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SCIENTIFIC OPINION

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

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

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ABSTRACT

This scientific opinion proposes toolboxes of welfare indicators, and their corresponding outcomes of consciousness, unconsciousness or death, for developing monitoring procedures at slaughterhouses for poultry stunned using electrical waterbaths and gas mixtures or slaughtered without stunning. For waterbath stunning, the opinion proposes a toolbox of indicators for assessing consciousness in poultry at two key stages of monitoring: (a) between the exit from the waterbath stunner and neck cutting and (b) during bleeding. For gas stunning, the opinion proposes a toolbox of indicators for assessing consciousness in poultry at two key stages of monitoring: (a) during shackling and (b) during bleeding. For slaughter without stunning, a toolbox is proposed for confirming death prior to entering scald tanks. Various activities—including a systematic literature review, an online survey and stakeholders' and hearing experts' meetings—were conducted to gather information about the specificity, sensitivity and feasibility of the indicators. On the basis of such information, a methodology was developed to select the most appropriate indicators to be used in the monitoring procedures. The frequency of checking differs according to the role of each person with responsibility for ensuring poultry welfare. The personnel will have to check all the birds and confirm that they are not conscious following stunning with electrical waterbaths or gas mixtures and that they are dead before entering scald tanks. For the animal welfare officer, a mathematical model for the sampling protocols is proposed, giving some allowance to set the sample size of birds that he/she needs to check at a given throughput rate (total number of birds slaughtered in the slaughterhouses) and threshold failure rate (number of potential failures—birds that are conscious after stunning). 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, death, 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, stunning methods and slaughter without stunning. 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 poultry after stunning with waterbaths or gas mixtures and the assessment of death in poultry during slaughter without stunning. The Panel on Animal Health and Welfare agreed that, although it is traditional to look for outcomes of unconsciousness in poultry following stunning, the risk of poor welfare can be detected better if bird 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. A toolbox of selected indicators is proposed to check for signs of consciousness in poultry after stunning with waterbaths or gas mixtures; a different toolbox of indicators is proposed for confirming death of the birds following slaughter without 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 slaughter with stunning of poultry, the major interest is to detect the undesired outcome, namely the presence of consciousness in birds. The toolbox proposes indicators and their outcomes. In the case of slaughter without stunning, the interest is to detect whether the animals become unconscious and to detect when the animal dies, as this determines the start of the next operational phase at the slaughterline. However, the indicators applied for this task also have to correctly detect animals as conscious or alive. The toolbox proposes 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 slaughter with prior stunning using electrical waterbath:

After stunning of the birds prior to slaughter the indicators should be repeatedly checked to detect signs of consciousness through the two key stages of monitoring during the slaughter process: between the exit from the waterbath stunner and neck cutting (key stage 1) and during bleeding (key stage 2).

The recommended indicators in Toolbox 1 (for monitoring between the exit from the waterbath stunner and neck cutting) are tonic seizures, breathing and spontaneous blinking. Additionally, the corneal or palpebral reflex and vocalisations may be used. In Toolbox 2 (for monitoring during bleeding) the recommended indicators are wing flapping and breathing. In addition, the corneal or palpebral reflex, spontaneous swallowing and head shaking may also be used.

Toolboxes for slaughter with prior stunning using gas mixtures:

After stunning of the birds with gas mixtures prior to slaughter, the indicators should be repeatedly checked to detect signs of consciousness through the two key stages of monitoring during the slaughter process: between the exit from the gas stunner and the entrance to the scalding tank, especially during shackling (key stage 1) and during bleeding (key stage 2).

The recommended indicators in Toolbox 3 (for monitoring between the exit from the gas stunner and neck cutting, especially during shackling) are breathing, muscle tone, wing flapping and spontaneous blinking. Additionally, the corneal or palpebral reflex and vocalisations may be used.

In Toolbox 4 (for monitoring during bleeding) the recommended indicators are wing flapping, muscle tone and breathing. In addition, the corneal or palpebral reflex may also be used.

Toolboxes for slaughter without stunning:

In the case of slaughter without stunning, all birds should be checked to confirm death before undergoing scalding. Moreover, consciousness or life in checked animals should be correctly identified. On this basis, the indicators were selected for the toolbox.

The recommended indicators in Toolbox 5 (for monitoring death before scalding) are breathing, the corneal or palpebral reflex, pupil size and bleeding. Additionally, muscle tone may be used.

The personnel performing stunning, and/or bleeding will have to check all birds to rule out the presence of consciousness following electrical waterbath or gas stunning or confirm death during slaughter without stunning. The person in charge of monitoring the overall bird welfare at slaughter (i.e. animal (poultry) welfare officer) has to check a certain sample of slaughtered birds 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 threshold failure rate (number of potential failures—birds that are conscious after electrical waterbath or gas 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 particular circumstances and needs of the slaughterhouse so requires.

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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.

Article 5 also requires operators, when animals are slaughtered without stunning, to carry out systematic checks to ensure that the animals do not present any signs of consciousness or sensibility before being released from restraint and do not present any sign of life before undergoing dressing or scalding.

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.

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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.

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

  (1) penetrative captive bolt for bovine animals,

  (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 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 if staff is an important risk factors).

  – 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

[6] Wording used for the stunning methods refers to Annex I to Regulation (EC) No 1099/2009.

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, in general, as proposed in other opinions concerning bovines, pigs, and sheep and goats, 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.

However, the pre-slaughter handling, stunning and slaughter procedures used in poultry are different from those practised in red meat slaughterhouses. In slaughterhouses using electrical waterbath stunning for poultry, conscious birds are manually shackled (hung upside down on metal shackles) prior to stunning and passed through electrified water baths; the birds' necks are then cut mechanically. In slaughterhouses using gas stunning, conscious birds contained in transport crates or tipped on to a conveyor are passed through gas chambers to render them unconscious prior to shackling manually and the birds' necks are cut mechanically. Owing to mechanical neck cutting, it is proposed to monitor the state of consciousness and unconsciousness in birds at two key stages: (1) between the exit from the electrical waterbath stunner and neck cutting or during shackling after gas stunning; and (2) during bleeding until death occurs.

In slaughterhouse conditions, live birds can enter scald tanks under two scenarios. Firstly, inadequately stunned birds and those that have failed to make contact with the electrified waterbath, because of wing flapping or because they are runts, would also miss the neck cutter by holding their heads up. Occasionally, effectively stunned birds also miss the neck cutting machine because they miss the rails that guide the neck towards the blade(s). Hence, if these birds are not slaughtered manually, they will enter the scald tank live and possibly conscious. Secondly, adequately stunned birds could have a poor neck cut and hence enter the scald tank alive but unconscious. In view of these potential scenarios, all birds must be checked at key stages 1 and 2, as a precaution. It is worth mentioning that a common practice in poultry slaughterhouses is that birds are visually checked after they exit from the neck cutting machines and manually cut in the event of failures.

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 pre-slaughter handling, stunning, shackling, hoisting and/or bleeding (hereafter referred to as the 'personnel') of animals 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, re-stun the animal. 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.

Finally, the person in charge of the overall animal welfare at slaughter (i.e. animal (poultry) welfare officer) should be able to monitor the birds during the entire process, from stunning to bleeding, and ensure that they do not show any signs of consciousness and sensibility and also that death occurs before the birds enter the scald tank. 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 changes are ambiguous, abolition of 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 neck cutting can also be recognised under the field conditions from the characteristic changes in the behaviour of poultry (e.g. spontaneous blinking, wing flapping, spontaneous swallowing, head shaking), 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 external stimulus such as blinking response to touching the cornea (corneal reflex), response to pain stimulus such as comb 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 poultry (e.g. see EFSA, 2004; Raj *et al*., 2006a, b, c).

At all of the key stages, monitoring is carried out to identify birds that are improperly stunned, and therefore attention is focused on the indicator of consciousness. Effectively stunned birds are expected to remain unconscious throughout key stage 2 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 the 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.

The slaughter of animals without prior stunning is regulated by Article 4 (4) of Regulation (EC) No 1099/2009. Slaughter without stunning induces gradual loss of consciousness and consequently death as a result of the brain being deprived of nutrients and oxygenated blood and onset of brain ischaemia. According to the Regulation, people performing slaughter without stunning are also required to have a certificate of competence. The Regulation also stipulates that scalding of poultry shall begin after the onset of death. Therefore, it is important to define indicators that can be used to recognise death following slaughter without stunning while simultaneously recognising as such any animal still conscious or alive.

Conditions of slaughter without stunning of poultry may vary depending upon local circumstances. However, most birds intended for slaughter without stunning are shackled prior to slaughter, but their necks may be cut manually or mechanically. Failure to cut both carotid arteries and inadequate bleed-out time will lead to birds remaining alive when entering scald tanks.

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).

1.3. Physiology of electrical waterbath stunning

Electrical stunning of poultry using a waterbath with a current of sufficient magnitude induces immediate loss of consciousness through the induction of generalised epileptiform activity in the brain (Raj *et al*., a, b, c). The neurophysiological basis of the generalised epileptiform activity and the associated loss of consciousness is well documented in the scientific literature (see EFSA, 2004, report for details). Since the induction of generalised epileptiform activity in poultry is dependent on the frequency (Hz) of current used in the waterbath stunners, certain minimum currents appropriate to the frequency are stipulated in the Regulation 1099/2009). Depending on the electrical frequency applied, the waterbath stunning can also induce cardiac arrest. This applies to low frequencies (e.g. 50 Hz sine wave alternating current) only.

Successful induction of epileptiform activity in the brain induces a tonic seizure. During tonic seizures, the birds show tetanus (arched and stiff neck, wings held tightly close to body), breathing is absent and the eyeballs are fixed. The tonic seizure is usually followed by clonic seizures, which are mild compared with those seen red meat species and difficult to recognise as birds are hanging on shackles during the stunning process.

The tonic seizures are followed by loss of muscle tone, which can be recognised from drooping wings. Additionally, reflexes that would require brain control are also abolished. For example, the palpebral (elicited by touching the 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. comb pinch) are also abolished during the period of unconsciousness.

Ineffective waterbath electrical stunning of poultry can occur for various reasons (e.g. intermittent contact of shackle with the earth bar, intermittent immersion of head in the waterbath as a result of wing flapping or pre-stun shocks at the entrance to the waterbath stunner), and, as a consequence, the bird may not experience the generalised epileptiform activity required to achieve unconsciousness. This situation will lead to different behavioural manifestations and retention of reflexes, which can be recognised from the absence of tonic–clonic seizures and the presence of breathing (including laboured breathing). Ineffectively stunned birds and those recovering consciousness will show spontaneous blinking, spontaneous swallowing (deglutition reflex triggered by water from the stunner or blood from the neck-cutting wound entering the mouth during bleeding; Raj *et al*., 2006a) or positive eye reflexes (palpebral, corneal and pupillary). The eyeballs in poultry are fixed in the socket and, for this reason, eye movements (e.g. rotation of eyeball as in red meat species) are not possible in ineffectively stunned birds; however, movement of the third eyelid (nictitating membrane) can be seen instead. Head righting (attempt to raise head), head shaking or wing flapping after electrical stunning is also a sign of consciousness.

Effectively stunned, i.e. unconscious, birds are bled out by the cutting of both carotid arteries in the neck, usually by the use of an automatic, rotating knife (also known as killing or neck-cutting machines) located on the line after the waterbath stunner. Prompt and accurate neck cutting of effectively stunned birds results in rapid onset of death, and therefore birds do not show signs of recovery of consciousness at any of the key stages of monitoring. This means that when stunning has been effective and the duration of unconsciousness induced by the stunning method was longer than the total time between the end of stunning and the neck cutting (stun-to-neck cutting interval) plus the time it takes for bird to die through blood loss, the bird will remain unconscious until death occurs. On the other hand, ineffective stunning or prolonged stun-to-neck cutting interval and/or inappropriate/inadequate neck cutting will lead to birds showing signs of recovery of consciousness.

Inappropriate neck cutting includes a cut that only severs the veins or one artery only, which is not enough to facilitate rapid bleeding (see EFSA, 2004, for details).

Induction of cardiac arrest in waterbath stunners produces relaxed carcasses, which manifests as drooping wings and dilated pupils in birds at the exit from the stunners.

1.4. Physiology of gas stunning

Exposure of poultry to gas mixtures contained in a chamber leads to gradual loss of consciousness and sensibility owing to the inhibition of brain function, as evidenced from the abolition of spontaneous and evoked electrical activity, recorded using EEG or ECoG.

The neurophysiological basis of this effect varies depending on the gases involved and their relative concentration, and is documented in the scientific literature (see EFSA, 2004, report for details). Depending on the gas concentration and the duration of exposure, gas stunning can be either reversible or irreversible (see Regulation 1099/2009).

Several different methods of gas stunning of poultry can be used, involving different gas combinations. As set out in Regulation 1099/2009, these include (a) carbon dioxide at high concentration, (b) carbon dioxide in two phases, (c) carbon dioxide combined with inert gases such as argon or nitrogen or (d) inert gases alone.

Successful induction of unconsciousness using gas stunning results in a bird that loses posture, sometimes (e.g. depending on gas combinations used) displays head shaking, leg paddling and wing flapping during the stunning process, and lies flat and relaxed on belly, side or back when exiting the chamber. After stunning, the body of the bird is completely relaxed, breathing is absent and the eyeballs are fixed.

The birds are then shackled and, if stunning has been successful, the birds' bodies will remain completely relaxed and without muscle tone until death is achieved by bleeding. Additionally, reflexes that would require brain control are also abolished. For example, the palpebral (elicited by touching the 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. comb pinch) are also abolished during the period of unconsciousness.

Ineffective gas stunning of poultry can occur for various reasons and, as a consequence, the depth of unconsciousness may be insufficient or the duration of unconsciousness may not last until the end of bleeding. Ineffective stunning or recovery of consciousness can be recognised during shackling from the presence of muscle tone (e.g. neck tension), breathing (including laboured breathing), spontaneous blinking, the corneal or palpebral reflex or wing flapping. Birds recovering consciousness during bleeding can be recognised from wing flapping, the presence of breathing, the corneal or palpebral reflex, eye movements, spontaneous swallowing and head shaking.

1.5. Physiology of slaughter without stunning

Slaughter without stunning does not induce immediate loss of consciousness in any type of animals. In other words, birds are gradually rendered unconscious by the severance of carotid arteries as brain perfusion becomes insufficient to sustain normal function, eventually leading to death. The times to onset of unconsciousness and to death can be highly variable between different species (turkey vs. broilers) and between individual birds (e.g. turkey hens vs. turkey toms). The rate of bleeding may not always be profuse or uninterrupted if severance of the carotid arteries is incomplete (poor cut), which will lead to poor welfare, and therefore continuous and systematic monitoring of all birds slaughtered without stunning is required.

Monitoring of bird welfare during slaughter without stunning is mainly focused on detecting live birds prior to scalding, and live birds can be recognised from the presence of breathing or of the corneal and palpebral reflexes, pupils that are not fully dilated, continued bleeding, or the presence of muscle tone and body movements. The literature suggests that the longest time to onset of unconsciousness in broilers, defined as the time to loss of posture or the end of bleeding, is 26 and 45 seconds respectively (Barnett *et al*., 2007). Although similar data concerning slaughter without stunning of turkeys are lacking, the literature suggests that turkeys are more resilient to the effect of brain ischaemia and therefore times to onset of unconsciousness and death are expected to be significantly longer.

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 poultry after stunning.
>
> **Indicators B,** designed to detect—in the poultry slaughtered without stunning—signs of death before undergoing scalding.

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

The Working Group agreed that, although it is traditional to look for outcomes of unconsciousness in poultry following stunning, the risk of poor welfare can be detected better if bird welfare monitoring is focused on detecting consciousness, i.e. ineffective stunning or recovery of consciousness. Therefore, the indicators were phrased neutrally (e.g. eye movements) and the outcomes were phrased either suggesting unconsciousness (e.g. absence of third eyelid movements) or suggesting consciousness (e.g. presence of third eyelid movements). 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 as from mandate's ToRs	**Checking state of**	**Outcome related to poultry welfare**
Poultry	Stunning with waterbath	Key stage 1 = between exit from the waterbath and neck cutting	A	Consciousness and unconsciousness	Consciousness
		Key stage 2 = during bleeding	A	Consciousness and unconsciousness	Consciousness
	Stunning with gas mixtures	Key stage 1 = during shackling	A	Consciousness and unconsciousness	Consciousness
		Key stage 2 = during bleeding	A	Consciousness and unconsciousness	Consciousness
	Slaughter without stunning	Key stage 1 = Prior to scalding	B	Life and death	Life

The indicators investigated in this opinion were selected based on previous EFSA opinions (EFSA, 2004, 2006) and amended in Working Group discussion on the basis of feedbacks from (i) a stakeholder meeting at which interested parties were consulted by 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 toolboxes of selected indicators for the other species) 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/neck cutting 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 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.

2.1.2.2. Sensitivity and specificity during slaughter without stunning

In contrast, during slaughter without stunning, all the animals are alive and conscious when neck cutting is performed. However, as with stunning, the purpose of slaughter is to induce death (i.e. kill for human food), and it is therefore imperative to confirm death in birds prior to scalding. Therefore, it is of major interest to detect unconsciousness and death in all animals. The use of indicators for detecting unconsciousness or death is a test with positive or negative outcome, where the positive outcome causes the animal to be considered as conscious or alive, and the negative outcome is the confirmation of unconsciousness or death, respectively.

So, similar to slaughter with stunning, sensitivity is calculated as the number of conscious or live animals considered conscious or alive based on the outcome of the indicator (E in Table 3) divided by the number of conscious or alive animals (E + G), respectively, multiplied by 100 (in short, the percentage of animals truly still conscious or alive that the indicator tests conscious or alive).

Specificity is calculated as the percentage of unconscious or dead animals (F + H) that the indicator tests as unconscious or dead (H), respectively.

An indicator for slaughter without stunning is considered to be 100 % sensitive if it detects all animals still conscious or alive as conscious or live animals. An indicator is considered to be 100 % specific if it detects unconsciousness or death in animals, when animals truly became unconscious or dead, respectively.

Table 3: Sensitivity and specificity of indicators during slaughter without stunning

Slaughter without stunning		**Truth: the animal is still conscious (alive)**	
		Yes	**No**
Is the animal considered conscious (alive), based on the outcome of the indicator?	**Yes**	**E**	F
	No	G	**H**

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 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 Animal Health and Animal Welfare Panel of EFSA 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 and of the slaughter process without stunning. 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, having a data distribution of easy = 3; normal = 6; 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. 11/186 for waterbath stunning); if the uncertainty rating was omitted, answers were re-set to the lowest uncertainty weight (i.e. 1 = 'not sure'; 3/175). If a respondent's answer to all or the priming sequence of 'not show/respond to' (i.e. 'breathing', 'comb and pinch') questions reversed the logic (i.e. "5 % of truly unconscious animals will not show eye movements") and the same question was rather consistently answered by other respondents (i.e. here 19/20 respondents rated above 80 %), then the corresponding values in the data record were reversed as '100 % minus rating' (8/175). Ratings were not reversed if variability across the respondents was too large for particular indicators to conclude logical inconsistency. Particularly for waterbath stunning data, the answers of one respondent were excluded as they indicated a misinterpretation of the questionnaire (8/175).

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 poultry industry, one representative from the red meat industry and two representatives from European Commission) 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. The experts invited to this meeting had previous access to the draft opinion on poultry, and the proposed toolboxes of indicators for poultry, 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 8). 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 captive bolt stunning situation, 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]

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

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

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

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 this 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.

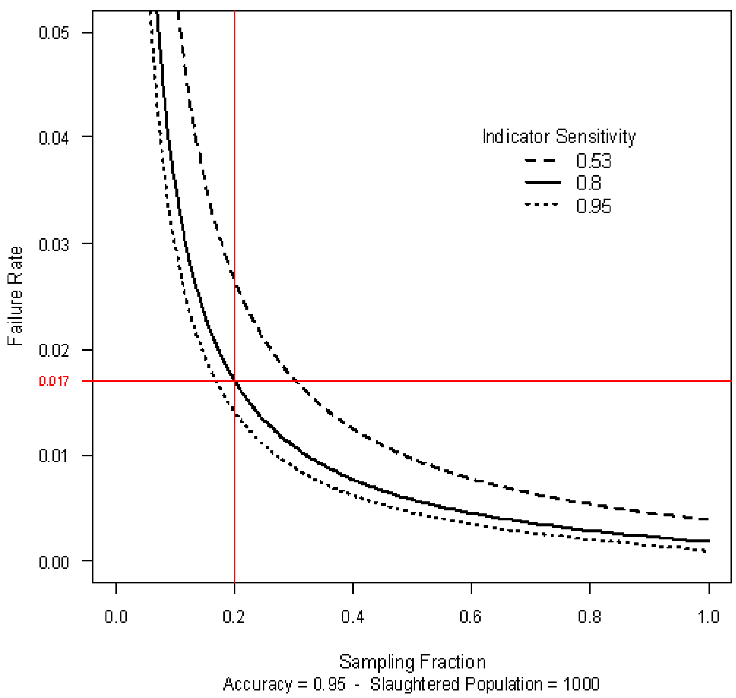


Figure 1: Example graph of the relationship between the parameters defining a sampling protocol (SF and detectable threshold FR for fixed values of accuracy (here 95 %) and slaughter population (here 1 000 animals) and various scenarios for indicator sensitivity)

[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/.

In Figure 21, 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 failure rate (FR) and the sampling fraction (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

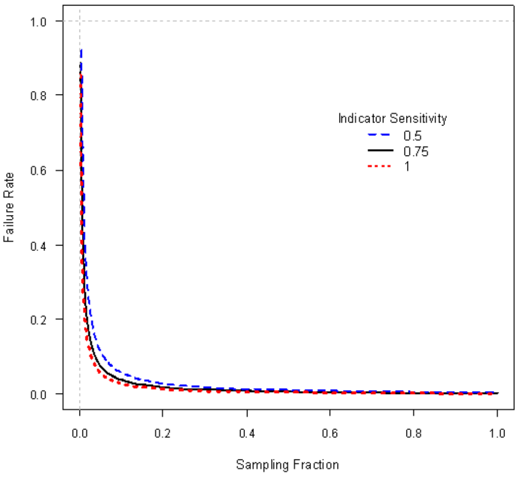
The results of the statistical modelling are summarised in Figure 2.

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 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 Figure 2a, b and c.

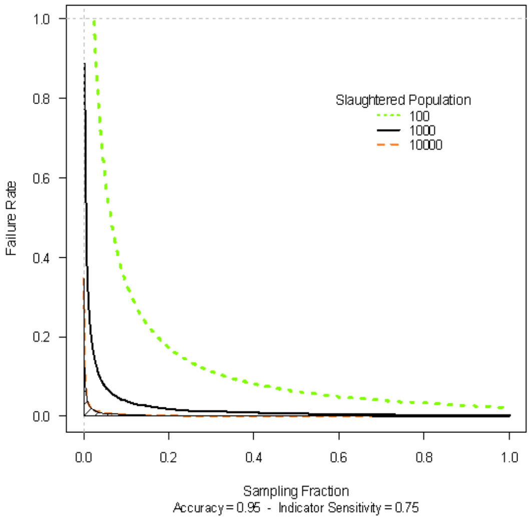
[9] Proportion of mis-stunned animals (see section 2.3.1).

[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).

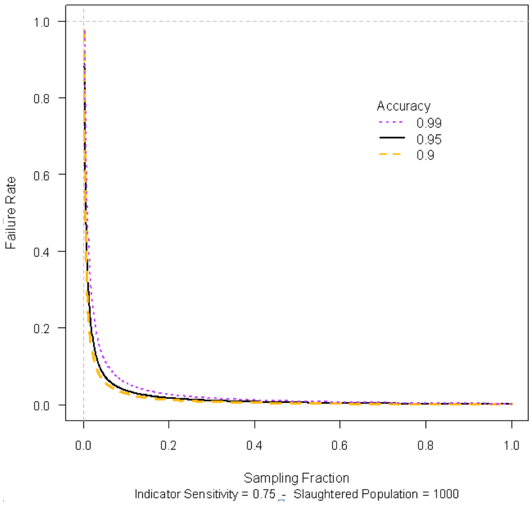
[11] Percentage of situations in which the sampling protocol was applied and served its purpose, i.e. raising an alarm if there were more ineffectively stunned animals than the prescribed failure rate would allow (see section 2.3.1.)



(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, 1 000, 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 4a, b and c shows numerical examples of failure rates for three levels of indicator sensitivity, sample fraction and sampling protocol accuracy.

Table 4: 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 and death in poultry are reported in Table 5.

Table 5: Total number of answers and the outcomes of unconsciousness and death of indicators most used for poultry as collected through questionnaire 1 of the stakeholder meeting

Species/method	Total No of answers	Outcome of unconsciousness of most used indicators[12]	Outcome of death of most used indicators[13]
Chicken and turkeys—waterbath stunning	29	No wing flapping No corneal reflex No vocalisation	
Chicken and turkeys—gas stunning	43	No corneal reflex Completely related body No vocalisation	
Chicken and turkeys—slaughter without stunning	9	No attempts to raise the head No wing flapping	Cessation of spontaneous movement Absence of breathing End of bleeding

Experts responded that they observe the outcomes of the indicators between 10 and 30 seconds after stunning or after neck cutting. The main problem encountered in checking most of the indicators is access to the animal. Another common problem is the difficulty of evaluating the indicators in different animal categories. Several indicators are normally used by the experts to assess the state of unconsciousness and death 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

The systematic literature review concluded that the publications considered in the evaluation of the gas stunning, electrical waterbath stunning and slaughter without stunning of chickens and turkeys did not meet the study evaluation criteria. Therefore, neither the sensitivity nor specificity of the indicators

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

was identified. In particular, three studies reported the use of EEG to assess unconsciousness in birds that were stunned using an electric waterbath (Prinz *et al*., 2010a, b, 2012). The authors collected data on the number of animals with the indicators of interest and two EEG measures; neither measure explicitly included the authors' definition of unconsciousness. Nevertheless, data were reported as the proportion of stunned animals with the indicator at < 10 seconds, 10–20 seconds, 20–30 seconds and 30–40 seconds. The study authors also reported the proportion of stunned animals that were unconscious. These data are reported in an external report commissioned by EFSA (O'Connor *et al.*, in press). Therefore, as discussed in section 3.6.1 of that report, this approach to reporting does not enable calculation of the sensitivity and specificity of indicators of unconsciousness. These proportions were reported in bar charts, and therefore the numbers were inferred. Further, the proportions in the figures were obtained from predicted models. However, the study authors did not discuss the assessment of model fit (Prinz *et al*., 2010a, b, 2012).

Regarding gas stunning of poultry, 10 studies reported the use of EEG to measure unconsciousness in poultry stunned using various gas methods (Raj *et al*., 1990, 1991, 1992a, b, c, 1998; Coenen *et al*., 2000, 2005, 2009; McKeegan *et al*., 2007; Gerritzen *et al*., 2013). However, no study explicitly reported an indicator requested by EFSA. For instance, a commonly reported outcome was eye closure, but this was not an indicator under investigation because effective gas stunning of poultry does not always result in closed eyes (Raj *et al*., 1990). In addition, these data were of little value because they were measured and averaged at a group level. As indicated in section 3.6.3 of the external report (O'Connor *et al.*, in press), without knowledge of the joint distribution, the information needed to assess sensitivity and specificity at the bird level cannot be estimated since translation of group-level time-based metrics require assumptions that are likely to be invalid. Therefore, none of these data can be used to estimate sensitivity and specificity of the indicators.

No studies were identified that used EEG-based measures of death compared with the indicators under investigation.

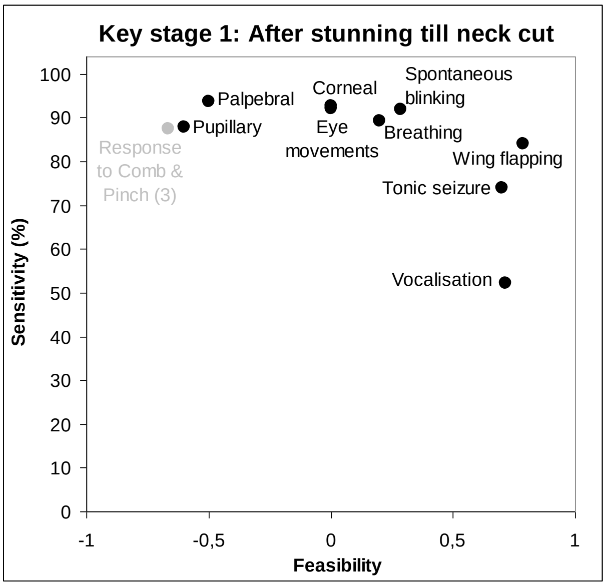
Many studies (so-called 'prevalence studies') report the proportion of stunned animals with outcomes of consciousness or unconsciousness, rather than the proportion of truly unconscious or conscious animals at a set time point with the outcome of the indicators. Such data cannot be translated into sensitivity and specificity. However, prevalence studies are used to describe the indicators in sections 3.7, 3.8 and 3.9 of this opinion.

3.3. Results from questionnaire 2 on electrical waterbath stunning

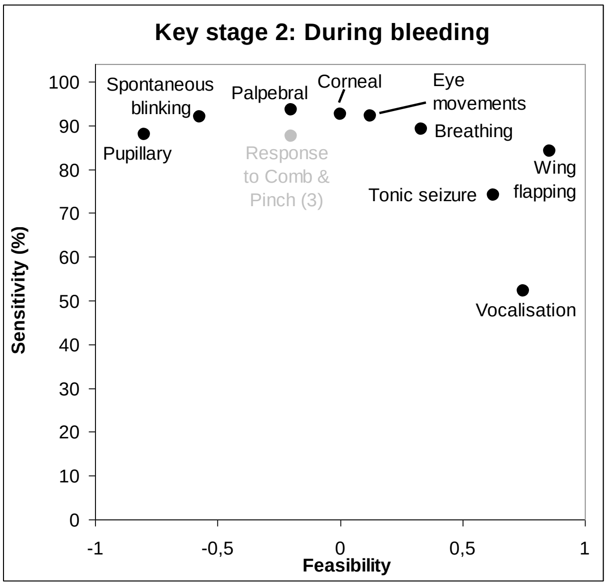
From the second questionnaire, namely the online survey, answers from around 82 experts were collected. Respondents could answer for more than one species or method, depending upon their work experience, so the total number of completed surveys was 84.

In total, 22 respondents said that they monitor the welfare of poultry following electrical waterbath stunning. Sixteen respondents answered for gas stunning procedures, and six respondents answered for slaughter without stunning.

The graphs in Figure 3a and b combine the estimates of feasibility and sensitivity for each indicator for electrical waterbath stunning at each key stage (key stage 1 = between the exit from the waterbath stunner and neck cutting, key stage 2 = during bleeding). Thus, the most indicators nearest the top-right indicators corner have high sensitivity and high feasibility. In the graphs the sensitivity value is identical but the feasibility score changes according to the respondent ratings.



(a) Between the exit from the waterbath stunner and neck cutting

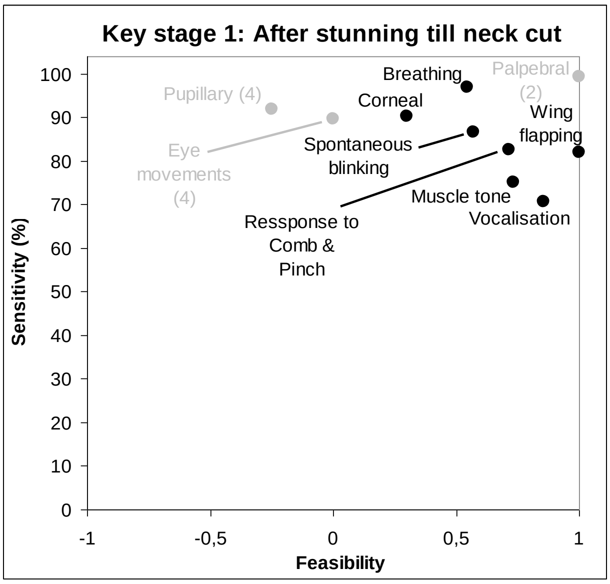


(b) During bleeding

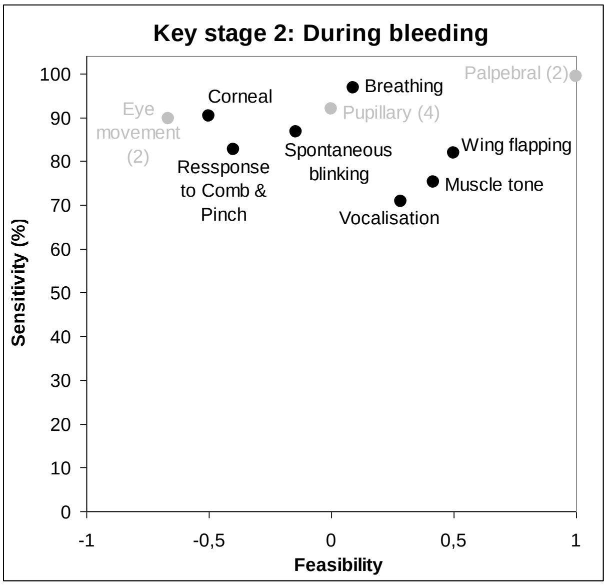
Figure 3: Graphical combination of feasibility score and sensitivity for electrical waterbath resulting from questionnaire 2 for each indicator at (a) key stage 1 = after stunning till neck cut and (b) key stage 2 = during bleeding. Grey symbols/items are indicators with minimum number of data points

3.4. Results from questionnaire 2 on gas stunning

The graphs in Figure 4a and b combine the estimates of feasibility and sensitivity for each indicator for gas stunning at each key stage (key stage 1 = between stunning and neck cut, key stage 2 = during bleeding).



(a) Between stunning and neck cutting



(b) During bleeding

Figure 4: Graphical combination of feasibility score and sensitivity for gas stunning resulting from questionnaire 2 for each indicator at (a) key stage 1 = after stunning till neck cut and (b) key stage 2 = during bleeding. Grey symbols/items are indicators with minimum number of data points

3.5. Results from questionnaire 2 on slaughter without stunning

Five respondents who said that they are experienced with slaughter without stunning in poultry contributed response data. The respondents reported the feasibility of assessing indicators prior to scalding and the sensitivity and specificity of five indicators. However, for the five indicators included in the questionnaire, in total 12 ratings were received. Thus, the data did not support further quantitative considerations.

3.6. Results from working group discussion

In view of the fact that the process of shackling, stunning and slaughter (neck cutting) of poultry is different from that of red meat species, and the anatomy of poultry is different from that of mammals, the Working Group agreed that description of some of the indicators may have to be modified. It was also agreed that new indicators, other than those included in the systematic literature review or in questionnaire 2, need to be considered for inclusion in the toolboxes as new information becomes available.

3.7. Description of indicators for electrical waterbath 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 electrical waterbath stunning. Some of these outcomes occur spontaneously following stunning (e.g. tonic seizures) whereas some other outcomes will have to be intentionally provoked (e.g. 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 an applied stimulus (e.g. 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.

3.7.1. Tonic seizure

3.7.1.1. Description

In key stage 1, effective electrical stunning leads to **onset of tonic seizure**. The tonic seizure as seen in stunned, shackled birds can be recognised from the occurrence of an arched and stiff neck (i.e. necks appear parallel to the ground in birds hanging from the shackle line) and wings held tightly close to the body.

The tonic seizure will cease rapidly following neck cutting and, therefore, is not applicable at key stage 2. In addition, induction of cardiac arrest at stunning in a waterbath stunner would lead to absence of tonic seizure. Cardiac arrest at stunning can be recognised from drooping wings and complete loss of muscle tone; however, induction of cardiac arrest with a 50 Hz current is not practised nowadays because of the detrimental effects of this electrical frequency on carcass and meat quality. Therefore, loss of muscle tone or relaxed body is not considered to be relevant to waterbath stunning of poultry.

3.7.1.2. Feasibility

From questionnaire 2, tonic seizure was rated as easy ($n = 7$) or normal ($n = 3$) to assess at key stages 1 and 2 ($n = 10$). No respondent found it difficult to assess. For key stage 2, 2 out of 10 respondents reported that this indicator is not applicable, probably because the tonic seizure will end prior to or very soon after neck cutting. However, tonic seizure was rated as easy ($n = 5$) or normal ($n = 3$) to assess during bleeding by the remaining eight respondents.

3.7.1.3. Sensitivity and specificity

The outcome of consciousness for this indicator is the absence of tonic seizure. Hence, the sensitivity is the percentage of conscious birds which do not show tonic seizure, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 78 % ($n = 9$). The specificity is calculated as a percentage of birds showing tonic seizures, out of all truly unconscious birds. This was estimated to be 77 % ($n = 13$).

3.7.2. Wing flapping

3.7.2.1. Description

Wing flapping is expected only in conscious birds and can be used as an indicator at all key stages of monitoring. However, not all the conscious birds will show wing flapping, and hence absence of wing flapping does not always mean that the bird is unconscious. Birds showing wing flapping must be re-stunned. Since unconscious birds will not show wing flapping, this indicator is not applicable to monitoring unconsciousness.

3.7.2.2. Feasibility

From questionnaire 2, wing flapping was rated as easy or normal to assess at key stage 1 by 13 out of 15 respondents, and one respondent found it difficult to assess. Furthermore, one respondent found it not applicable. At key stage 2, a total of 14 out of 15 found it easy or normal to assess, and one respondent found it not applicable.

3.7.2.3. Sensitivity and specificity

The positive outcome of wing flapping, namely the presence of wing flapping, is the sign of consciousness. Therefore, the sensitivity is the percentage of birds which show wing flapping, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 76 % ($n = 13$). The specificity is calculated as a percentage of birds showing no wing flapping, out of all truly unconscious birds. This was estimated to be 69 % ($n = 13$), which is low, probably because wing flapping may be difficult to differentiate from wing movements occurring during tonic seizures.

3.7.3. Breathing

3.7.3.1. Description

In key stage 1, effective electrical stunning will lead to immediate onset of apnoea, i.e. **absence of breathing**, which can be used to monitor the effectiveness of electrical waterbath stunning. Ineffective electrical stunning can be recognised from the sustained/**presence of breathing, including laboured breathing**.

An effectively stunned and neck-cut bird will remain unconscious until death occurs in key stage 2 and therefore is not expected to show any signs of breathing. For this reason, breathing as an indicator is not applicable at this stage. On the other hand, birds 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 they will have to be re-stunned.

3.7.3.2. Feasibility

In questionnaire 2, breathing was rated as easy or normal to assess at key stage 1 by 12 out of 16 respondents, and three respondents found it difficult to assess. Furthermore, one respondent found it not applicable at this stage. For key stage 2, a total of 10 out of 15 respondents found it easy to assess, and four found it difficult to assess.

3.7.3.3. Sensitivity and specificity

The positive outcome of breathing, namely the presence of rhythmic breathing, is the sign of consciousness. Therefore, the sensitivity is the percentage of birds which show rhythmic breathing, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 89 % ($n = 13$). The specificity is calculated as a percentage of birds showing no rhythmic breathing, out of all truly unconscious birds. This was estimated to be 79 % ($n = 13$).

3.7.4. Response to comb or toe pinching

3.7.4.1. Description

Ineffective electrical stunning and recovery of consciousness due to poor stunning and/or bleeding can be recognised from the **response to comb or toe pinch** at all key stages of monitoring. Birds showing a positive response to painful stimulus at any stage must be re-stunned.

3.7.4.2. Feasibility

In questionnaire 2, response to comb or toe pinching was rated as easy or normal to assess at key stage 1 by one out of five respondents, and two respondents found it difficult to assess. At key stage 2, a total of four out of five respondents found it normal to assess, and one respondent found it difficult to assess.

3.7.4.3. Sensitivity and specificity

The positive outcome of response to comb or toe pinching, namely the presence of such a response, is the sign of consciousness. Therefore, the sensitivity is the percentage of birds which show a response to pinching, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 88 % (n = 3). The specificity is calculated as a percentage of birds showing no response to comb or toe pinching, out of all truly unconscious birds. This was estimated to be 99 % (n = 4). The percentage of conscious birds estimated not to show a response to comb or toe pinching was reported to be 12 %. The sensitivity figure is reasonably good and the specificity figure is very high, but it should be kept in mind that only three respondents answered this question.

3.7.5. Vocalisation

Vocalisation is expected only in conscious birds and can be used as an indicator in all key stages of monitoring. However, not all conscious birds will vocalise, and hence absence of vocalisation does not always mean that the bird is unconscious. Birds showing vocalisation must be re-stunned. Since unconscious birds will not vocalise, this indicator is not applicable to monitoring unconsciousness.

3.7.5.1. Feasibility

From questionnaire 2, vocalisation was rated as easy or normal to assess at key stage 1 by seven out of eight respondents, and one respondent found it not applicable at this stage. For key stage 2, a total of seven out of eight respondents found it easy to assess whereas one respondent found vocalisation difficult to assess.

3.7.5.2. 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 birds which do vocalise, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 52 % (n = 7). The specificity is calculated as a percentage of birds showing no vocalisation, out of all truly unconscious birds. This was estimated to be 98 % (n = 8). The percentage of conscious birds estimated not to vocalise was reported to be 48 %. The low sensitivity figure indicates that respondents believe that it is quite common that birds do not vocalise, even if they have not been successfully stunned.

3.7.6. Eye movements

3.7.6.1. Description

In key stage 1, effective electrical stunning will produce **fixed eyes** (eyes wide open and glassy) and the eyes will remain fixed until death occurs. Birds that are not effectively stunned with an electric current or those recovering consciousness will show movement of the third eyelid, commonly known as the nictitating membrane. Birds showing eye movements must be re-stunned.

3.7.6.2. Feasibility

In questionnaire 2, eye movements were considered as easy or normal to assess at key stage 1 by six out of nine respondents, and two respondents found it difficult to assess. Furthermore, one respondent found it not applicable at this key stage. At key stage 2, a total of six out of nine respondents found it easy or normal and two respondents found it difficult to assess, whereas one found it not applicable.

3.7.6.3. Sensitivity and specificity

The positive outcome of eye movement is the sign of consciousness, namely the presence eye movement. Therefore, the sensitivity is the percentage of birds which do show eye movement after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 92 % (n = 6). The specificity is calculated as a percentage of birds showing no eye movement, out of all truly unconscious birds. This was estimated to be 82 % (n = 9).

3.7.7. Palpebral reflex

3.7.7.1. Description

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

3.7.7.2. Feasibility

In questionnaire 2, the palpebral reflex was rated as normal to assess at key stage 1 by two out of six respondents, and two respondents found it difficult to assess. Furthermore, two respondents found it not applicable at this key stage. At key stage 2, a total of four out of six found it normal and one respondent found it difficult to assess, whereas one found it not applicable.

3.7.7.3. Sensitivity and specificity

The positive outcome of palpebral reflex is the sign of consciousness, namely a positive palpebral reflex. Therefore, the sensitivity is the percentage of birds which show the palpebral reflex after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 94 % (n = 5). The specificity is calculated as a percentage of birds showing no palpebral reflex, out of all truly unconscious birds. This was estimated to be 82 % (n = 6).

3.7.8. Corneal reflex

3.7.8.1. Description

Effective electrical stunning will lead to abolition of corneal reflex. Effectively stunned and neck-cut birds show **no corneal reflex** during any key stage. On the other hand, ineffectively or poorly stunned birds and those recovering consciousness prior to sticking or during bleeding are expected to show a **positive corneal reflex** at any key stage. Birds showing a positive corneal reflex must be re-stunned.

3.7.8.2. Feasibility

In questionnaire 2, the corneal reflex was rated as easy or normal to assess at key stage 1 by 11 out of 18 respondents, and four respondents found it difficult to assess. Furthermore, three respondents found it not applicable at this key stage. At key stage 2, a total of 12 out of 18 found it easy or normal and four respondents found it difficult to assess, whereas two found it not applicable.

3.7.8.3. Sensitivity and specificity

The positive outcome of corneal reflex is the sign of consciousness, namely the positive corneal reflex. Therefore, the sensitivity is the percentage of birds which show corneal reflex after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 93 % (*n* = 13). The specificity is calculated as a percentage of birds showing no corneal reflex, out of all truly unconscious birds. This was also estimated to be 93 % (*n* = 15).

3.7.9. Spontaneous blinking

3.7.9.1. Description

Spontaneous blinking is expected only in conscious birds and can be used as an indicator in all key stages of monitoring. However, not all the conscious birds will show spontaneous blinking, and hence absence of blinking does not always mean that the bird is unconscious. Birds showing blinking must be re-stunned. Since unconscious birds will not show blinking, this indicator is not applicable to monitoring unconsciousness.

3.7.9.2. Feasibility

In questionnaire 2, spontaneous blinking was rated as easy or normal to assess at key stage 1 by seven out of eight respondents, and one respondent found it not applicable at this key stage. At key stage 2, a total of three out of eight found it normal and four respondents found it difficult to assess, whereas one found it not applicable.

3.7.9.3. Sensitivity and specificity

The positive outcome of spontaneous blinking is the sign of consciousness, namely the presence of spontaneous blinking. Therefore, the sensitivity is the percentage of birds which show spontaneous blinking after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 94 % (*n* = 6). The specificity is calculated as a percentage of birds showing no corneal reflex, out of all truly unconscious birds. This was estimated to be 92 % (*n* = 6).

3.7.10. Pupillary reflex

3.7.10.1. Description

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

3.7.10.2. Feasibility

In questionnaire 2, the pupillary reflex was rated as normal to assess at key stage 1 by two out of six respondents, while three respondents found it difficult to assess and one respondent found it not applicable at this key stage. For key stage 2, only one out of six respondents rated it normal, four found it difficult to assess and one still found it not applicable.

3.7.10.3. Sensitivity and specificity

The positive outcome of pupillary reflex is the sign of consciousness, namely a positive pupillary reflex. Therefore, the sensitivity is the percentage of birds which show the pupillary reflex after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 88 % (*n* = 5). The specificity is calculated as a percentage of birds showing no corneal reflex, out of all truly unconscious birds. This was also estimated to be 78 % (*n* = 5).

3.7.11. Spontaneous swallowing

Although not included in questionnaire 2, the Working Group agreed that there are additional indicators reported in the scientific literature and these could be included for monitoring electrical waterbath stunning. Spontaneous swallowing (deglutition reflex) of blood entering the mouth during bleeding has been reported in the majority of the birds that have been ineffectively stunned, i.e. that fail to show epileptiform activity in the EEG or recover consciousness during bleeding owing to failure to cut both carotid arteries in the neck (Raj *et al*., 2006a, b, c). Such a reflex can also be triggered in ineffectively stunned birds as a result of water entering the mouth during immersion in the waterbath stunners and can be seen clearly. Therefore, spontaneous swallowing was included in Toolbox 2, although the sensitivity, specificity and feasibility had not been estimated in questionnaire 2. Low feasibility scores assigned to other indicators presented in Table 7 also prompted inclusion of swallowing.

3.7.12. Head shaking

Although not included in questionnaire 2, the Working Group agreed that there are additional indicators reported in the scientific literature and these could be included for monitoring electrical waterbath stunning. Head shaking during bleeding (probably triggered by the entry of blood into nostrils) has been reported in majority of the birds that have been ineffectively stunned, i.e. that fail to show epileptiform activity in the EEG or recover consciousness during bleeding owing to failure to cut both carotid arteries in the neck (Raj *et al*., 2006a, b, c) and the indicator can be seen clearly. Therefore, head shaking was included in Toolbox 2, although the sensitivity, specificity and feasibility had not been estimated in the questionnaire 2. Low feasibility scores assigned to other indicators presented in Table 6 also prompted inclusion of swallowing.

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

Table 6: Summary of information on sensitivity, specificity and feasibility of indicators and outcomes of consciousness for electrical waterbath stunning from questionnaire 2

Indicators after electrical waterbath 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 score		
						After stunning	During neck cutting	During bleeding
Tonic seizures	Absence	78	71 (44, 80, 100)	77	83 (77, 90, 100)	0.70	0.70	0.63
Wing flapping	Presence	76	82 (58, 95, 100)	69	72 (38, 90, 99)	0.79	0.86	0.86
Breathing	Presence	89	86 (84, 100, 100)	79	74 (45, 100, 100)	0.20	0.00	0.33
Response to comb pinch	Presence	88	87 (n.a. (*n* = 3))	99	100 (99, 100, 100)	–0.67	–0.67	–0.20
Vocalisation	Presence	52	51 (20, 30, 90)	98	98 (96, 100, 100)	0.71	0.75	0.75
Eye movements	Presence	92	88 (88, 95, 100)	82	85 (86, 98, 99)	0.00	–0.25	0.13
Palpebral reflex	Presence	94	88 (82, 100, 100)	82	84 (98, 100, 100)	–0.50	–0.40	–0.20
Corneal reflex	Presence	93	89 (90, 100, 100)	93	91 (88, 98, 100)	0.00	–0.47	0.00
Spontaneous blinking	Presence	94	87 (82, 95, 98)	92	91 (90, 95, 99)	0.29	–0.29	–0.57
Pupillary reflex	Presence	88	85 (80, 95, 100)	78	74 (48, 90, 92)	–0.60	–0.80	–0.80
Spontaneous swallowing	Presence	(b)	(b)	(b)	(b)	(b)	(b)	(b)

Head shaking	Presence	(b)	(b)	(b)	(b)	(b)	(b)	(b)

(a): In questionnaire 2, tonic seizures as an indicator was intended to be 'presence of tonic seizures' and was an outcome of unconsciousness. n.a.—not applicable as fewer than five responses available.
(b): These indicators were added following Working Group discussions and were not included in questionnaire 2; therefore values for their sensitivity and specificity are not available.

3.8. Description of indicators for gas stunning and overview of their performance

3.8.1. Muscle tone

3.8.1.1. Description

In key stage 1, effective gas stunning leads to a loss of **muscle tone**, which can be recognised from a relaxed body including drooping wings. As birds are manually shackled during key stage 1, muscle tone is generally considered a feasible indicator at this point in time. A relaxed body can be seen at both key stages.

3.8.1.2. Feasibility

From questionnaire 2, muscle tone was rated as easy or normal to assess at key stage 1 by all 15 respondents. For key stage 2, 11 out of 14 found it easy or normal to assess, one found it difficult too assess and two found it not applicable.

3.8.1.3. Sensitivity and specificity

The positive outcome of muscle tone, namely the presence of muscle tone, is the sign of consciousness. Hence, the sensitivity is the percentage of birds which show a certain level of muscle tone, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 75 % (n = 10). The specificity is calculated as a percentage of birds showing loss of muscle tone, out of all truly unconscious birds. This was estimated to be 99 % (n = 10).

3.8.2. Wing flapping

3.8.2.1. Description

Wing flapping is expected only in conscious birds and can be used as an indicator in all key stages of monitoring. However, not all the conscious birds will show wing flapping and hence absence of wing flapping does not always mean that the bird is unconscious. Birds showing wing flapping must be re-stunned. Since unconscious birds will not show wing flapping this indicator is not applicable to monitoring unconsciousness/.

3.8.2.2. Feasibility

From questionnaire 2, wing flapping was rated as easy to assess at key stage 1 by all 11 respondents. At key stage 2, 7 out of 11 found it easy to assess, one rated it as normal to assess, two as difficult to assess and one respondent found it not applicable.

3.8.2.3. Sensitivity and specificity:

The positive outcome of wing flapping, namely the presence of wing flapping, is the sign of consciousness. Therefore, the sensitivity is the percentage of birds which show a certain level of wing flapping, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 82 % (n = 9). The specificity is calculated as the percentage of birds showing no wing flapping, out of all truly unconscious birds. This was estimated to be 99 % (n = 11), which is considerably higher than the 76 % estimated for electrical waterbath stunning. The higher specificity for wing flapping in gas-stunned poultry is because effective gas stunning of poultry results in a completely relaxed body and therefore wing flapping can be easily recognised.

3.8.3. Breathing

3.8.3.1. Description

In key stage 1, effective gas stunning will lead to apnoea, i.e. **absence of breathing**, which can be used to monitor the effectiveness of stunning. Ineffective gas stunning can be recognised from the sustained/**presence of breathing, including laboured breathing**.

In key stage 2, unconscious birds will continue to manifest apnoea, and therefore breathing is not applicable in this situation. In contrast, birds recovering consciousness whilst hanging on the shackle line will attempt to breathe, which may begin as **regular gagging, leading to resumption of breathing**; these birds will have to be re-stunned.

3.8.3.2. Feasibility

In questionnaire 2, breathing was rated as easy or normal to assess at key stage 1 by 10 out of 11 respondents, and one respondent found it difficult to assess. At key stage 2, a total of 9 out of 11 found it easy or normal to assess and two respondents found it difficult to assess.

3.8.3.3. Sensitivity and specificity

The positive outcome of breathing, namely the presence of rhythmic breathing, is the sign of consciousness. Therefore, the sensitivity is the percentage of birds which show rhythmic breathing, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 97 % (n = 3). The specificity is calculated as a percentage of birds showing no rhythmic breathing, out of all truly unconscious birds. This was estimated to be 100 % (n = 3). It is worth mentioning that specificity for breathing was estimated to be 79 % for electrical waterbath stunning, and this relatively lower specificity might be the reason why it may be difficult to differentiate breathing movements occurring in the vent region from the contraction and relaxation of cloaca occurring in unconscious birds.

3.8.4. Response to comb or toe pinching

3.8.4.1. Description

Ineffective gas stunning and recovery of consciousness as a result of poor stunning and/or bleeding can be recognised from the **response to comb or toe pinch** at all key stages of monitoring. Birds showing a positive response to a painful stimulus at any stage must be re-stunned.

3.8.4.2. Feasibility

In questionnaire 2, response to comb or toe pinching was rated as easy or normal to assess at key stage 1 by all seven respondents. For key stage 2, three out of seven found it normal to assess, two found it difficult to assess and two respondents found it not applicable.

3.8.4.3. Sensitivity and specificity

The positive outcome of response to comb or toe pinching, namely the presence of such a response, is the sign of consciousness. Therefore, the sensitivity is the percentage of birds which show a response to pinching, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 83 % (n = 3). The specificity is calculated as a percentage of birds showing no response to comb or toe pinching, out of all truly unconscious birds. This was estimated to be 100 % (n = 3).

3.8.5. Vocalisation

Vocalisation is expected only in conscious birds and can be used as an indicator in all key stages of monitoring. However, not all the conscious birds will vocalise, and hence absence of vocalisation does not always mean that the bird is unconscious. Birds showing vocalisation must be re-stunned. Since unconscious birds will not vocalise, this indicator is not applicable to monitoring unconsciousness.

3.8.5.1. Feasibility

From questionnaire 2, vocalisation was rated as easy or normal to assess at key stage 1 by all seven respondents. At key stage 2, a total of five out of seven respondents found it easy or normal to assess, whereas two respondents found it difficult to assess.

3.8.5.2. 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 birds which do vocalise, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 71 % ($n = 4$). It is worth noting that vocalisation had a sensitivity of only 52 % for electrical waterbath stunning because birds entering waterbaths vocalise loudly, making it difficult to hear other birds that exit the stunner at the same time. The specificity is calculated as a percentage of birds showing no vocalisation, out of all truly unconscious birds. This was estimated to be 100 % ($n = 4$). The percentage of conscious birds that do not vocalise is estimated to be 29 %. The low sensitivity figure indicates that the respondents believe that it is quite common that birds do not vocalise, even if they have not been successfully stunned.

3.8.6. Eye movements

3.8.6.1. Description

In key stage 1, effective gas stunning will produce **fixed eyes** (eyes wide open and glassy) and eyes will remain fixed until death occurs. Birds that are not effectively stunned by the gas or those recovering consciousness will show movement of the third eyelid, commonly known as the nictitating membrane. Birds showing eye movements must be re-stunned.

3.8.6.2. Feasibility

In questionnaire 2, eye movements were considered as easy or normal to assess at key stage 1 by five out of seven respondents, and two respondents found it difficult to assess. For key stage 2, one out of seven respondents found it normal to assess, five found it difficult to assess and one found it not applicable.

3.8.6.3. Sensitivity and specificity

The positive outcome of eye movement is the sign of consciousness, namely the presence eye movement. Therefore, the sensitivity is the percentage of birds which do show eye movement after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 90 % ($n = 2$). The specificity is calculated as a percentage of birds showing no eye movement, out of all truly unconscious birds. This was estimated to be 100 % ($n = 2$). It is worth mentioning that specificity for eye movements was estimated to be 82 % for electrical waterbath stunning, and this relatively lower specificity might be because electrical immobilisation, rather than effective stunning, in waterbath stunners will also lead to absence of eye movements.

3.8.7. Palpebral reflex

3.8.7.1. Description

Effective gas stunning will lead to abolition of palpebral reflex. Effectively stunned and neck-cut birds show **no palpebral reflex** at any key stage. On the other hand, ineffectively or poorly stunned birds and those recovering consciousness prior to sticking or during bleeding are expected to show a **positive palpebral reflex** at any key stage. Birds showing a positive palpebral reflex must be re-stunned.

3.8.7.2. Feasibility

In questionnaire 2, the palpebral reflex was rated as easy to assess at key stage 1 by all three respondents. At key stage 2, a total of two out of three found it easy to assess, whereas one found it not applicable.

3.8.7.3. Sensitivity and specificity

The positive outcome of palpebral reflex is the sign of consciousness, namely the positive palpebral reflex. Therefore, the sensitivity is the percentage of birds which show palpebral reflex after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 99 % (n = 1). The specificity is calculated as a percentage of birds showing no palpebral reflex, out of all truly unconscious birds. This was estimated to be 100 % (n = 1).

3.8.8. Corneal reflex

3.8.8.1. Description

Effective gas stunning will lead to abolition of corneal reflex. Effectively stunned and neck-cut birds show **no corneal reflex** at any key stage. On the other hand, ineffectively or poorly stunned birds and those recovering consciousness prior to sticking or during bleeding are expected to show a **positive corneal reflex** at any key stage. Birds showing a positive corneal reflex must be re-stunned.

3.8.8.2. Feasibility

In questionnaire 2, the corneal reflex was rated as easy or normal to assess at key stage 1 by 9 out of 10 respondents, and one respondent found it difficult to assess. At key stage 2, a total of 3 out of 10 found it easy or normal to assess, and five respondents found it difficult, whereas two respondents found it not applicable.

3.8.8.3. Sensitivity and specificity

The positive outcome of corneal reflex is the sign of consciousness, namely a positive corneal reflex. Therefore, the sensitivity is the percentage of birds which show a corneal reflex after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 90 % (n = 5). The specificity is calculated as the percentage of birds showing no corneal reflex, out of all truly unconscious birds. This was estimated to be 93 % (n = 5). The percentage of conscious birds estimated not to show a positive corneal reflex was 10 %.

3.8.9. Spontaneous blinking

3.8.9.1. Description

Spontaneous blinking is expected only in conscious birds and can be used as an indicator in all key stages of monitoring. However, not all conscious birds will show spontaneous blinking, and hence the absence of blinking does not always mean that a bird is unconscious. Birds showing blinking must be re-stunned. Since unconscious birds will not show blinking, this indicator is not applicable to monitoring unconsciousness.

3.8.9.2. Feasibility

In questionnaire 2, spontaneous blinking was rated as easy or normal to assess at key stage 1 by all seven respondents. At key stage 2, a total of five out of seven found it easy or normal to assess and two respondents found it difficult to assess.

3.8.9.3. Sensitivity and specificity

The positive outcome of spontaneous blinking is the sign of consciousness, namely the presence of spontaneous blinking. Therefore, the sensitivity is the percentage of birds which show spontaneous blinking after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 87 % (*n* = 3). The specificity is calculated as a percentage of birds showing no corneal reflex, out of all truly unconscious birds. This was estimated to be 94 % (*n* = 3).

3.8.10. Pupillary reflex

3.8.10.1. Description

Effective gas stunning will lead to abolition of pupillary reflex. Effectively stunned and neck-cut birds show **no pupillary reflex** during any key stage. On the other hand, ineffectively or poorly stunned birds and those recovering consciousness prior to sticking or during bleeding are expected to show a **positive pupillary reflex** at any key stage. Birds showing a positive pupillary reflex must be re-stunned.

3.8.10.2. Feasibility

In questionnaire 2, the pupillary reflex was rated as easy or normal to assess at key stage 1 by two out of four respondents, and two respondents found it difficult to assess. For key stage 2, the pupillary reflex was rated as easy or normal to assess at key stage 1 by two out of four respondents, whereas one found it difficult to assess and one found it not applicable.

3.8.10.3. Sensitivity and specificity

The positive outcome of pupillary reflex is the sign of consciousness, namely a positive pupillary reflex. Therefore, the sensitivity is the percentage of birds which show the pupillary reflex after stunning, out of all truly conscious birds. This was estimated by questionnaire 2 respondents to be 92 % (*n* = 3). The specificity is calculated as the percentage of birds showing no corneal reflex, out of all truly unconscious birds. This was also estimated to be 99 % (*n* = 3).

A summary of the information on indicator sensitivity, specificity and feasibility collected from questionnaire 2 and the systematic literature review is presented in Table 7.

Table 7: Summary of information on sensitivity, specificity and feasibility of indicators and outcomes of consciousness for gas stunning from questionnaire 2

Indicators after gas 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**		
						Specificity (%)	**During neck cutting**	**During bleeding**
Muscle tone	Relaxed body [(a)]	75	72 (48, 80, 100)	99	99 (99, 100, 100)	0.73	0.13	0.42
Wing flapping	Presence of	82	74 (46, 85, 100)	99	99 (100, 100, 100)	1.00	0.82	0.50
Breathing	Presence of	97	93 (90, 100, 100)	100	100 (100, 100, 100)	0.55	–0.18	0.09
Response to comb pinch	Presence of	83	79 (50, 96, 100)	100	100 (100, 100, 100)	0.71	–0.67	–0.40
Vocalisation	Presence of	71	64 (18, 90, 99	100	100 (100, 100, 100)	0.86	0.14	0.29
Eye movements	Presence of	90	87 (n.a. (*n* = 4))	100	100 (99, 100, 100)	0.00	–0.83	–0.67
Palpebral reflex	Presence of	99	100 (n.a. (*n* = 2))	100	100 (n.a. (*n* = 3))	1.00	–1.00	1.00
Corneal reflex	Presence of	90	89 (78, 99, 100)	93	91 (100, 100, 100)	0.30	–0.78	–0.50
Spontaneous blinking	Presence of	87	84 (74, 90, 100)	94	92 (96, 100, 100)	0.57	–0.71	–0.14
Pupillary reflex	Presence of	92	86 n.a. (*n* = 4)	100	100 n.a. (*n* = 4)	–0.25	–0.67	0.00

(a): In questionnaire 2, muscle tone as an indicator was referred to as ‘relaxed body’ and was an outcome of unconsciousness. n.a.—not applicable as fewer than five responses were available.

3.9. Description of indicators for slaughter without stunning and overview of their performance

Regulation 1099/2009 requires that unconsciousness be established prior to releasing animals from the restraint while death must be established in animals prior to carcass dressing or scalding. Since poultry are usually shackled prior to slaughter without stunning and are not released from the shackles (restraint), checking for unconsciousness is not applicable. However, death should be confirmed systematically in all the birds prior to scalding.

This list of indicators is intended for use to confirm death before the birds enter the scalding tank.

3.9.1. Breathing

3.9.1.1. Description

Loss of consciousness following slaughter without stunning will eventually lead to death in birds, which can be recognised from **permanent cessation of breathing**. **Rhythmic breathing** can be recognised from the regular abdominal (vent) movement. Since the trachea is also severed at the time of neck cutting at slaughter without stunning, the absence of breathing cannot be assessed from the air movement at the external nostrils and beak, and will have to be confirmed by the absence of any abdominal movements suggestive of breathing.

3.9.1.2. Feasibility

Only 2two of the respondent considered breathing as an indicator and therefore it is not possible to assess its feasibility. However, the experts felt that cessation of breathing is used in poultry slaughterhouses.

3.9.1.3. Sensitivity and specificity

The negative outcome of the indicator ‘breathing’, i.e. the sign of death, is the cessation of breathing. None of the respondent considered breathing as an indicator and therefore it is not possible to assess its sensitivity or specificity. However, the experts felt that cessation of breathing is used in poultry slaughterhouses.

3.9.2. Corneal reflex

3.9.2.1. Description

The **corneal reflex** is a blinking response elicited by touching or tapping the cornea. Death following slaughter without stunning can be determined from the **absence of the corneal reflex**.

3.9.2.2. Feasibility

From questionnaire 2, the corneal reflex was rated (n = 1) as easy to assess.

3.9.2.3. Sensitivity and specificity

The negative outcome of the indicator ‘corneal reflex’, i.e. the sign of death, is absence of the corneal reflex. Therefore, the specificity is the percentage of dead birds showing no corneal reflex immediately after killing, out of all truly dead birds. Data from questionnaire 2 were inconclusive (n = 2).

3.9.3. Pupil size

3.9.3.1. Description

Dilated pupils (midriasis) are an indicator of onset of brain death, which requires close examination of the eyes.

3.9.3.2. Feasibility

From questionnaire 2, one out of three respondents found dilated pupils easy to assess, one found it normal to assess and one found it not applicable.

3.9.3.3. Sensitivity and specificity

The negative outcome of the indicator 'pupils', i.e. the sign of death, is dilated pupils. Therefore, the specificity is the percentage of dead birds which show dilated pupils immediately after killing, out of all truly dead birds. This was estimated by questionnaire 2 respondents to be 97 % ($n = 3$). The sensitivity is calculated as the percentage of live birds observed without dilated pupils, out of all truly alive birds. This was estimated to be 96 % ($n = 3$).

3.9.4. Muscle tone

3.9.4.1. Description

Complete and irreversible loss of muscle tone leads to a **relaxed body of the bird,** which can be recognised from the limp carcass, and is an indicator of death.

3.9.4.2. Feasibility

From questionnaire 2, a relaxed body was considered ($n = 5$) to be easy to assess by two respondents and normal to assess by two2 of the respondents.

3.9.4.3. Sensitivity and specificity

The negative outcome of the indicator 'body relaxation', i.e. the sign of death, is a relaxed body. Therefore, the specificity is the percentage of dead birds which show a relaxed body immediately after killing, out of all truly dead birds. This was estimated by questionnaire 2 respondents to be 97 % ($n = 5$). The sensitivity is calculated as the percentage of alive birds showing certain maintenance of muscle tone, out of all truly alive birds. This was estimated to be 97 % ($n = 5$).

3.9.5. Bleeding

3.9.5.1. Description

Slaughter eventually leads cessation of bleeding, with only minor dripping, from the neck cut wound, and therefore **end of bleeding** in both carotid arteries and jugular veins can be used as an indicator of death.

3.9.5.2. Feasibility

From questionnaire 2, end of bleeding was rated ($n = 3$) as easy to assess by one and normal to assess by two respondents.

3.9.5.3. Sensitivity and specificity

The negative outcome of the indicator 'bleeding', i.e. the sign of death, is the end of bleeding. Therefore, the specificity is the percentage of dead birds which stop bleeding after killing, out of all truly dead birds. This was estimated by questionnaire 2 respondents to be 94 % ($n = 3$). The sensitivity is calculated as the percentage of live birds observed to bleed, out of all truly alive birds. This was estimated to be 86 % ($n = 3$).

3.9.6. Cardiac activity

3.9.6.1. Description

Onset of death leads to permanent **absence of cardiac activity** (absence of heart beat), which can be ascertained using a stethoscope.

3.9.6.2. Feasibility

From questionnaire 2, cardiac activity was rated (n = 3) as normal to assess by one and as difficult to assess by two of the experts.

3.9.6.3. Sensitivity and specificity

The negative outcome of the indicator 'cardiac activity', i.e. the sign of death, is the absence of a heart beat. Therefore, the specificity is the percentage of dead birds without cardiac activity after killing, out of all truly dead birds. Data from questionnaire 2 were inconclusive (n = 2).

3.9.7. Pulse rate

3.9.7.1. Description

Onset of death leads to permanent loss of pulse. Pulse can be ascertained physically by pressing the (uncut) arteries in an extremity (e.g. femoral), and absence of pulse can be used to confirm death in birds.

3.9.7.2. Feasibility

From questionnaire 2, pulse rate was considered (n = 1) as normal to assess by the only expert who responded to this question.

3.9.7.3. Sensitivity and specificity

The negative outcome of the indicator 'pulse rate', i.e. the sign of death, is the absence of a pulse. Therefore, the specificity is the percentage of dead birds without pulse after killing, out of all truly dead birds. No responses as to the specificity were given by questionnaire 2 respondents. The sensitivity is calculated as the percentage of live birds showing a positive pulse rate out of all live birds. No responses as to the sensitivity were given by questionnaire 2 respondents.

4. Discussion

4.1. Introduction

As previously described, this scientific opinion proposes welfare indicators to be used for monitoring during the slaughtering process of poultry. In order to allow effective monitoring, the birds 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 unconsciousness or death in birds. 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 (not exclusively) from questionnaire 2, which was especially aimed at obtaining estimated values for their sensitivity, specificity and feasibility. 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, prevalence studies (Hindle *et al*., 2010) 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 3.7, 3.8 and 3.9 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). A short description of the physiology and elicitation of the indicators or evoking a conscious response is also presented in the glossary.

Indicators additional to those recommended in the toolboxes can also be used if considered necessary. Although the questionnaire was structured and presented to the respondents in such a way as to avoid confusion between sensitivity, specificity and feasibility, close examination of the data revealed that the sensitivity ratings given to some of the indicators may have been influenced by the feasibility of checking under the different scenarios. For example, vocalisation was given a higher sensitivity rating under gas stunning (71 %) than under electrical waterbath stunning (52 %).

It became apparent from the results that the respondents rated the feasibility of assessing indicators during neck cutting as very low because that neck cutting is performed mechanically in poultry and people responsible for monitoring welfare can check for consciousness only after the birds have been through the neck-cutting machine, that is, during bleeding. The Working Group agreed that, on the basis of feasibility, the key stages of monitoring the welfare of poultry at slaughter could be limited to two. The key stages for waterbath stunning are (1) between the exit from the stunner and neck cutting and (2) during bleeding. The key stages for gas stunning are (1) during shackling and (2) during bleeding. Slaughter without stunning of poultry is also normally performed after shackling them while alive. Therefore, only one key stage, i.e. during bleeding, is applicable to confirm death prior to scalding.

It should also be noted that the size, i.e. number of 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 also with external hearing experts on 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. The external experts advised that provision of a limited number of indicators as recommended and a few more as additional indicators was confusing and too prescriptive. In addition, they 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 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 a 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 certificate should also include monitoring animal welfare.

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, might have a good feasibility (ease of use) in slaughterhouses. The experts of the Working Group also agreed that indicators given low sensitivity and specificity at present by the respondents to 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 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 in 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 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 but that the highest sensitivity of the selected indicators be considered when planning the required sample size. 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. Monitoring procedures for electrical waterbath stunning

4.2.1. Combination of selected indicators (the 'toolboxes')

4.2.1.1. Toolbox for key stage 1 (Toolbox 1 = between the exit from the waterbath stunner and neck cutting)

This opinion recommends the following indicators (and their outcomes of consciousness) for inclusion in the toolbox at key stage 1: tonic seizures, breathing and spontaneous blinking (these are presented above the dashed line in the flow chart). Additional indicators—corneal or palpebral reflex and vocalisations—are also proposed (these are presented below the dashed line in the flow chart), but their sensitivity or feasibility is low 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 seizures

This indicator has a relatively high feasibility and sensitivity and can be used as an indicator following electric waterbath stunning, especially in key stage 1.

Breathing

According to the respondents to questionnaire 2, breathing has a high sensitivity and, even though it does not have very high feasibility, nevertheless, it was considered an important indicator.

Spontaneous blinking

It is not easy to observe spontaneous blinking according to the respondents to questionnaire 2: the feasibility is low. However, because of its high sensitivity it is included in this toolbox as an important indicator.

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

Corneal or palpebral reflex

In questionnaire 2, the corneal and palpebral reflexes were both considered highly sensitive but lowly feasible as they are not easy to observe, and it is therefore not realistic to propose this as a prime indicator. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness. 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.

Vocalisations

Spontaneous vocalisations have a relatively low sensitivity. Even though the feasibility of observing them is high, they should not be relied upon as a key indicator. However, this indicator can be very useful in addition to others.

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, due to the limited or no access to the animal (see paragraph 3.4): eye movements, response to comb or toe pinching, wing flapping and pupillary reflex.

4.2.1.2. Toolbox for key stage 2 (Toolbox 2 = during bleeding)

This opinion proposes the following indicators to be included in the toolbox at key stage 2: wing flapping and breathing (these are presented above the dashed line in the flow chart). Additional indicators—corneal or palpebral reflex, spontaneous swallowing and head shaking—are also proposed (these are presented below the dashed line in the flow chart), but their sensitivity or feasibility is low and they should not be relied upon solely.

The reasons for this are as follows.

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

Wing flapping

Wing flapping is an indicator with moderate sensitivity and high feasibility, which can be observed during bleeding.

Breathing

According to the respondents to questionnaire 2, breathing has a high sensitivity, but low feasibility. Because of its relatively high sensitivity, it was nevertheless considered an important indicator.

<u>Additional indicators (below the dashed line in the flow chart)</u>

Corneal or palpebral reflex

In questionnaire 2, the corneal and palpebral reflexes were both considered highly sensitive but lowly feasible as they are not easy to observe, and therefore it is not realistic to propose this as a prime indicator. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Spontaneous swallowing

Spontaneous swallowing (deglutition reflex) of blood entering the mouth during bleeding has been reported in majority of the birds that recover consciousness during bleeding owing to failure to cut both carotid arteries in the neck. Therefore, even though this indicator was not included in questionnaire 2, the Working Group advises that spontaneous swallowing be included as an additional indicator in Toolbox 2.

Head shaking

Head shaking during bleeding (probably triggered by the entry of blood into the nostrils) has been reported in the majority of birds that recover consciousness during bleeding owing to failure to cut both carotid arteries in the neck. Therefore, even though this parameter was not included in questionnaire 2, the Working Group recommended it as an additional indicator in Toolbox 2.

<u>Indicators not considered in the flow chart</u>

The following indicators were not included in the flow chart because of their low sensitivity or feasibility ratings, due to the limited or no access to the animal (see section 3.4): eye movements, response to comb or toe pinching, vocalisation, tonic seizure and the pupillary reflex.

4.2.2. Flow chart for the use of the toolbox indicators at slaughter with electrical waterbath 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 the section 3.3 for the definitions and selection process of the indicators and refer to section 3.7 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 two 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 poultry following electrical waterbath stunning. Following the stun, and prior to shackling (key stage 1), it is recommended that the four indicators listed above the dashed line in blue Toolbox 1 are be used to recognise consciousness. The 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 cutting.

In Toolbox 2, the two recommended indicators are presented above the dashed line, and these can be used to check for signs of consciousness at key stage 2. There are 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. 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 it can be concluded there is no risk of regained consciousness.

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.

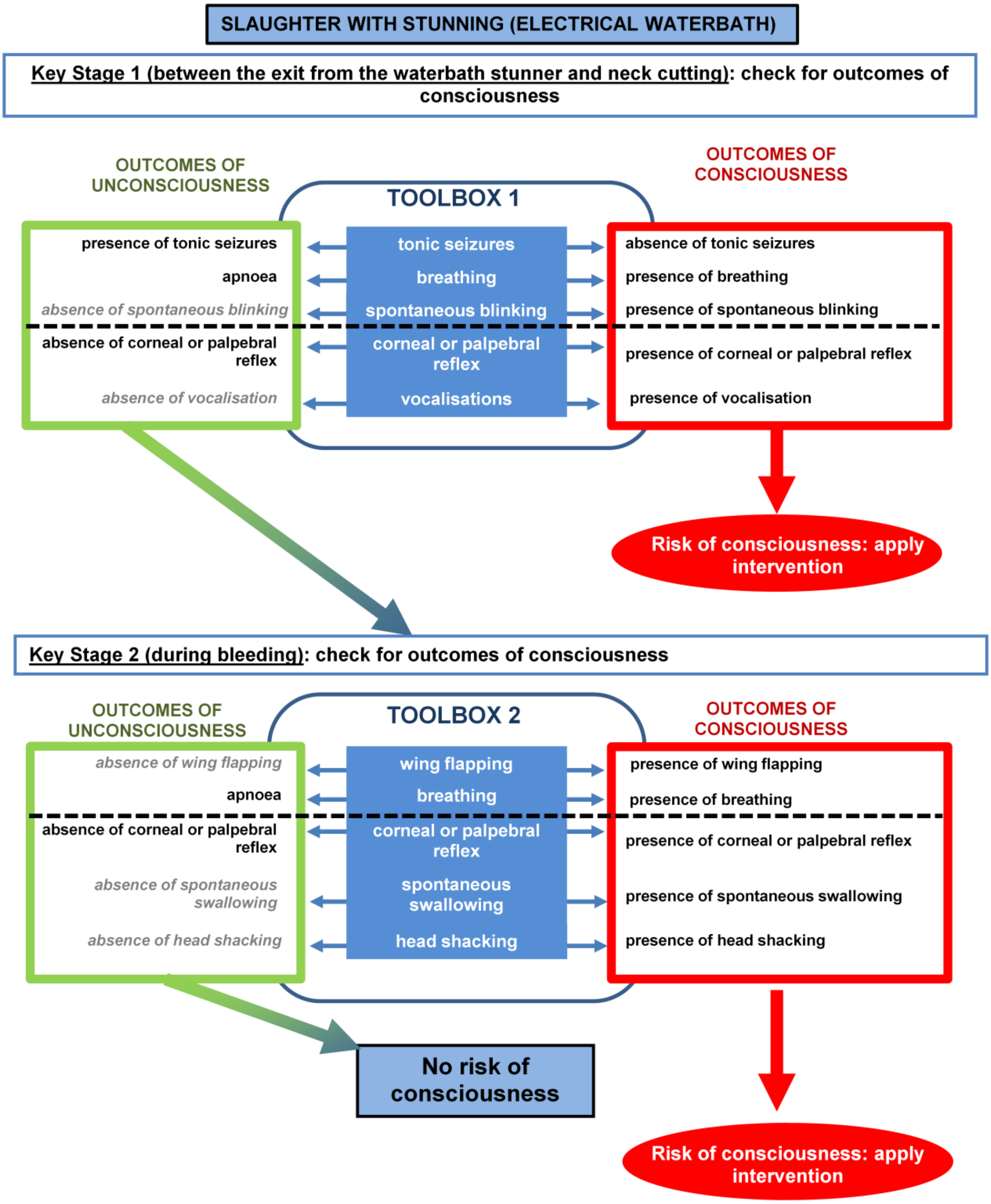


Figure 5: Toolbox of indicators that are considered suitable to be used for detection of conscious animals at each key stage of the procedure of electrical waterbath stunning in poultry

4.3. Monitoring procedures for gas stunning

4.3.1. Combination of selected indicators (the "toolbox")

4.3.1.1. Toolbox for key stage 1 (Toolbox 3 = during shackling)

This opinion recommends the following indicators (and their outcomes of consciousness) for inclusion in the toolbox at key stage 1: breathing, muscle tone, wing flapping, spontaneous blinking (these are presented above the dashed line in the flow chart). Additional indicators—corneal or palpebral reflex—are also proposed (these are presented below the dashed line in the flow chart), but the sensitivity or feasibility of some is relatively low 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)

Breathing

According to the respondents to questionnaire 2, breathing has high sensitivity, and the feasibility is good (0.55), and it was therefore considered an important indicator.

Muscle tone

The muscle tone of birds stunned using gas mixtures is a sensitive indicator with good feasibility and can be used while the birds are being shackled.

Wing flapping

Wing flapping is an indicator with high sensitivity and feasibility, which can be observed when birds are being shackled.

Spontaneous blinking

The feasibility of the indicator 'spontaneous blinking' is reasonable and it has a relatively high sensitivity. It was suggested during Working Group discussions that it be included in this toolbox as an important indicator that is commonly used for checking consciousness.

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

Corneal or palpebral reflex

In questionnaire 2, the corneal and palpebral reflexes were both considered highly sensitive but lowly feasible as they are not easy to observe, and is therefore not realistic to propose this as a prime indicator. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of consciousness.

Vocalisations

Spontaneous vocalisations have relatively low sensitivity. Even though the feasibility of observing them is high, they should not be relied upon as a key indicator. However, this indicator can be very useful in addition to others.

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): response to comb or toe pinching, spontaneous swallowing, eye movements and the pupillary reflex.

4.3.1.2. Toolbox for key stage 2 (Toolbox 4 = during bleeding)

This opinion proposes the following indicators to be included in the toolbox at key stage 2: wing flapping, muscle tone and breathing (these are presented above the dashed line in the flow chart). An additional indicator—palpebral or corneal reflex—is also proposed (these are presented below the dashed line in the flow chart), but it 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)

Wing flapping

Wing flapping is an indicator with a relatively high sensitivity and medium feasibility, which can be observed during bleeding.

Muscle tone

Muscle tone does not have high sensitivity but does have reasonable feasibility and can be used as an additional indicator.

Breathing

According to the respondents to questionnaire 2, breathing has high sensitivity, but the feasibility of observing it during bleeding is low. Because of its high sensitivity it was nevertheless considered an important indicator.

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

Corneal or palpebral reflex

In questionnaire 2, the corneal and palpebral reflexes were both considered highly sensitive but lowly feasible as they are not easy to observe, and it is therefore not realistic to propose this as a prime indicator. However, the presence of the corneal or palpebral reflex should be used as a warning signal to check for other outcomes of 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): vocalisation, response to comb or toe pinching, spontaneous blinking, pupillary reflex and eye movements.

4.3.2. Flow chart for the use of the toolbox indicators at slaughter with gas stunning

A flow chart was designed to support the understanding of the use of the indicators and is shown in Figure 6. Please refer to section 3.4 for the definitions and selection process of the indicators and refer to section 3.8 and Table 7 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 7 illustrates this opinion’s recommendations regarding the two 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 poultry following gas stunning. Following the stun, and prior to shackling (key stage 1), it is recommended that the four indicators listed above the dashed line in blue Toolbox 3 be used to recognise consciousness. The indicators below the dashed line also can 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 cutting.

In Toolbox 4, two recommended indicators are presented above the dashed line, and these can be used to check for signs of consciousness at key stage 2. There are additional indicators below the dashed line in Toolbox 4, 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 no indicator 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.

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), 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.

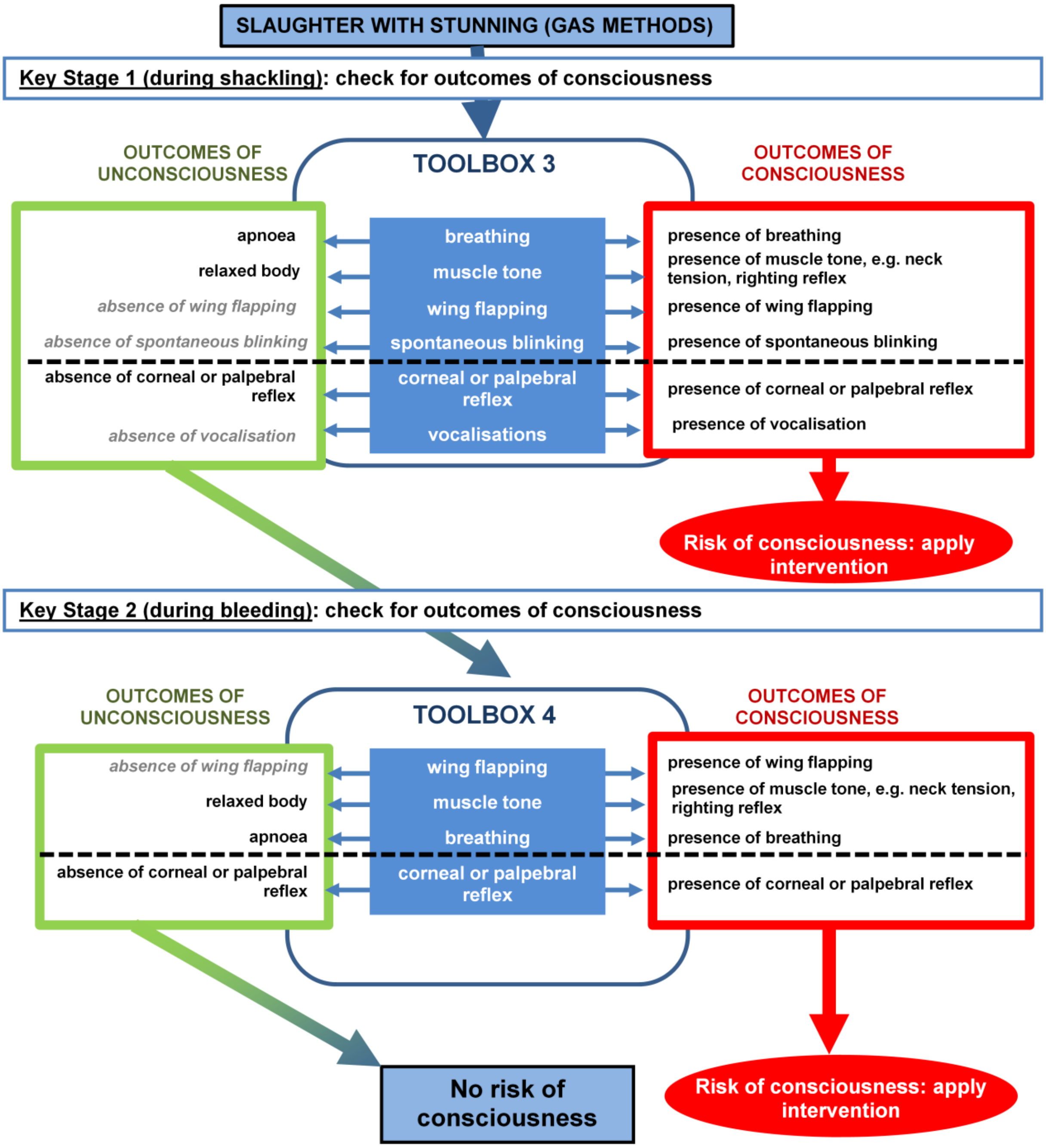


Figure 6: Toolbox of indicators that are considered suitable to be used for detection of conscious animals at each key stage of the procedure of gas stunning in poultry

4.3.3. Sampling protocol for electrical waterbath stunning and gas 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 birds, all birds (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 birds by all personnel involved in stunning and slaughter.

4.3.3.1. Risk factors and welfare consequences

The final welfare consequence of failed electrical or gas stunning is the risk of conscious or not fully unconscious birds being shackled, neck cut 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 8. 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 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 bird as soon as the overall proportion of poorly stunned animals exceeds the set 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 birds 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 8: Risk factors to bird welfare associated with electrical waterbath stunning or gas stunning of poultry.

Component	Risk factor	Risk of poor stunning [(a)]	Risk of poor assessment [(a)]
STAFF	Competence	√	√
	Experience	√	√
	Fatigue	√	√
EQUIPMENT	Maintenance	√	
	Features (e.g. for waterbath stunning: poor water conductivity during waterbath stunning; e.g. for gas stunning: short exposure time to gas mixtures)	√	
	Presence of records of maintenance (e.g. cleaning)	√	
RECORDS OF THE CHECKS	Conformity in the past	√	√
ANIMALS	Body weight	√	√
	Species/hybrid/temperament	√	√
ESTABLISHMENT	Line speed	√	√

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

4.3.3.2. 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 the literature as normal, tightened and reduced inspections).

<u>'Standard' sampling protocol</u>

The standard operating procedure for slaughter of poultry will involve a sampling fraction of 100 % by slaughterhouse personnel, as the operators check each animal for indicators of consciousness immediately after stunning, before cutting 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. 10 000 birds), and a threshold failure rate of 0.01, the sampling fraction will be about 3 %. Therefore 1 in every 30 birds will need to be monitored. However, if the slaughter population is set at one working week (at the same daily throughput, so 50 000 animals), then the sampling fraction will be less than 1 %: so not more than 1 in every 150 animals has to be sampled. 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 animal welfare officer.

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 the 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 of 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 referred to in the standard sampling protocol was set to 10 000 animals, then for the time till the next 1 000 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 with the monitoring protocol (the other factors of the model, slaughtered population and test sensitivity, being unchanged).

4.4. Monitoring procedures for slaughter without stunning

4.4.1. Combination of selected indicators (the 'toolboxes')

As explained in section 2.1.2.2, since, in the case of slaughter without stunning, unconsciousness and death are induced gradually, indicators checking the state of unconsciousness and death were selected based on their specificity to detect unconscious animals out of all unconscious animals, and preferably based on their sensitivity to detect animals still truly conscious as conscious. The sensitivity of the indicators was considered together with the feasibility of each stage. Here, the specificity—the number of unconscious animals detected out of all unconscious animals—would be less relevant for the purpose of monitoring welfare as no further processing can occur as long as the outcome of the checked indicator suggests consciousness, regardless whether that outcome is true or false.

As explained in section 4.2.1, with the purpose of detecting unconscious and dead animals in the slaughterline, indicators can be used in parallel.

4.4.1.1. Toolbox for indicators related to death at slaughter without stunning (Toolbox 5)

This opinion recommends the following indicators and outcomes of death for inclusion in the toolbox: breathing, corneal or palpebral reflex, pupil size, bleeding. An additional indicator—muscle tone—is also proposed, but its sensitivity is low and it should not be relied upon solely.

The reasons for this are as follows.

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

Breathing

Data from questionnaire 2 were inconclusive since only two respondents gave answers for this indicator. It was nevertheless considered an important indicator.

Corneal or palpebral reflex

Data from questionnaire 2 were inconclusive since only one respondent gave answers for this indicator. 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 the corneal and palpebral reflexes. Therefore, these two eye reflexes are to be used in combination. It was nevertheless considered an important indicator.

Pupil size

The few respondents to questionnaire 2 rated pupils as having high sensitivity, specificity and feasibility. It was therefore considered an important indicator.

Bleeding

The few respondents to questionnaire 2 rated bleeding as having high sensitivity, specificity and feasibility. It was therefore considered an important indicator.

<u>Additional indicators (below the dashed line in the flow chart)</u>

Muscle tone

The few respondents to questionnaire 2 rated muscle tone as having high sensitivity, specificity and moderate feasibility. It was considered an additional indicator.

<u>Indicators not considered in the flow chart</u>

The following indicators were not included in the flow chart because of their low sensitivity or feasibility ratings (see section 3.4): wing flapping, response to nose or comb pinching, vocalisations, eye movements, pupillary reflex, cardiac activity and pulse rate.

4.4.2. Flow chart for the use of the toolbox indicators at slaughter without stunning

A flow chart was designed to support understanding the use of the indicators.

Figure 7: The flow chart in Figure 7 illustrates this opinion's recommendations regarding important outcomes of consciousness or unconsciousness and the course of action to be taken when outcomes of consciousness and life are detected in poultry slaughtered without stunning. Please refer to the section 3.5 for the definitions and selection process of the indicators. Following neck cutting, it is recommended that the four indicators listed above the dashed line in blue Toolbox 5 are checked. The indicators below the dashed line may also be checked, but they may become difficult to ascertain under certain conditions (severe restraint and rotation). If the indicators suggest that the animal is still conscious (red box) and bleeding, then the animals should not be processed further.

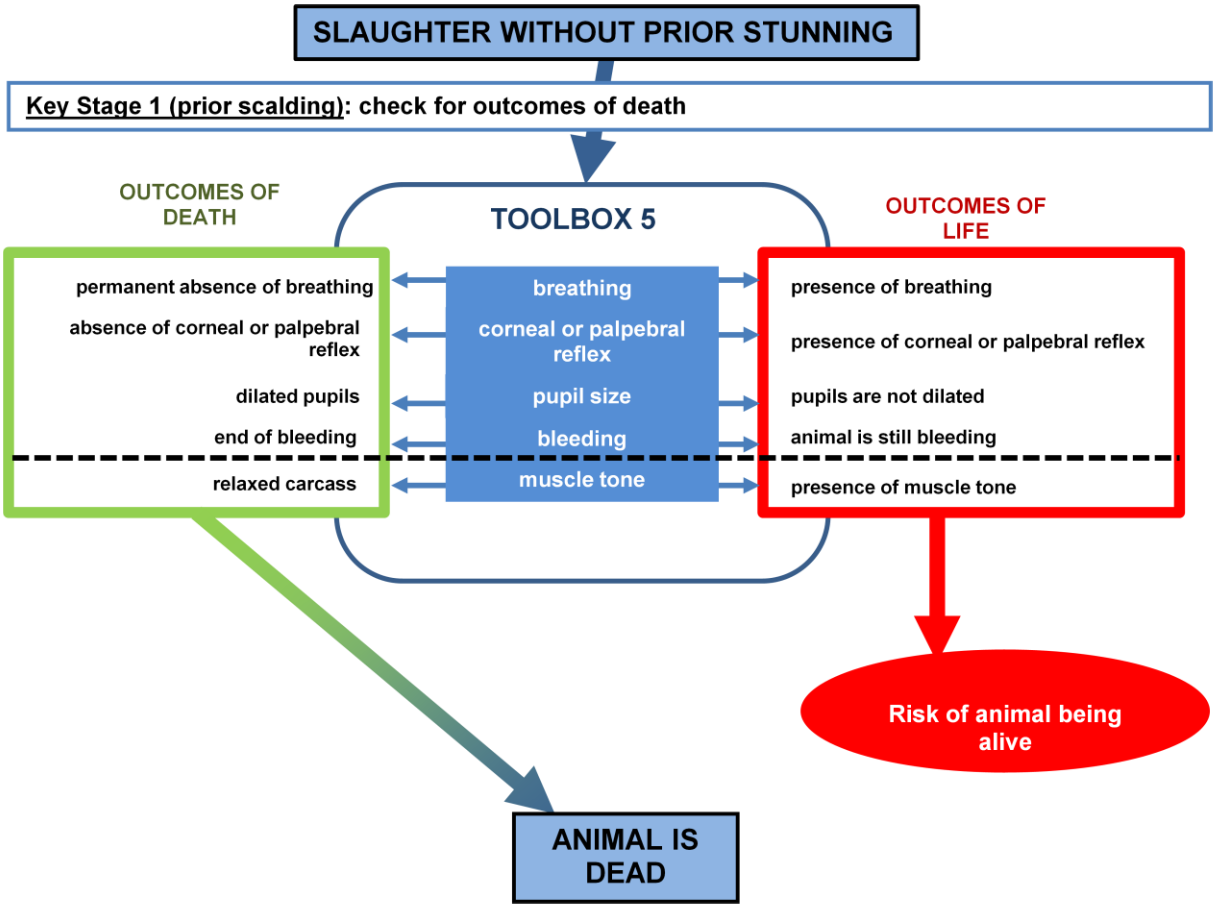


Figure 8: Toolbox of indicators and their outcomes as applicable prior to scalding for slaughter without stunning in poultry that are considered suitable to be used for confirmation of animals becoming dead as well as detection of animals still alive

4.4.3. Sampling protocol for slaughter without stunning

According to Regulation (EC) 1099/2009, when poultry are killed without prior stunning, persons responsible for slaughtering shall carry out systematic checks to ensure that the animals present signs of unconsciousness before scalding. Therefore, the personnel responsible for slaughtering should carry out monitoring in all animals slaughtered without stunning.

4.4.3.1. Risk factors and welfare consequences

The legislation requires the inspection frequency of animals being slaughtered without stunning to be 100 %. Although several aspects of the neck-cutting procedure are likely to affect the time to unconsciousness or death, their presence or absence does not affect the frequency of inspection, nor any further actions required by slaughter personnel.

Risk factors affecting the quality of neck cutting have been described in the DIALREL report (von Holleben *et al*., 2010). For instance, if both carotid arteries are not correctly cut during manual or mechanical neck cutting, this may lead to a delayed time to onset of death.

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, a lack of knowledge and understanding of the physiological basis of the indicators may have contributed to the respondents of the questionnaires rating some indicators as low on sensitivity, specificity and feasibility.

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 pre-slaughter handling, stunning, shackling, hoisting and/or bleeding, and (ii) the animal welfare officer, the person responsible for overall animal welfare at slaughter.

8) 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.

9) For detecting consciousness in poultry after waterbath and gas 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).

10) Since unconsciousness should be confirmed from the stunning application until death, this opinion recognises two key stages for monitoring welfare at slaughter: (i) between the exit from the waterbath stunner and neck cutting (for waterbath stunning) or during shackling (for gas stunning) and (ii) during bleeding.

11) The opinion concludes that a set of indicators (a minimum of two indicators) to be used to detect conscious animals following waterbath stunning in poultry should consist of:

Key stage 1: tonic seizures, breathing and spontaneous blinking. Additional indicators—corneal or palpebral reflex and vocalisations—are also proposed, but they should not be relied upon solely.

Key stage 2: wing flapping and breathing. In addition, the corneal or palpebral reflex, spontaneous swallowing and head shaking may also be used.

12) The opinion concludes that a set of indicators (a minimum of two indicators) to be used to detect conscious animals following gas stunning in poultry should consist of:

Key stage 1: breathing, muscle tone, wing flapping and spontaneous blinking. Additional indicators—corneal or palpebral reflex and vocalisations—are also proposed, but they should not be relied upon solely.

Key stage 2: wing flapping, muscle tone and breathing. In addition, the corneal or palpebral reflex may also be used.

13) In order to develop sampling protocols for monitoring consciousness in poultry after waterbath or gas 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.

14) In waterbath and gas stunning of poultry, there are two types of risk factors: (i) associated to stun quality and (ii) associated to the quality of the monitoring. Only the latter have an effect on the sampling protocol.

15) 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.

CONCLUSIONS ON POULTRY SLAUGHTER WITHOUT STUNNING

16) In the case of slaughter without stunning, it is important to routinely check indicators that have high feasibility and both high specificity and sensitivity in detecting conscious and live animals, respectively.

17) For monitoring poultry during slaughter without stunning, the sensitivity of an indicator (ability of an indicator to detect live animals as live) is relevant for animal welfare whereas specificity (ability of an indicator to detect dead animals as dead) is more related to the logistics (the personnel of the slaughterhouse have to wait longer before performing carcass dressing).

18) The opinion concludes that that the indicators to be used to detect dead birds prior to scalding following slaughtering without stunning are breathing, corneal or palpebral reflex, pupil size and bleeding. In addition, muscle tone can be used, but it should not be relied upon solely.

19) In slaughter without stunning, there are two types of risk factors: (i) associated with neck cutting quality and (ii) associated with the quality of the monitoring. However, none of them affects the sampling protocols since all animals have to be checked as required by Regulation (EC) 1099/2009.

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 using 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 two key stages: (i) between the exit from the waterbath stunner and neck cutting (for waterbath stunning) or during shackling (for gas stunning)and (ii) during bleeding.

RECOMMENDATIONS ON POULTRY ELECTRICAL WATERBATH STUNNING AND GAS STUNNING

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

9) A toolbox composed of the following indicators should be checked to determine consciousness of animals after waterbath stunning in poultry at both key stages of the process, to ensure that animals remain unconscious until death occurs.

Key stage 1: tonic seizures, breathing, spontaneous blinking. Additional indicators—corneal or palpebral reflex and vocalisations—are also proposed, but they should not be relied upon solely.

Key stage 2: wing flapping, breathing. In addition, the corneal or palpebral reflex, spontaneous swallowing and head shaking may also be used.

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

Key stage 1: breathing, muscle tone, wing flapping and spontaneous blinking. Additional indicators—corneal or palpebral reflex and vocalisations—are also proposed, but they should not be relied upon solely.

Key stage 2: wing flapping, muscle tone and breathing. In addition, the corneal or palpebral reflex may also be used.

11) In order to develop sampling protocols for monitoring consciousness in poultry after stunning:

- 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.

12) In waterbath and gas stunning of poultry, 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, until the risk is identified and rectified. All animals should be tested during a period represented by one-tenth of the slaughtered population.

13) 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.

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 POULTRY SLAUGHTER WITHOUT STUNNING

15) According to Council Regulation (EC) 1099/2009, all birds must be mechanically restrained for the purpose of slaughter without stunning and unconsciousness should be established before releasing them from the restraint and death should be confirmed before scalding. Since poultry are usually shackled prior to slaughter without stunning and are not released from the shackles (restraint), checking for unconsciousness is not applicable. Therefore, a toolbox of indicators for the determination of death was presented in the flow chart and these should be used during slaughter without stunning. It is recommended that breathing, the corneal or palpebral reflex, pupil size and bleeding should be checked. In addition, muscle tone can also be checked. Their outcomes of death should be confirmed before the animal is further processed.

16) For slaughter without stunning, 100 % of the animals need to be assessed for unconsciousness and death by checking appropriate indicators, i.e. those in Toolbox 5.

The animal welfare officer should confirm unconsciousness and death of the animals as well as the skill and aptitude of the operator in checking the indicators.

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GLOSSARY

DEFINITIONS OF THE INDICATORS OF CONSCIOUSNESS IN POULTRY AFTER GAS STUNNING

Breathing: effective stunning will result in apnoea (absence of breathing). Ineffectively stunned birds and those recovering consciousness will start to breathe in a pattern commonly referred to as rhythmic breathing, which involves a respiratory cycle of inspiration and expiration. Rhythmic breathing can be recognised from the regular abdominal movement, in both unshackled and shackled birds.

Corneal reflex: the corneal reflex is elicited by touching or tapping the cornea with a thin, blunt object. Ineffectively stunned birds and those recovering consciousness will blink in response to the stimulus. Unconscious birds 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 gas stunning. Correctly stunned birds will show fixed eyes that are wide open. Ineffectively stunned birds and those recovering consciousness may show nystagmus (spontaneous rapid side-to-side movements of the eyeballs) or rotation of the eyeball. Rotation of eyeball can be recognised from the appearance of mostly sclera, with little or no iris/cornea being visible.

Head shaking: birds shake their heads from side to side to get rid of blood or water entering the nostrils.

Muscle tone: unconscious birds will show a general loss of muscle tone and a completely relaxed body. Effective stunning will result in a completely relaxed and flaccid body, with no neck tension. Ineffectively stunned birds on the other hand, may be recumbent, but show tension in the neck or other body parts, or even show attempts to regain posture.

Palpebral reflex: the palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Ineffectively stunned birds and those recovering consciousness will blink in response to the stimulus.

Pupillary reflex: the pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Live and conscious birds will show pupillary constriction (miosis) in response to light. Deeply unconscious birds will not show a positive reflex.

Responses to a comb or toe pinch: the absence of response to a painful stimulus such as a hard pinch or tweak to the comb or the toe indicates unconsciousness following stunning.

Spontaneous blinking: bird opens/closes eyelid on its own (fast or slow) without stimulation.

Spontaneous swallowing: birds try to swallow blood or water in the mouth.

Vocalisation: conscious birds may vocalise, and therefore purposeful vocalisation can be used to recognise ineffective stunning or recovery of consciousness. However, not all the conscious birds may vocalise.

Wing flapping: effective stunning will result in a complete absence of body movements. In insufficiently stunned birds wing flapping, which is not necessarily vigorous, may be seen. Wing flapping is characterised by rhythmic flapping with both wings, and should not be confused with rapid trembling of the entire body of the bird.

DEFINITIONS OF THE INDICATORS OF CONSCIOUSNESS IN POULTRY AFTER ELECTRICAL WATHERBATH STUNNING

Breathing: effective stunning will result in apnoea (absence of breathing). Ineffectively stunned birds and those recovering consciousness will start to breathe in a pattern commonly referred to as rhythmic breathing, which involves a respiratory cycle of inspiration and expiration. Rhythmic breathing can be recognised from the regular abdominal movement, also in shackled birds.

Corneal reflex: the corneal reflex is elicited by touching or tapping the cornea with a thin, blunt object. Ineffectively stunned birds and those recovering consciousness will blink in response to the stimulus. Unconscious birds 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 electrical stunning. Correctly stunned birds will show fixed eyes that are wide open. Ineffectively stunned birds and those recovering consciousness may show nystagmus (spontaneous rapid side-to-side movements of the eyeballs) or rotation of the eyeball. Rotation of eyeball can be recognised from the appearance of mostly sclera, with little or no iris/cornea being visible.

Palpebral reflex: the palpebral reflex is elicited by touching or tapping a finger on the inner/outer eye canthus or eyelashes. Ineffectively stunned birds and those recovering consciousness will blink in response to the stimulus.

Pupillary reflex: the pupillary reflex can be elicited by focusing/shining a torch light at the pupils. Live and conscious birds will show pupillary constriction (miosis) in response to light. Deeply unconscious birds will not show a positive reflex.

Responses to a comb or toe pinch: the absence of response to a painful stimulus such as a hard pinch or tweak to the comb or the toe indicates unconsciousness following stunning.

Spontaneous blinking: bird opens/closes eyelid on its own (fast or slow) without stimulation.

Tonic seizures: effective electrical head-to-body stunning will result in tonic seizure, which in shackled birds can be recognised by arched neck and wings held tightly to the body. A followed clonic seizure occurring is rarely identified in shackled birds.

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

Wing flapping: effective stunning will result in the complete absence of body movements. In insufficiently stunned birds, wing flapping, which is not necessarily vigorous, may be seen. Wing flapping is characterised by rhythmic flapping with both wings, and should not be confused with rapid trembling of the entire body of the bird.

DEFINITIONS OF THE INDICATORS OF DEATH IN POULTRY AFTER SLAUGHTER WITHOUT STUNNING

Bleeding: slaughter eventually leads cessation of bleeding, with only minor dripping, from the neck cut wound, and therefore end of bleeding can be used as an indicator of death. It should be stressed that this indicator is valid only if there has first been proper bleeding, as it is focused on cessation of bleeding, not absence of bleeding (which may indicate an improper neck cut).

Cardiac activity: onset of death leads to permanent absence of cardiac activity (absence of heart beat), which can be ascertained using a stethoscope where possible.

Muscle tone: onset of death leads to a complete and irreversible loss of muscle tone, which can be recognised from the limp carcass.

Pulse rate: onset of death leads to permanent loss of pulse, which can be ascertained physically by pressing the arteries in an extremity where possible.

Pupil size: dilated pupils (midriasis) are an indication of death.