity of 80 % (solid line), a sampling fraction of 20 % (i.e. sample size of 200 animals from a slaughter population of 1 000 animals) will be able to detect, with 95 % accuracy, a threshold failure rate of 2 % (i.e. more than 20 conscious animals out of 1 000 animals slaughtered in this example) or greater. The dotted lines illustrate how this relationship changes with indicators of varying sensitivity.

Different scenarios were considered assuming alternative model parameters for the specification of the sampling protocol. In detail the following scenarios were considered:

- accuracy: 0.90, 0.95, 0.99
- slaughter population: 100, 1 000, 10 000
- test sensitivity: 0.5, 0.75, 1

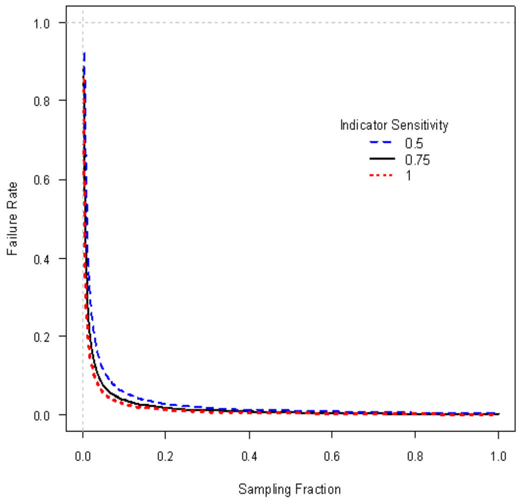
In order to compare the impact of these three parameters on the relationship between the threshold FR and the SF, the other two of them were set at fixed values. Then combinations of FR and SF were evaluated, to identify those that would trigger an alarm with the required accuracy, and those that would not. These critical combinations constitute the line graph exactly representing the desired accuracy level, e.g. in Figure 1. All 3×3 combinations were explored. Further details about the calculations can be found in the SAS Technical Report (EFSA SAS Unit, 2013).

2.3.2. The resulting model for the sampling protocol

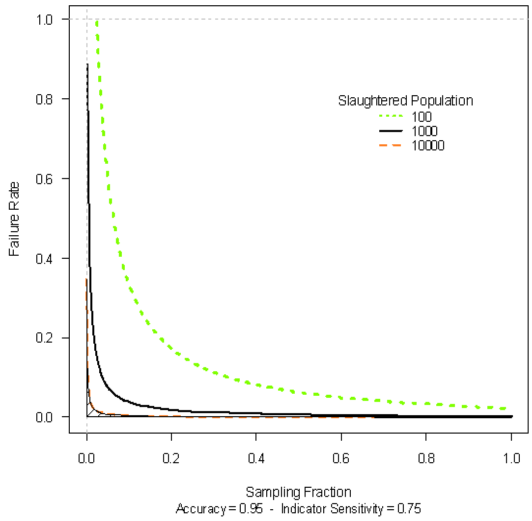
The results of the statistical modelling are summarised in Figure 2.

Using the five parameters of the model presented in Equation 1, it is possible to calculate each of them if the other four are specified. To illustrate the influence of the different parameters, the full range of threshold FR[9] and SF were combined with (a) the sensitivity of the indicator, (b) the slaughter population of the slaughterhouse[10] and (c) the desired accuracy of the sampling protocol,[11] whilst keeping the other two parameters constant. The impacts of different indicator sensitivity, slaughter population and accuracy values are presented in Figures 2a, b and c.

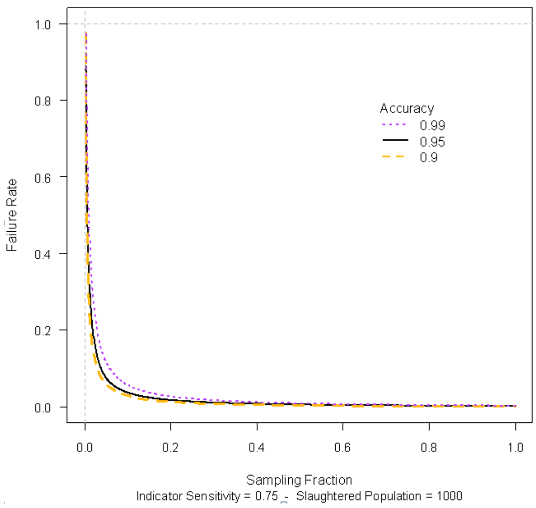
[9] Proportion of mis-stunned animals (see section 2.3.1).
[10] The total number of animals being stunned during a given period according to the type of the slaughterhouse and the species slaughtered (see section 2.3.1).
[11] Percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed failure rate would allow (see section 2.3.1).



(a) The effect of SF on threshold FR for three levels of indicator sensitivity (0.5, 0.75, 1), given a slaughter population of 1 000 animals and an accuracy of 0.95



(b) The effect of SF on threshold FR for three levels of slaughter population (100, 1000, 10 000) and accuracy (c), given an accuracy of 0.95 and indicator sensitivity of 0.75



(c) The effect of SF on threshold FR for three levels of accuracy (0.9, 0.95, 0.99), given a slaughter population of 1 000 animals and indicator sensitivity of 0.75

Figure 2: The effect of SF on threshold FR for three levels of indicator sensitivity (a), slaughter population (b) and accuracy (c), given a slaughter population of 1 000 animals (a, c), an accuracy of 0.95 (a, b) and an indicator sensitivity of 0.75 (b, c). Each *x*–*y*-coordinate in the diagrams represents one possible particular sampling protocol

Those sampling protocols that fall below the line describing that combination of parameters will not be able to meet the purpose of detecting if threshold FR is exceeded; those protocols above the line graph will meet the required purpose and raise an alarm.

Table 3a, b and c shows numerical examples of threshold failure rates for three levels of indicator sensitivity, sample fraction and sampling protocol accuracy.

Table 3: The effect of SF on threshold FR for three levels of (a) indicator sensitivity, given a slaughter population of 1 000 animals and accuracy of 0.95; (b) slaughter population, given an accuracy of 0.95 and indicator sensitivity of 0.75; and (c) accuracy, given a slaughter population of 1 000 animals and indicator sensitivity of 0.75

(a) The effect of SF on threshold FR for three levels of indicator sensitivity (0.5, 0.75, 1), given a slaughter population of 1 000 animals and accuracy of 0.95

Sampling fraction	**Threshold failure rate**		
	Indicator sensitivity = 0.5	**Indicator sensitivity = 0.75**	**Indicator sensitivity = 1**
0.1	0.058	0.038	0.028
0.2	0.028	0.018	0.013
0.3	0.018	0.012	0.008
0.4	0.013	0.008	0.006
0.5	0.01	0.006	0.004
0.6	0.008	0.005	0.003
0.7	0.007	0.004	0.002
0.8	0.006	0.003	0.002
0.9	0.005	0.003	0.001
1	0.004	0.002	NA

(b) The effect of SF on threshold FR for three levels of slaughter population (100, 1 000, 10 000 animals), given an accuracy of 0.95 and indicator sensitivity of 0.75

Sampling fraction	**Threshold failure rate**		
	***n* = 100**	***n* = 1 000**	***n* = 10 000**
0.1	0.34	0.04	0
0.2	0.17	0.02	0
0.3	0.11	0.01	0
0.4	0.08	0.01	0
0.5	0.06	0.01	0
0.6	0.05	0.01	0
0.7	0.04	0	0
0.8	0.03	0	0
0.9	0.03	0	0
1	0.02	0	0

(c) The effect of SF on threshold FR for three levels of accuracy (0.9, 0.95, 0.99), given a slaughter population of 1 000 animals and indicator sensitivity of 0.75

Sampling fraction	Threshold failure rate		
	Accuracy = 0.9	Accuracy = 0.95	Accuracy = 0.99
0.1	0.029	0.038	0.058
0.2	0.014	0.018	0.028
0.3	0.009	0.012	0.018
0.4	0.006	0.008	0.013
0.5	0.005	0.006	0.01
0.6	0.004	0.005	0.008
0.7	0.003	0.004	0.006
0.8	0.003	0.003	0.005
0.9	0.002	0.003	0.004
1	0.002	0.002	0.003

3. Results

3.1. Results from stakeholder meeting

From the stakeholder meeting held on January 30 2013, about 60 completed questionnaires were collected. Most of the experts provided information for more than one species and method: the total number of answers and the most used signs of unconsciousness in pigs are reported in Table 4.

Table 4: Total number of answers and the outcomes of unconsciousness of indicators most used for pigs as collected through questionnaire 1 of the stakeholder meeting

Species/method	Total No of answers	Outcome of unconsciousness of most used indicators[12]
Pigs—head-only electrical stunning	46	Immediate onset of tonic seizures, followed by clonic seizures Immediate collapse Immediate and sustained absence of rhythmic breathing
Pigs—carbon dioxide stunning	23	No corneal reflex Completely relaxed body No attempts to raise the head

Experts responded that they observe the outcomes of the indicators between 10 and 30 seconds after stunning or after sticking. The main problem encountered in checking most of the indicators is access to the animal. Several indicators are normally used by the experts to assess the state of unconsciousness in animals. However, there was no harmonised list of indicators, either species or method specific, or scientific rationale.

3.2. Results from systematic literature review

No studies on head-only electrical stunning in pigs met the criteria set for the systematic review. Two studies used EEG-based measures of unconsciousness in pigs exposed to high (90 %) concentrations of carbon dioxide (CO_2) and indicators under investigation (Martoft *et al*., 2002; Llonch *et al*., 2013). Llonch *et al*. (2013) described the proportion of animals with muscular excitation, gasping or vocalisation as a proportion of all animals exposed to 90 % CO_2. The authors also reported that

[12] Indicators used to check the state of consciousness and unconsciousness.

rhythmic breathing, the corneal reflex and sensitivity to pain disappeared by the end of the gas exposure.

Martoft *et al*. (2002) reported the proportion of animals with a positive pinch response at the end of gassing (60 seconds). However, the denominator was the number of animals gassed rather than the number of unconscious animals (as confirmed by EEG). The authors used two possible definitions of unconsciousness: time to lowest depth of anaesthesia and time to lowest mean 95 % spectral edge. However, no measures of dispersion were reported and it was not possible to determine if it was appropriate to assume that all pigs were 'unconscious' at the end of the 60-second gassing period.

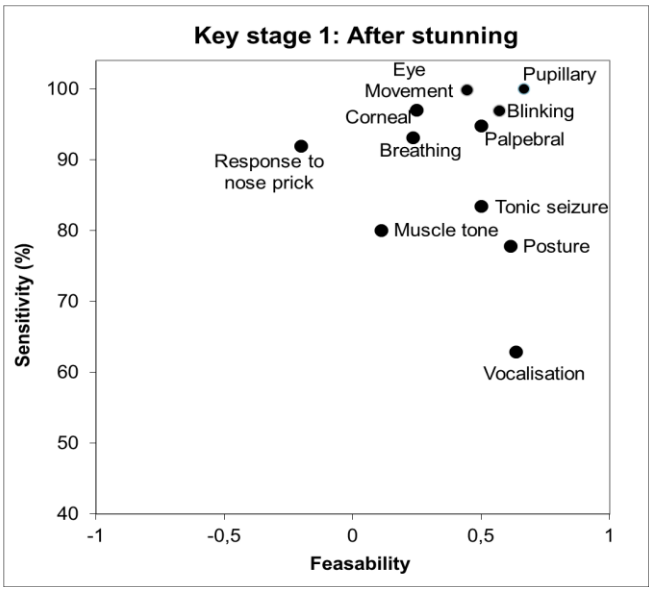
As a result, no data from these studies could be used to calculate individual level or group sensitivity and specificity.

3.3. Results from questionnaire 2 on head-only electrical stunning

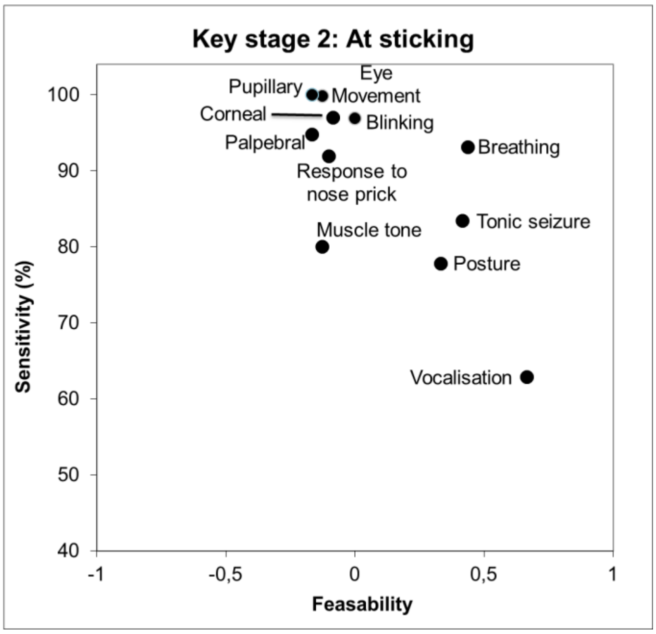
From the second questionnaire, namely the online survey, answers from around 82 experts were collected. Respondents could answer for more than one species or method, depending upon their work experience, so the total number of completed surveys was 84.

In total, 35 respondents said that they monitor welfare of pigs following stunning. Of these, 19 experts responded to the survey on head-only electrical stunning but 16 experts answered only the feasibility as well as the sensitivity/specificity question, thus providing valid answers suitable for analysis (see section 2.2.3).

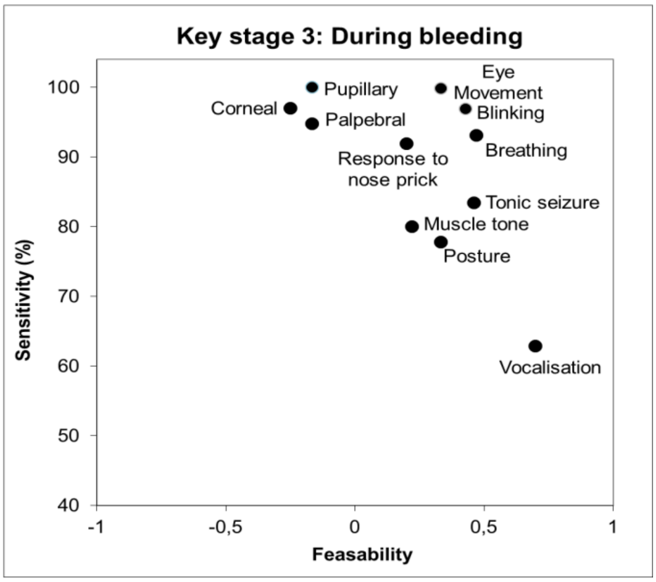
The graphs in Figure 3a, b and c combine the feasibility score and estimate of sensitivity for each indicator for head-only electrical stunning at each key stage (key stage 1 = immediately after stunning, key stage 2 = at sticking, key stage 3 = during bleeding). Thus, indicators nearest the top-right corner have high sensitivity and high feasibility. In all three graphs the sensitivity value is identical but the feasibility score changes according to the respondent ratings.



(a) Immediately after stunning



(b) During sticking



(c) During bleeding

Figure 3: Graphical combination of feasibility score and sensitivity resulting from questionnaire 2 for each indicator at (a) key stage 1 = immediately after stunning, (b) key stage 2 = during sticking and (c) key stage 3 = during bleeding

3.4. Description of indicators for head-only electrical stunning and overview of their performance

The combined efforts of the above activities led to the following overview of indicators and outcomes of consciousness and unconsciousness.

The following paragraphs discuss the indicators and their outcomes mentioned above in relation to their relevance in identifying consciousness at key stages of monitoring slaughter with head-only electrical stunning. Some of these outcomes occur spontaneously following stunning (e.g. collapse of the animal without regaining posture, tonic–clonic seizure or apnoea) and sticking whereas some other outcomes will have to be intentionally provoked (e.g. the corneal reflex). The Working Group agreed that the risk of poor welfare can be detected better if animal welfare monitoring is focused on detecting consciousness. The presence of certain outcomes (e.g. vocalisation) or a positive response of the animal to applied stimulus (e.g. the corneal reflex) is most relevant. In addition to this, the sensitivity, specificity and feasibility of the indicators are presented, based on information gathered in the different activities described in this opinion. Depending on all these aspects, some indicators may not be applicable to monitoring at certain key stages.

3.4.1. Posture

3.4.1.1. Description

In key stage 1 (i.e. after stunning), unconsciousness is manifested as **immediate collapse** (indication of unconsciousness) of the animal and, if the stunning is ineffective, the animal will either **fail to collapse** or **attempt to regain posture** (indication of consciousness). Pigs showing these signs of ineffective electrical stunning will require immediate re-stun.

Depending upon the stunning system used, either an effectively stunned pig will be shackled, hoisted and presented for sticking (bleeding or slaughter) or sticking will be performed in pigs lying horizontally on a conveyor, which is key stage 2. An unconscious pig at this stage will be in tonic–clonic seizure on the overhead shackle or lying on the conveyor and is therefore not expected to show any changes in its **posture.** Hence posture as an indicator is **not applicable** (n.a.) in this situation. In contrast, a pig recovering consciousness whilst hanging on the overhead shackle or lying on a conveyor will **attempt to regain posture**, which will be manifested as **arching of the neck or body**; such an animal will have to be re-stunned.

3.4.1.2. Feasibility

In questionnaire 2, immediate collapse was rated as an indicator that is easy or normal to assess at stunning (key stage 1) by 62 % and 38 % respectively (*n* = 13).

3.4.1.3. Sensitivity and specificity

The positive outcome of the indicator ‘posture’ is the sign of consciousness, namely the failure to collapse. Therefore, the sensitivity is the percentage of animals which maintain upright posture immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 78 % (*n* = 10). The specificity is calculated as the percentage of animals immediately losing posture, out of all truly unconscious animals. This was estimated to be 97 % (*n* = 13). The reason for the relatively low sensitivity is probably because immediate loss of posture without loss of consciousness might be present due to electro-immobilisation, which can be induced with a current lower than that necessary to induce generalised epileptiform activity in the brain.

3.4.2. Breathing

3.4.2.1. Description

In key stage 1, effective electrical stunning of pigs will lead to **immediate onset of apnoea** (absence of breathing), which can be used to monitor the effectiveness of stunning. Ineffective stunning can be recognised from the sustained/**presence of breathing, including laboured breathing**. In key stage 2, unconscious pigs will continue to manifest apnoea. In contrast, a pig recovering consciousness whilst lying on a conveyor or hanging on the overhead shackle will attempt to breathe, which may begin as **regular gagging leading to resumption of breathing**; such an animal will have to be re-stunned.

An effectively stunned and stuck pig will remain unconscious until death occurs in key stage 3 and therefore is not expected to show any signs of breathing. On the other hand, a pig recovering consciousness whilst hanging on the overhead shackle and bleeding will attempt to breathe, which may begin as **regular gagging leading to resumption of breathing**, and such an animal will have to be re-stunned.

3.4.2.2. Feasibility

Breathing was rated as easy or normal to assess at key stage 1 by 41 % in each case (*n* = 17); at key stage 2 by 50 % and 44 % (*n* = 16) respectively; and during key stage 3 by 53 % and 41% (*n* = 17) respectively. This is probably because it may not be possible to recognise breathing in animals shackled and hoisted on to the overhead rail. Also, occasional or irregular gagging may occur in unconscious animals just prior to the onset of brain death.

3.4.2.3. Sensitivity and specificity

The positive outcome of breathing is the sign of consciousness, namely the resumption of breathing. Therefore, the sensitivity is the percentage of animals which show presence of breathing, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 93 % (*n* = 13). The specificity is calculated as the percentage of animals showing apnoea, out of all truly unconscious animals. This was estimated to be 86 % (*n* = 17).

3.4.3. Tonic-clonic seizures

3.4.3.1. Description

In key stage 1, effective electrical stunning leads to the **onset of tonic–clonic seizures** soon after collapse, which may be recognised from the tetanus. The tonic seizure lasts for several seconds and is followed by clonic seizures lasting for seconds and leading to loss of muscle tone, usually before the animal is shackled, hoisted and presented for sticking, and, therefore, tonic seizure as an indicator is not applicable at key stages 2 and 3. However, tonic-clonic seizures may be present at sticking (key stage 2) if the stun-to-stick interval is short, especially when pigs are stuck (bled out) on a conveyor after exiting an automatic stunning system. It is also important to note that the intensity and duration of these seizures are affected by the waveform and frequency of electrical current used for head-only stunning of pigs.

3.4.3.2. Feasibility

From questionnaire 2, tonic-clonic seizures were rated as easy or normal to assess at key stage 1 by 56 % and 38 % of experts, respectively (*n* = 16). The reason for these low ratings could be that tonic muscle contractions would occur during the application of an electrical current across the head, irrespective of the outcome, i.e. consciousness or unconsciousness.

3.4.3.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of tonic seizures is the sign of consciousness, namely the absence of tonic-clonic seizures. Therefore, the sensitivity is the percentage of animals which do not show the onset of tonic-clonic seizures immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 83 % (*n* = 14). The specificity is calculated as the percentage of animals showing onset of tonic seizures, out of all truly unconscious animals. This was estimated to be 91 % (*n* = 16).

3.4.4. Muscle tone

3.4.4.1. Description

Effectively stunned animals will show tonic–clonic seizures followed by **loss of muscle tone**, which can be recognised from **floppy ears and relaxed jaw**. Animals which regain muscle tone may manifest **stiff (upright) ears and jaws and the righting reflex** (e.g. severe kicking, head lifting, body arching). These signs are more visible after sticking, when the animals are hanging from the overhead rail, especially at key stages 2 and 3. Animals showing any of these signs of muscle tone must be re-stunned.

3.4.4.2. Feasibility

From questionnaire 2, muscle tone was rated as easy to assess at key stage 1 by 22 % (*n* = 9), at key stage 2 by 13 % (*n* = 8) and during key stage 3 by 44 % (*n* = 9) of experts.

3.4.4.3. Sensitivity and specificity

The positive outcome of muscle tone, namely the presence of muscle tone, is the sign of consciousness. Therefore, the sensitivity is the percentage of animals which show a certain level of muscle tone, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 80 % (*n* = 7). The specificity is calculated as the percentage of animals showing loss of muscle tone, out of all truly unconscious animals. This was estimated to be 66 % (*n* = 8). The specificity is low because, after electrical stunning, animals exhibited muscle tone during the tonic–clonic seizure.

3.4.5. Response to nose prick or ear pinch

3.4.5.1. Description

Ineffective electrical stunning and recovery of consciousness can be recognised from the **response to nose prick or ear pinch** at all key stages of monitoring. Animals showing a positive response to painful stimulus at any stage must be re-stunned.

3.4.5.2. Feasibility

From questionnaire 2, response to nose prick or ear pinch was considered (*n* = 10) as easy or normal by only a minority of experts: 20 % and 40 % respectively at key stage 1; 40 % and 10 % respectively at key stage 2; and 50 % and 20 % respectively during key stage 3. Many (up to 50 %) of the experts reported that response to nose prick or ear pinch is difficult at any of the key stages of monitoring. Lack of access to animals could be one of the reason why response to a painful stimulus, i.e. nose prick or ear pinch, cannot be assessed during bleeding.

3.4.5.3. Sensitivity and specificity

The positive outcome of the response to nose prick or ear pinch is the sign of consciousness, namely the presence of a response to nose prick or ear pinch. Therefore, the sensitivity is the percentage of animals which do respond to nose prick or ear pinch immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 92 % (*n* = 9). The specificity is calculated as the percentage of animals showing no response to nose prick or ear pinch, out of all truly unconscious animals. This was estimated to be 91 % (*n* = 10).

3.4.6. Vocalisations

3.4.6.1. Description

Vocalisation is expected only in conscious animals and can be used as an indicator in all key stages of monitoring. However, not all the conscious animals may vocalise (e.g. electrically immobilised pigs), and hence absence of vocalisation does not always mean that the animal is unconscious. Animals showing vocalisation must be re-stunned. Since unconscious animals will not vocalise, this indicator is not applicable to monitoring unconsciousness.

3.4.6.2. Feasibility

From questionnaire 2, vocalisation was considered to be easy or normal to assess, respectively, at key stage 1 by 73 % and 18 %, at key stage 2 by 63 % and 9 %, and during key stage 3 by 72 % and 9 % of the experts (*n* = 11). Some experts reported that vocalisation is difficult to assess at all of the key stages, but the reason was not stated.

3.4.6.3. Sensitivity and specificity

The positive outcome of vocalisation is the sign of consciousness, namely the presence of vocalisation. Therefore, the sensitivity is the percentage of animals which do vocalise immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 63 % (*n* = 10). The specificity is calculated as the percentage of animals showing no vocalisation, out of all truly unconscious animals. This was estimated to be 91 % (*n* = 11). The reason for the low sensitivity is that vocalisation is a spontaneous behaviour and thus not all conscious animals may vocalise.

3.4.7. Eye movements

3.4.7.1. Description

In key stage 1, effective electrical stunning of pigs will produce **fixed eyes** (eyes wide open and glassy) or eyeballs may be obscured owing to rotation into the eye socket. Pigs that are not effectively stunned or those recovering consciousness will show **eye movements**, which can be used to recognise consciousness during all three key stages. Animals showing any eye movements must be re-stunned.

3.4.7.2. Feasibility

From questionnaire 2, eye movements were considered as easy to assess at key stage 1 (*n* = 9) by 44 %, at key stage 2 (*n* = 8) by 25 % and during key stage 3 (*n* = 9) by 44 % of the experts. Eye movements were rated as normal to assess at stunning (*n* = 9) by 56 %, at sticking (*n* = 8) by 38 % and during bleeding (*n* = 9) by 44 % of the experts. Eye movements as an indicator were also rated as difficult to assess at sticking (*n* = 8) by 38 % and during bleeding (*n* = 9) by 11 % of the experts. It may be difficult or impossible to observe eye movements at the time of sticking or bleeding because of the orientation of the animal, i.e. with the operator facing the brisket, at these two key stages.

3.4.7.3. Sensitivity and specificity

The positive outcome of eye movement is the sign of consciousness, namely the presence of eye movements. Therefore, the sensitivity is the percentage of animals which show eye movement immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 100 % (*n* = 7). The specificity is calculated as the percentage of animals showing fixed eyes, out of all truly unconscious animals. This was estimated to be 90 % (*n* = 9).

3.4.8. Palpebral reflex

3.4.8.1. Description

Effective electrical stunning leads to abolition of palpebral reflex. Effectively stunned and stuck animals show **no palpebral reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show **positive palpebral reflex** at any key stage. Animals showing a positive palpebral reflex must be re-stunned.

3.4.8.2. Feasibility

From questionnaire 2, the palpebral reflex was rated as easy and normal to assess at key stage 1 by 67 % and 17 % respectively, at key stage 2 by 33 % and 17 % respectively and during key stage 3 by 17 % and 50 % of experts respectively (*n* = 6). A number of experts considered the palpebral reflex as difficult to assess at stunning (17 %), at sticking (50 %) and during bleeding (33%); the reason for these ratings is unclear. It is possible to suggest that inaccessibility/lack of access to animals during bleeding has been taken into consideration for rating eye reflexes (palpebral and corneal reflexes and spontaneous blinking) as difficult.

3.4.8.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of palpebral reflex is the sign of consciousness, namely the presence of a palpebral reflex. Therefore, the sensitivity is the percentage of animals which show palpebral reflex immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 95 % (*n* = 5). The specificity is calculated as the percentage of animals showing no palpebral reflex, out of all truly unconscious animals. This was estimated to be 91 % (*n* = 6).

3.4.9. Corneal reflex

3.4.9.1. Description

Effective electrical stunning leads to abolition of corneal reflex. Effectively stunned and stuck animals show **no corneal reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show

positive corneal reflex at any key stage. Animals showing a positive corneal reflex must be re-stunned.

3.4.9.2. Feasibility

From questionnaire 2, the corneal reflex was rated (*n* = 12) as easy to assess at key stage 1 by 42 %, at key stage 2 by 33 % and during key stage 3 by 25 % of experts. The corneal reflex was rated as normal to assess at stunning by 42 %, at sticking by 25 % and during bleeding by 25 % of experts. A number of experts also considered the corneal reflex as difficult to assess at stunning (17 %), at sticking (42 %) and during bleeding (50 %), and the reason for these high ratings could be the inaccessibility of animals.

3.4.9.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of corneal reflex is the sign of consciousness, namely the positive corneal reflex. Therefore, the sensitivity is the percentage of animals which show corneal reflex immediately after stunning, out of all conscious animals. This was estimated by questionnaire 2 respondents to be 97 % (*n* = 11). The specificity is calculated as the percentage of animals showing no corneal reflex, out of all truly unconscious animals. This was estimated to be 84 % (*n* = 12).

3.4.10. Blinking

3.4.10.1. Description

Spontaneous blinking is expected only in conscious animals and can be used as an indicator at all key stages of monitoring. However, not all the conscious animals will show spontaneous blinking, and hence absence of blinking does not always mean that the animal is unconscious. Animals showing blinking must be re-stunned.

3.4.10.2. Feasibility

From questionnaire 2, spontaneous blinking was rated (*n* = 7) as easy to assess at key stage 1 by 57 %, at key stage 2 by 29 % and during key stage 3 by 43 % of experts. Spontaneous blinking was rated as normal to assess at stunning by 43 %, at sticking by 43 % and during bleeding by 57 % of experts. A number of experts also considered spontaneous blinking difficult to assess at sticking (29 %).

3.4.10.3. Sensitivity and specificity

The positive outcome of blinking is the sign of consciousness, namely the presence of spontaneous blinking. Therefore, the sensitivity is the percentage of animals which show spontaneous blinking immediately after stunning, out of all conscious animals. This was estimated by questionnaire 2 respondents to be 97 % (*n* = 7). The specificity is calculated as the percentage of animals observed to show no spontaneous blinking, out of all unconscious animals. This was estimated to be 89 % (*n* = 7).

3.4.11. Pupillary reflex

3.4.11.1. Description

Effective electrical stunning leads to abolition of pupillary reflex. Effectively stunned and stuck animals show **no pupillary reflex** at any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show **positive pupillary reflex** at any key stage. Animals showing a positive pupillary reflex must be re-stunned.

3.4.11.2. Feasibility

From questionnaire 2, pupillary reflex was rated (*n* = 5) as easy to assess at key stage 1 by 67 %, at key stage 2 by 17 % and during key stage 3 by 17% of experts. The pupillary reflex was rated as normal to assess at stunning by 33 %, at sticking by 17 % and during bleeding by 50 % of experts.

Some of the experts also considered the pupillary reflex as difficult to assess at sticking (50 %) and during bleeding (33 %). The main reason for these ratings could be the lack of access to the animal.

3.4.11.3. Sensitivity and specificity

The positive outcome of the pupillary reflex is the sign of consciousness, namely the positive pupillary reflex. Therefore, the sensitivity is the percentage of animals which show a pupillary reflex immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 100 % (n = 5). The specificity is calculated as the percentage of animals observed to show no pupillary reflex, out of all truly unconscious animals. This was estimated to be 74 % (n = 6).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 is presented in Table 5.

Table 5: Summary of the data from questionnaire 2 regarding sensitivity, specificity and feasibility of indicators and outcomes of consciousness after head-only electrical stunning of pigs

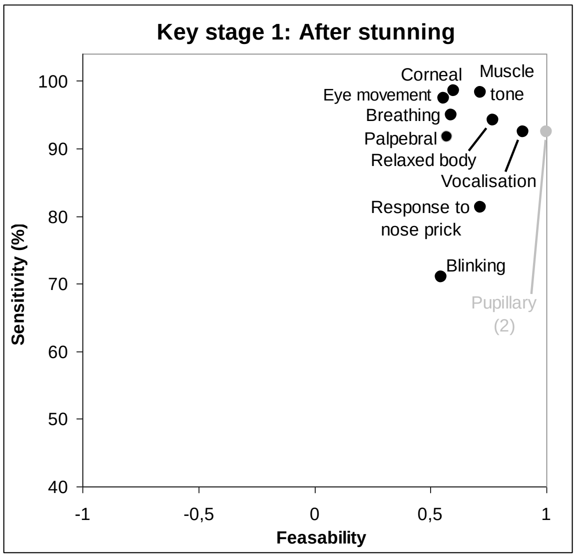
Indicators after head-only electrical stunning	Outcomes of consciousness	Sensitivity (%)	Data [(a)] (without uncertainty, average (20th, 50th and 80th percentiles)	Specificity (%)	Data [(a)] (without uncertainty, average (20th, 50th and 80th percentiles)	Feasibility		
						After stunning	At sticking	During bleeding
Posture [(b)]	Failure to collapse or attempt to regain posture	78	80 (74, 100, 100)	97	97 (94, 100, 100)	0.62	0.33	0.33
Breathing	Presence	93	92 (90, 98, 100)	86	85 (76, 95, 100)	0.24	0.44	0.47
Tonic/clonic seizures	No tonic/clonic seizures	83	79 (50, 99, 100)	91	89 (90, 100, 100)	0.50	0.42	0.46
Muscle tone	Righting reflex	80	74 (52, 100, 100)	66	71 (30, 95, 100)	0.11	–0.13	0.22
Response to nose prick or ear pinch	Presence	92	91 (88, 98, 100)	91	86 (58, 100, 100)	–0.20	–0.10	0.20
Vocalisations	Presence	63	58 (24, 55, 92)	91	89 (95, 100, 100)	0.64	0.67	0.70
Eye movements	Presence	100	100 (100, 100, 100)	90	84 (74, 98, 100)	0.44	–0.13	0.33
Palpebral reflex	Presence	95	96 (94, 100, 100)	91	87 (75, 94, 100)	0.50	–0.17	–0.17
Corneal reflex	Presence	97	97 (98, 100, 100)	84	80 (58, 96, 100)	0.25	–0.08	–0.25
Spontaneous blinking	Presence	97	96 (92, 100, 100)	89	89 (83, 95, 100)	0.57	0.00	0.43
Pupillary reflex	Presence	100	100 (100, 100, 100)	74	74 (40, 98, 100)	0.67	–0.17	–0.17

(a): For all data, n was between 5 and 17 respondents.
(b): In questionnaire 2, posture as an indicator was referred to its outcome of unconsciousness, namely ‘immediate collapse’.

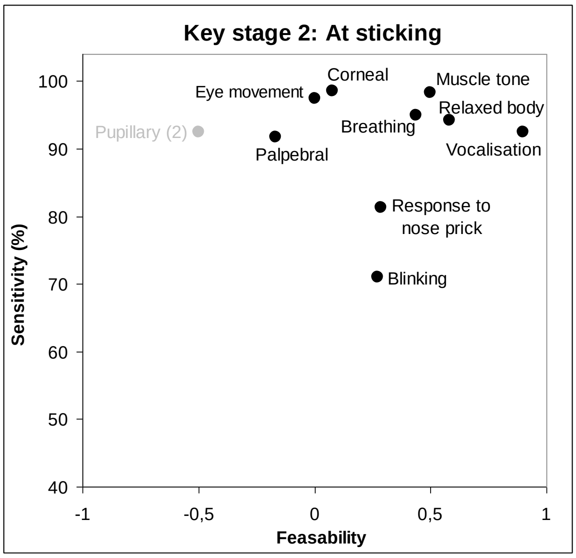
3.5. Results from questionnaire 2 on carbon dioxide stunning

From the second questionnaire, namely the online survey, 35 respondents said that they monitor welfare of pigs following stunning. Of these, 19 experts responded to the survey on carbon dioxide stunning, of whom 17 answered the feasibility as well as the sensitivity/specificity question, thus providing valid answers suitable for analysis (see section 2.2.3).

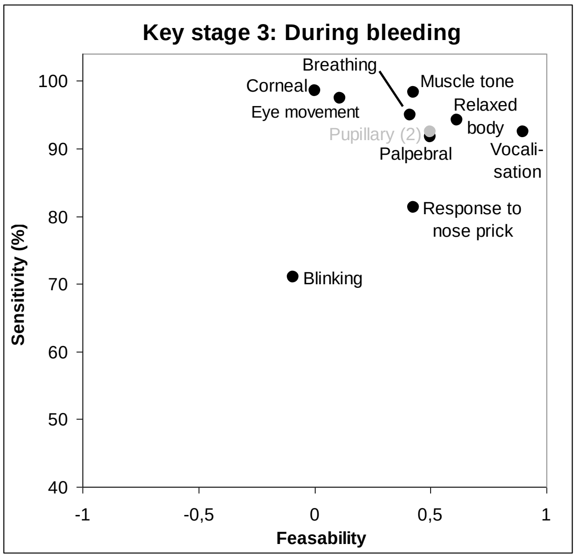
The graphs in Figure 4a, b and c combine the feasibility score and estimate of sensitivity for each indicator for carbon dioxide stunning at each key stage (key stage 1 = between end of stunning and sticking, key stage 2 = at sticking, key stage 3 = during bleeding). Thus, the indicators nearest the top-right corner have high sensitivity and high feasibility. In all three graphs the sensitivity value is identical but the feasibility score changes according to the respondent ratings.



(a) Immediately after stunning



(b) During sticking



(c) During bleeding

Figure 4: Graphical combination of feasibility score and sensitivity resulting from questionnaire 2 for each indicator at (a) key stage 1 = immediately after stunning, (b) key stage 2 = during sticking and (c) key stage 3 = during bleeding. Grey labelled indicators are based on a small number of respondents

3.6. Description of indicators for carbon dioxide stunning and overview of their performance

3.6.1. Posture

3.6.1.1. Description

In key stage 1 (i.e. between end of stunning and shackling), unconsciousness is manifested as **permanent collapse or loss of posture** (indication of unconsciousness) of the animal and, if the exposure to carbon dioxide is ineffective/inadequate, the animal will show **attempts to regain posture** (indication of consciousness). Pigs showing these signs of ineffective stunning will require immediate re-stun using a back-up method.

A pig that has been effectively stunned with carbon dioxide will be shackled, hoisted and presented for sticking, or bled out horizontally while lying on a conveyor, which is key stage 2. An unconscious pig at this stage will be hanging flaccidly on the overhead shackle or lying relaxed on the conveyor and is therefore not expected to show any changes in its **posture.** Hence posture as an indicator is **not applicable** (n.a.) in this situation. In contrast, a pig recovering consciousness whilst on a conveyor or hanging on the overhead shackle will **attempt to regain posture**, which will be manifested as **arching of the neck or body and/or convulsions**; such an animal will have to be re-stunned using a back-up method.

A pig that has been effectively stunned and stuck will remain unconscious during bleeding until death occurs in key stage 3 and therefore is not expected to show any change in **posture** (n.a). On the other hand, a pig recovering consciousness whilst hanging on the overhead shackle and bleeding will **attempt to regain posture**, which may be **manifested as arching of the neck or body and/or convulsions**, and such an animal will have to be re-stunned using a back-up method.

3.6.1.2. Feasibility

In questionnaire 2, completely relaxed body was rated as an indicator that is easy or normal to assess at key stage 1 by 85 % and 8 % respectively.

3.6.1.3. Sensitivity and specificity

The positive outcome of relaxed body is the sign of consciousness, namely the maintenance of some degree of muscle tone or manifestation of convulsions. Therefore, the sensitivity is the percentage of animals which show muscle tone or convulsions at the exit of the chamber, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 94 % (n = 11). The specificity is calculated as the percentage of animals observed to immediately lose posture, out of all truly unconscious animals. This was estimated to be 100 % (n = 13).

3.6.2. Muscle tone

3.6.2.1. Description

Effective stunning of pigs with carbon dioxide leads to **loss of muscle tone**, which can be recognised from **floppy ears and relaxed jaw and completely relaxed body**. These signs are more visible when the animals are hanging from the overhead rail. Muscle tone can be used at all three key stages. On the other hand, ineffectively stunned animals and those recovering consciousness at all key stages will retain or recover certain levels of **muscle tone,** manifested as **stiff (upright) ears and jaws, and**

righting reflex (e.g. severe kicking, head lifting, body arching). Animals showing any of these signs of muscle tone must be re-stunned using a back-up method.

3.6.2.2. Feasibility

From questionnaire 2, loss of muscle tone was rated as easy to assess at key stage 1 by 71 %, at key stage 2 by 43 % and during key stage 3 by 57 % of experts (*n* = 7). Loss of muscle tone was rated as normal at key stage 1 by 16 %, at key stage 2 by 43 % and at key stage 3 by 29 % of experts. One of the expert considered loss of muscle tone as not applicable at either key stage 2 (14 %) or key stage 3 (14 %). The main reason for these ratings could be the lack of access to animals.

3.6.2.3. Sensitivity and specificity

The positive outcome of muscle tone is the sign of consciousness, namely the retention of muscle or convulsions. Therefore, the sensitivity is the percentage of animals which show muscle tone or convulsions after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 98 % (*n* = 6). The specificity is calculated as the percentage of animals observed to show loss of muscle tone, out of all truly unconscious animals. This was estimated to be 100 % (*n* = 8).

3.6.3. Breathing

3.6.3.1. Description

In key stage 1, effectively stunned pigs can be recognised from the **absence of breathing** (apnoea). Ineffective/inadequate exposure to carbon dioxide can be recognised from the sustained/**presence of breathing, including laboured breathing**.

In key stage 2, unconscious pigs will continue to manifest apnoea. In contrast, a pig recovering consciousness whilst hanging on the overhead shackle will attempt to breathe, which may begin as **regular gagging before leading to resumption of breathing**; such an animal will have to be re-stunned using a back-up method.

An effectively stunned and stuck pig will remain unconscious until death occurs in key stage 3 and therefore is not expected to show any signs of breathing. On the other hand, a pig recovering consciousness whilst hanging on the overhead shackle and bleeding will attempt to breathe, which may begin as **regular gagging before leading to resumption of breathing** and such an animal will have to be re-stunned using a back-up method.

3.6.3.2. Feasibility

Breathing was rated (*n* = 17) as easy and normal to assess, respectively at key stage 1 by 59 % and 41 %; at key stage 2 by 53 % and 29 %; and during key stage 3 by 59 % and 24 %. However, 18 % and 12 % of the experts rated breathing as difficult to assess during key stages 2 and 3, respectively. This is probably because it may be difficult to recognise breathing in animals shackled and hoisted on to the overhead rail. In addition, some people may be unable to distinguish between irregular and regular gagging, and the former would also occur in unconscious pigs before the onset of brain death.

3.6.3.3. Sensitivity and specificity

The positive outcome of breathing is the sign of consciousness, namely the resumption of breathing. Therefore, the sensitivity is the percentage of animals which show the presence of breathing, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 95 % (*n* = 15). The specificity is calculated as the percentage of animals observed to show apnoea, out of all truly unconscious animals. This was estimated to be 97 % (*n* = 17).

3.6.4. Response to nose prick or ear pinch

3.6.4.1. Description

Ineffective stunning and recovery of consciousness due to poor stunning can be recognised from the **response to nose prick or ear pinch** at all key stages of monitoring. Animals showing a positive response to a painful stimulus at any stage must be re-stunned using a back-up method.

3.6.4.2. Feasibility

From questionnaire 2, response to nose prick or ear pinch was considered as easy or normal to assess by, respectively, 71 % and 29 % at key stage 1, 43 % and 43 % at key stage 2, and 57 % and 29 % during key stage 3. Some (14 %) of the experts reported that response to nose prick or ear pinch is difficult to assess at key stages 2 and 3. The reason why response to a painful stimulus, i.e. nose prick or ear pinch, cannot be performed at sticking is unclear but carcases may not be accessible during bleeding.

3.6.4.3. Sensitivity and specificity

The positive outcome of the response to nose prick or ear pinch is the sign of consciousness, namely a positive response to nose prick or ear pinch. Therefore, the sensitivity is the percentage of animals which do respond to nose prick or ear pinch immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 81 % ($n = 7$). The specificity is calculated as the percentage of animals observed to show no response to nose prick or ear pinch, out of all truly unconscious animals. This was estimated to be 100 % ($n = 7$). The reason for the limited sensitivity, i.e. about 20 % of conscious animals lacking response to painful stimulus, could be associated with the inability of the animal to perceive pain owing to the analgesic effect of the gas lasting longer than the duration of unconsciousness.

3.6.5. Vocalisations

3.6.5.1. Description

Vocalisation is expected only in conscious animals and can be used as an indicator in all key stages of monitoring. However, not all conscious animals will vocalise, and hence absence of vocalisation does not always mean that the animal is unconscious. Animals showing vocalisation must be re-stunned using a back-up method. Since unconscious animals will not vocalise, this indicator is not applicable to monitoring unconsciousness.

3.6.5.2. Feasibility

From questionnaire 2, vocalisation was considered to be easy or normal to assess by, respectively 90 % and 10 % of the experts ($n = 10$) at all the key stages of monitoring.

3.6.5.3. Sensitivity and specificity

The positive outcome of vocalisation is the sign of consciousness, namely the presence of vocalisation. Therefore, the sensitivity is the percentage of animals which do vocalise immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 93 % ($n = 8$). The specificity is calculated as the percentage of animals observed to show no vocalisation, out of all truly unconscious animals. This was estimated to be 100 % ($n = 10$). It is worth noting that vocalisation had a lower sensitivity for electrical stunning (63 %) because, in addition to the noisy environment, conscious pigs entering the restrainers used for manual or mechanical electrical stunning will vocalise loudly, making it difficult to hear vocalisation of other pigs after stunning. Moreover, the higher sensitivity in gas-stunned pigs could be related to the fact that recovery of gas stunning often starts with gasping movements that may be accompanied by guttural sounds. Respondents might incorrectly interpret such guttural sounds as vocalisations.

3.6.6. Eye movements

3.6.6.1. Description

In key stage 1, effective stunning of pigs with carbon dioxide will produce **fixed eyes** (eyes wide open and glassy) and eyes will remain so until death occurs. Pigs that are not effectively stunned or those recovering consciousness will show **eye movements, including nystagmus** (or rotation of the eyeball), which can be used to recognise consciousness during all the three key stages. Animals showing any eye movements must be re-stunned using a back-up method.

3.6.6.2. Feasibility

From questionnaire 2, eye movements were considered as easy to assess at key stage 1 by 56 %, at key stage 2 by 11 % and during key stage 3 by 33 % of the experts (*n* = 9). Eye movements were rated as normal to assess at key stage 1 by 29 %, at key stage 2 by 67 % and during key stage 3 by 44 % of the experts. Eye movements as an indicator were also rated as difficult to assess at key stage 2 by 11 % and during key stage 3 by 22 % of the experts. It may be difficult or impossible to observe eye movements at key stages 2 and 3 because of the orientation of the animal, i.e. with the operator facing the brisket, at these two key stages.

3.6.6.3. Sensitivity and specificity

The positive outcome of eye movement is the sign of consciousness, namely the presence of eye movement. Therefore, the sensitivity is the percentage of animals which show eye movement immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 97 % (*n* = 8). The specificity is calculated as the percentage of animals observed to show fixed eyes, out of all truly unconscious animals. This was estimated to be 99 % (*n* = 9).

3.6.7. Palpebral reflex

3.6.7.1. Description

Effective stunning of pigs with carbon dioxide leads to abolition of palpebral reflex. Effectively stunned and stuck animals show **absence of the palpebral reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show **presence of the palpebral reflex** at any key stage. Animals showing a positive palpebral reflex must be re-stunned using a back-up method.

3.6.7.2. Feasibility

From questionnaire 2, the palpebral reflex was rated as easy and normal to assess at key stage 1 by 71 % and 14 % respectively, at key stage 2 by 14 % and 43 % respectively, and during key stage 3 by 57 % and 14 % of experts respectively (*n* = 7). A number of experts considered the palpebral reflex as difficult to assess at key stage 1 (14%), at key stage 2 (29 %) and during key stage 3 (14 %), and one expert considered palpebral reflexes as not applicable at key stages 2 and 3; the reason for these ratings is unclear. It is possible to suggest that inaccessibility/lack of access to animals during bleeding has been taken into consideration in rating eye reflexes (palpebral and corneal reflexes and spontaneous blinking) as difficult to assess.

3.6.7.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of the palpebral reflex is the sign of consciousness, namely a positive palpebral reflex. Therefore, the sensitivity is the percentage of animals which show a palpebral reflex immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 92 % (*n* = 7). The specificity is calculated as the percentage of animals observed to show no palpebral reflex, out of all truly unconscious animals. This was estimated to be 98 % (*n* = 7).

3.6.8. Corneal reflex

3.6.8.1. Description

Effective stunning of pigs with carbon dioxide leads to abolition of the corneal reflex. Effectively stunned and stuck animals show **absence of the corneal reflex** during any key stage. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show **presence of the corneal reflex** at any key stage. Animals showing a positive corneal reflex must be re-stunned using a back-up method.

3.6.8.2. Feasibility

From questionnaire 2, the corneal reflex was rated as easy to assess at key stage 1 (*n* = 15) by 57 %, at key stage 2 (*n* = 15) by 33 % and during key stage 3 (*n* = 14) by 36 % of experts. The corneal reflex was rated as normal at key stage 1 by 27 %, at key stage 2 by 27 % and during key stage 3 by 21 % of experts. A number of experts also considered the corneal reflex as difficult to assess at key stage 1 (7 %), at key stage 2 (27 %) and during key stage 3 (36 %), and a further 13 % and 7 % said that it is not applicable at key stages 2 and 3 respectively. The reasons for these ratings were not described. However, it may be difficult or impossible to observe the corneal reflex at the time of sticking or bleeding because of the orientation of the animal, i.e. with the operator facing the brisket, at these two key stages.

It is possible to suggest that inaccessibility/lack of access to animals during bleeding has been taken into consideration in rating eye reflexes (palpebral and corneal reflexes and spontaneous blinking) as difficult to assess.

3.6.8.3. Sensitivity and specificity

From questionnaire 2, the positive outcome of corneal reflex is the sign of consciousness, namely the positive corneal reflex. Therefore, the sensitivity is the percentage of animals which show the corneal reflex immediately after stunning, out of all conscious animals. This was estimated by questionnaire 2 respondents to be 99 % (*n* = 13). The specificity is calculated as the percentage of animals observed to show no corneal reflex, out of all truly unconscious animals. This was estimated to be 99 % (*n* = 15).

3.6.9. Blinking

3.6.9.1. Description

Spontaneous blinking is expected only in conscious animals and can be used as an indicator at all key stages of monitoring. However, not all the conscious animals will show spontaneous blinking, and hence absence of blinking does not always mean that the animal is unconscious. Animals showing blinking must be re-stunned using a back-up method. Since unconscious animals will not show blinking, this indicator is not applicable to monitoring unconsciousness/insensibility.

3.6.9.2. Feasibility

From questionnaire 2, spontaneous blinking was rated (*n* = 11) as easy to assess at key stage 1 by 55 %, at key stage 2 by 36 % and during key stage 3 by 36 % of experts. Spontaneous blinking was rated as normal at key stage 1 by 46 %, at key stage 2 by 55 % and during key stage 3 by 18 % of experts. A number of experts also considered spontaneous blinking as difficult to assess at key stages 2 (9 %) and 3 (45 %), but the reason for these ratings is unclear. It is possible to suggest that inaccessibility/lack of access to animals during bleeding has been taken into consideration in rating eye reflexes (palpebral and corneal reflexes and spontaneous blinking) as difficult to assess.

3.6.9.3. Sensitivity and specificity

The positive outcome of blinking is the sign of consciousness, namely the presence of spontaneous blinking. Therefore, the sensitivity is the percentage of animals which show spontaneous blinking immediately after stunning, out of all conscious animals. This was estimated by questionnaire 2 respondents to be 71 % (*n* = 9). The specificity is calculated as the percentage of animals observed to show no spontaneous blinking, out of all unconscious animals. This was estimated to be 99 % (*n* = 11). The reason for the medium sensitivity is because blinking is a spontaneous behaviour and may not occur in all conscious animals.

3.6.10. Pupillary reflex

3.6.10.1. Description

Effective stunning of pigs with carbon dioxide leads to abolition of the pupillary reflex. Effectively stunned and stuck animals show **absence of the pupillary reflex** at all key stages. On the other hand, ineffectively or poorly stunned animals and those recovering consciousness prior to sticking or during bleeding are expected to show **presence of pupillary reflex** at all key stages. Animals showing a positive pupillary reflex must be re-stunned using a back-up method.

3.6.10.2. Feasibility

From questionnaire 2, the pupillary reflex was rated (*n* = 2) as easy to assess at key stage 1 by 100 %, at key stage 2 by 0 % and during key stage 3 by 50 % of experts. The pupillary reflex was rated as normal to assess at stunning by 0 %, at key stage 1 by 50 % and during key stage 3 by 50 % of experts. One expert considered the pupillary reflex difficult to assess at key stage 2 (50 %). The main reason for this rating could be the lack of access to animals.

3.6.10.3. Sensitivity and specificity

The positive outcome of the pupillary reflex is the sign of consciousness, namely the positive pupillary reflex. Therefore, the sensitivity is the percentage of animals which show the pupillary reflex immediately after stunning, out of all truly conscious animals. This was estimated by questionnaire 2 respondents to be 93 % (*n* = 2). The specificity is calculated as the percentage of animals observed to show no pupillary reflex, out of all truly unconscious animals. This was estimated to be 100 % (*n* = 2).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 is presented in Table 6.

Table 6: Summary of the data from questionnaire 2 regarding sensitivity, specificity and feasibility of indicators and outcomes of consciousness after carbon dioxide stunning of pigs

Indicators after carbon dioxide stunning	**Outcomes of consciousness**	**Sensitivity (%)**	**Data (without uncertainty, average (20th, 50th and 80th percentiles)**	**Specificity (%)**	**Data (without uncertainty, average (20th, 50th and 80th percentiles)**	**Feasibility**		
						After stunning	**At sticking**	**During bleeding**
Posture	Relaxed body	94	91 (80, 100, 100)	100	100 (100, 100, 100	0.77	0.58	0.62
Muscle tone	Righting reflex	98	98 (100, 100, 100)	100	99 (99, 100, 100)	0.71	0.50	0.43
Breathing	Regular gagging	95	93 (98, 100, 100)	97	96 (92, 100, 100)	0.59	0.44	0.41
Response to nose prick or ear pinch	Presence of	81	73 (50, 80, 100)	100	100 (100, 100, 100)	0.71	0.29	0.43
Vocalisation	Presence of	93	88 (70, 100, 100)	100	100 (100, 100, 100)	0.90	0.90	0.90
Eye movements	Presence of	97	94 (100, 100, 100)	99	98 (98, 100, 100)	0.56	0.00	0.11
Palpebral reflex	Presence of	92	91 (91, 100, 100)	98	98 (96, 100, 100)	0.57	–0.17	0.50

Corneal reflex	Presence of	99	98 (95, 100, 100)	99	99 (97, 100, 100)	0.60	0.08	0,00
Spontaneous blinking	Presence of	71	70 (38, 80, 100)	99	99 (100, 100, 100)	0.55	0.27	–0.09
Pupillary reflex	Presence of	93	85 n.a. [(a)]	100	100 n.a. [(a)]	1.00	–0.50	0.50

(a): n.a.: not applicable owing to *n* = 2 with data (70, 100) and (10, 100); other data with *n* between 6 and 17 respondents.

4. Discussion

4.1. Introduction

As previously described, this scientific opinion proposes welfare indicators to be used for monitoring stunning and slaughter of pigs after head-only electrical stunning or exposure to high concentrations of carbon dioxide. In order to allow effective monitoring, the animals must be able to express behaviours and reflexes associated with consciousness. Owing to the scarcity of scientific publication involving simultaneous assessment of EEG indicators of unconsciousness and welfare indicators (such as physical reactions and reflexes), the systematic literature review was not very productive and therefore much of the information for the selection of the indicators comes from questionnaire 2, which was especially aimed at obtaining estimated values for the sensitivity, specificity and feasibility of the indicators. The indicators proposed in the toolboxes were selected based on sensitivity, specificity and feasibility as derived from various activities and on an expert consultation process (public consultation and technical meeting with experts from interested parties on 3 September 2013). In addition, studies from the literature (e.g. Rodriguez *et al.*, 2008) were evaluated in order to strengthen the scientific basis for inclusion of some indicators in the toolbox (e.g. the corneal reflex). Similarly, the model proposed for the sampling protocols was discussed with interested parties. The description of indicators in sections 3.4 and 3.5 also contains some basic information about elicitation of reflexes and responses, and how to use the indicators. This is particularly relevant for indicators that warrant evoking a response from the animals (e.g. the corneal reflex). Indicators additional to those recommended in the toolboxes can also be used if considered necessary. A short description of the physiology and elicitation of the indicators or evoking a conscious response is also presented in the glossary.

It should also be noted that the sample size, i.e. respondents to the questionnaire, was small and mainly from small to medium-sized slaughterhouses; nevertheless, it indicates the existing knowledge, understanding and skill levels.

The outcomes of questionnaire 2 and the systematic review were discussed with external hearing experts in a meeting held on 3 September 2013. During the meeting, consensus was achieved on a set of recommended indicators to be included in each toolbox. Furthermore, for each toolbox, additional indicators were identified which can be used, but have lower sensitivity or feasibility, and are therefore not sufficient by themselves. In addition, the experts argued that skill levels in slaughterhouses and the feasibility of assessing the indicators may vary from slaughterhouse to slaughterhouse and therefore the toolbox should have more indicators. The external experts also felt that provision of indicators alone is not helpful in the decision making, and therefore a flow chart should be considered.

The outcomes of questionnaire 2 and discussion with hearing experts suggested that the reason for the low sensitivity and specificity ratings given to some outcomes of consciousness could be that the overall practice is to look at the outcomes of unconsciousness, which is the expected outcome of stunning, rather than detection of consciousness as a poor welfare outcome. Misconceptions with regard to the physiological basis of indicators were also inferred. These misconceptions need to be eliminated to harmonise welfare monitoring in slaughterhouses. It is also suggested that the sensitivity and specificity of these indicators would improve as people acquire relevant knowledge, skill and experience in assessing them. The feasibility scores reported in this opinion are also based upon limitations of the existing infrastructure, which is not necessarily designed and constructed with welfare monitoring as a priority. Therefore, it is suggested that the feasibility of monitoring these indicators would also improve if welfare monitoring is taken into consideration during the design, layout and construction of new, or following structural change to existing, slaughterhouses.

The monitoring procedures are intended for use by the FBO in order to prevent negative welfare outcomes for the animals. The FBO, as a licence holder of a slaughterhouse, and employees with responsibility for animal welfare, including those designated as animal welfare officers, should undergo proper training and assessment of competence in welfare monitoring before licences are granted. For this to occur, any training, assessment and certification programmes implemented by the Member States should include welfare monitoring and the contents of such education/training courses should be harmonised. Within the scope of the Regulation (EC) 1099/2009, standard operating procedures should be implemented by the FBO and Member States/Competent Authorities should develop guides to good practice. These instruments should include welfare monitoring protocols/procedures for all key stages.

In addition, the regulation requires that the personnel handling, stunning or bleeding have a certificate of competence, and awarding of such a certificate should also include monitoring animal welfare.

4.2. Monitoring procedures for stunning of pigs

For the creation of the toolboxes of indicators to be used in the monitoring procedures, indicators and their outcomes were selected by the working group members based on their knowledge regarding the validity, feasibility and indicator sensitivity. The specificity is not relevant for the toolbox considered to address potential welfare issues using consciousness as outcome (see section 2.1.2).

Indicators with high sensitivity and feasibility ratings in the questionnaire were selected for the toolbox. Some additional indicators that were given relatively lower ratings for sensitivity or feasibility were also included because the hearing experts and the Working Group thought that some of these indicators, such as vocalisation in electrically stunned pigs, might have a good feasibility (ease of use) in slaughterhouses. The experts of the Panel on Animal Health and Welfare also agreed that indicators given low sensitivity and specificity at present by the respondents of the questionnaire might have potential for improvement in the future through education, training and assessment of personnel with responsibility for monitoring and ensuring welfare at slaughter (i.e. award of the Certificate of Competence). Similarly, indicators with low feasibility at present could be improved by changes in the design and layout or changes to existing practice. It was also thought that the toolbox should contain practical guidance with regard to recognition of consciousness and the decision-making process.

Indicators can be used either in parallel or in series. If two or more indicators are used in series, the second indicator is checked conditional on the outcome of the first indicator applied; if two or more indicators are used in parallel, they are performed simultaneously and therefore the animal is considered conscious when at least one of the indicators is positive.

For the purpose of detecting conscious animals on the slaughterline, indicators should be used in parallel. Indicators from the toolbox must be checked simultaneously on each sampled animal. To rule out consciousness, it is necessary that none of the indicators selected from the toolbox shows the outcome of consciousness. In practice, however, action may already have been taken, if there is evidence of consciousness, before all indicators have been checked.

When applying more than one indicator, it seems reasonable to expect an increase in the probability of detecting conscious animals, i.e. higher overall sensitivity of the monitoring protocol. If the outcomes of the checked indicators are independent of each other, then the overall sensitivity indeed increases. However, this possible increase in sensitivity will be reduced if the outcomes of the indicators are correlated, e.g. because of a common physiological basis or the checking procedure itself. The exact quantification of this correlation is not yet possible owing to a lack of scientific information. But it can be shown that the combined sensitivity of two or more indicators is at least equal to the highest sensitivity of either or any alone (Gardner *et al*., 2000). Therefore, and in the absence of a quantified correlation between indicator outcomes, it is recommended that more than one indicator be used for monitoring. The sensitivity estimate during sample size calculations would be based on the indicator with the highest sensitivity, out of all of the indicators that have been selected.

This approach may lead to an oversampling, which, on the other hand, is in line with the precautionary principle needed to protect the welfare of animals.

4.2.1. Combination of selected indicators (the 'toolboxes') for head-only electrical stunning

Toolbox for key stage 1 (Toolbox 1 = immediately after head-only electrical stunning)

This opinion recommends the following indicators (and their outcomes of consciousness) for inclusion in Toolbox 1 at key stage 1: tonic-clonic seizures, the palpebral or corneal reflex and breathing (these are presented above the dashed line in the flow chart). Additional indicators—spontaneous blinking, posture and vocalisations—are also proposed (these are presented below the dashed line in the flow chart), but their sensitivity or feasibility is lower and they should not be relied upon solely.

The reasons for this approach are presented in the following paragraphs.

Recommended indicators (above the dashed line in the flow chart)

Tonic-clonic seizures

The scientific rationale is that tonic–clonic seizures occur as a consequence of the induction of generalised epileptiform activity in the brain. This indicator is routinely used in pig slaughterhouses and therefore was considered highly sensitive, specific and feasible at key stage 1 in questionnaire 2.

Breathing

This indicator is feasible at key stage 1 and highly sensitive according to questionnaire 2 and can be used.

Corneal or palpebral reflex

In questionnaire 2, both the corneal and palpebral reflexes were considered to be highly sensitive. It was also suggested during Working Group discussions that people performing checks usually touch the whole eye, intending to provoke blinking in conscious animals, and may not always make a distinction between the corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination.

Additional indicators (below the dashed line in the flow chart)

Spontaneous blinking

The presence of spontaneous blinking was considered as a highly sensitive but not so specific indicator by the respondents to questionnaire 2. This indicator was also rated as highly feasible during key stage 1. The presence of spontaneous blinking should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Posture

The respondents to questionnaire 2 considered posture to have modest sensitivity for the outcome of unconsciousness 'immediate collapse'. This is probably because immediate collapse may occur in animals without rendering them unconscious following electrical stunning, for example as a result of immobilisation. The experts felt that failure to collapse or an attempt to regain posture is a reliable outcome of consciousness and immediate collapse is a reliable outcome of unconsciousness. These outcomes are routinely used in pig slaughterhouses to recognise effective head-only electrical stunning.

Vocalisations

Vocalisation was considered as a highly feasible indicator at key stage 1, although not very sensitive because not all conscious animals may vocalise (e.g. electrically immobilised animals will remain conscious but may not vocalise). However, the presence of vocalisations such as grunting is a clear sign of consciousness warranting intervention (re-stunning).

<u>Indicators not considered in Toolbox 1</u>

The following indicators were not included in the flow chart because of their low sensitivity or feasibility at key stage 1, due to the limited or no access to the animal (see section 3.4).

Muscle tone

Loss of muscle tone occurs after tonic-clonic seizures. In an animal which has not been stuck, loss of muscle tone indicates the beginning of recovery of consciousness. In contrast, loss of muscle tone in bleeding animals indicates unconsciousness. Therefore, this indicator is applicable only at key stages 2 and 3.

Pupillary reflex

The respondents to questionnaire 2 rated this as a highly sensitive indicator but very low on feasibility at key stage 1 because the animals may not be accessible.

Eye movements

The respondents to questionnaire 2 rated eye movements as a highly sensitive indicator but with moderate feasibility at key stage 1 because the eyes may be obscured or the animals may not be accessible.

Response to nose prick or ear pinch

The respondents to questionnaire 2 rated this as a highly sensitive indicator but very low on feasibility at key stage 1 because the animals may not be accessible. However, after electrical stunning, consciousness appears before sensitivity to pain. Therefore, the response to nose prick or ear pinch will indicate that the animal is conscious, but lack or absence to nose prick or ear pinch may occur also in conscious animals (Velarde *et al*., 2002).

Toolbox for key stage 2 (Toolbox 2 = during sticking)

This opinion recommends the following indicators to be included in the Toolbox 2 at key stage 2: breathing, tonic-clonic seizures and muscle tone (these are presented above the dashed line in the flow chart). In addition, corneal or palpebral reflexes, spontaneous blinking and vocalisations may also be used (these are presented below the dashed line in the flow chart).

The reasons for this are as follows.

<u>Recommended indicators (above the dashed line in the flow chart)</u>

Breathing

This indicator is feasible and highly sensitive and feasible at key stage 2 according to questionnaire 2 and can be used.

Tonic-clonic seizures

This indicator was rated as feasible during key stage 2 by the respondents to questionnaire 2. This is because tonic seizures could be present at sticking if the stun-to-stick interval is short.

Muscle tone

This indicator is sufficiently feasible at key stage 2 and highly sensitive according to questionnaire 2. Recovery of different degrees of muscle tone may be manifested as leg kicking, head-righting reflex and arching of the back, and these signs can be used to recognise consciousness. Loss of muscle tone can be used to recognise unconscious animals only if tonic-clonic seizures are no longer present.

Additional indicators (below the dashed line in the flow chart)

Corneal or palpebral reflex

In questionnaire 2, corneal and palpebral reflexes were considered as, respectively, highly and moderately sensitive. However, it was suggested during Working Group discussions that people performing checks usually touch the whole eye, intending to provoke blinking in conscious animals, and may not always make a distinction between corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. The feasibility of both indicators is reduced in comparison with key stage 1, when the animal’s head is covered in blood following sticking. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Spontaneous blinking

The presence of spontaneous blinking was considered as a highly sensitive but not so specific indicator by the respondents to questionnaire 2. This indicator was also considered highly feasible during key stage 2. The presence of spontaneous blinking should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Vocalisations

Vocalisation was considered as a highly feasible indicator at key stage 2, although not very sensitive because not all conscious animals may vocalise (e.g. electrically immobilised animals will remain conscious but may not vocalise). However, the presence of vocalisations such as grunting is a clear sign of consciousness warranting intervention (re-stunning).

Indicators not considered in the Toolbox 2

The following indicators were not included in the flow chart because of their low sensitivity or feasibility at key stage 2, owing to limited or lack of access to the animal (see section 3.4): eye movements, posture, response to nose prick or ear pinch and pupillary reflexes.

Toolbox for key stage 3 (Toolbox 3 = during bleeding).

This opinion proposes the following indicators to be included in the Toolbox 3 at key stage 3: breathing and muscle tone (these are presented above the dashed line in the flow chart). In addition vocalisations, corneal or palpebral reflexes and spontaneous blinking may also be used (this is presented below the dashed line in the flow chart).

The reasons for this are as follows.

Recommended indicators

Breathing

This indicator is feasible at key stage 3 and highly sensitive according to questionnaire 2 and can be used.

Muscle tone

This indicator is feasible at key stage 3 and highly sensitive according to questionnaire 2. Recovery of different degrees of muscle tone may be manifested as leg kicking, the head-righting reflex and arching of the back, and these signs can be used to recognise consciousness. When electrically-stunned animals are bled out during tonic phase, the loss of muscle tone after the clonic activity during bleeding would confirm the persistence of unconsciousness in animals.

Additional indicators (below the dashed line in the flow chart)

Vocalisations

Vocalisation was considered as a highly feasible at key stage 3, although not very because not all the conscious animals may vocalise (e.g. electrically immobilised animals will remain conscious but may not vocalise). However, the presence of vocalisations such as grunting is a clear sign of consciousness warranting intervention (re-stunning).

Corneal or palpebral reflex

In questionnaire 2, corneal and palpebral reflexes were considered as highly sensitive. It was also suggested during Working Group discussions that people performing checks usually touch the whole eye, intending to provoke blinking in conscious animals, and may not always make a distinction between the corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. The feasibility of both indicators is reduced in comparison with key stage 1, when the animal's head is covered in blood following sticking. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Spontaneous blinking

The presence of spontaneous blinking was considered as a highly sensitive but not so specific indicator by the respondents to questionnaire 2. This indicator was also considered highly feasible during key stage 3. The presence of spontaneous blinking should be taken as a clear sign of consciousness warranting intervention (re-stunning).

Indicators not considered in the flow chart

The following indicators were not recommended and not considered to be in the flow chart for key stage 3 owing to their low performance or their low feasibility because of limited access to the animal (see section 3.4): tonic or clonic seizures, response to nose prick or ear pinch, posture, eye movements and pupillary reflex.

4.2.2. Flow chart for the use of the toolbox indicators at slaughter with head-only electrical stunning

A flow chart was designed to support the understanding of the use of the indicators and is shown in Figure 5. Please refer to section 3.3 for the definitions and selection process of the indicators and refer to section 3.4 and Table 5 for the sensitivity of each indicator (that is used to calculate the sample size). Please refer to the SAS Technical Report (EFSA SAS Unit, 2013) for further details on the practical calculation of the sample size.

The flow chart in Figure 5 illustrates this opinion's recommendations regarding the three key stages of monitoring, the recommended outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness are detected in pigs following head-only electrical stunning. Following the stun, and prior to shackling (key stage 1), it is recommended that the three indicators listed above the dashed line in blue Toolbox 1 be used to recognise consciousness. The three indicators below the dashed line can also be used to check for signs of consciousness, but their sensitivity or feasibility is low and they should not be relied upon solely. If the animal shows any of the signs of consciousness (red box), then appropriate intervention should be applied. If no indicator suggests that the animal is conscious, i.e. all performed checks resulted in outcomes of unconsciousness (green box), then the animal can be shackled and bled out by a chest stick.

In Toolbox 2, three recommended indicators are presented above the dashed line, and these can be used to check for consciousness at key stage 2. There are three additional indicators below the dashed line in Toolbox 2, and these may also be used to check for outcomes of consciousness, but with low sensitivity or feasibility. If the animal shows any of the outcomes of consciousness (red box), then appropriate intervention should be applied. If no indicator suggests consciousness, i.e. all performed checks resulted in outcomes of unconsciousness (green box), then the animal should continue to be checked during bleeding (key stage 3).

The blue Toolbox 3 recommends two indicators and provides three additional indicators to be used to check for consciousness. If any one outcome of these indicators suggests consciousness (red box), then appropriate intervention should be applied. If no indicator presented in Toolbox 3 suggests consciousness, i.e. all performed checks resulted in outcomes of unconsciousness (green box), then it can be concluded there is no risk of regained consciousness.

Out of the recommended indicators above the dashed line, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

Please note that, in the case of those indicators which rely on the animal manifesting certain behaviour suggestive of consciousness (e.g. spontaneous blinking, vocalisations), the outcomes of unconsciousness are presented in grey as a reminder of the limited predictive value of the indicator, i.e. the percentage of non-vocalising animals that are truly unconscious out of all non-vocalising animals). Nevertheless, the outcome of consciousness suggests that the animal is conscious and is a ‘warning signal’ requiring an intervention.

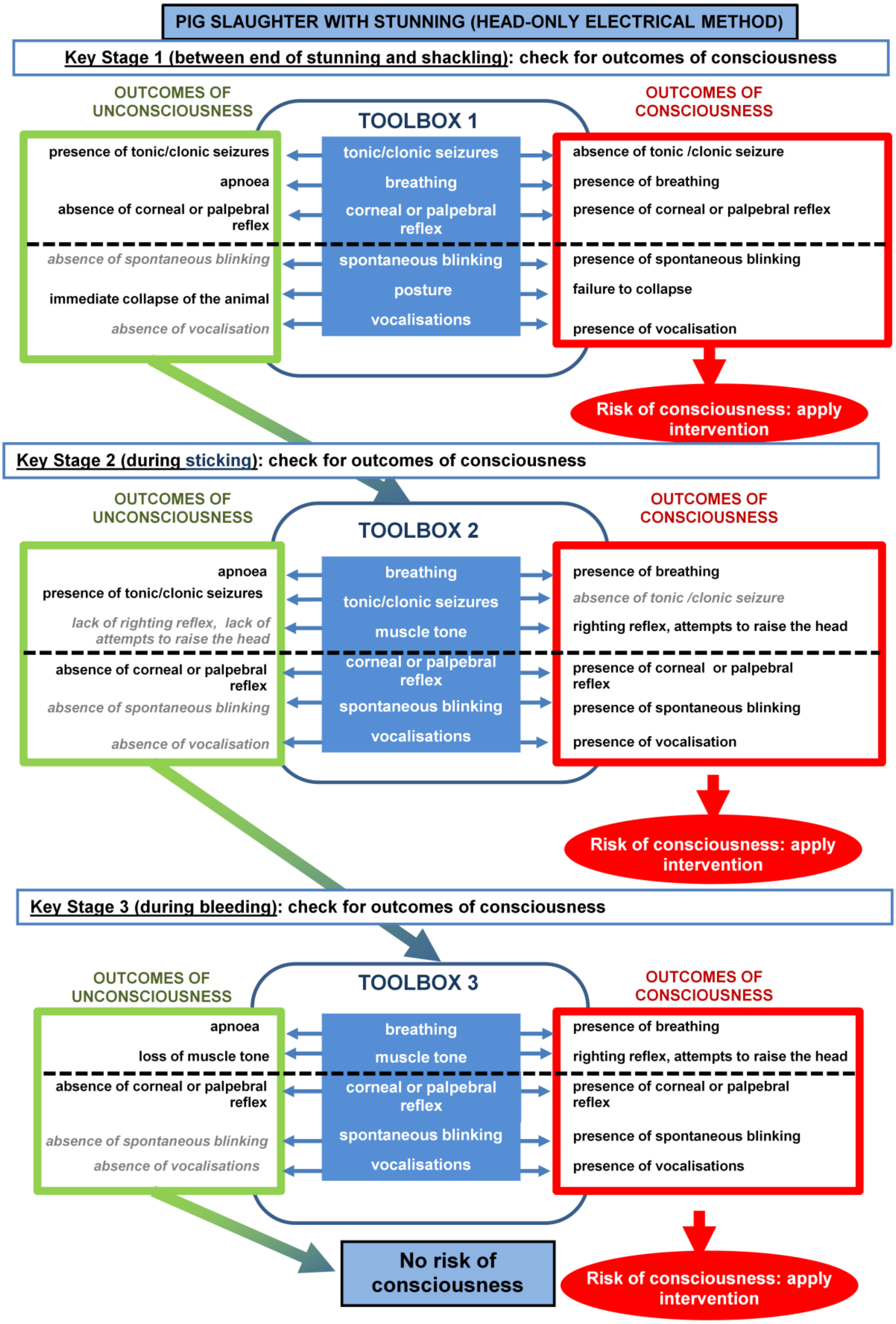


Figure 5: Toolbox of indicators that are considered suitable to be used for detection of conscious animals at each key stage of the procedure of head-only electrical stunning in pigs

4.2.3. Combination of selected indicators (the 'toolboxes') for carbon dioxide stunning at high concentration

Toolbox for Key Stage 1 (Toolbox 4 = between end of carbon dioxide exposure and sticking)

This opinion recommends the following indicators (and their outcomes of consciousness) for inclusion in Toolbox 4 at key stage 1: muscle tone, breathing and corneal/palpebral reflexes (these are presented above the dashed line in the flow chart). Additional indicators—response to nose prick or ear pinch and vocalisations—are also proposed (these are presented below the dashed line in the flow chart), but their sensitivity or feasibility is lower and they should not be relied upon solely.

The reasons for this approach are presented in the following paragraphs.

Recommended indicators (above the dashed line in the flowchart)

Muscle tone

This indicator was rated as highly sensitive, specific and feasible at key stage 1 according to questionnaire 2. Recovery of different degrees of muscle tone may be manifested as the righting reflex, attempts to raise the head, leg kicking and arching of the back, and these signs can be used to recognise consciousness.

Breathing

This indicator is highly feasible at key stage 1 and highly sensitive and specific according to questionnaire 2 and can be used.

Corneal or palpebral reflex

In questionnaire 2, corneal and palpebral reflexes were considered as highly sensitive. It was also suggested during Working Group discussions that people performing checks usually touch the whole eye, intending to provoke blinking in conscious animals, and may not always make a distinction between the corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination.

Additional indicators (below the dashed line in the flow chart)

Vocalisations

Vocalisation is highly sensitive, specific and feasible at key stage 1 according to questionnaire 2 and can be used to recognise consciousness. It is worth noting that vocalisation is audible at exit from the gas stunning equipment and therefore the feasibility is high.

Response to nose prick or ear pinch

This indicator was considered low on feasibility but highly sensitive and specific according to questionnaire 2 and can be used in key stage 1.

Indicators not considered in the flow chart

The following indicators were not included in the flow chart because of their low sensitivity or feasibility, because of limited or no access to the animal (see section 3.4): eye movements, posture, spontaneous blinking and pupillary reflex.

Toolbox for Key Stage 2 (Toolbox 5 = during sticking after carbon dioxide stunning)

This opinion recommends the following indicators to be included in the Toolbox 5 at key stage 2: muscle tone, breathing and vocalisations (these are presented above the dashed line in the flow chart).

In addition, the corneal or palpebral reflexes and response to nose prick or ear pinch may also be used (this is presented below the dashed line in the flow chart).

The reasons for this are as follows.

Recommended indicators (above the dashed line in the flow chart)

Muscle tone

Muscle tone was considered highly sensitive, specific and feasible at key stage 2 according to questionnaire 2. Recovery of different degrees of muscle tone may be manifested as leg kicking, the head-righting reflex and arching of the back, and these signs can be used to recognise consciousness.

Breathing

This indicator is highly feasible at key stage 2 and highly sensitive and specific according to questionnaire 2 and can be used.

Vocalisations

Vocalisation is highly sensitive, specific and feasible at key stage 2 according to questionnaire 2 and can be used to recognise consciousness.

Additional indicators (below the dashed line in the flow chart)

Corneal or palpebral reflex

In questionnaire 2, the corneal and palpebral reflexes were considered as highly sensitive. It was suggested during Working Group discussions that people performing checks usually touch the whole eye, intending to provoke blinking in conscious animals, and may not always make a distinction between the corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. The feasibility of both indicators is reduced in comparison with key stage 1, when the animal's head is covered in blood following sticking. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Response to nose prick or ear pinch

This indicator is moderately feasible at key stage 2 and highly sensitive and specific according to questionnaire 2 and can be used to recognise consciousness.

Indicators not considered in the flow chart

The following indicators were not included in the flow chart because of their low sensitivity or feasibility ratings, because of limited or no access to the animal (see section 3.4): posture and the pupillary reflex.

Toolbox for key stage 3 (Toolbox 6 = during bleeding).

This opinion proposes the following indicators to be included in the Toolbox 6 at key stage 3: muscle tone and breathing (these are presented above the dashed line in the flow chart). In addition, corneal or palpebral reflex and vocalisations may also be used (this is presented below the dashed line in the flow chart).

The reasons for this are as follows.

Recommended indicators

Muscle tone

Muscle tone was considered highly sensitive, specific and feasible at key stage 3 according to questionnaire 2. Recovery of different degrees of muscle tone may be manifested as leg kicking, the head-righting reflex and arching of the back, and these signs can be used to recognise consciousness.

Breathing

Breathing was considered highly feasible at key stage 3 and highly sensitive and specific according to questionnaire 2 and can be used.

Additional indicators (below the dashed line in the flow chart)

Corneal or palpebral reflex

In questionnaire 2, the corneal and palpebral reflexes were considered as highly sensitive. It was suggested during Working Group discussions that people performing checks usually touch the whole eye, intending to provoke blinking in conscious animals, and may not always make a distinction between the corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. The feasibility of both indicators is reduced in comparison with key stage 1, when the animal's head is covered in blood following sticking. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Vocalisations

Vocalisation is sensitive, specific and feasible at key stage 3 according to questionnaire 2 and can be used to recognise consciousness.

Indicators not considered in the flow chart

The following indicators were not recommended and not considered to be in the flow chart for their low performance or their low feasibility due to the limited access to the animal (see section 3.4): response to nose prick or ear pinch, posture, eye movements and the pupillary reflex.

4.2.4. Flow chart for the use of the toolbox indicators at slaughter with carbon dioxide

A flow chart was designed to support the understanding of the use of the indicators and is shown in Figure 5. Please refer to section 3.5 for the definitions and selection process of the indicators and refer to section 3.5 and Table 6 for the sensitivity of each indicator (that is used to calculate the sample size). Please refer to the SAS Technical Report (EFSA SAS Unit, 2013) for further details on the practical calculation of the sample size.

The flow chart in Figure 5 illustrates this opinion's recommendations regarding the three key stages of monitoring, the recommended outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness are detected in pigs following carbon dioxide stunning. Following the stun, and prior to shackling (key stage 1), it is recommended that the three indicators listed above the dashed line in blue Toolbox 4 be used to recognise consciousness. The two indicators below the dashed line also can be used to check for signs of consciousness, but their sensitivity is low and they should not be relied upon solely. If the animal shows any of the signs of consciousness (red box), then appropriate intervention should be applied.

If all indicators suggest that the animal is unconscious (green box), then the animal can be shackled and bled out by a neck cut or chest stick. In Toolbox 5, three recommended indicators are presented above the dashed line, and these can be used to check for consciousness at key stage 2. There are two additional indicators below the dashed line in this Toolbox 5, and these may also be used to check for outcomes of consciousness, but with low sensitivity. If the animal shows any of the outcomes of consciousness (red box), then appropriate intervention should be applied.

If all the indicators suggest unconsciousness (green box), then the animal should continue to be checked during bleeding (key stage 3). The blue Toolbox 6 recommends indicators to be used to check for consciousness. If any one outcome of these indicators suggests consciousness (red box), then appropriate intervention should be applied. If the indicators presented in Toolbox 6 suggest unconsciousness (green box), then it can be concluded there is no risk of regained consciousness.

Out of the recommended indicators above the dashed line, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

Please note that, in the case of those indicators which rely on the animal manifesting certain behaviour suggestive of consciousness (e.g. spontaneous blinking, vocalisations), the outcomes of unconsciousness are presented in grey as a reminder of the limited predictive value of the indicator, i.e. the percentage of non-vocalising animals that are truly unconscious out of all non-vocalising animals). Nevertheless, the outcome of consciousness suggests that the animal is conscious and is a 'warning signal' requiring an intervention.

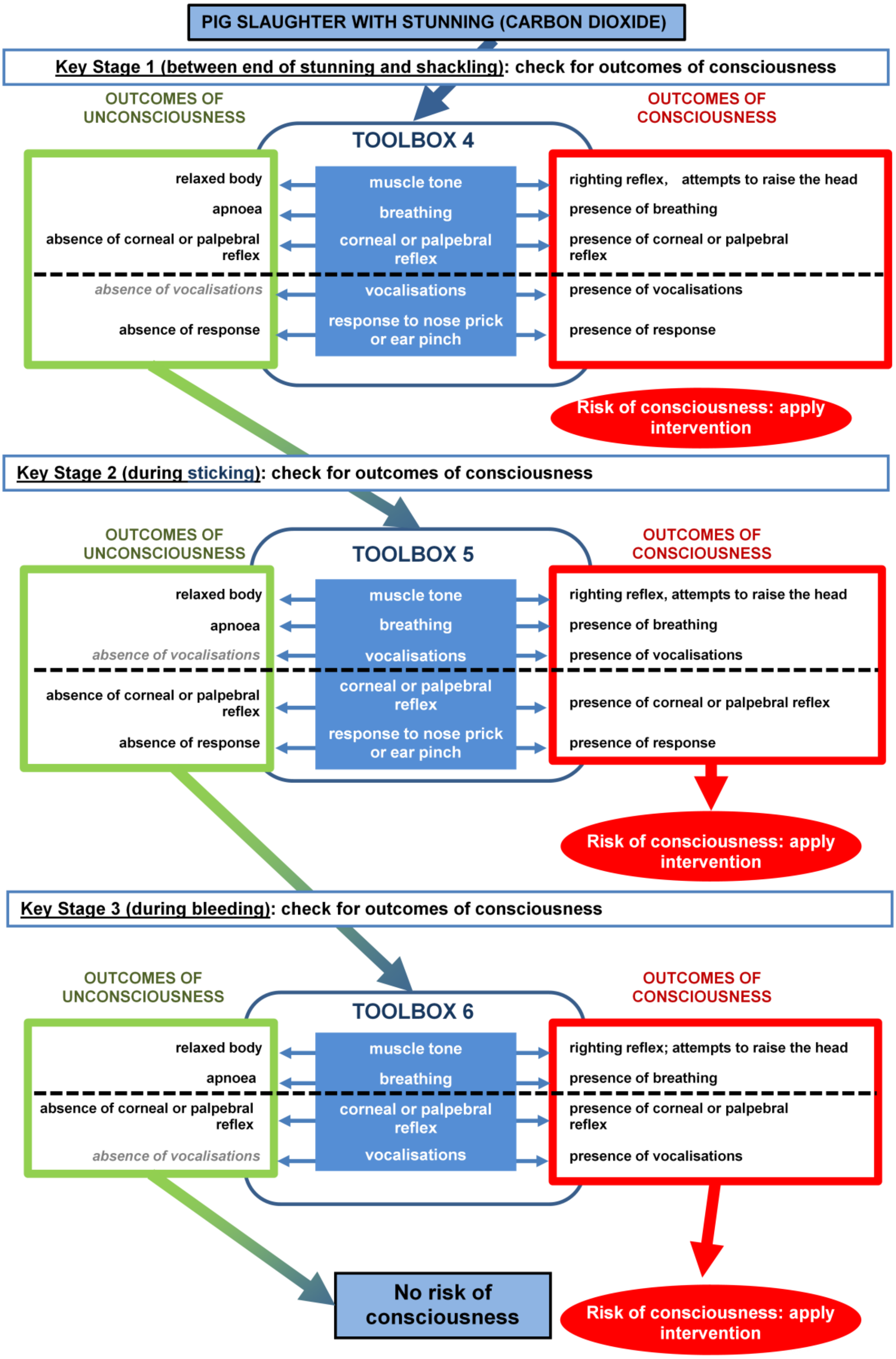


Figure 6: Toolbox of indicators that are considered suitable to be used for detection of conscious animals at each key stage of the procedure of carbon dioxide stunning in pigs

4.2.5. Sampling protocol for head-only electrical and carbon dioxide stunning

Independent of the sampling protocol specified in section 3.2.3 and discussed below, but in line with the duties of the personnel, who should process only unconscious animals, all animals (SF 100 %) should be monitored to prevent poor welfare outcomes. The indicators suggested in the flow chart are aimed at achieving effective monitoring of welfare of the animals by all the personnel involved in stunning and slaughter.

4.2.5.1. Risk factors and welfare consequences

The final welfare consequence of failed stunning is the risk of conscious or not fully unconscious animals being shackled, stuck or scalded. This risk needs to be reduced to zero, by ensuring proper stunning routines and monitoring of stun efficacy.

In order to develop a monitoring protocol, the mandate from the Commission requests EFSA to estimate the optimal frequency with which animals should be checked for signs of consciousness following stunning.

This frequency should take into account risk factors associated with the stunning procedure.

The most common risk factors involved in the welfare of animals during slaughter are listed in Table 7. The EFSA opinion on stunning and slaughter (EFSA, 2004) provides a detailed list of risk factors associated with the stunning procedure. They have been linked to two categories: those risk factors that affect the quality of the stun and those that affect the quality of the assessment.

The two types of risk factors have a different effect on the sampling protocol.

Risk factors that reduce the quality of the stun

When the quality of the stun is reduced, the probability of an animal not being properly stunned increases. This will increase the number of conscious animals which are presented to the operator for checking, i.e. increased threshold failure rate. The model-based sampling procedure developed in Chapter 2 is designed to detect any increase in this proportion of mis-stunned animals: in particular, the system will detect at least one conscious animal as soon as the overall proportion of poorly stunned animals exceeds the set threshold failure rate. Therefore, in the case of risk factors affecting the quality of the stun, the frequency of sampling does not have to be increased even though the number of animals that are mis-stunned increases. These risk factors do not necessitate a change in the sampling fraction.

Risk factors that reduce the sensitivity of the indicators used

Factors reducing the effectiveness of the assessment of consciousness will increase the likelihood that conscious animals are processed as if they were unconscious. This, of course, is an undesirable situation from an animal welfare point of view. If we deal with the indicators as if they were a diagnostic test, the ‘effectiveness’ of an indicator is expressed by the sensitivity, i.e. the probability of correctly classifying a truly conscious animal as conscious. It is intuitive that the lower this probability (i.e. the sensitivity of the indicator), the greater the number of animals that have to be tested in order to achieve a consistent level of confidence. This relationship is quantified through the model developed in Chapter 2.

The quantification of these sensitivity values is based on the knowledge and experience of a pool of stakeholders who were asked to complete questionnaire 2 (see section 3.3). Therefore, the resulting figures have to be referred to as ‘regular’ or ‘average’ for the situation. As a consequence, it is plausible to assume that under certain circumstances or ‘risk factors’ (e.g. the employment of new personnel) the same indicator may perform worse than under regular circumstances. Quantitatively speaking, when dealing with these different conditions, the sensitivity reference values may no longer hold thus the sample size required under these circumstances will be larger. These risk factors will therefore affect the monitoring procedure, because they alter the sensitivity of the indicator.

Table 7: Risk factors to animal welfare associated with head-only electrical and carbon dioxide stunning of pigs

Component	Risk factor	Risk of poor stunning [(a)]	Risk of poor assessment [(a)]
STAFF	Competence (e.g. poor tong position)	√	√
	Experience	√	√
	Fatigue	√	√
EQUIPMENT	maintenance	√	
	Features (e.g. for head-only electrical stunning: dirty or corroded stunning tongs; e.g. for gas stunning: too many animals in the cradle)	√	
	Presence of records of maintenance (e.g. cleaning)	√	
RECORDS OF THE CHECKS	Conformity in the past	√	√
ANIMALS	Body weight	√	√
	Category/breed/temperament	√	√
ESTABLISHMENT	Line speed	√	√

(a): The choice of risk category is based on expert opinion only.

4.2.5.2. Different scenarios for the sampling protocols

The risk factors described in the previous paragraph may require changes to the sampling protocol applied in the slaughterhouse. Three levels of sampling can be identified: standard, reinforced and light (also referred to in literature as normal, tightened and reduced inspections).

<u>‘Standard’ sampling protocol</u>

The standard operating procedure for slaughter of pigs will involve a sampling fraction of 100 % by slaughterhouse personnel, as the operators check each animal for indicators of consciousness immediately after stunning, before sticking and during bleeding. In addition to this, the animal welfare officer will sample a fraction of all animals to monitor the effectiveness of the process, and will correct the operator or other aspects of the stunning process if necessary. The fraction sampled by the welfare officer can be calculated by the model, and is dependent on the indicator sensitivity, the slaughtered population, the maximum allowed threshold failure rate and the required accuracy, as described previously.

The larger the chosen slaughter population, or the higher the threshold failure rate, the lower the resulting sampling fraction will be. This means that the number of animals between two consecutively tested animals becomes larger. For example, if we take a required accuracy of 95 %, and an indicator with a sensitivity of 90 %, then the following calculation illustrates the effects of a risk manager’s decision regarding threshold failure rate and slaughter population. Given a slaughter population of number of animals killed on one day (e.g. 500 animals), and a threshold failure rate of 0.01, the sampling fraction will be 50 %. Therefore, one in every two animals will need to be monitored. However, if the slaughter population is set at one working week (at the same daily throughput, so 2 500 animals), then the sampling fraction will be 13 %: so one in every eight animals. An appropriate decision on the criterion for defining a slaughter population and threshold failure rate would therefore help in achieving the requirements of the legislation on animal welfare at slaughter.

It goes without saying that the sampling protocol itself should not be a reason to delay the procedure. If slaughterhouse personnel identify a mis-stunned animal, they should take immediate remedial action. Subsequently, the personnel should identify the reason for the poor stun and implement remedial action. They should then inform the FBO or AWO.

If the animal welfare officer identifies a mis-stunned animal during execution of the sampling procedure, he or she should take remedial action and instigate the reinforced sampling protocol.

‘Reinforced’ sampling protocol

If one of the above-mentioned risk factors is present, which suggests reduction in the sensitivity of the indicator applied by the personnel, the welfare officer will need to implement back-up sampling. This can be done by concentrating the sampling efforts in a shorter time following the introduction of the risk factor, until the risk is identified and rectified. The degree to which the sampling needs to be increased is determined by the incurred reduction in indicator sensitivity. However, because the reduction in indicator sensitivity is not known a pragmatic approach is required. This is to test all animals during a period represented by one-tenth of the slaughtered population. For example, if the slaughtered population as defined by the standard sampling protocol is set to 200 animals, then for the time until the next 20 animals are processed, i.e. one-tenth of the slaughter population, all animals have to be retested.

‘Light’ sampling protocol

There are no circumstances under which the sampling frequency (sample fraction) of the welfare officer can be relaxed, as a reduction in the sampling fraction will immediately reduce the accuracy by which a given excess threshold failure rate may be detected by the monitoring protocol (the other factors of the model, slaughtered population and test sensitivity, being unchanged).

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS

1) From the stakeholder meeting it was learned that several indicators are currently used by experts to assess unconsciousness and death in animals. However, there is no harmonised list of indicators, either species or method specific, nor is there a scientific rationale. This highlights the need to develop a scientifically based set of indicators and monitoring protocols.

2) The systematic literature review revealed that no study has explicitly reported the sensitivity and specificity of the indicators in unconscious animals—as determined by measuring brain activity using electroencephalography (EEG). Therefore, there is a scarcity of scientific publications reporting correlation between unconsciousness or death ascertained by EEG and the behavioural and physiological indicators to detect unconsciousness and death that could be used in slaughterhouse conditions.

3) The feasibility of monitoring any welfare indicator may vary depending upon the design and layout of the slaughter plant. Therefore, the feasibility of monitoring these indicators can be improved if welfare monitoring is taken into consideration during the design, layout and construction of a new, or following structural change to existing, slaughterhouses.

4) Stakeholders need to be aware that this opinion provides a methodology and a scientifically valid approach to determining the sample size and sampling protocols. In this regard, the sensitivity, specificity and feasibility of indicators that are relevant to the skill level and facilities of the slaughterhouse should be ascertained and used in estimating appropriate sample size and protocols.

5) The level of competence of the staff influences the feasibility, sensitivity and specificity of the indicators. Therefore, lack of knowledge and understanding of physiological basis of indicators may have contributed to some ratings being low on sensitivity, specificity and feasibility by the respondents of the questionnaires.

6) Sampling protocols suggested in this opinion are based on sensitivity assessment for indicators involving expert survey because there are no (or few) controlled studies under laboratory conditions which determine the sensitivity of the indicators based on correlation with the EEG parameters.

7) In a slaughterhouse, consciousness, unconsciousness and death of the animals are checked throughout the process by two different categories of operators: (i) the ‘personnel’, namely the person(s) performing stunning, shackling, hoisting and/or bleeding, and (ii) the animal welfare officer, the person responsible for overall animal welfare at slaughter.

8) In order to develop sampling protocols for monitoring consciousness in pigs after stunning, indicator(s) sensitivity, threshold failure rate (i.e. tolerance level) for acceptable proportion of mis-stunning, the size of the slaughter population, the sampling frequency (i.e. sample fraction) and the desired accuracy of the sampling protocol are required.

9) During stunning of pigs, there are two types of risk factors: (i) those associated with stun quality and (ii) those associated with the quality of the monitoring. Only the latter have an effect on the sampling protocol.

10) Risk factors related to the quality of monitoring may require changes to the sampling protocol applied in the slaughterhouse, from a ‘standard’ to a ‘reinforced’ sampling protocol.

11) To reduce welfare risks due to poor stunning, it is important to detect the animals that are not properly stunned or recover consciousness after stunning. Therefore, it is most important to check periodically indicators with high sensitivity and feasibility in detecting conscious animals.

12) For detecting consciousness in pigs following stunning, the sensitivity of the indicators (ability of an indicator to detect conscious animals as conscious) is relevant for animal welfare whereas specificity (ability of an indicator to detect unconscious animals as unconscious) is more related to the logistics (personnel have to re-stun the animal).

13) Since unconsciousness should be confirmed from the stunning application until death, this opinion recognises three key stages for monitoring welfare at slaughter: (i) immediately after stunning (between end of stunning and shackling), (ii) at sticking and (iii) during bleeding.

CONCLUSIONS ON HEAD-ONLY ELECTRICAL STUNNING IN PIGS

14) The opinion concludes that a set of indicators (a minimum of two indicators) to be used to detect conscious animals following head-only electrical stunning should consist of:

Key stage 1 (between end of electrical stunning and shackling): tonic–clonic seizures, breathing and the corneal or palpebral reflex. Additional indicators—spontaneous blinking, posture, and vocalisations—are also proposed, but their sensitivity is low and they should not be relied upon solely.

Key stage 2 (at sticking): breathing, tonic/clonic seizures and muscle tone. In addition, the corneal or palpebral reflex, spontaneous blinking and vocalisations may also be used.

Key stage 3 (during bleeding): breathing and muscle tone. In addition, vocalisations, the corneal or palpebral reflex and spontaneous blinking can also be used.

CONCLUSIONS ON CARBON DIOXIDE STUNNING OF PIGS

15) The opinion concludes that a set of indicators (a minimum of two indicators) to be used to detect conscious animals following carbon dioxide stunning should consist of:

Key stage 1 (between end of carbon dioxide stunning and shackling): muscle tone, breathing and the corneal or palpebral reflex. Additional indicators—vocalisation and response to nose prick or ear pinch – may also be used.

Key stage 2 (at sticking): muscle tone, breathing and vocalisations. In addition, the corneal or palpebral reflex and response to nose prick or ear pinch may also be used.

Key stage 3 (during bleeding): muscle tone and breathing. In addition, the corneal or palpebral reflex and vocalisations may also be used.

RECOMMENDATIONS

GENERAL RECOMMENDATIONS

1) A scientifically based and harmonised set of indicators for use in standard operating procedures in slaughterhouses as well as in monitoring protocols is needed.

2) Further scientific studies should be carried out to determine the correlation between the state of consciousness/unconsciousness and death—as measured by brain activity on electroencephalography—and the behavioural and physiological indicators used to detect

unconsciousness and death in order to collect valid information on indicator sensitivity and specificity.

3) In a controlled laboratory conditions the sensitivity of the indicators should be determined by correlation to EEG parameters, according to the "Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing" (EFSA AHAW Panel, 2013).

4) The level of competence of slaughterhouse staff, which determines the feasibility, sensitivity and specificity of the indicators, should be improved through harmonised education, training and assessment throughout the EU. Until such time as any improvement in sensitivity or specificity resulting from personnel training is objectively demonstrated, the values given in this opinion for calculating the sample size should be considered as a minimum requirement.

5) The procedure of approval of the design, layout and construction of a new slaughterhouse, or of a structural change to existing slaughterhouses, should include as a criterion the feasibility of welfare monitoring throughout the slaughtering process.

6) The animal welfare officer should monitor the effectiveness of the entire stunning and slaughter process, and correct personnel behaviour or other aspects of the slaughter process if necessary.

7) Since unconsciousness should be confirmed from the stunning application until death, this opinion also suggests checking that the animal is not conscious at each of the three key stages: (i) immediately after stunning (between end of stunning and shackling), (ii) at sticking and (iii) during bleeding.

8) During slaughter with stunning, indicators to detect conscious animals should be used to recognise failures (i.e. poor welfare) and apply intervention.

9) In order to develop sampling protocols for monitoring consciousness in stunning of pigs:

- Slaughterhouse 'personnel' should sample 100 % of the animals immediately after stunning, during neck cutting and during bleeding.

- The animal welfare officer should periodically sample the slaughter population and the sampling fraction can be calculated using the statistical model proposed in this opinion (here referred to as 'standard' sampling protocol). This fraction is dependent on the test sensitivity, the slaughtered population, the maximum allowed threshold failure rate and the required accuracy.

10) The 'standard' monitoring protocol should be reinforced (here referred to as 'reinforced' sampling protocol) when a conscious animal is detected, or when a risk factor affecting the quality of the monitoring is identified, and rectified. All animals should be tested during a period represented by one-tenth of the slaughtered population.

11) It is recommended that the animal welfare officer should not reduce the sampling frequency (sample fraction), as a reduction in sampling fraction (here referred to as 'light' sampling protocol) will immediately reduce the accuracy of the monitoring protocol.

12) In order to allow effective monitoring, the animals must be able to express behaviours and reflexes associated with consciousness. Consequently, procedures, processes or treatments that could mask the expression of such behaviours (such as electrical immobilisation or electrical stimulation) should not be used prior to confirmation of death in animals.

RECOMMENDATIONS ON HEAD-ONLY ELECTRICAL STUNNING OF PIGS

13) A toolbox composed of the following indicators should be checked to determine consciousness of animals after stunning at all three key stages of the process, to ensure that animals remain unconscious until death occurs.

Key stage 1 (between end of electrical stunning and shackling): tonic–clonic seizures, breathing and the corneal or palpebral reflex. Additional indicators—spontaneous blinking, posture and vocalisations—are also proposed, but their sensitivity is low and they should not be relied upon solely.

Key stage 2 (at sticking): breathing, tonic/clonic seizures and muscle tone. In addition, the corneal or palpebral reflex, spontaneous blinking and vocalisations may also be used.

Key stage 3 (during bleeding): breathing and muscle tone. In addition, vocalisations, the corneal or palpebral reflex and spontaneous blinking can also be used.

14) Of the recommended indicators above the dashed line in the flow chart, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

RECOMMENDATIONS ON CARBON DIOXIDE STUNNING OF PIGS

15) A toolbox composed of the following indicators should be checked to determine consciousness of animals after stunning at all three key stages of the process, to ensure that animals remain unconscious until death occurs.

Key stage 1 (between end of carbon dioxide stunning and shackling): muscle tone, breathing and the corneal or palpebral reflex. Additional indicators—response to nose prick or ear pinch and vocalisations—may also be used.

Key stage 2 (at sticking): muscle tone, breathing and vocalisations. In addition, the corneal or palpebral reflex and response to nose prick or ear pinch may also be used.

Key stage 3 (during bleeding): muscle tone and breathing. In addition, the corneal or palpebral reflex and vocalisations may also be used.

16) Of the recommended indicators above the dashed line in the flow chart, a minimum of two indicators relevant to each key stage should be employed for an effective monitoring of the process.

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GLOSSARY

GLOSSARY FOCUSED ON KEYWORDS

Outcome of a welfare indicator: the result of check performed using an indicator based on which the animal is considered conscious or unconscious, or alive or dead.

Welfare indicator: an observation used to obtain information on an animal's state of consciousness/unconsciousness or life/death. In this opinion, all indicators are animal-based observations.

GLOSSARY FOCUSED ON THE MODEL

Accuracy of the sampling protocol: the percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed threshold failure rate would allow. This corresponds to confidence level in freedom from disease methodologies.

Sampling fraction: the proportion of the slaughter population which is assessed in the sampling protocol.

Sensitivity of the indicators: as defined previously, this is the percentage of truly conscious animals detected as conscious by the indicator. This corresponds to diagnostic test sensitivity in freedom from disease methodologies.

Slaughter population: a group of animals slaughtered under the same circumstances as determined by risk factors (see Table 7).

Threshold failure rate for proportion of mis-stunned animals: the minimum proportion of animals that are ineffectively stunned, which will still be detected by the sampling protocol. This corresponds to design prevalence in freedom from disease methodologies.

GLOSSARY FOCUSED ON INDICATORS

DESCRIPTIONS/DEFINITIONS OF THE INDICATORS

Breathing: effective electrical stunning will result in immediate onset of apnoea (absence of breathing). Ineffectively stunned animals and those recovering consciousness will start to breathe in a pattern commonly referred to as rhythmic breathing, which may begin as regular gagging and involves respiratory cycle of inspiration and expiration. Rhythmic breathing can be recognised from the regular flank and/or mouth and nostrils movement. Recovery of breathing, if not visible through these movements, can be checked by holding a small mirror in front of the nostrils or mouth to look for the appearance of condensation due to expiration of moist air.

Corneal reflex: the corneal reflex is elicited by touching or tapping the cornea. Ineffectively stunned animals and those recovering consciousness will blink in response to the stimulus. Unconscious animals may also intermittently show a positive corneal reflex.

Eye movements: eye movements and the position of the eyeball can be recognised from close examination of eyes after stunning. Correctly stunned animals will show fixed eyes, and this can be recognised from wide open and glassy eyes with clearly visible iris/cornea in the middle. Eyeballs may be obscured in some animals owing to rotation into the eye socket following effective head-only electrical stunning. Ineffectively stunned animals and those recovering consciousness will show eye movements.

Muscle tone: head-only electrically stunned animals will show general loss of muscle tone after the termination of tonic–clonic seizures coinciding with the recovery of breathing and the corneal reflex if not previously stuck. Loss of muscle tone can be recognised from the completely relaxed legs, floppy ears and tail, and relaxed jaws with protruding tongue. Ineffectively stunned animals and those recovering consciousness will show a righting reflex and attempts to raise the head.

Palpebral reflex: the palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Correctly stunned animals will not show a palpebral reflex. Ineffectively stunned animals and those recovering consciousness will blink in response to the stimulus.

Posture: effective head-only electrical stunning will result in immediate collapse or loss of posture in animals that are not restrained or prevented from doing so. Ineffectively stunned animals, on the other hand, will fail to collapse or will attempt to regain posture after collapse.

Pupillary reflex: the pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Correctly stunned animals will not show pupillary reflex. Ineffectively stunned animals will show pupillary constriction (miosis) in response to light.

Responses to a nose prick or ear pinch: response to a painful stimulus such as a pin prick to the muzzle (area between external nostrils) or the ear with a sharp instrument indicates consciousness following stunning using an electric current.

Spontaneous blinking: conscious animals may show spontaneous blinking and therefore this sign can be used to recognise ineffective stunning or recovery of consciousness after electrical stunning. However, not all the conscious animals may show spontaneous blinking.

Tonic– seizures: effective head-only electrical stunning leads to the onset of tonic-clonic seizures soon after immediate collapse of the animal. The tonic seizure, which may be recognised from the tetanus, lasts for several seconds and is followed by clonic seizures lasting for seconds and leading to loss of muscle tone.

Vocalisations: conscious animals may vocalise, and therefore purposeful vocalisation can be used to recognise ineffective stunning or recovery of consciousness after electrical stunning. However, not all conscious animals may vocalise.