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SCIENTIFIC OPINION

Scientific Opinion on the electrical requirements for waterbath stunning equipment applicable for poultry[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Commission requested that EFSA review relevant new scientific references on electrical stunning of poultry and to recommend, if necessary, new electrical requirements applicable for waterbath stunning equipment. A systematic literature review was conducted to determine those electrical parameters that would deliver an effective stun so that birds would be rendered unconscious and insensible until death. Inspection data from slaughterhouse inspections conducted both in Member States in and non-Member States were included. Many of the published studies did not allow a comprehensive analysis due to different study designs and incomplete data. There are few observational studies in abattoirs to determine the numbers of birds that are effectively stunned, however, the inspection data from the Food and Veterinary Office (FVO) did not identify major problems but, for practical reasons, they used non-EEG (electro-encephalogram) methods to ascertain the effectiveness of a stun. At the present time, an EEG is the most reliable indicator of unconsciousness and insensibility. Clinical somatosensory indicators are not as reliable. The aim of a stunning system is to achieve a 100% effective stun, and the most effective electrical parameters in use can achieve an effectiveness of up to 96% as measured using EEG ascertainment methods (100% were reported as unconscious using non-EEG methods). It is recommended that the Regulation should indicate minimum current for each bird, frequency and current type as well as the wave characteristics - duty cycle and waveform. There should be better surveillance and monitoring of the electrical parameters in use at abattoirs and, in addition, methods that allow the accurate measurement of actual electrical current flowing through each bird should be further developed. Research on effective stunning should be validated by the measurement of EEG activity and related to clinical measures that are easier to use in practice.

KEY WORDS

Waterbath, electrical stunning parameters, poultry, slaughter, insensibility.

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1 On request from the Commission, Question No EFSA-Q-2011-00801, adopted on 23 May 2012.

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SUMMARY

Following a request from the Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on the electrical parameters of waterbath stunning equipment applicable for poultry.

The parameters for electrical waterbath stunning of poultry are presented in Table 2 of Chapter II of Annex I to Regulation (EC) No 1099/2009. The electrical requirements stated in this table are based on two previous EFSA Opinions (EFSA, 2004, 2006). The Commission has received information and requests from the UK and Dutch Authorities to amend the values stated in the Table 2, based on new scientific research untaken since the adoption of these previous EFSA Opinions.

The UK Authority requested that the high range frequency band in the Table 2 should cover 600 to 800 Hz and that it should not extend to 1500 Hz, due to the concerns that frequencies above 800 Hz will lead to electro-immobilisation and will not produce an effective stun. The request also mentioned changing the mid-range frequency band in Table 2 to cover 200 to 600 Hz (instead of 200 to 400 Hz as currently drafted in the Regulation), for reasons relating to the resulting meat quality. In addition Table 2 should explicitly specify current type.

The Dutch Authority was concerned that Table 2 of the Regulation refers to the electrical parameters as ‘average’ values per animal, and that this risks animals not being stunned effectively. If, however, the values were specified as ‘minimum current for each bird’, that would be acceptable. The Dutch Authority further state that in practice the current can only be measured as an average value at the abattoir, as devices for measuring effective currents for each bird are not widely available. In addition, they suggested specifying details regarding wave characteristics (waveform and duty cycle/pulse width).

Based on these requests from the UK and Dutch Authorities, the AHAW Panel was asked:

1. to review the relevant new scientific references on electrical stunning of poultry and in particular the ones provided by the British and Dutch Authorities; and

2. to recommend, if necessary, new electrical requirements applicable for waterbath stunning equipments than the ones laid down in Table 2 of Chapter II of Annex I to Regulation (EC) No 1099/2009.

In order to perform a comprehensive review of the scientific references on electrical stunning of poultry, a systematic literature review was carried out to collect available data on electrical parameters used in waterbath stunning of poultry and outcome on unconsciousness and insensibility. Inspection data from slaughterhouse inspections conducted both in Member and non-Member States were added in order to complement the findings from the systematic literature review.

The data were stratified first by current type (alternating current (AC) or direct current (DC)) and then by species and category. Different electrical ‘treatment’ classes were determined comprising different combinations of waveform (for AC current type only, as DC current type consisted of only one waveform type; pulsed waves), current (mA) and frequency (Hz) were formed. A comparison between the electrical treatment classes was then performed against the following measurement/outcome.

- Percentage of animals reported as stunned (unconsciousness ascertainment using electro-encephalogram (EEG) methods (i.e. EEG epileptiform activity, EEG suppression, absence of somatosensory evoked responses (SEP).

- Percentage of animals reported as stunned (unconsciousness ascertainment using non-EEG methods (i.e. neck tension recovery, corneal reflex, comb pinch).

- Mean duration of unconsciousness (unconsciousness ascertainment using both EEG and Non-EEG methods).

- Percentage of animals with cardiac arrest.

The conclusions and recommendations are as follows:

Conclusions:

1 Legislation requires that there is always an effective stun that lasts until the bird dies. This depends on there being a good stunning procedure and an effective method of killing at the abattoir. In order to determine whether or not this is the case, there have to be accurate measures of unconsciousness and insensibility, and its irreversibility until death.

2 A stun is effective if it renders the bird rapidly unconscious and insensible for a period of at least 45 seconds. It is also effective if it results in death of the bird from cardiac arrest as then the action of stunning does not result in poor welfare. However, it is uncertain whether currents that induce cardiac arrest may cause momentary pain and distress for a few second.

3 The aim of a stunning system is to achieve a 100% effective stun. The most effective electrical parameters in present use can achieve an effectiveness of up to 96 %as measured by EEG methods and 100%, reported as unconscious using non-EEG methods. A wide variety of electrical variables were tested but neither AC nor DC currents that have been tried give a 100 % stun rate, when using EEG methods of ascertainment.

4 Given the speed of procedures in present stunning systems, recovery of consciousness, as indicated by epileptiform activity followed by a continuous isoelectric EEG, before death by bleeding out, will not occur before subsequent events in the slaughter process if the duration of unconsciousness and insensibility achieved by the stun is 45 seconds or more.

5 At the present time, the most reliable way to determine if a bird is unconscious and insensible is by looking for particular patterns in the EEG. Somatosensory reflexes (comb-pinch response) and muscle tone and direct observations (rhythmic breathing, seizures) are not sufficiently reliable indicators of insensibility at high frequencies. At AC currents above 120 mA and at frequencies up to 200 Hz, the absence of corneal reflex is closely associated with suppressed EEG and would be a more, reliable indicator of insensibility. Evidence from EEG phases linked to physical observations is not feasible at an abattoir.

6 A wide variety of waveforms have been used in experimental studies. However, few of these have been studied in depth and repeated in different laboratories, and so their effectiveness needs to be reliably established.

7 Most studies on electrical parameters for water-bath stunning are laboratory-scale studies and difficult to extrapolate directly to large-scale field conditions, mainly because the current flowing through each bird cannot be guaranteed. Few of the waveforms used in experimental studies have been systematically studied under practical abattoir conditions, so their effectiveness needs to be reliably established.

8 With present stunning equipment used in slaughterhouses it is not possible to accurately measure and to adjust the amount of actual current that the individual bird receives during electrical water-bath stunning. The actual current that a bird receives during electrical water-bath stunning will vary according to (i) the number of birds in the waterbath at any one time, (ii) the individual variances in impedance of each bird, which will mean that some birds are not effectively stunned.

9 Problems occur in the abattoir with waterbath stunning because birds may move at critical stages, electrical contacts may be poor, with the result that some birds are inadequately stunned.

10 Electrical parameters (current, frequency) can be varied in many abattoirs, for example, if it appears that carcasses are having to be downgraded because of meat quality or birds are being inadequately stunned.

11 AC waveforms are more effective than pulsed DC in terms of inducing epileptiform activity, but industry commonly uses DC to achieve better meat quality.

12 Many of the published studies did not allow a comprehensive analysis due to different study designs and incomplete data.

13 For broilers using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns AC sine wave 101-150 mA, 50-200 Hz; AC square wave 101-150 mA; 50-400 Hz and DC pulsed wave (duty cycle 1:1; 50 % pulse width) 1-200 mA; 50-600 Hz produced a stun that was effective in 94-96 % birds. Use of an AC sine wave at 400 Hz and 600 Hz did not produce an effective stun. Application of an AC square wave at 600 Hz has not been measured.

14 For turkeys, using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns AC sine/clipped sine wave 1-250 mA, 50-200 Hz, produced a stun that was effective in 92 % of birds.

15 For laying hens, using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns AC sine/clipped sine wave, 1-100 mA, 50-200 Hz produced a stun that was effective in 50 % of birds. Using the same waveform and current but increasing the frequency to 201-400 Hz and above 600 Hz, no birds were effectively stunned.

16 There are few observational studies in abattoirs to determine the numbers of birds that are effectively stunned. When done on a large scale the FVO did not identify major problems but, for practical reasons, they used non-EEG methods for ascertaining the effectiveness of a stun.

17 The requests from the Dutch and UK Authorities highlight problems with Table 2 in the proposed regulations as it does not clearly state if the current type pertains to AC or DC, or both, and also does not specify all electrical parameters relevant for waterbath stunning.

18 The Regulation does not include details on waveform, or clearly specify the current type, or include details on duty cycle/pulse width (for DC currents), or specify minimum currents for each bird.

19 Evidence for supporting an increase in the mid-band frequency range for broilers to 600Hz using an AC current at 150-200 mA was not found.

20 It may not be practical at the present time to measure EEG routinely in the abattoir. However laboratory studies do show that current flow through individual birds at a specified frequency can be used with confidence to predict the EEG. Thus the effectiveness of the stun can be assessed under abattoir conditions from accurate measurement of current flow through individual birds.

21 When waterbath stunning is used, it is not possible to ensure that all birds are stunned.

Recommendations:

1 The Regulation should indicate minimum current for each bird, frequency and current type as well as the wave characteristics - duty cycle and waveform.

2 There should be better surveillance and monitoring of the electrical parameters in use at abattoirs and, in addition, methods that allow the accurate measurement of actual electrical current flowing through each bird should be further developed.

3 Research on effective stunning should be validated by the measurement of EEG activity and related to clinical measures which are easier to use in practice.

4 There is an urgent need to develop electrical methods that guarantee 100 % stun.

5 Unless the problems described in this opinion for all existing waterbath stunning methods can be resolved, other stunning methods should be used.

Further research:

- In order to standardise experimental electrical waterbath studies and reporting, further research should employ EEG activity assessments in their design, and correlate the results of these with practical measures of unconsciousness and insensibility.

- Any new electrical stunning systems developed should be tested under abattoir conditions. It would be unnecessary to measure EEG if a system could measure and guarantee adequate current flow in each bird.

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BACKGROUND AS PROVIDED BY THE COMMISSION

Table 2 of Chapter II of Annex I to Regulation (EC) No 1099/2009[4] on the protection of animals at the time of killing sets out the average values per animal of electrical requirement which must be used when stunning chickens, turkeys, ducks and geese and quail using waterbath equipment. These requirements have been based on two previous EFSA opinions on the subject adopted respectively in 2004 and 2006.

Article 4(2) of Regulation (EC) No 1099/2009 allows Annex I to be amended to take account of scientific and technical progress on the basis of an EFSA opinion and in accordance with the procedures laid down in Article 25 of this Regulation (comitology).

The Commission recently received information from the British and Dutch authorities which may justify amending these parameters (see Annex I to Regulation (EC) No 1099/2009). In order to evaluate the relevance of these demands, the Commission would like to request the EFSA to review its previous opinions on the subject in the light of the references provided by the British and Dutch authorities as well as of any new relevant scientific development.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

The Commission therefore considers it opportune to request the EFSA to give an independent view on the electrical requirements for stunning equipment applicable for poultry.

1. Review the relevant new scientific references on electrical stunning of poultry and in particular the ones provided by the British and Dutch authorities;

2. Recommend, if necessary, new electrical requirements applicable for waterbath stunning equipments than the ones laid down in Table 2 of Chapter II of Annex I to Regulation (EC) No 1099/2009.

[4] Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing (Text with EEA relevance) OJ L 303, 18.11.2009, p. 1–30.

ASSESSMENT

1. Introduction

The Commission has requested EFSA to review its previous opinions on the killing of poultry using electrical methods (EFSA, 2004, 2006) in the light of the references provided by the UK and Dutch Authorities as well as any new relevant scientific developments. It should be noted that while research is carried out on individual birds in a laboratory, very few reports were found on what actually happens at the abattoir. It is obvious however, that one very important factor influencing the choice of the stunning and killing method is the quality of the meat resulting from the slaughter.

In section 1 we review the communications from the Dutch and UK Authorities. Section 2 describes the processes that occur in the abattoir and the criteria and reasoning behind the definition of an 'effective stun' that persists for long enough for birds to be insensible throughout the slaughter process, as well as the recognition of an effective stun. In order to answer the terms of reference (ToR), a systematic review of the recent literature was carried out which is described in section 3, and the results are summarised in section 4. Findings from inspections of poultry slaughterhouses by the Food and Veterinary Office of the European Union (FVO) are also reported in section 4. In section 5, the results of the systematic literature review are discussed in relation to some of the suggestions made by the UK and Dutch Authorities. Section 6 provides conclusions and recommendations and further research suggestions are given in section 7.

1.1. Requests by Dutch and UK Authorities

The concerns of the UK and Dutch Authorities mainly concern Electrical Requirements for waterbath stunning equipment (proposed Regulation implementation date 01 January 2013) given in Regulation (EC) No 1099/2009, Chapter II of Annex I:

Table 2 Electrical requirements for waterbath stunning equipment

(average values per animal)

Frequency (Hz)	Chickens	Turkeys	Ducks and Geese	Quails
<200 Hz	100 mA	250 mA	130 mA	45 mA
From 200 to 400 Hz	150 mA	400 mA	Not permitted	Not permitted
From 400 to 1500 Hz	200 mA	400 mA	Not permitted	Not permitted

1.1.1. Request from the UK Authority (Department of Environment Food and Rural Affairs)

The UK Authority has requested that the Table should clearly identify the current type, as this is presently not specified, and because research shows that different electrical parameters are required to achieve an effective stun when using AC or pulsed DC waveforms. They state that the values currently specified in the Table broadly correlate with research findings in relation to AC currents but in order to ensure appropriate welfare standards are maintained, it is important to ensure that the electrical parameters clearly relate to AC currents and that a separate table of requirements should be included for any other current type.

In addition, the UK Authority request a change to the mid-range frequency band in the Table 2 to cover the range 200 to 600 Hz, as industry has highlighted a significant reduction in meat quality associated with a 400 Hz/150 mA frequency and current combination, compared with existing industry averages. The UK Authority state that using a higher frequency of 600 Hz, combined with a current of

150 mA in broilers, would achieve an effective stun as indicated from research data, and also reduce meat loss from down-grading. Furthermore, results from a questionnaire sent to the UK poultry industry by the UK Authority, highlight that all but one of the respondents would have to modify their current parameters significantly in order to comply with the electrical parameters set out in Table 2. However, if the mid-range band was amended to reflect this proposed range, then some companies would not have to adjust their currents and frequencies.

In addition, the UK Authority requests that the high range frequency band in the Table 2 should cover 600 to 800 Hz and not be extended to 1500 Hz, due to the concern that frequencies above 800 Hz will lead to electro-immobilisation and not produce an effective stun. In the past varying assessment methods have been used to assess insensibility and unconsciousness, making comparisons between different electrical parameters difficult. The UK Authority therefore suggests that in the future EEG activity assessment for unconsciousness and insensibility be included in any study to help ensure that research papers on waterbath electrical stunning are directly comparable.

1.1.2. Request from Dutch Authority (Ministry of Agriculture, Nature and Food Quality)

The Dutch Authority, like the UK Authority, find the proposed legislation to be misleading as it does not detail frequency and other wave characteristics of the current.

The Dutch Authority request also states that in Table 2 of Chapter II of Annex I to Regulation (EC) No 1099/2009, the parameters are defined as an 'average' value per animal and that by using an average this risks some animals not being stunned effectively. The Dutch Authority has therefore asked that the figures used be the 'minimum' value per animal to reflect the important difference between 'minimum' and 'average' current values. In practice, the current that is measured (and which can be read on a display at the abattoir) in a multiple-bird stunning waterbath indicates the total current that flows through all the birds in the waterbath, and can be used to calculate only the average current for each bird. As variation in impedance (resistance) between the birds is significant, it is not possible to ensure a standard constant current for each bird that is stunned which can result is some birds not being effectively stunned. This problem could be resolved by increasing the average current in the recommendations, but further research would be needed to ensure that all birds with a high impedance are stunned effectively. They also point out that the corresponding voltages to achieve these higher currents would be too high to ensure good meat product quality.

The Dutch Authority also provide results from research it has commissioned, carried out at Wageningen University, on electrical parameters for the use of multiple-bird waterbath stunners and new stunning techniques. They state that the results of this work are relevant for the implementation of the new regulation on the protection of animals at the time of killing, as the research indicates that electrical parameters stated in Table 2 are not adequately ensuring an effective stun for broilers.

The study investigated the present situation in slaughterhouses, together with physiological measurements on individual birds under laboratory conditions, and the efficacy of alternative waveforms. The research included investigations into alternative locations for electrode placement as well as an alternative method based on transcranial magnetic stimulation (TMS). However these alternative methods are not used in conventional waterbath stunning practices in abattoirs and so have not been described further in this opinion (Hindle et al., 2009).

1.1.2.1. Present Situation in Slaughterhouses in The Netherlands

In this study the electrical parameter settings at 10 Dutch slaughterhouses (broilers, hens and ducks) were measured and recorded using a hand-held oscilloscope which measures current (mA) and voltage. The oscilloscope was placed at strategic points on the shackle-line directly adjacent to the waterbath. At eight of these slaughterhouses, a prototype of a stand-alone in-line measuring device was hung from the shackle instead of a bird and was run through the waterbath for each setting. It was concluded that there were large differences between slaughterhouses in the settings for waterbath stunning parameters for broilers, hens and ducks. Differences were seen with varying numbers of birds in the waterbath, stunning duration, and the voltage and frequency applied (Hindle et al., 2009).

1.1.2.2. Results of research under controlled laboratory conditions with individual birds

The Dutch study included measurements on individual birds, where the efficacies of various electrical settings on inducing unconsciousness were analysed using reflex response measurements, EEG and ECG measurements to determine brain and heart activity, respectively. Broilers and hens were stunned in a single waterbath at frequencies of 50, 400 and 1000 Hz, and ducks at 50 and 400 Hz, using an AC voltage stunner with a modified square wave, for 5 seconds. All stuns were performed to assess the effectiveness of regulatory current levels (i.e.100 mA at 50 Hz for broilers/laying hens and 130 mA for ducks) and minimum current levels recommended in the previous EFSA opinion (EFSA, 2004) or varied with frequency to produce a current that would provide an effective stun (defined as failure to recover within 1 minute post-stunning or death). Each bird was weighed after bleeding and, in the case of broilers, carcasses were examined for meat quality.

From this study it was concluded that at similar voltages for individual broilers, hens and ducks there are differences in current which indicate differences in impedance that influence the delivery of an effective stun. Bodyweight did not have a significant effect and was not a reliable predictor for differences in impedance. The use of a frequency of 50 Hz applied for 5 seconds delivering a current of 100 mA produced an effective stun in most birds (as ascertained by comb pinching, corneal reflex and suppression EEG frequencies or death). At 400Hz, one broiler (2%) remained conscious after receiving a current just above the minimum level (150 mA) recommended in the previous EFSA opinion (EFSA, 2004). Higher frequencies required higher levels of current to produce an effective stun in broilers but this also reduced meat quality.

1.1.2.3. Alternative waveforms

Nine square AC waveforms varying in duty cycle were tested as alternatives to a standard sine wave to stun broilers in individual waterbath experiments. The EEG and ECG were measured and, after bleeding, the carcass of each bird was examined for meat quality. Seven of these waveforms were not successful in delivering an effective stun but two were selected to study further. From these studies it was concluded that alternative waveforms can be effective in producing an effective stun but that the higher currents needed to deliver them will reduce meat quality. Thus these alternative waveforms are not recommended because of the problem of reduced meat quality.

The recommendations from this research carried out by Wageningen University are as follows.

1. The present legal standards for electrical stunning of poultry must be adapted to include specification of frequency, and wave characteristics (waveform and duty cycle).

2. Measurement of the application settings in practice must be performed in-line at animal level.

3. Use of the conventional electrical waterbath stunning in its present form is to be strongly discouraged because of the inability to guarantee that each bird receives sufficient current for an effective stun

Furthermore, the following aspects should be developed further for practical application.

A. Alternative pathways for application of stunning

B. Individual application of an electric stun

C. Alternative electrical stunning methods

These two communications from the Dutch and UK Authorities raise important issues for the effective stunning of poultry at an abattoir and the processes involved, and the scientific evidence for their effectiveness is reviewed in the Sections 3 and 4.

2. The key issues

There are key scientific issues in relation to the electrical stunning using a waterbath.

1. The relevant slaughter processes (2.1)

2. The determination of unconsciousness and insensibility (section 2.2)

3. The duration of unconsciousness and insensibility (section 2.3)

4. The electrical parameters producing unconsciousness and insensibility for sufficiently long enough to prevent return of consciousness and sensibility following stunning until death (section 2.4)

5. The contribution that the variation between bird species and sizes within a species or batch makes to an effective stun when using multiple birds at the same time in a waterbath (section 2.5)

6. Other factors that affect the efficiency of stunning to be considered (section 2.6)

2.1. Description of the relevant slaughter processes

Most of the data given below refer to the slaughter of broiler chickens but where there are important differences these are noted.

At the abattoir, birds are removed from transport crates and hung upside down by pulling their legs into metal leg shackles (‘shackling‘), at roughly 15-20 cm intervals, that are attached to a continuously moving conveyer line. The shackles are attached to hooks (sometimes with the addition of a breast support) which take the birds to an electrified waterbath where they are stunned, followed by a neck cutter, a bleeding-out area, a hot (50-55C) water tank (so-called ‘scald-tank’) and finally de-feathering and further processing. The line moves at rates in excess of 5,000 to 12,000 + birds/hour (~ 200 birds/minute or ~ 3 birds/second) in high throughput slaughterhouses For turkeys and geese the rates vary between 600 and 3600 birds/hour (Paul Berry, Technical Ltd, personal communication, 2012). The time taken between shackling and entering the waterbath varies between 30 to 120 seconds depending on the species (chicken, duck, turkey) and category (broiler, hen) of bird, but at least 15 to 30 seconds are required for the birds to ‘settle’ on the line and to stop leg, wing and neck (escape) movements before entering the waterbath. In the past, the time between shackling and entering the waterbath was up to 3 minutes for chickens and 6 minutes for turkeys (EFSA, 2004) but faster times are now being achieved (around 1 minute for both species) (Pinillos, 2010).

At any one time around 10 to 25 birds enter the waterbath and are immersed up to the base of the neck, so that the heads and necks are in the water (Gregory and Wotton, 1991). The contact of the head and neck with the water completes the electric circuit between the water (positive electrode) and the line hook and shackle which acts as the earth or negative bar electrode, so that an electric current passes through the bird’s head and body. Regulation 1099/2009 stipulates that contact must be maintained for 4 seconds but it can be longer depending on the line speed (which can be adjusted by the operators based on the type of current used by the abattoir) (Pinillos, 2010). Within a few seconds of leaving the waterbath the birds are moved to a neck-cutting machine where the major blood vessels are cut; the birds then die through loss of blood supply to the brain.

An individual is positioned after the neck cutter to ensure that all birds have their necks cut. If any bird appears to have missed the cutter (often for reasons related to variation in the size of the birds in a flock, or birds moving and missing the cutter) or are not bleeding sufficiently, this individual will perform a manual neck cut. It is assumed that by the time the birds reach the scald tank, they will be dead. Random checks on the process are performed by the Animal Welfare Officer and official veterinary service and any problems are reported to the appropriate Food Business Organisation. The dead birds then continue to move along the line from the scald tank to complete the remaining processing stages.

2.2. The determination of unconsciousness and insensibility

2.2.1. Neurophysiological bases of stunning

In order to achieve an effective stun, a sufficient amount of electric current has to pass through each bird to induce a generalised epileptic fit (EEG data) which will make it unconscious and insensible. An effective stun will lead to a period of epileptiform EEG activity, where the neurones are firing in a hyper-synchronised way (approximate duration 15 seconds). This is rapidly followed by a period of a profoundly suppressed or a quiescent or an isoelectric EEG which can be linked to insensibility (for a period of at least 30 seconds), and is indicative of neuronal fatigue in the brain (Schütt-Abraham et al.,, 1983; Gregory and Wotton, 1987). Somatosensory evoked potentials in the brain are abolished during this period of profoundly suppressed EEG (EFSA, 2004) indicating insensibility which lasts for approximately 45 seconds.

2.2.2. Tests for unconsciousness and insensibility

The legislation requires that each bird is unconscious and insensible until death. Various measures of unconsciousness and insensibility or death have been used to demonstrate insensibility. These range from simple tests that an abattoir worker can use to more sophisticated equipment used in research: somatosensory (physical) methods such as eye prick (corneal reflex indicated by movement of the nictitating membrane, sometimes called the third eyelid reflex), palpebral reflex (blink reflex), pupillary dilation, toe pinch (withdrawal response), wing flapping, absence of neck tension, comb/head pinch response; cardiac measures such as ventricular fibrillation and cardiac arrest measured by an electrocardiogram (ECG); rhythmic respiratory movement by direct observation or indirectly through movement of the cloaca or vent; and neurological measures in the brain, using superficial or deep recording electrodes, such as somatosensory, visual and auditory evoked responses (SERs, VERs, AERs), and electro-encephalogram (EEG) waveform or electro-corticogram (ECoG) waveforms (EFSA, 2004, 2006; Raj and O'Callaghan, 2004). (EEC and ECoG can be considered to be the same for the purposes in this opinion).

Conclusion 1: Legislation requires that there is always an effective stun that lasts until the bird dies.

Conclusion 2: Assurance of an effective stun depends on accurate scientific measures being made of unconsciousness and insensibility and its irreversibility until death.

Digital recorders have recently become available and have replaced analogue equipment and this change has improved the accuracy of measurements of EEG and ECG as well as decreasing the time at which they can be recorded after leaving the stunner. Effective stunning can be confirmed by the observation of a generalised epileptiform (grand mal) brain activity together with convulsions, followed by a continuous isoelectric EEG. However, under abattoir conditions it is not possible to monitor EEG, SEPs or ECG. Furthermore, evidence for a good correlation between physical observations with the EEG activity phases is limited. With other measures such as absence of muscle tone or corneal reflex, one cannot be absolutely sure that a bird is unconscious and insensible as reflexes and muscle tone are also absent in conscious birds (Raj, 2003). Therefore, one cannot use muscle tone to reliably to determine the effectiveness of electrical stunning nor stun duration based on the return of neck tension. Birds that have lost their neck tension may not be stunned but electro-immobilised through muscle depolarisation (Wenzlawowicz and von Holleben, 2001; Raj, 2003).

Conclusion 3: The effectiveness and duration of electrical stunning cannot be based on muscle tone.

Conclusion 4: Birds that have lost their neck tension may not be stunned but electro-immobilised.

It is important to note, that when insufficient current passes through a bird only certain areas of the brain are affected. Consequently it is possible to induce partial epilepsy that leaves a bird conscious and sensible even though it shows seizures and convulsions that are indistinguishable from those shown after an adequate stun (Schütt-Abraham et al., 1983; Raj et al., 2006a). Therefore, the occurrence of seizures and convulsions is not a reliable indicator of unconsciousness and insensibility.

Conclusion 5: The occurrence of seizures and convulsions is not a reliable indicator of unconsciousness and insensibility.

Corneal reflex testing is a method used in slaughterhouses to assess stunning effectiveness since it is usually lost for a short period following current flow (Wenzlawowicz and von Holleben, 2001). Prinz (2009) measured the association between the corneal reflex and EEG in broilers using AC and DC stunning procedures at currents from 60-150 mA (AC) and 80 – 150 mA (DC) and frequencies from 70-1500 Hz. At AC currents above 120 mA the corneal reflex was closely associated with suppressed EEG at frequencies up to 200 Hz and both indicated an effective stun in over 95 % of birds. However one cannot conclude from this high correlation that absence of a corneal reflex guarantees an effective stun for every bird. The corneal reflex is located in the brain stem and can be elicited even under deep anaesthesia. Thus positive corneal responses are a sign of overall brain function and not necessarily related to consciousness (Gregory, 1989; Prinz, 2009). At lower currents and higher frequencies the corneal reflex was not correlated with suppressed EEG (Prinz, 2009). With DC currents, the corneal reflex was not a reliable indicator of unconsciousness and insensibility at any current or frequency. Overall, these observations show that the presence or absence of a corneal reflex is not a reliable indicator of unconsciousness and insensibility, The recovery of corneal reflexes after stunning, together with the return of spontaneous breathing and eye blinking, can be signs of a progressive recovery of brain function (Wenzlawowicz and von Holleben, 2001; Prinz et al., 2010a). On the other hand, a continuing absence of a corneal reflex could be an indicator for approaching brain death or severe brain impairment (Gregory, 1989).

Conclusion 6: At AC currents above 120 mA and at frequencies up to 200 Hz, the corneal reflex is closely associated with suppressed EEG. At lower currents and at higher frequencies (≥400 Hz) the corneal reflex is not correlated with suppressed EEG and is not a reliable indicator of unconsciousness and insensibility. With DC currents the corneal reflex is not a reliable indicator at any current or frequency.

The comb pinch reflex is generally lost immediately after electrical stunning for up to 3 minutes, but it may also be absent in birds that show obvious signs of recovery of consciousness. Consequently, a negative comb-pinch reflex does not indicate insensibility, but a positive reflex means that a bird is not unconscious and would be aware of painful stimuli (Wenzlawowicz and von Holleben, 2001).

Conclusion 7: A negative comb-pinch response does not necessarily indicate unconsciousness or insensibility, but a positive reflex means that a bird is not unconscious.

Rhythmic breathing is usually lost after stunning and its return can indicate a bird may be regaining sensibility but its absence does not necessarily mean that a bird is insensible. It was not well correlated with EEG activity and was more related to cardiac function than consciousness (Prinz 2009).

Conclusion 8: Absence of rhythmic breathing is not a good indicator of insensibility, but its return may indicate a bird is regaining consciousness.

Cardiac arrest or cardiac (ventricular) fibrillation may occur in some birds during stunning. These birds are very rapidly rendered insensible and die and so will eliminate the chances of recovery of consciousness and sensibility (Gregory and Wotton, 1986). Induction of cardiac arrest is current dependent and low currents do not reliably produce arrest (30-60mA compared with >140 mA both with AC 50 Hz) (Gregory and Wotton, 1987). Raj et al., (2006b) found that 4 out of 5 and 2 out of 10 birds given a pulsed DC current at 150 and 200 mA, average current, respectively, at 200 Hz, resulted in cardiac arrest. However, these birds showed no epileptiform EEGs indicative of effective stunning, and were likely to be immobilised and conscious for a very short time until the brain becomes sufficiently hypoxic to lose consciousness. Therefore, induction of cardiac arrest without a generalised epileptiform activity in the brain should not be considered as an effective and 'immediate' stun Cardiac arrest may be important for some forms of slaughter as animals will be 'restrained' but not recover.

Conclusion 9: Short-term cardiac arrest is not a reliable indicator of unconsciousness and insensibility.

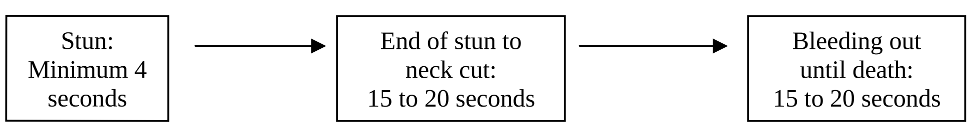
Several studies have shown that the production of an epileptiform hyperactivity followed by suppressed EEG activity is well correlated with unconsciousness and insensibility (see above). Other measures, such as reflexes or direct observations on the birds, that are frequently used in abattoirs are far more variable and their presence or absence is not well correlated with the ability of a bird to experience pain and distress during the slaughter processes (see also Table 6, section 4 which shows the lack of correlation between EEG and non-EEG ascertainment methods).

Conclusion 10: Only EEG activity can be used as a reliable measure of unconsciousness and insensibility.

2.3. The duration of unconsciousness and insensibility

It is obviously important that a bird does not recover from stunning prior to the blood vessels in its neck being cut, and that sufficient time is given for a bird to lose enough blood so that it becomes irreversibly unconscious and dies. The interval between stunning and death will vary according to the length and speed of the line and the number of blood vessels that are severed, but it is usually takes less than 90 seconds (around 20 seconds). The number of blood vessels cut will vary depending upon the make and model of the automatic neck cutter and the way the machine is set up. Some machines cut a vertebral artery at the back of the head, others aim to cut one carotid artery and one jugular vein on one side of the neck (unilateral neck cut) and others aim to cut two carotids and two jugular veins (ventral cut).

At a technical hearing in September 2011, attended by two technical hearing experts in electrical waterbath stunning, the duration of unconsciousness induced by electrical stunning, and defined by the experts present should be at least 44 seconds from the start of stunning, to death by bleeding out, as described in the flow diagram below:



These timings are supported by those described in the previous EFSA opinion (EFSA, 2004) which specifies that the duration of unconsciousness induced by stunning should last longer than 45 seconds (20 seconds stun to neck cut, plus 25 seconds to achieve brain ischaemia through blood loss).

The bleeding duration depends on the number of blood vessels severed by the cut. The EC Regulation (No. 1099/2009) states that for stunning methods which do not result in instantaneous death, the stun should be followed as quickly as possible by a procedure ensuring death, such as bleeding out (in which the two carotid arteries or vessels from which they arise shall be systematically severed), before the birds regain consciousness. Furthermore, the Regulation also states that birds shall not be slaughtered by means of automatic neck cutters unless it can be ascertained whether or not the neck cutters have effectively severed both blood vessels.

If the neck vessels are not cut properly (e.g. are cut on one side only) then the bird takes longer to bleed-out, and may even recover some degree of consciousness and sensibility before death if the electric stun does not last long enough. Recent work has shown that cutting causes pain as evidenced from EEG recordings (EFSA, 2004; 2006; Adams and Sheridan, 2008; Mellor et al., 2009). Recovery of consciousness before death by bleeding out will not occur if the duration of unconsciousness and insensibility achieved by the stun is 40 seconds or longer as indicated by an epileptiform activity followed by a continuous isoelectric EEG (Schütt-Abraham et al., 1983). It is important to note that birds recovering from being stunned show a staged reversal in sensibility in that the cortex of the brain recovers first, followed by spinal cord synapses, followed by the neuromuscular junctions. Thus, an animal that is regaining or has regained sensibility may be aware but unable to move, making it difficult to recognise signs indicating regained sensibility in the absence of EEG monitoring.

Conclusion 11: Recovery of consciousness will not occur before subsequent events in the slaughter process if the duration of unconsciousness and insensibility achieved by the stun is 45 seconds or more, as indicated by an epileptiform activity followed by a continuous isoelectric EEG.

2.4. The electrical parameters needed to produce unconsciousness and insensibility for sufficiently long enough to prevent return of consciousness following stunning

Induction of a generalised epileptiform fit or stun leading to an isoelectric EEG depends upon five key parameters:

1. Minimum current
2. Minimum voltage
3. Frequency of current
4. Current type (AC/DC)
5. Waveform of the electricity

Whatever electrical parameters are used, the aim is to render a bird unconscious and insensible in a way that is rapid and continues throughout the slaughter process.

In order to optimise current flow to achieve an effective stun, voltage, waveform and frequency can be varied at the abattoir to deliver sufficient current. An alternating current (AC) is often used, whereas some slaughterhouses apply a pulsed direct current. The form of an AC current can vary; some plants apply clipped or rectified waveforms, but a sinusoidal wave is the traditional form. More recently, modern commercial stunners are using a rectangular waveform. While there seem to be a wide variety of waveforms, very few have been studied in depth and repeated in other laboratories. The efficacy of any waveform needs to be reliably established in a way that can translate to practical situations in the abattoir including meat quality and adequacy of the stun. The application time of the current under commercial conditions is usually around 10 seconds (Prinz, 2010b).

AC waveforms: are typically sine waves that can be varied. The frequency of the wave may be increased from the normal mains value of 50/60 Hz to 2000 Hz or more. The shape of the wave may be clipped, or it may be modified from the typical sine shape to square waves. It can also be rectified and the application time and frequency can be varied. The current and voltage are often expressed as the root mean square (RMS) current and voltage.

Electrical frequency (Hz) and amount of RMS current (mA) determine the effectiveness of stunning. The effectiveness of the current is determined by its frequency and waveform, and these are varied at the abattoir to achieve an adequate stun. In general, higher frequencies are less effective at stunning and require a greater current to be effective but lower frequencies tend to produce better meat quality at a given current (Gregory and Wilkins, 1989; Gregory et al., 1995). Higher currents also tend to produce longer periods of unconsciousness and insensibility (Gregory and Wotton, 1990).

DC waveforms: the voltage (typically lower than AC) that produces the current has a variable effect on the birds according to their resistance. DC currents are normally pulsed and the average current may be expressed as 'pulsed' with a description of the 'duty cycle' (i.e. a ratio of 'on to off' times (mark space ratio) e.g. 50% (1:1), 33% (1:2). Current may also be expressed as 'peak current' or 'average current'. The longer the time 'off' within each cycle the less effective is the stun (Raj et al., 2006c). The currents used with DC are lower than with AC values (HSA, 2011; Paul Berry, Technical Ltd, Personal Communication, 2012).

The relationships between the various electrical parameters (waveforms, currents levels and frequency) used to electrically stun poultry and the occurrence of epilepsy in the EEG are not known (Wenzlawowicz and Holleben, 2001). AC waveforms are more effective than pulsed DC in terms of inducing epileptiform activity, however industry is more inclined to use DC for reasons related to meat quality. The inefficiency of pulsed DC at inducing epileptiform activity is probably because the current flows in a positive direction only, whereas the AC flows both in positive and negative directions. In addition, compared with pulsed DC, sine wave AC has a relatively slower rate of voltage change and longer excursion distance (Raj, 2006c, 2006d). Direct brain cell stimulation studies have shown that AC current-induced electrical fields, affect the neuronal cell axis both parallel and perpendicular to the electrical field, whereas DC current induced electrical fields affect only the cell axis parallel to the electrical field. It is believed that electrical fields perpendicular to the cell axis are much more effective in affecting neuronal function than those parallel to the cell axes (Raj, 2006).

Conclusion 12: The effectiveness of any waveform needs to be reliably established in a way that can translate to practical situations in the abattoir.

Conclusion 13: Electrical parameters (current, frequency) can be varied in many abattoirs, for example if it appears that carcasses are having to be downgraded because of meat quality or birds are being inadequately stunned.

Conclusion 14: The relationships between the various electrical parameters (waveforms, currents levels and frequency) used to electrically stun poultry and the occurrence of epilepsy in the EEG have not been fully evaluated.

Conclusion 15: AC waveforms are more effective than pulsed DC in terms of inducing epileptiform activity, but industry commonly uses DC to achieve better meat quality.

2.5. The contribution that the variation between birds species and sizes within a species or batch makes to an adequate stun when using multiple birds at the same time in a waterbath

The characteristics of the electric current are altered according to the species of bird (e.g. chicken, turkey, duck, geese, quail). This is necessary because of the differences in resistance (or impedance for AC currents) between the different types of birds. This is compounded when several birds of the same species and type are stunned at the same time in a waterbath (possibly 10-25 birds). However, the fewer birds in a waterbath at any one time, the less will be the variation and this is being used in practice but no scientific assessments have been made. Given a constant voltage and good electrical contacts, the current passing through each bird will vary inversely with the resistance of each bird i.e. the greater the resistance the lower will be the current passing through a given bird and so the greater is the chance of an inadequate stun. Conversely, when stunning birds in groups as in waterbath stunning, those birds with a lower resistance will receive a higher current which will be more effective for stunning but can result in a greater chance of a poorer meat quality (Wotton and Wilkins, 2004).

When there are several birds in the waterbath at the same time, each bird will have a different resistance. At present, electrical measurements taken in an abattoir only indicate the average current flowing through a bird and do not measure the actual current flowing through each bird. In-line constant current devices have been used in research projects to measure and control the current passing through each bird but they are not used in practice mainly because of the prohibitive costs involved. However, inspectors at abattoirs are using such equipment, fitted with an electrical resistor and attached to a shackle, to measure the actual current passing through individual shackles containing birds. As electrical parameters can be varied at the abattoir this may result in either an effective stun or poor meat quality, or *vice versa*. In the future it may be possible to develop a practical method for in-line monitoring of each bird.

Conclusion 16: For a constant voltage, the current passing through each bird will vary inversely with its resistance. When several birds are in the waterbath at the same time the range of bird size and body form present will affect the efficacy of the stun.

2.6. Other factors that affect the efficiency of stunning to be considered

a) The efficiency/conductivity of the contact between the shackle and the leg of the bird can be a problem and is critically important. The leg is covered by keratinised skin that can vary in thickness and it has a resistance to the passage of electrical current (Hewson and Russell, 1991; Wotton and Gregory, 1991). The resistance has been found to vary between: 1000-2600 Ω for broilers; 1900-7000Ω for laying hens; 800-5700 Ω for turkeys; 1100-2400 Ω for ducks; and 1200-4100 Ω for geese (Wotton and Wilkins, 2004). Birds vary in degree of feather and keratinisation with older laying hens having more feathers and a drier keratinised leg than broilers. Male broilers have thicker legs than female broilers.

b) There is a build up of scale and leg 'secretions' on the stainless steel shackle and the contact between the shackle and the earthing plate or bar (sometimes called a rubbing bar) that forms the negative electrode) may be impaired. This contact also varies along the line e.g. around corners. The cleaning agents used to maintain good electrical contacts along the line may not always be effective.

c) For current to flow through a bird, its head and neck have to make good contact with the water in the waterbath and the height of the water has to be sufficient for this to happen. However, some birds (particularly ducks and geese) may hold their heads clear of the water, and also short birds in a line, may not make contact and be stunned. The size of the waterbath can influence stunning insofar as its length will determine the duration of exposure to the stunning current for a given line speed. Furthermore, the number of birds that can be placed into the waterbath at any one time can affect the efficacy of a stun due to the variability in resistance (impedance) of each bird and the reduced chance of an adequate stun for a constant voltage applied to more than one bird.

Conclusion 17: Problems occur in the abattoir with waterbath stunning because birds may move at critical stages, electrical contacts may be poor, with the result that some birds are inadequately stunned.

3. Approach

In order to perform a comprehensive review of the scientific references on electrical stunning of poultry, a systematic literature review (SLR) was carried out. Inspection data from slaughterhouse inspections conducted both in Member States and non-Member States have also been added in order to complement the findings from the systematic literature review.

3.1. Systematic literature review data

The SLR methodology is a formalised approach to conducting a critical review of the literature and has been applied to policy making in many areas, including food safety regulation. The methodology is based on the key principles of transparency, comprehensiveness and quality assessment (EFSA, 2010).

The systematic review consists of four steps (i) literature search, (ii) relevance screening, (iii) eligibility/quality assessment and data extraction and (iv) data analysis and summation.

3.1.1. Methodology

The goal of the SLR was to retrieve all relevant primary research publications displayed in peer-reviewed documents i.e. journal articles, conference proceedings articles and PhD theses, in a structured and reproducible way and to then extract the information/data in a uniform way from each paper. Initially, subject matter experts were invited to a technical hearing, to discuss and identify relevant parameters and details that would need to be reported in relevant studies, in order to develop the data extraction parameters of the SLR. The detailed systematic review protocol, including a list of the references found relevant and used for data extraction, can be found in Appendix 1.

One review question was defined for the systematic review:

Which electrical parameters for waterbath stunning equipment for poultry induce appropriate stunning (i.e. immediate onset of unconsciousness and insensibility until death)?

3.1.2. Literature search and strategy

Electronic literature searches were carried out in the databases CAB abstracts (1910 to present), CCC (1998 to present), FSTA (1969 to present), Web of Science (1975 to present) and PubMed (1946 to present).

A total number of 1138 references were retrieved and checked for duplicates. Duplicate citations were removed by electronic and manual scanning using the EndNote X1.0.1 (Bld 2682) electronic database. Following the removal of duplicate references, 710 citations were uploaded (including one reference provided by the WG chairman) to the Web-based systematic review software DistillerSR (Evidence Partners, Ottawa, Canada), for relevance screening.

3.1.3. Relevance criteria

The relevance of retrieved references was assessed by screening their title and abstracts independently by two reviewers, following the a priori set relevance criteria:

Criterion 1: The reference is a primary research paper OR a PhD thesis OR a conference proceedings article containing a full description of a primary research study

Criterion 2: The language of the article body is in English OR French OR German (this selection reflects the language capacities of the reviewers

Criterion 3: The reference is testing a hypothesis regarding OR describing the electrical parameters used for waterbath stunning for poultry (broilers, hens, turkey, ducks geese, quail), and describing their effect on the consciousness of stunned birds.

For each question there were three possible responses: ‘Yes’, ‘No’ and ‘Cannot tell’. A ‘No’ response for any of the three questions resulted in the reference not advancing to the next stage of the review, if both reviewers were in agreement. Any disagreement between the two reviewers was discussed and when no consensus was reached, the record was included for the full text review.

When references were considered relevant, the full text was obtained for data extraction. For identification purposes each reference was given unique four figure identification number (RefID), during the data extraction phase.

3.1.4. Data extraction and collating the data

At this stage of the review, data on parameters and measurements listed below, which were determined as relevant to electrical waterbath stunning at the technical hearing meeting, were extracted from the relevant papers by one reviewer:

- Species type
- Sample size (number of animals studied)
- Number of animals simultaneously immersed in waterbath
- Duration of exposure to the waterbath
- Depth of immersion
- Electrical details (voltage, current, current type, frequency, wave form)
- Time elapsed between leaving the waterbath and the neck cutting
- How was unconsciousness ascertained?
- Length of observation after stunning
- Number of animals unconscious after stun
- Duration of unconsciousness
- Number of animals with cardiac arrest/ventricular fibrillation after stunning

The quantitative data extracted, were collated into an Excel spreadsheet and then presented in graphical plots and tables. Each experiment within a single reference was given its own unique identifier (ranging from 1 to 199).

3.1.5. Data presentation

Plots were developed using the extracted data. The data were first stratified by current type (AC or DC) and then by species. Different electrical 'treatment[5]' classes were determined (see Tables 1-3), comprising of different combinations of waveform (for AC current type only, as DC current type consisted of only one waveform type; pulsed waves), current (mA) and frequency (Hz) (see Section 2.4 for descriptions of the waveform types). Although the symbols used in Tables 1-3 are the same, they denote different electrical parameter ranges for each species, as indicated. Where data were not present with regards to any of these three electrical parameters then the experiment was excluded from the plot. A comparison between the electrical treatment classes was then performed against the following measurement/outcome, if the information was available:

- Percentage of animals reported as stunned (unconsciousness ascertainment using EEG methods (i.e. EEG epileptiform activity, EEG suppression, absence of SEP – see Section 2.2 for details on these methods).

- Percentage of animals reported as stunned (unconsciousness ascertainment using non-EEG methods (i.e. neck tension recovery, corneal reflex, comb pinch - see Section 2.2 for details on these methods).

[5] Technical term referring to the application of different electrical parameters.

• Mean duration of unconsciousness (unconsciousness ascertainment using both EEG and Non-EEG methods).

• Percentage of animals with cardiac arrest.

Table 1. Electrical Treatment Classes: Current Type AC: Species: Broilers, Laying hens, Ducks

Wave form	**Symbol**
Sine/clipped sine	Alpha
Square/rectangular	Beta
Rectified sine	Gamma
RMS Current (mA)	**Symbol**
1-100	A
101-150	B
151-200	C
201-400	D
Frequency (Hz)	**Symbol**
50-200	I
201-400	II
401-600	III
≥601	IV

Table 2. Electrical Treatment Classes: Current Type AC: Species: Turkeys

Wave form	**Symbol**
Sine/clipped sine	Alpha
Square/rectangular	Beta
Rectified	Gamma
RMS Current (mA)	**Symbol**
1-250	A
251-400	B
Frequency (Hz)	**Symbol**
50-200	I
201-400	II
401-600	III
≥601	IV

Table 3. Electrical Treatment Classes: Current Type DC: Species: Turkeys, Broilers, Laying hens, Ducks

Average Current (mA)	**Symbol**
1-100	A
101-200	B
201-400	C*
Frequency (Hz)	**Symbol**
50-200	I
201-600	II
≥601	III

*peak current

3.1.6. Inspections of poultry slaughterhouses in Member States and non-Member states by the FVO

Data relating to stunning of poultry in slaughter houses, compiled by the Food and Veterinary Office (FVO) from inspections of 13 slaughterhouses, from 2006 to 2011, and obtained by observing broilers ducks and quail being slaughtered and ascertaining the effectiveness of the stun by looking for critical signs (e.g. wing flapping, neck contractions and return of rhythmic breathing), are presented in section 4.

3.1.7. UK Food Standards Agency monitoring

In the UK, the Food Standards Agency record birds that have been through the waterbath but missed the neck cutter. These are referred to as 'red birds' due to their appearance after emerging from the scald tank. Data are collected and presented in the results section on 'red birds' that have missed the neck cutter and which will have arrived at the scalding tank alive and may or may not have been conscious.

4. Results and findings

4.1. Systematic literature review

Twenty-one relevant records were retrieved, from which data were extracted, resulting in 208 individual experiments; 199 experiments captured quantitatively for potential analyses and 9 described in narrative format.

A breakdown of the 199 experiments captured quantitatively is provided in the following tables:

Table 4: AC current type – number of experiments

Current type AC	Turkeys	Broilers	Laying Hens	Ducks
Total number of experiments:	**38**	**88**	**8**	**1**
Number using EEG ascertainment methods	9	61	5	1
Number using non-EEG ascertainment methods	29	27	3	0
Total number of experiments giving complete details*:	23	12	3	0
Number using EEG ascertainment methods	0	1	0	0
Number using non-EEG ascertainment methods	23	11	3	0
Number of experiments in which the percentage of birds stunned was not derivable	0	8	0	0
Number of experiments in which mean duration of unconsciousness was not given	12	68	5	0
Number of experiments not included in plots due to incomplete electrical treatment class details (current, frequency or waveform)	4	10	0	1
Number of experiments in 'percentage of animals stunned (EEG)' plot	5	59	5	0
Number of experiments in 'percentage of animals stunned (non-EEG)' plot	23	11	3	0
Number of experiments in 'mean duration of unconsciousness' plot	23	20	3	0
Number using EEG ascertainment methods	0	1	0	0
Number using non-EEG ascertainment methods	23	19	3	0
Number of experiments in 'percentage of animals with cardiac arrest' plot	26	29	3	0

* Current type, current, Frequency, waveform, % stunned, mean duration of unconsciousness

Table 5: DC current type – number of experiments

Current Type: DC	**Turkeys**	**Broilers**	**Laying Hens**	**Ducks**
Total number of experiments:	**6**	**56**	**2**	**0**
Number using EEG ascertainment methods	0	38	2	0
Number using non-EEG ascertainment methods	6	18	0	0
Total number of experiments giving complete details*:	6	6	0	0
Number using EEG ascertainment methods	0	0	0	0
Number using non-EEG ascertainment methods	6	6	0	0
Number of experiments in which the percentage of birds stunned was not derivable	0	12	0	0
Number of experiments in which mean duration of unconsciousness was not given	0	38	2	0
Number of experiments not included in plots due to incomplete electrical treatment class details (current, frequency or waveform)	0	0	2	0
Number of experiments in 'percentage of animals stunned (EEG)' plot	0	38	0	0
Number of experiments in 'percentage of animals stunned (non-EEG)' plot	6	6	0	0
Number of experiments in 'mean duration of unconsciousness' plot	6	18	0	0
Number using EEG ascertainment methods	0	0	0	0
Number using non-EEG ascertainment methods	6	18	0	0
Number of experiments in 'percentage of animals with cardiac arrest' plot	4	11	0	0

* Current type, current, Frequency, waveform, % stunned, mean duration of unconsciousness

There were no references on quail or geese found meeting the relevance criteria set during the SLR.

4.1.1. Analysis of results

Analysis of the effectiveness of stunning procedures has been based on the following measures: % of birds unconscious, ascertained by either EEG or non-EEG methods, duration of unconsciousness (all based on non-EEG methods with the exception of broiler AC treatment class, Alpha-A-I) and cardiac arrest.

The effectiveness of stunning methods for turkeys, broilers and laying hens, using AC current, based on the EEG methods of ascertainment are illustrated in Figures 1, 2 and 3, respectively..

Overall summaries (based on median values, due to the different weight of each record) of all measurements (based on median values) for turkeys, broilers and laying hens are summarised in Tables 6 to 10. There were no results for ducks. Figures containing all plots of all measurements for each species are presented in Appendix 2 and individual data listings (including the reference identification number) that were used to prepare the plots are presented in Appendix 3.

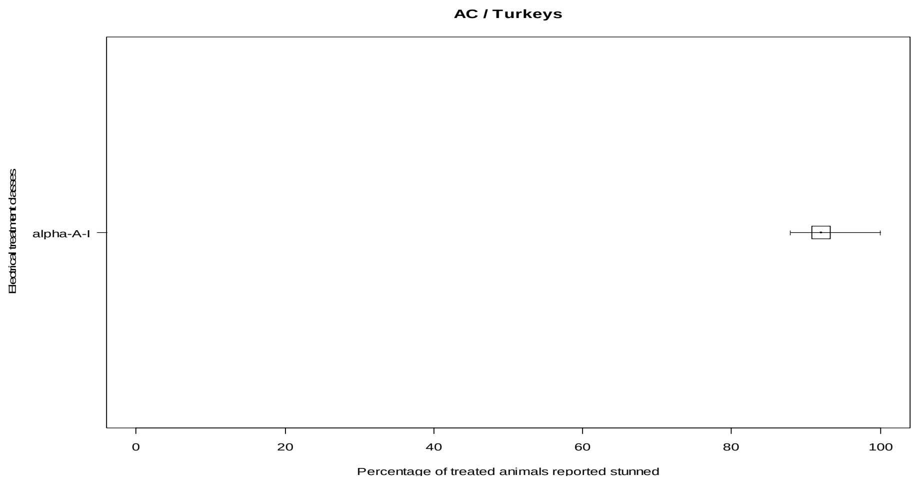


Figure 1. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

Inspection of Figure 1, for turkeys, using EEG methods for ascertainment of unconsciousness and insensibility, shows that the following patterns (Alpha-A-I) AC sine/clipped sine wave 1-250mA, 50-200Hz, produced a stun that was effective in 92% of birds.

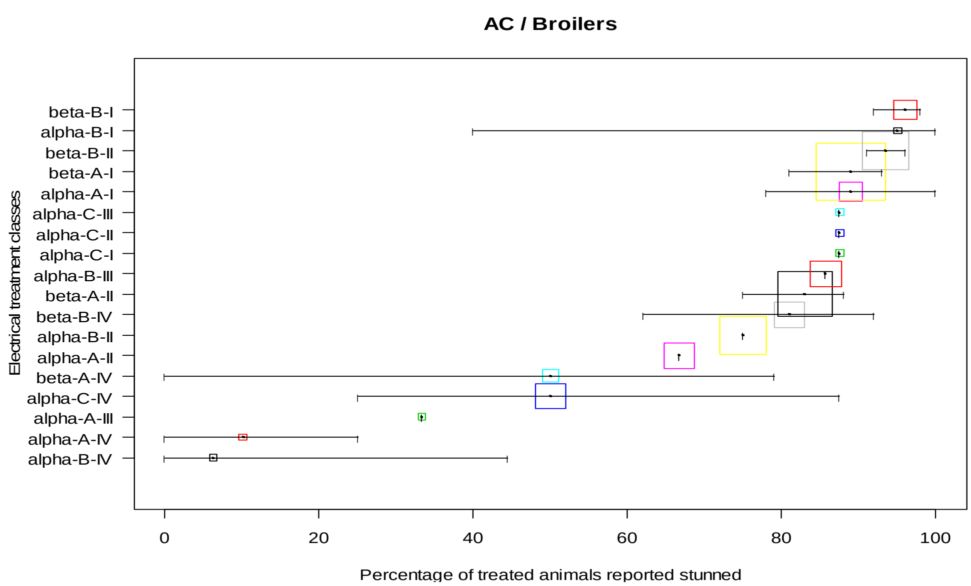


Figure 2. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

Inspection of Figure 2 (and Table 8) reveals that, when using EEG methods for ascertaining unconsciousness, the only treatment classes that achieved an effectiveness up to 96 % were Alpha-B-I (AC sine/clipped sine wave, 101-150 mA, 50-200 Hz), Beta-B-I (AC square/rectangular wave, 101-150 mA, 50-200 Hz) and Beta-B-II (AC square/rectangular wave, 101-150 mA, 201-400Hz). Alpha-B-III (AC sine/clipped sine wave, 101-150 mA, 401-600 Hz) was significantly less effective. Beta-B-III was not measured.

In addition, for broilers using DC current, treatment combinations achieved an effectiveness of 95% (ascertained with EEG methods) using pulsed waveforms were A-I (1-100 mA/50-200 Hz) and B-II (101-200 mA/201-600 Hz) (Table 9).

The number of treatment classes for broilers (both AC and DC), using EEG methods for ascertainment of unconsciousness) are more numerous and allow a complete overview of relationships between the electrical parameters and the outcome of the incidence of unconsciousness. Where this occurred it can be seen that for a given current, increasing the frequency results in a decrease in the number of birds reported as unconscious (as ascertained by EEG methods; Tables 8 and 9).

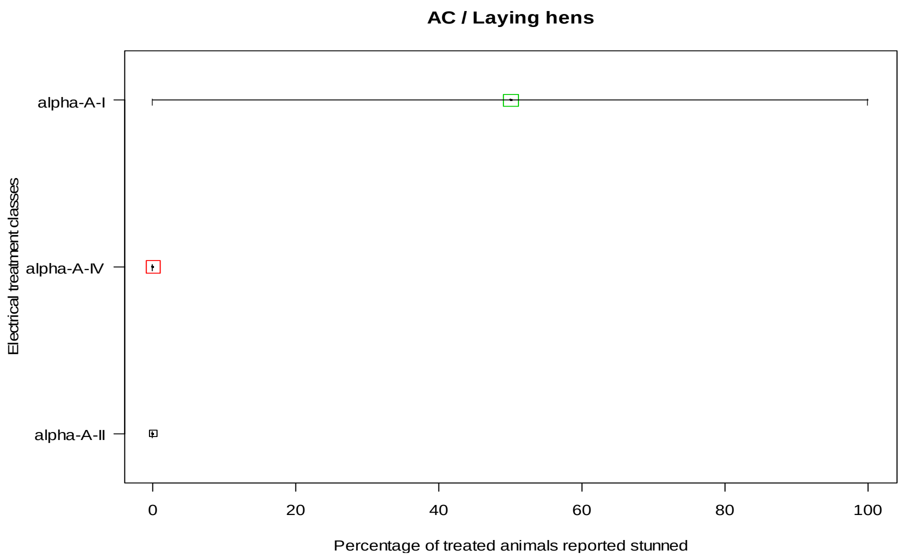


Figure 3. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

Inspection of Figure 3 reveals that for laying hens, using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns Alpha-A-I (AC sine/clipped sine wave, 1-100 mA, 50-200 Hz) produced a stun that was effective in 50% of birds. Using the same waveform and current but increasing the frequency to 201-400 Hz and above 600 Hz (Alpha-A-II and Alpha IV respectively), no birds were effectively stunned.

Table 6: Turkeys (AC) **-** Summary of stunning measurements (median values)

Current type: AC / Species: Turkey	**Stunning Measurement**			
Electrical Treatment Class/Combination	**% birds Unconscious: EEG ascertainment methods**	**% birds Unconscious: non-EEG ascertainment methods**	**Unconsciousness Duration (seconds)**	**% birds with cardiac arrest**
Alpha-A-I (Sine/Clipped sine/1-250mA/50-200Hz)	92	31	44	97
Alpha-A-II (Sine/Clipped sine/1-250mA/201-400Hz)	-	51	45	49
Alpha-A-III (Sine/Clipped sine/1-250mA/401-600Hz)	-	100	35	33
Gamma-A-I (Rectified sine/1-250mA/50-200Hz)	-	67	33	33

Refer to Appendix 2 - Figures 1-8, for plot representations of these data.
- = no measurement for electrical treatment class

Table 7: Turkeys (DC) **-** Summary of stunning measurements (median values)

Current type: DC / Species: Turkey	**Stunning Measurement**			
Electrical Treatment Class/Combination	**% birds Unconscious: EEG ascertainment methods**	**% birds Unconscious: non-EEG ascertainment methods**	**Unconsciousness Duration (seconds)**	**% birds with cardiac arrest**
B-II (101-200mA/201-600Hz)	-	97	61	3
B-III (101-200mA/≥601Hz)	-	100	40	3

Refer to Appendix 2 - Figures 25-30, for plot representations of these data.
- = no measurement for electrical treatment class

Table 8: Broilers (AC) **-** Summary of stunning measurements (median values)

Current type: AC / Species: Broiler	**Stunning Measurement**			
Electrical Treatment Class/Combination	**% birds Unconscious: EEG ascertainment methods**	**% birds Unconscious: non-EEG ascertainment methods**	**Unconsciousness Duration (seconds)**	**% birds with cardiac arrest**
Alpha-A-I (Sine/Clipped sine/1-100mA/50-200Hz)	89	26	57*	61
Alpha-A-II (Sine/Clipped sine/1-100mA/201-400Hz)	67	-	-	-
Alpha-A-III (Sine:Clipped sine/1-100mA/401-600Hz)	33	-	-	-
Alpha-A-IV (Sine/Clipped sine/1-100mA/≥600Hz)	10	-	-	-
Alpha-B-I (Sine/Clipped sine/101-150mA/50-200Hz)	95	12	53	90
Alpha-B-II	75	-	-	-

(Sine/Clipped sine/101-150mA/201-400Hz)				
Alpha-B-III (Sine/Clipped sine/101-150mA/401-600Hz)	86	-	52	-
Alpha-B-IV (Sine/Clipped sine/101-150mA/≥601Hz)	6	-	57	-
Alpha-C-I (Sine/Clipped sine/151-200mA/50-200Hz)	88	-	-	-
Alpha-C-II (Sine/Clipped sine/151-200mA/201-400Hz)	88	-	-	-
Alpha-C-II (Sine/Clipped sine/151-200mA/401-600Hz)	88	-	-	-
Alpha-C-IV (Sine/Clipped sine/151-200mA/≥601Hz)	50	-	-	-
Beta-A-I (Square/rectangular/1-100mA/50-200Hz)	89	-	-	80
Beta-A-II (Square/rectangular/1-100mA/201-400Hz)	83	-	-	-
Beta-A-IV (Square/rectangular/1-100mA/≥601Hz)	50	-	-	-
Beta-B-I (Square/rectangular/101-150mA/50-200Hz)	96	-	-	80
Beta-B-II (Square/rectangular/101-150mA/201-400Hz)	94	-	-	-
Beta-B-IV (Square/rectangular/101-150mA/≥601Hz)	81	-	-	-
Gamma-B-I (Rectified sine/101-150mA/50-200Hz)	-	100	42	2

Refer to Appendix 2 - Figures 9-16, for plot representations of these data.
- = no measurement for electrical treatment class
*includes one mean duration values based on EEG methods.

Table 9: Broilers (DC) **-** Summary of stunning measurements (median values)

Current type: DC / Species: Broiler	**Stunning Measurement**			
Electrical Treatment Class/Combination	**% birds Unconscious: EEG ascertainment methods**	**% birds Unconscious: non-EEG ascertainment methods**	**Unconsciousness Duration (seconds)**	**% birds with cardiac arrest**
A-I (1-100mA/50-200Hz)	95	-	-	-
A-II (1-100mA/201-600Hz)	92	-	29	-
A-III (1-100mA/≥601Hz)	60	-	-	-
B-I (101-200mA/50-200Hz)	80	-	-	80
B-II (101-200mA/201-600Hz)	95	100	50	2
B-III (101-200mA/≥601Hz)	51	100	43	-
C-I (201-400mA/50-200Hz)	73	-	-	45

Refer to Appendix 2 - Figures 31-38, for plot representations of these data.
- = no measurement for electrical treatment class

Table 10: Laying Hens (AC) - Summary of stunning measurements (median values)

Current type: AC / Laying Hen	Stunning Measurement			
Electrical Treatment Class/Combination	% birds Unconscious: EEG ascertainment methods	% birds Unconscious: non-EEG ascertainment methods	Unconsciousness Duration (seconds)	% birds with cardiac arrest
Alpha-A-I (Sine/Clipped sine/1-100mA/50-200Hz)	50	65	53	35
Alpha-A-II (Sine/Clipped sine/1-100mA/201-400Hz)	0	-	-	-
Alpha-A-IV (Sine/Clipped sine/1-100mA/≥601Hz)	0	-	-	-
Alpha-B-I (Sine/Clipped sine/101-150mA/50-200Hz)	-	23	56	77

Refer to Appendix 2 - Figures 17-24, for plot representations of these data.
- = no measurement for electrical treatment class

4.1.2. Results in narrative format

Results from experiments where outcomes were reported in a way that did not allow capture for quantitative data for presentation in plots are presented below.

AC: Waveform Sine/Clipped sine:

Broilers/Laying hens

Current: 1 to 75 mA

Gregory and Wotton, (1989) (Ref ID 254), found that at a frequency of 50 Hz, epileptiform activity was noted for 56% of non-ventricular-fibrillating birds, for an average duration of 18 seconds. Ventricular fibrillation occurred in 13 % of birds; 86 % of which developed epileptiform activity for an average duration of 15 seconds.

AC: Waveform Square/rectangular

Broilers

Average Current: 101 to 250 mA

Hindle et al. (2010) (Ref ID 1311) found that increasing the frequency from 50 Hz (current range 45 to 229 mA) to 400 Hz (current range 54 to 274 mA) and 1000 Hz (current range 65 to 444 mA) resulted in a decrease in the incidence of deaths (92 %, 48 % and 28 %, respectively) and an increase in the proportion of unconscious birds (2 %, 39 %, 66 %, respectively). Recovery of consciousness was assessed by a response to a comb pinch and supported by the re-occurrence of EEG – alpha waves (8 to 13 Hz) and beta (>13 Hz) waves.

Laying Hens

Average Current: 76 to 125 mA

Hindle et al. (2010) (Ref ID 1311) found that increasing the frequency from 50 Hz (current range 40 to 151 mA) to 400 Hz (48 to 136 mA), and 1000 Hz (current range 43 to 219 mA) resulted in a decrease in the incidence of deaths (44 %, 6 % and 0 %, respectively) and an increase in the proportion of unconscious birds (54 %, 83 %, 100 %, respectively). Recovery of consciousness was assessed by a response to a comb pinch and supported by the reoccurrence of EEG - alpha waves (8 to 13 Hz) and beta (>13 Hz) waves.

Ducks

Average Current: 151 to 175 mA

Hindle et al. (2010) (Ref ID 1311) found that increasing the frequency from 50 Hz (current range 77 to 243 mA) to 400 Hz (current range 64 to 362 mA) resulted in a decrease in the incidence of deaths (39 % and 10 %, respectively) and an increase in the proportion of unconscious birds (61 % and 84 %, respectively). Recovery of consciousness was assessed by a response to a head pinch/touching of eyelids and supported by the reoccurrence of EEG - alpha waves (8 to 13 Hz) and beta (>13 Hz) waves.

Broilers

DC: Waveform Pulsed

Peak current 400mA

Raj et al. (2006c) (Ref ID 1433) investigated the effect of 3 different pulse widths (10 %, 30 % and 50 % of the current cycle) using a peak current of 400 mA at a frequency of 200Hz. For pulse width of 10 %, 30 % and 50 %, the proportion of birds stunned were 13 %, 73 % and 80 %, respectively (as ascertained by epileptiform EEG activity. This indicated that for DC current pulse widths of 30 % or 50 % of the current cycle were more effective than using a pulse width of 10 % of the current cycle, to stun broilers.

4.2. Data from Slaughterhouse Inspections

4.2.1. Inspections of poultry slaughterhouses in Member States and non-Member States by the FVO

At the first slaughterhouse inspected, broilers regaining rhythmic breathing were observed during bleeding and approximately 5% of broilers were still actively moving when entering the scald tank, the neck was cut dorsally, however no values for electrical parameters were given.

During the inspection of a second slaughterhouse, involving electrical waterbath stunning of quail, where electrical parameters used were 200 mA, 66 V, 200 Hz for groups of 18 birds (equating to a current of 11 mA per bird), rhythmic breathing appeared soon after stunning, wing flapping was noted in 90 % of birds after the neck cutting and approximately 5 % of birds regained consciousness before entering the scald tank. For 15 days over a 2 month period the official veterinary report stated that the stunning parameters were lower than those applied at the FVO inspection and that 13 to 97 % (average 83.55%) of birds regained consciousness after neck cutting and 4 to 36 % (average 18.85 %) of birds were conscious when entering the scald tank when 160 to 170 mA for groups of 18 birds (approximately 9 mA per bird) were used. These current values are lower than the value of 45 mA stated in Table 2 of the Regulation (EC) No 1099/2009.

Signs of recovery (breathing, wing flapping) of consciousness during bleeding were noted in broilers during an inspection of a slaughterhouse when an AC current using the combination 355 Hz/120 mA or 355 Hz/95 to 115 mA per bird was used at an average exposure time of over 4 seconds. These values are below the current specified in Table 2 of the Regulation (EC) No 1099/2009 and the ranges proposed by the UK authority for the frequency used.

In another six slaughterhouses using different electrical setting combinations (where the exposure time was >4 seconds, unless otherwise stated), no signs of recovery of consciousness during bleeding (breathing, wing flapping) were noted when the combinations used were AC 300 Hz/130 mA (ducks); 200 Hz/119 mA – exposure time 11 seconds (broilers); 400 Hz/141 to 150 mA (broilers); 400 Hz/146 to 150 mA (broilers); 355 Hz to 140 mA (broilers); 400 Hz/172 mA – exposure time 11 seconds (broilers); 400 Hz/ 122-138 mA (broilers) and DC 275 Hz/160 mA or 165 mA (broilers). These combinations are mainly compliant with the values in Table 2 of the Regulation (EC) No 1099/2009, and the ranges proposed in the UK authority request. In some cases the current values were just below the minimal value of 150 mA stated for a frequency of 201 to 400 Hz. However, the combination of the parameters applied to the ducks (AC 300Hz/130 mA) used a much higher frequency that that stated in Table 2 of the Regulation (EC) No 1099/2009 (≤200 Hz), for a current of 130 mA.

An inspection of another poultry slaughter plant, where the electrical settings were 350 Hz at 110 mA per bird, approximately 5 % of broilers showed signs of consciousness, in particular rhythmic breathing. In another poultry slaughterhouse, where the stunning parameters varied between 129 to 148 mA per bird at a frequency of 1000 Hz, very frequent wing flapping at the time of automatic neck cutting was noted. The electrical parameters used in both cases are not compliant with Table 2 of the Regulation (EC) No 1099/2009 as the current values were lower than the minimum current to be applied when using a frequency of 350 or 1000 Hz for any species of bird.

At an inspection of electrical waterbath stunning of ducks, birds were exposed to a stunning current of 130 mA (compliant with the current values for ducks in Table 2 of the Regulation (EC) No 1099/2009, but the frequency was not stated), and were not effectively stunned as 5 % of birds had neck contractions after bleeding and approximately 2 % showed a palpebral reflex. It was also noted that not all the ducks' heads had been fully immersed at the time of stunning. During the inspection of another slaughterhouse, where ducks leaving the waterbath showed no signs of consciousness and were dead on entry to the scald tank, there were signs of recovery in around 10 % of birds during the bleed out period (the ducks had been bled by incising blood vessels manually via the inside of the mouth).

4.2.2. UK Food Standards Agency monitoring

In the UK, the Food Standards Agency has recorded the number of birds that were ineffectively stunned or missed the neck cutter (it is not clear whether they also missed being stunned). These are referred to as 'red birds' due to their appearance after emerging from the scald tank, and are often the smaller birds on the line. It is estimated that in the UK they number 0.005%, i.e. 1:100,000 birds (FSA, 2010).

5. Discussion

The study designs in the references reviewed for data extraction differed, with particular regard to the various ways of measuring unconsciousness and insensibility, i.e. physical observations and muscle tone versus EEG analysis, and this non-uniformity resulted in findings not being directly comparable. Furthermore, evidence for a correlation between physical observations with the EEG activity phases is limited.

Many experiments did not provide enough information to allow for a full evaluation. It was not possible to perform statistical analyses on the quantitative data extracted as the numbers of records in the electrical treatment classes formed were too small to allow statistical analysis of the factors which influence the stun outcome. Instead, the data were presented in tables and plots to show the different outcomes and measurements from the various electrical treatment classes against the percentage of birds stunned, mean duration, and occurrence of cardiac arrest. However, there were limitations with this method of presentation as some experiments were excluded due to incomplete electrical parameter details or due to a lack of information on the duration of the stun or it was not possible to derive information on the percentages of birds stunned. Many experiments provided data only on one or two of the outcomes investigated, so it was not possible to form a complete overview for a particular electrical treatment (i.e. the percentage of birds stunned together with the duration of the stun).

There were papers on geese and quail used previously in the EFSA opinion (EFSA, 2004) that were present in the search results from the systematic literature review. However, these papers were not deemed relevant for the review question as they did not specify the effects of the electrical parameters on unconsciousness, but instead focused on meat/carcase quality or cardiac arrest.

Conclusion 1: Many of the published studies did not allow a comprehensive analysis due to different study designs and incomplete data.

The available data did highlight some combinations which resulted in high numbers of broilers rendered unconscious when ascertained by EEG methods. For AC currents (see Table 8), a sine/clipped sine wave, 101-150 mA, 50-200 Hz combination (Alpha-B-I) resulted in a high proportion of birds (95 %) being stunned and this was also true when using an AC square/rectangular wave, 101-150 mA, at frequencies from 50 to 400 Hz (Beta-B-I and Beta-B-II) (94-96 %).

For broilers, DC pulsed waves (see Table 9) electrical combinations of 1-100mA/50-200Hz (A-I) and 101-200 mA/201-600 Hz (B-II) also resulted in a high number of broilers being stunned (95 %). A trend was also noted that at a given current, (AC and DC) increasing the frequency resulted in a lower number of birds being stunned (as measured by EEG methods) (see Table 8).

The lack of comprehensive data and the failure of many studies to use EEG as a method of determining unconsciousness and insensibility meant that there were some electrical parameters that appeared to induce 100 % unconsciousness and insensibility and that the estimation of duration was largely based on non-EEG measurements.

Conclusion 2: AC currents between 101-150 mA at 50-200 Hz with a sine/clipped sine wave and 101-150 mA at 50-400 Hz using a square/rectangular wave resulted in 94-96 % broilers being rendered unconscious based on EGG measurements.

Conclusion 3: DC currents between 1-200 mA at 50-600 Hz, resulted in 95 % of broilers being rendered unconscious based on EGG measurements.

Conclusion 4: Neither AC or DC currents that have been tried give a 100 % stun rate, when using EEG methods of ascertainment.

Given that billions of birds are slaughtered each year it is possible that even if a vey low percentage of birds were not effectively stunned this could result in considerable numbers of birds being aware of subsequent slaughter processes.

Although limited information was available with respect to some of the electrical parameters and the time at which physical observations were noted after the stunning procedure, the FVO inspection data (based on non-EEG measurements of unconsciousness) do provide some indication of stunning outcomes in slaughterhouses. Ineffective electrical waterbath stunning outcomes (indicated by physical observations, including wing flapping, return of breathing or neck contraction) were noted during inspections of slaughterhouses both in Member States and non-Member States. Physical observations were noted and occurred when the electrical combinations used were outside the ranges specified in Table 2 of the Regulation (EC) No 1099/2009 as well as the ranges proposed in the UK Authority request. For some inspections where the electrical parameters were closely related to those in Table 2 of the Regulation (EC) No 1099/2009 and the ranges proposed in the UK Authority request, no signs of recovery such as wing flapping, return of breathing or neck contraction were noted.

Conclusion 5: There are few observational studies in abattoirs to determine the numbers of birds that are effectively stunned. When done on a large scale the FVO did not identify major problems but, for practical reasons, they used non-EEG methods for ascertaining the effectiveness of a stun.

The Dutch authority request that Table 2 of the Regulation should refer to ‘the minimum values per animal', instead of 'average values per animal'. However, practical methods for measuring actual current values per bird are not available for use in slaughterhouses, even though it is known that the amount of current that each bird receives varies, resulting in some birds not being effectively stunned.

In addition, the research performed for the Dutch Authority recommends that additional electrical parameters should be specified regarding wave characteristics (waveform and duty cycle/pulse width).

The UK Authority requests clarification of current type and waveform used in the Table 2 of the regulation.

Conclusion 6: The Regulation does not include details on waveform, or clearly specify the current type, include details on duty cycle/pulse width (for DC currents), or specify minimum currents for each bird.

The UK authority also suggests lowering the high frequency range given in Table 2 of the Regulation (EC) No 1099/2009, from 1500 Hz to 800 Hz in order to improve welfare, but noting that above 800 Hz could lead to electro-immobilisation instead of insensibility. A change to the mid-range frequency band in Table 2 to cover the range 200 to 600 Hz was suggested, as industry had highlighted a significant reduction in meat quality associated with a 400 Hz/150 mA frequency and current combination, compared with existing industry averages. They state that using a higher frequency of 600 Hz in the mid-band of Table 2, combined with a current of 150 mA in broilers, would achieve an effective stun as indicated from research data, and also reduce meat loss from down-grading.

- From the systematic literature review EFSA was not able to comment on electro-immobilisation as this was not specifically addressed in the literature. However, a trend was apparent for broilers for both AC and DC currents, where at a given current increasing the frequency resulted in a lower number of birds being stunned (as measured by EEG methods).

- Broilers rendered unconscious was less than 90 % for AC currents between 150 mA to 200 mA and combined with frequencies of 600 Hz (Alpha-C-II (sine/clipped sine/151-200 mA/401-600 Hz – 88 %; Alpha-B-III (sine/clipped sine/101-150 mA/401-600 Hz – 86 %).

Conclusion 7: Evidence for supporting an increase in the mid-band frequency range for broilers to 600 Hz using an AC current at 150-200 mA was not found.

The current Table 2 in the Regulation (EC) No 1099/2009 lacks specification of current type and waveform and the frequency ranges presently overlap. The frequencies could be made mutually exclusive (e.g. ≤ 200 Hz, 201-400 Hz, 401-1500 Hz), as originally stated in the EFSA report (EFSA, 2004).

6. Conclusions and recommendations

CONCLUSIONS

1 Legislation requires that there is always an effective stun that lasts until the bird dies. This depends on there being a good stunning procedure and an effective method of killing at the abattoir. In order to determine whether or not this is the case, there have to be accurate measures of unconsciousness and insensibility, and its irreversibility until death.

2 A stun is effective if it renders the bird rapidly unconscious and insensible for a period of at least 45 seconds. It is also effective if it results in death of the bird from cardiac arrest as then the action of stunning does not result in poor welfare. However, it is uncertain whether currents that induce cardiac arrest may cause momentary pain and distress for a few second.

3 The aim of a stunning system is to achieve a 100 % effective stun. The most effective electrical parameters in present use can achieve an effectiveness of up to 96 %as measured by EEG methods and 100 %, reported as unconscious using non-EEG methods. A wide variety of electrical variables were tested but neither AC nor DC currents that have been tried give a 100 % stun rate, when using EEG methods of ascertainment.

4 Given the speed of procedures in present stunning systems, recovery of consciousness, as indicated by epileptiform activity followed by a continuous isoelectric EEG, before death by bleeding out, will not occur before subsequent events in the slaughter process if the duration of unconsciousness and insensibility achieved by the stun is 45 seconds or more.

5 At the present time, the most reliable way to determine if a bird is unconscious and insensible is by looking for particular patterns in the EEG. Somatosensory reflexes (comb-pinch response) and muscle tone and direct observations (rhythmic breathing, seizures) are not sufficiently reliable indicators of insensibility at high frequencies. At AC currents above 120 mA and at frequencies up to 200 Hz, the absence of corneal reflex is closely associated with suppressed EEG and would be a more, reliable indicator of insensibility. Evidence from EEG phases linked to physical observations is not feasible at an abattoir.

6 A wide variety of waveforms have been used in experimental studies. However, few of these have been studied in depth and repeated in different laboratories, and so their effectiveness needs to be reliably established.

7 Most studies on electrical parameters for water-bath stunning are laboratory-scale studies and difficult to extrapolate directly to large-scale field conditions, mainly because the current flowing through each bird cannot be guaranteed. Few of the waveforms used in experimental studies have been systematically studied under practical abattoir conditions, so their effectiveness needs to be reliably established.

8 With present stunning equipment used in slaughterhouses it is not possible to accurately measure and to adjust the amount of actual current that the individual bird receives during electrical water-bath stunning. The actual current that a bird receives during electrical water-bath stunning will vary according to (i) the number of birds in the waterbath at any one time, (ii) the individual variances in impedance of each bird, which will mean that some birds are not effectively stunned.

9 Problems occur in the abattoir with waterbath stunning because birds may move at critical stages, electrical contacts may be poor, with the result that some birds are inadequately stunned.

10 Electrical parameters (current, frequency) can be varied in many abattoirs, for example, if it appears that carcasses are having to be downgraded because of meat quality or birds are being inadequately stunned.

11 AC waveforms are more effective than pulsed DC in terms of inducing epileptiform activity, but industry commonly uses DC to achieve better meat quality.

12 Many of the published studies did not allow a comprehensive analysis due to different study designs and incomplete data.

13 For broilers using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns AC sine wave 101-150 mA, 50-200 Hz; AC square wave 101-150 mA; 50-400 Hz and DC pulsed wave (duty cycle 1:1; 50 % pulse width) 1-200 mA; 50-600 Hz produced a stun that was effective in 94-96 % birds. Use of an AC sine wave at 400 Hz and 600 Hz did not produce an effective stun. Application of an AC square wave at 600 Hz has not been measured.

14 For turkeys, using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns AC sine/clipped sine wave 1-250 mA, 50-200 Hz, produced a stun that was effective in 92 % of birds.

15 For laying hens, using EEG methods of ascertainment for unconsciousness and insensibility, the following patterns AC sine/clipped sine wave, 1-100 mA, 50-200 Hz produced a stun that was effective in 50 % of birds. Using the same waveform and current but increasing the frequency to 201-400 Hz and above 600 Hz, no birds were effectively stunned.

16 There are few observational studies in abattoirs to determine the numbers of birds that are effectively stunned. When done on a large scale the FVO did not identify major problems but, for practical reasons, they used non-EEG methods for ascertaining the effectiveness of a stun.

17 The requests from the Dutch and UK Authorities highlight problems with Table 2 in the proposed regulations as it does not clearly state if the current type pertains to AC or DC, or both, and also does not specify all electrical parameters relevant for waterbath stunning.

18 The Regulation does not include details on waveform, or clearly specify the current type, or include details on duty cycle/pulse width (for DC currents), or specify minimum currents for each bird.

19 Evidence for supporting an increase in the mid-band frequency range for broilers to 600Hz using an AC current at 150-200mA was not found.

20 It may not be practical at the present time to measure EEG routinely in the abattoir. However laboratory studies do show that current flow through individual birds at a specified frequency can be used with confidence to predict the EEG. Thus the effectiveness of the stun can be assessed under abattoir conditions from accurate measurement of current flow through individual birds.

21 When waterbath stunning is used, it is not possible to ensure that all birds are stunned.

RECOMMENDATIONS

1 The Regulation should indicate minimum current for each bird, frequency and current type as well as the wave characteristics - duty cycle and waveform.

2 There should be better surveillance and monitoring of the electrical parameters in use at abattoirs and, in addition, methods that allow the accurate measurement of actual electrical current flowing through each bird should be further developed.

3 Research on effective stunning should be validated by the measurement of EEG activity and related to clinical measures which are easier to use in practice.

4 There is an urgent need to develop electrical methods that guarantee 100% stun.

5 Unless the problems described in this opinion for all existing waterbath stunning methods can be resolved, other stunning methods should be used.

7. Further research

- In order to standardise experimental electrical waterbath studies and reporting, further research should employ EEG activity assessments in their design, and correlate the results of these with practical measures of unconsciousness and insensibility.

- Any new electrical stunning systems developed should be tested under abattoir conditions. It would be unnecessary to measure EEG if a system could measure and guarantee adequate current flow in each bird.

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Raj ABM, O'Callaghan M and Hughes SI, 2006c. The effects of pulse width of a direct current used in waterbath stunning and of slaughter methods on spontaneous electroencephalograms in broilers. Animal Welfare, 15, 25-30.

Schütt-Abraham, I, Wormuth HJ, Fessel, J and Knapp, J, 1983. Electrical stunning of poultry in view of animal welfare and meat production. In: Stunning of Animals for Slaughter. Ed Eikelenboom G. Martinus Nijhoff. The Hague, the Netherlands154 pp.

Wenzlawowicz, M. and von Holleben, K, 2001. Assessment of stunning effectiveness according to present scientific knowledge on electrical stunning of poultry in a waterbath. Archiv Für. Geflügelkunde. 65, 193–198.

Wotton, SB and Gregory, NG, 1991. How to prevent pre-stun electric shocks in waterbath stunners. Turkeys, 39, 15-30.

Wotton, SB and Wilkins LJ, 2004. Primary processing of poultry. In: Measuring and Auditing Broiler Welfare. Eds. Weeks CA, and Butterworth A. CABI, Wallingford, UK. 161-180.

Appendices

A. Appendix 1: Protocol: Systematic literature review concerning the electrical requirements for waterbath stunning equipment applicable for poultry

Background

Due to its methodological rigor and its transparent nature, systematic literature reviews can provide additional value for answering well formulated questions generated by risk assessment processes. Detailed guidance and examples for the conduct of key steps in the systematic review process are presented in figure 1 and described in the EFSA guidance for systematic reviews. The following steps are proposed for the systematic review the electrical requirements for waterbath stunning equipment applicable for poultry:

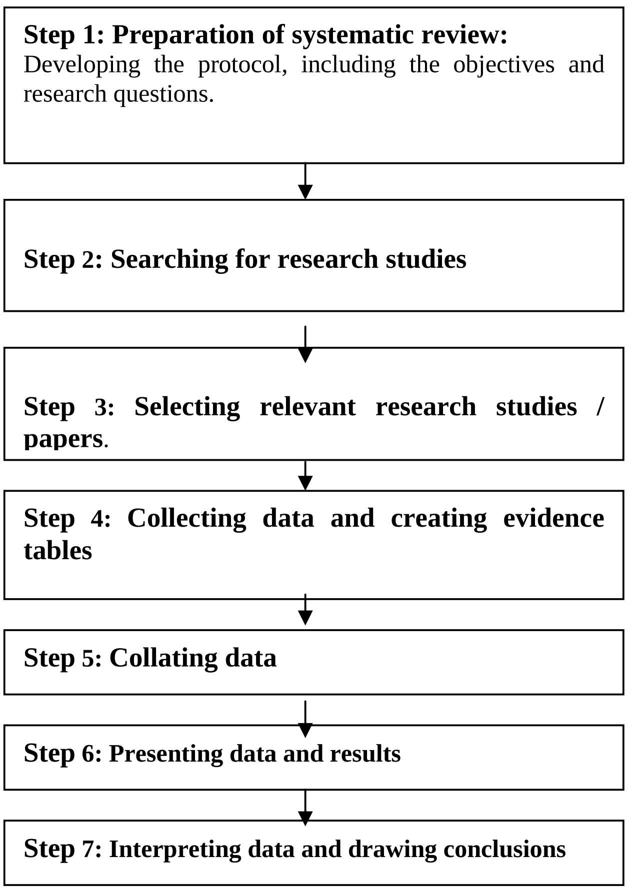


Figure 1: Seven core steps for performing a systematic literature review (adapted from Cochrane Handbook for Systematic Literature reviews of Interventions, Higgins and Green, 2009)

Objectives

Annex 1 of Commission Regulation (EC) 1099/2009 provides electrical requirements for waterbath stunning of poultry. These requirements are based on EFSA opinions (EFSA Journal, 2004, 45; EFSA Journal, 2006, 326, 1-18). The Commission has since received information from Dutch and UK authorities that might justify amending those requirements. EFSA has therefore been requested (in EFSA mandate EFSA-M-2011-0230) to give an independent view on electrical requirements for stunning equipment applicable for poultry, by reviewing its previous opinions in light of references provided by the British and Dutch as well as of any new relevant scientific developments.

The objective of the review is to provide a systematic overview of existing evidence in order to address the following Terms of Reference in the mandate:

- Review the relevant new scientific references on electrical stunning of poultry and in particular the ones provided by the British and Dutch authorities;
- Recommend, if necessary, new electrical requirements applicable for waterbath stunning equipments than the ones laid down in Table 2 of Chapter II of Annex I to Commission Regulation (EC) 1099/2009.

Pre-specified and standardised methods have been used to identify and appraise evidence, hereby documenting the review process adequately to allow others to critically appraise the criteria used in selection of papers, the interpretation of the results and, if necessary, to repeat or update the review.

To meet these objectives, one review question was defined for the systematic review:

Review question:

"Which electrical parameters for waterbath stunning equipment for poultry induce appropriate stunning (i.e. immediate onset of unconsciousness and insensibility until death)"

Searching for research studies (search strategy)

We aimed at retrieving primary research displayed in peer-reviewed documents, i.e. journal articles, conference proceeding articles and PhD. So we searched the following main scientific electronic databases in life science: CAB Abstracts, CCC, FSTA, Web of Sciences (WoS), and PubMed, using the search equations displayed in Table 1 (which uses the template recommended in the EFSA Guidance on pesticides[6]). That strategy was realised by an information specialist, and validated by the scientific manager. No limit was applied to the search (except that the search was performed with English terms).

In order to also retrieve available thesis, the following thesis repositories were also searched:

- DART database. Because it does not allow complex searches, it was searched with "waterbath", which retrieved nothing, and "stunning", which retrieved 15 results, all of them irrelevant.
- German thesis repository: here the 5 results for the search on "stunning", all irrelevant
- Indian thesis repository: no result neither for "waterbath" nor "stunning"
- French thesis repository: two irrelevant results (search performed: "poulet abattage")
- Canadian thesis repository: "waterbath" retrieved 2 irrelevant records and "stunning" retrieved 33 records, all irrelevant.

[6] Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under regulation (EC) No 1107/2009, EFSA Journal 9(2), 2092.

Table 1. Search strategy applied

Limits: no limit applied for this search (except that the search terms were in English) **Date of the search:** 01-02 Sept 2011 **Justification for choosing the sources:** major sources in this field				
Database 1: CAB Abstracts (WoK)	**Database 2:** CCC (WoK)	**Database 3:** FSTA (WoK)	**Database 4:** PubMed (NLM)	**Database 5:** Web of Science (WoK)
Date span of the DB: 1910-present	**Date span of the DB:** 1998-present	**Date span of the DB:** 1969-present	**Date span of the DB:** 1946	**Date span of the DB:** 1975-present
Search strategies used for this data requirement (including any limits): TS=(bantam OR Broiler OR capon OR chick* OR cock OR coturnix OR duck OR fowl OR "Gallus gallus" OR geese OR hen OR poult OR poultry OR quail OR turkey) AND TS=(waterbath OR (electric* AND (kill* OR slaughter* OR stun*)) OR electronarcosis OR electrocution) NOT TS=(kill* NEAR bacteria*)	**Search strategies used for this data requirement (including any limits):** TS=(bantam OR Broiler OR capon OR chick* OR cock OR coturnix OR duck OR fowl OR "Gallus gallus" OR geese OR hen OR poult OR poultry OR quail OR turkey) AND TS=(waterbath OR (electric* AND (kill* OR slaughter* OR stun*)) OR electronarcosis OR electrocution) NOT TS=(kill* NEAR bacteria*)	**Search strategies used for this data requirement (including any limits):** TS=(bantam OR Broiler OR capon OR chick* OR cock OR coturnix OR duck OR fowl OR "Gallus gallus" OR geese OR hen OR poult OR poultry OR quail OR turkey) AND TS=(waterbath OR (electric* AND (kill* OR slaughter* OR stun*)) OR electronarcosis OR electrocution) NOT TS=(kill* NEAR bacteria*)	**Search strategies used for this data requirement (including any limits):** (waterbath OR (electric* AND (kill* OR stun* OR slaughter*)) OR electronarcosis[MeSH]) AND ("bantam"[All Fields] OR Broiler[All Fields] OR "chickens"[MeSH Terms] OR "chickens"[All Fields] OR cock[All Fields] OR capon[All Fields] OR "coturnix"[MeSH Terms] OR "ducks"[MeSH Terms] OR fowl[All Fields] OR "Gallus gallus"[All Fields] OR "geese"[MeSH Terms] OR "hen"[All Fields] OR poult[All Fields] OR "poultry"[MeSH Terms] OR "quail"[MeSH Terms] OR "turkey"[MeSH Terms])	**Search strategies used for this data requirement (including any limits)** TS=(bantam OR Broiler OR capon OR chick* OR cock OR coturnix OR duck OR fowl OR "Gallus gallus" OR geese OR hen OR poult OR poultry OR quail OR turkey) AND TS=(waterbath OR (electric* AND (kill* OR slaughter* OR stun*)) OR electronarcosis OR electrocution) NOT TS=(kill* NEAR bacteria*)

Total number of records retrieved: 350	**Total number of records retrieved:** 158	**Total number of records retrieved:** 272	**Total number of records retrieved:** 143	**Total number of records retrieved:** 214

Selecting relevant research studies

After the search for references, their title and abstracts were screened for their relevance independently by two reviewers, following the *a priori* set relevance criteria. The languages of articles to be considered relevant were limited to German, English and French because of the reviewers' capacities.

Relevance criteria:

- Reference is a primary research paper OR a Ph.D. thesis OR a conference proceedings article containing a full description of a primary research study AND
- Language of article body is in English OR French OR German AND
- Reference is testing a hypothesis regarding OR describing the electrical parameters used for waterbath stunning for poultry (broilers, hens, turkey, ducks geese, quail), and describing their effect on consciousness of stunned birds.

Exclusion criteria:

- Reference is not a primary research paper, Ph.D. thesis, conference proceedings article containing a full description of a primary research study (e.g. review articles) OR
- Language of article body in a language other than English or French or German OR
- Reference is NOT testing a hypothesis regarding OR describing the electrical parameters used for waterbath stunning for poultry (broilers, hens, turkey, ducks geese, quail), and describing their effect on consciousness of stunned birds.

The screening for relevance of the papers was carried out using the DistillerSR programme. Disagreement between the reviewers was discussed. When no consensus was possible the record was included for review of the full text.

Collecting data from relevant studies

The full text of the relevant papers was reviewed by 1 AHAW staff member for data extraction.

The parameters on which data were extracted from the studies were:

- Species type
- Sample size (number of animals studied)
- Number of animals simultaneously immersed in waterbath
- Duration of exposure to the waterbath
- Depth of immersion
- Electrical details (voltage, current, current type, frequency, waveform)
- Time elapsed between leaving the waterbath and the neck cutting
- How was unconsciousness ascertained?
- Length of observation after stunning
- Number of animals unconscious after stun
- Duration of unconsciousness
- Number of animals with cardiac arrest/ventricular fibrillation after stunning

Collating the data from the relevant studies and presenting the results

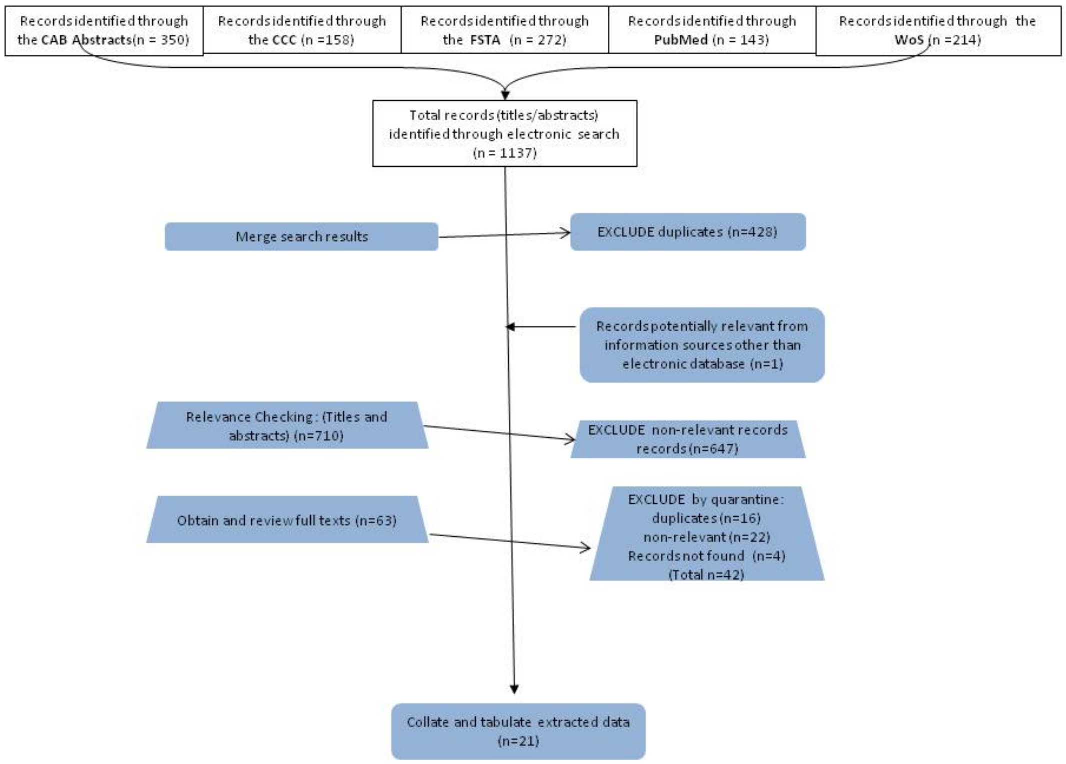
The extracted data were presented in plots or as narrative within the Results section.

Papers Included in Data Extraction

References	RefID
Beyssen C, Babile R, and Fernandez X, 2004. Electrocorticogram spectral analysis and somatosensory evoked potentials as tools to assess electrical stunning efficiency in ducks. British Poultry Science, 3, 409-415.	1179
Contreras CC, and Beraquet NJ, 2001. Electrical stunning, hot boning, and quality of chicken breast meat. Poultry Science, 4, 501-507.	1197
Gregory NG and Wotton SB, 1987. Effect of electrical stunning on the electroencephalogram in chickens. British Veterinary Journal, 2, 175-183.	1271
Gregory NG and Wotton SB, 1989. Effect of electrical stunning on somatosensory evoked potentials in chickens. British Veterinary Journal, 2, 159-164.	254
Gregory NG and Wotton SB, 1990. Effect of stunning on spontaneous physical activity and evoked activity in the brain. British Poultry Science, 1, 215-220.	239
Gregory NG and Wotton SB, 1991. Effect of depth of immersion in the waterbath on the effectiveness of electrical stunning in chickens. Research in Veterinary Science, 2, 200-202.	1279
Gregory NG and Wotton SB, 1991. Effect of a 350 Hz DC stunning current on evoked responses in the chicken's brain, Research in Veterinary Science, 2, 250-251.	1283
Gregory NG and Wotton SB, 1991. Effect of electrical stunning on somatosensory evoked responses in the turkey brain. British Veterinary Journal, 3, 270-274.	689
Gregory NG and Wotton SB, 1994. Effect of electrical stunning current on the duration of insensibility in hens. British Poultry Science, 3, 463-465.	1287
Hindle VA, Lambooij E, Reimert HG, Workel LD and Gerritzen MA, 2010. Animal welfare concerns during the use of the waterbath for stunning broilers, hens, and ducks. Poultry Science, 89, 401-412.	1311
Mouchoniere M, Pottier GI, and Fernandez X, 1999. The effect of current frequency during waterbath stunning on the physical recovery and rate and extent of bleed out in turkeys. Poultry Science, 77: 485-489.	1378
Mouchoniere M, Pottier GI, and Fernandez X, 2000. Effect of current frequency during electrical stunning in a waterbath on somatosensory evoked responses in turkey's brain, Research in Veterinary Science, 69, 53-55.	1379
Prinz, S, 2009. Waterbath stunning of chickens - effects of electrical parameters on the electroencephalogram and physical reflexes of broilers. Thesis (PhD), Radboud University Nijmegen, Nijmegen, The Netherlands. 158 pp.	1397
Raj A.B.M and O'Callaghan M, 2004. Effects of electrical waterbath stunning current frequencies on the spontaneous electroencephalogram and somatosensory evoked potentials in hens. British Poultry Science, 2, 230-236.	1405
Raj ABM, O'Callaghan M and Hughes SI, 2006. The effects of amount and frequency of pulsed direct current used in waterbath stunning and of slaughter methods on spontaneous electroencephalograms in broilers. Animal Welfare, 15, 19-24.	1433
Raj ABM, O'Callaghan M and Hughes SI, 2006. The effects of pulse width of a direct current used in waterbath stunning and of slaughter methods on spontaneous electroencephalograms in broilers. Animal Welfare, 15, 25-30.	1434
Raj ABM, O'Callaghan M and Knowles TG, 2006. The effects of amount and frequency of alternating current used in waterbath stunning and of slaughter methods on electroencephalograms in broilers. Animal Welfare, 15, 7-18.	1435
Wilkins LJ, Gregory NG, Wotton SB and Parkman ID, 1998. Effectiveness of electrical stunning applied using a variety of waveform-frequency combinations and consequences	1535

for carcase quality in broiler chickens. British Poultry Science, 39, 511-518.	
Wilkins LJ, Gregory NG and Wotton SB, 1999. Effectiveness of different electrical stunning regimens for turkeys and consequences for carcase quality. British Poultry Science, 40, 478-484.	1534
Wilkins LJ, Wotton SB, Parkman ID, Kettlewell PJ and Griffiths P, 1999. Constant current stunning effects on bird welfare and carcass quality. Journal of Applied Poultry Research, 8, 465-471.	1537
Wotton SB and Wilkins LJ, 1999. Effect of very low pulsed direct currents at high frequency on the return of neck tension in broilers. Veterinary Record, 145, 393-396.	1559

Overview of results



Timetable

Step	Deadline	Responsibility
1. Preparing the review -developing the review protocol -setting the logistics	July 2011	EFSA staff
2. Searching for research studies	July-September 2011	EFSA staff
3. Selecting studies for inclusion in the review (relevance check)	September 2011	EFSA staff
4., Collecting data from included studies	October to December 2011	EFSA staff
5. Synthesising data	December 2011 to April 2012	EFSA staff
6.Presenting data and results, interpreting results and drawing conclusions	January to May 2012	EFSA staff, supported by Working Group chair
7.Narrative description of methods, results, discussion and conclusions	May 2012	EFSA staff, supported by Working Group chair

B. APPENDIX 2: DATA PLOTS

Current Type: AC / Species: Turkey

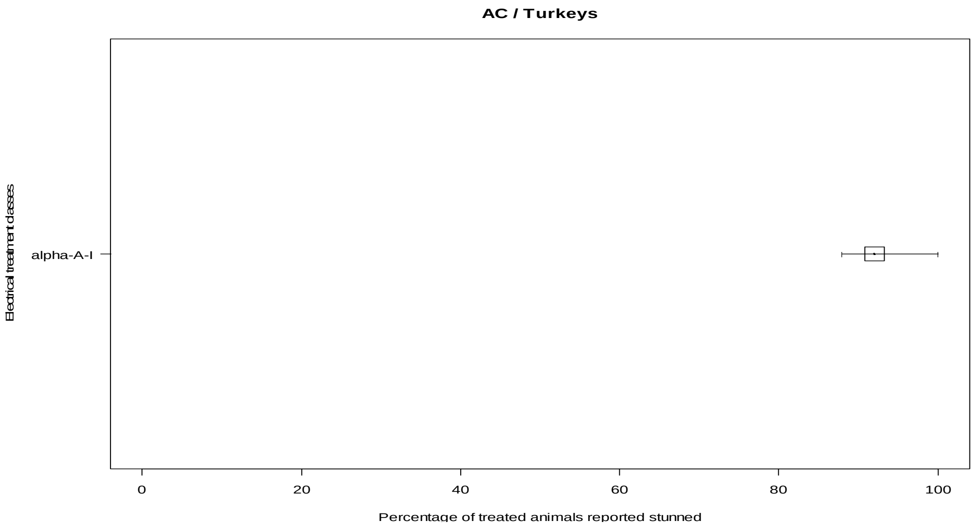


Figure 1. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

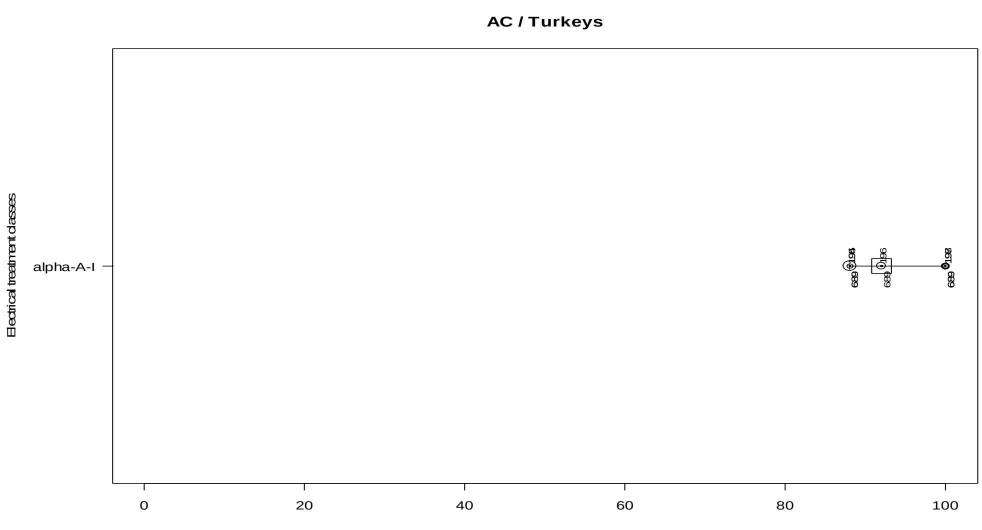


Figure 2. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles =

individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

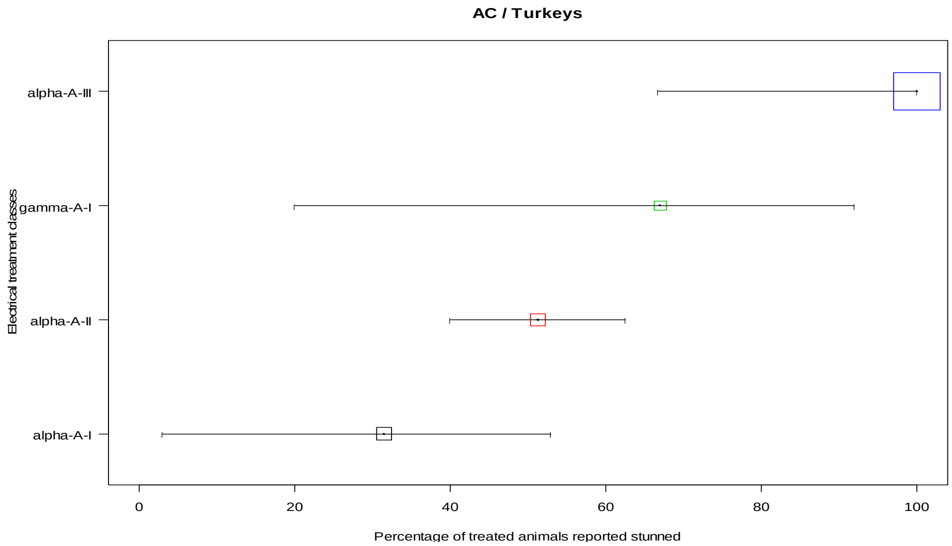


Figure 3. Percentage of treated animals reported stunned, using non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

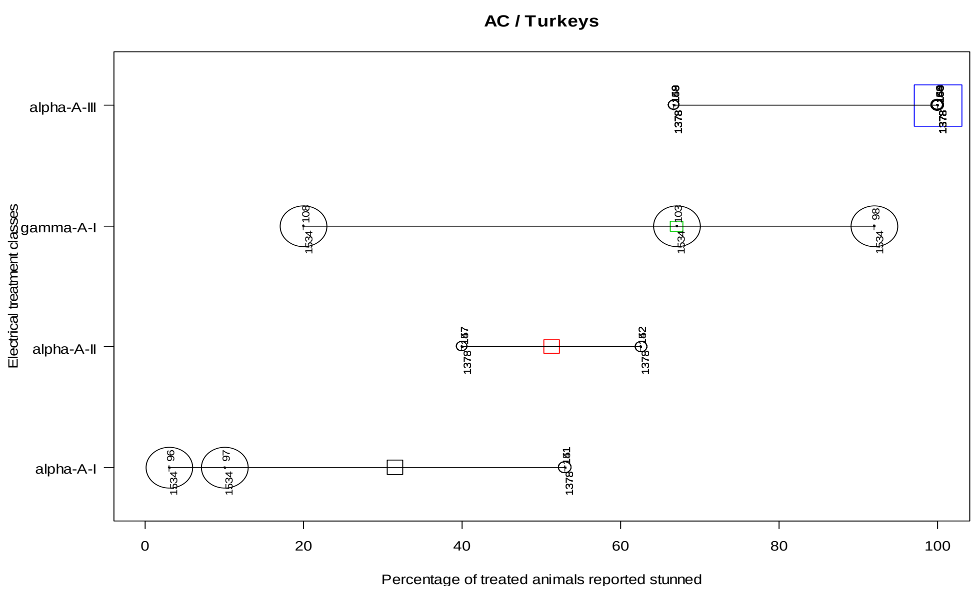


Figure 4. Percentage of treated animals reported stunned, using Non-EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles =

individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

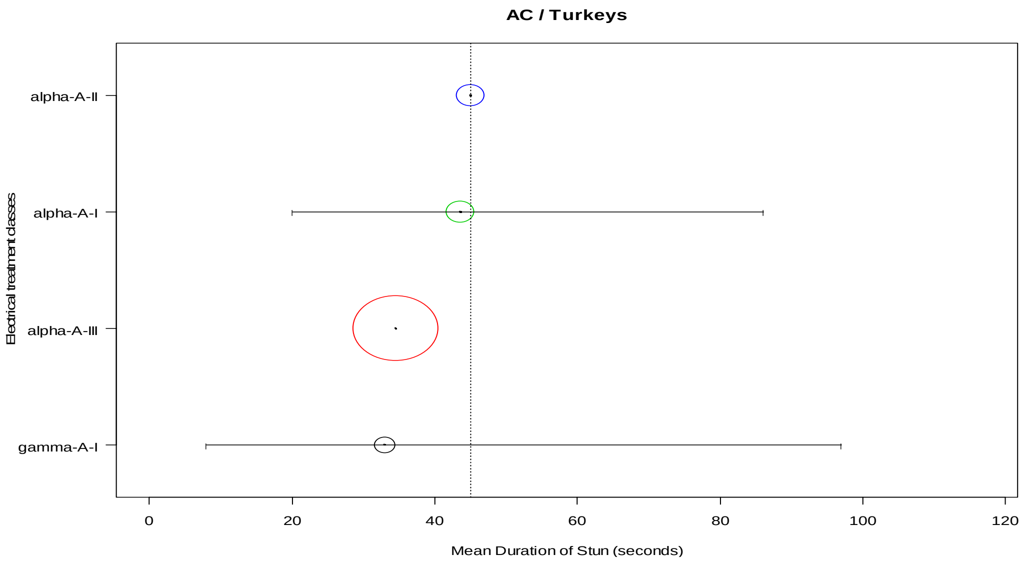


Figure 5. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). Circles' centre = median of the mean values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Dotted vertical line = 45 seconds.

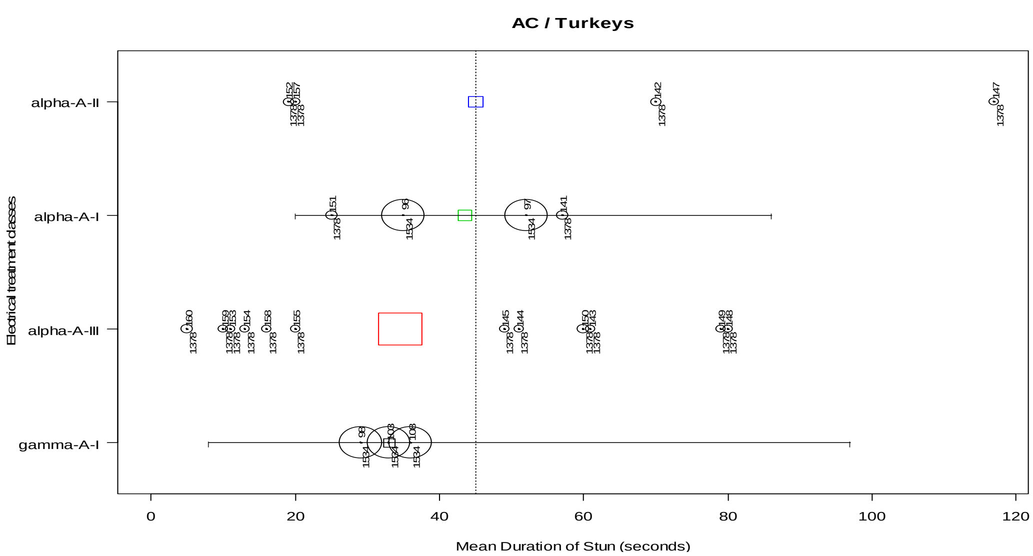


Figure 6. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment - Individual experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). . Squares' centre = median of the mean values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of

stun) observed in the studies within each treatment class. Circles = individual studies/records. Circles' centre = observed mean value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record). Dotted vertical line = 45 seconds.

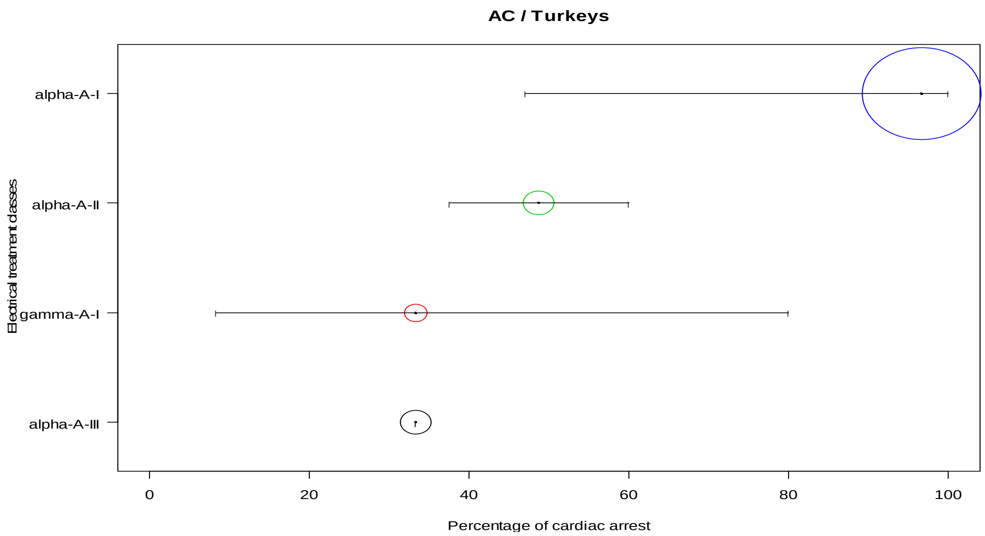


Figure 7. Percentage of treated animals reported with cardiac arrest - Summary of experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). Circles' centre = median of the percentage values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies.

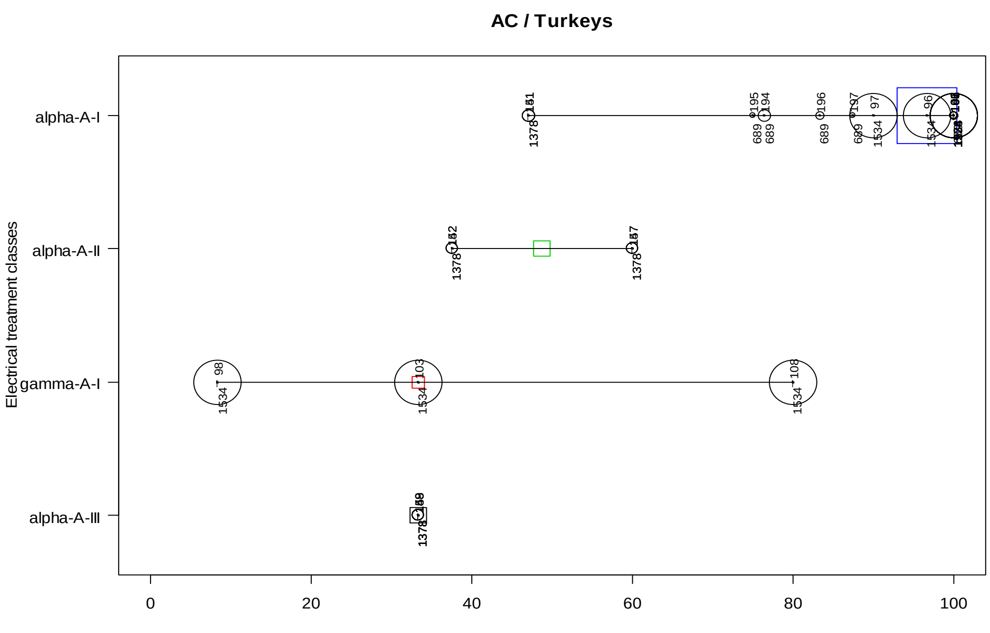


Figure 8. Percentage of treated animals reported with cardiac arrest - Individual experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 2). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

Current Type: AC / Species: Broilers

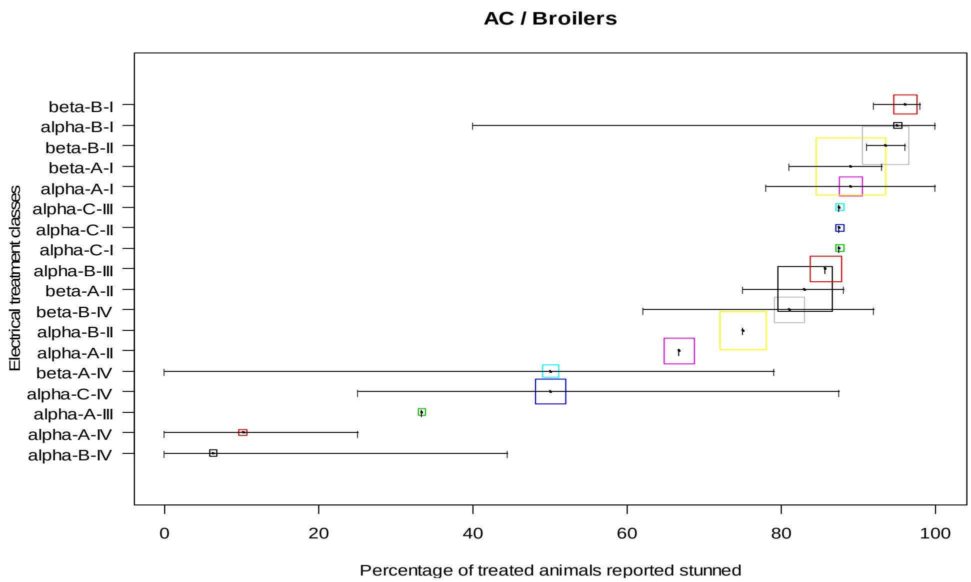


Figure 9. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

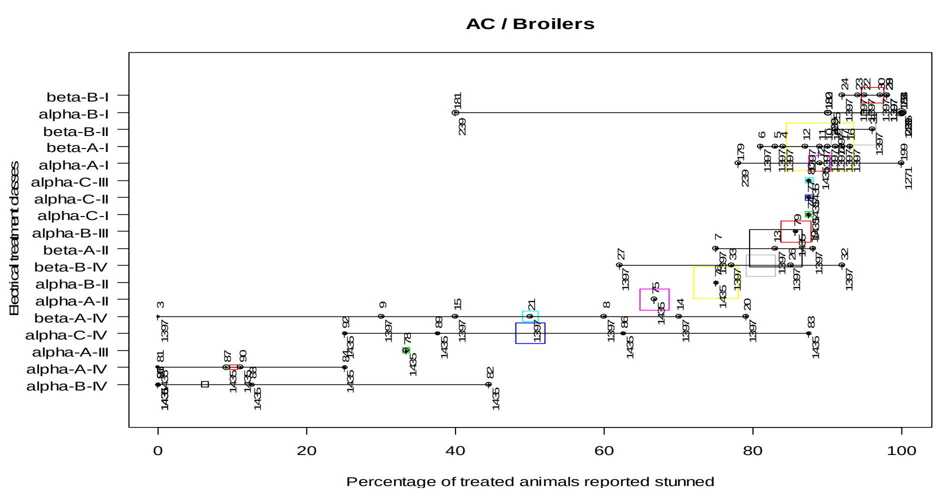


Figure 10. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

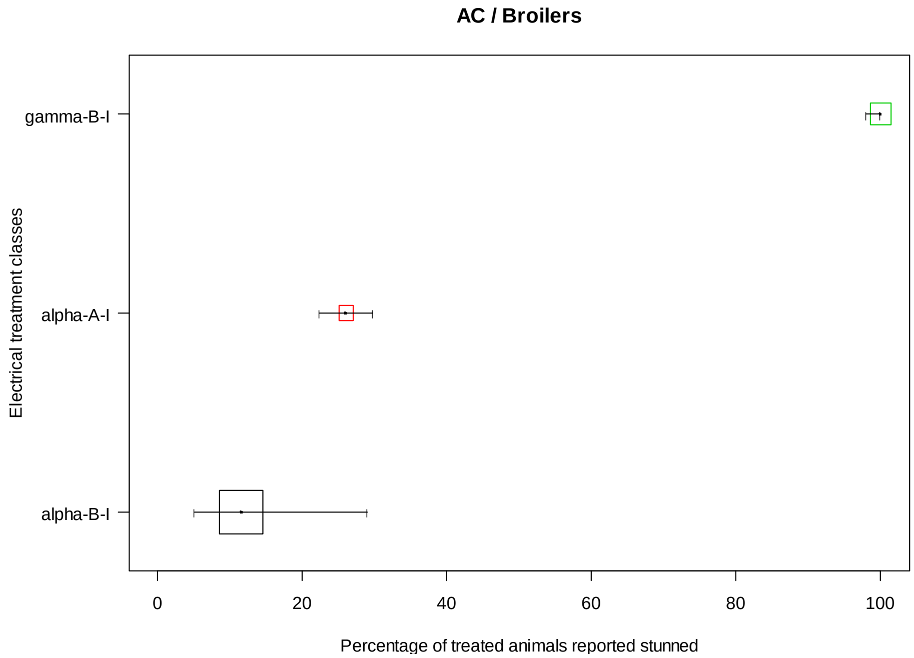


Figure 11. Percentage of treated animals reported stunned, using non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

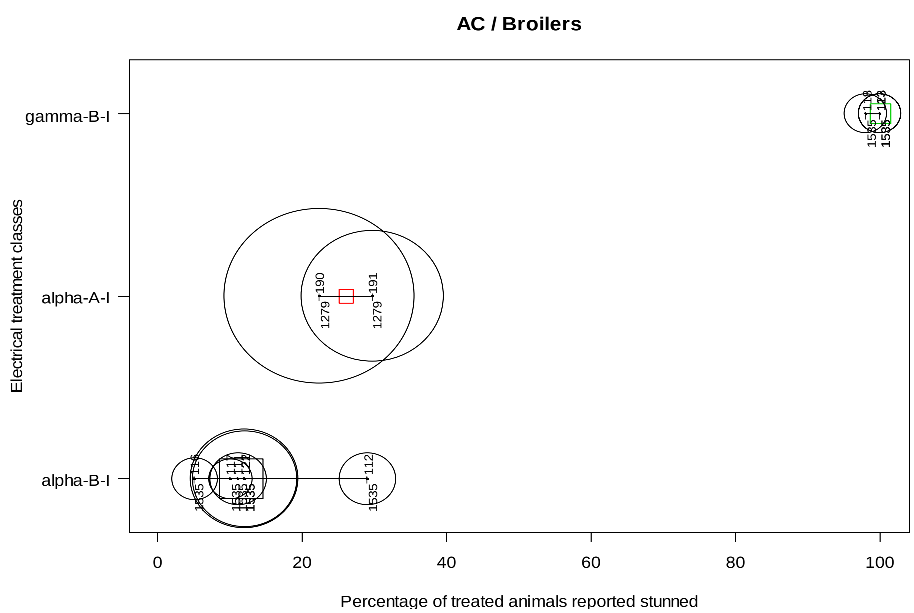


Figure 12. Percentage of treated animals reported stunned, using Non-EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the

minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

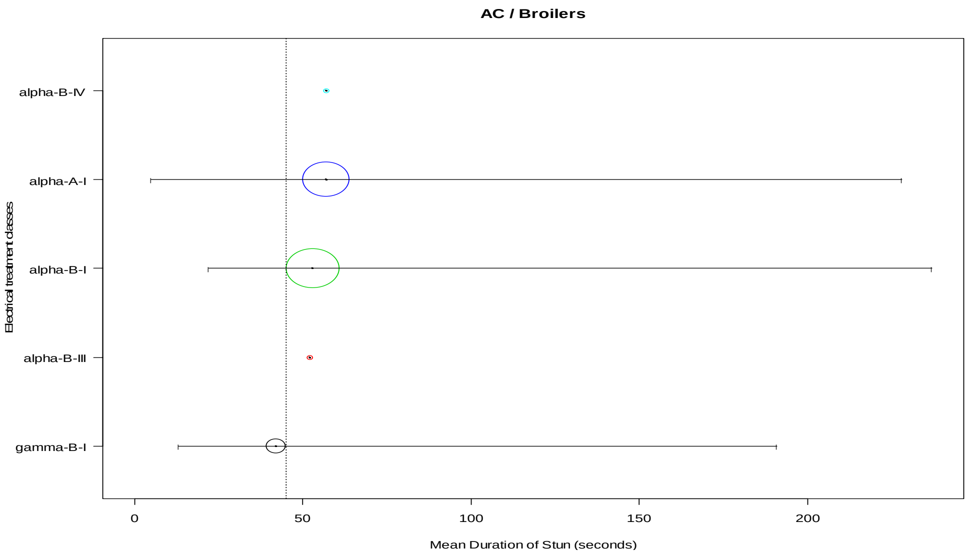


Figure 13. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Circles' centre = median of the mean values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Dotted vertical line = 45 seconds.

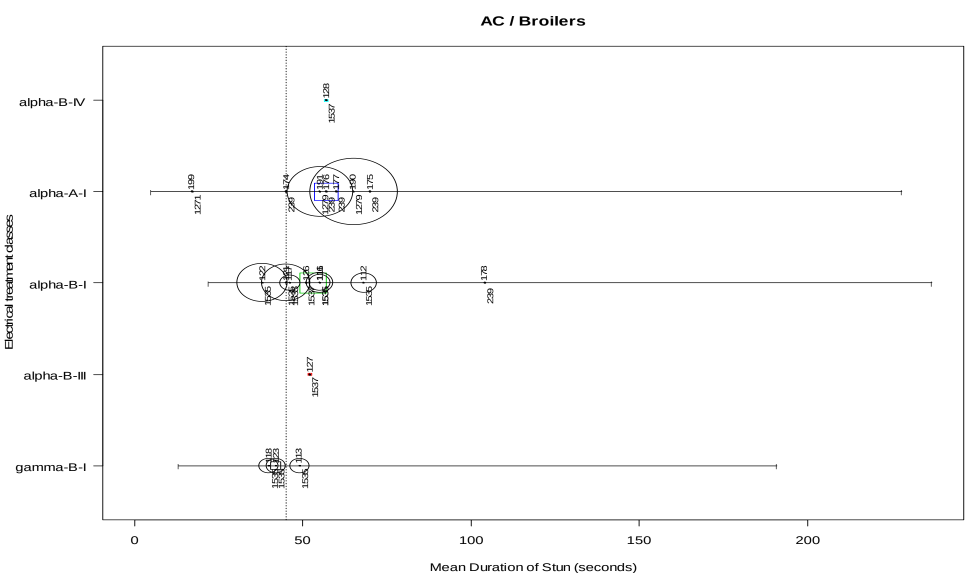


Figure 14. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment - Individual experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the mean values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of

stun) observed in the studies within each treatment class. Circles = individual studies/records. Circles' centre = observed mean value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record). Dotted vertical line = 45 seconds.

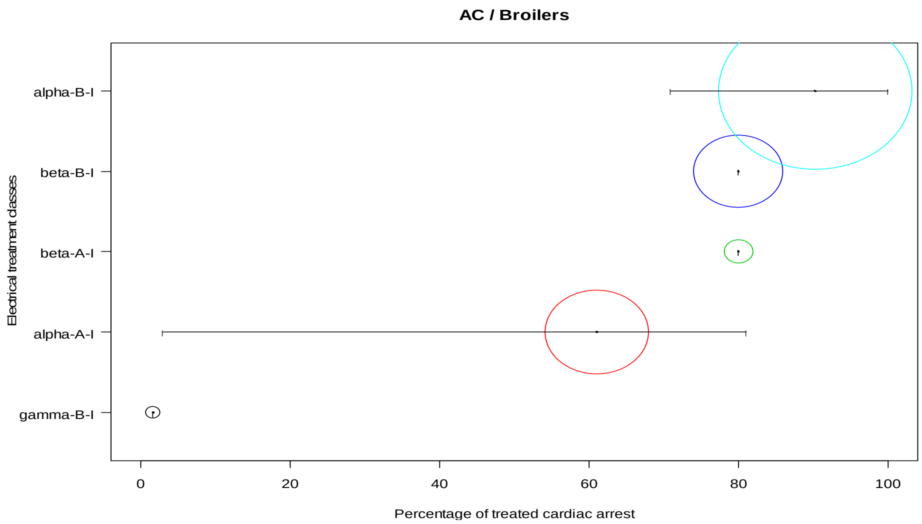


Figure 15. Percentage of treated animals reported with cardiac arrest - Summary of experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Circles' centre = median of the percentage values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies.

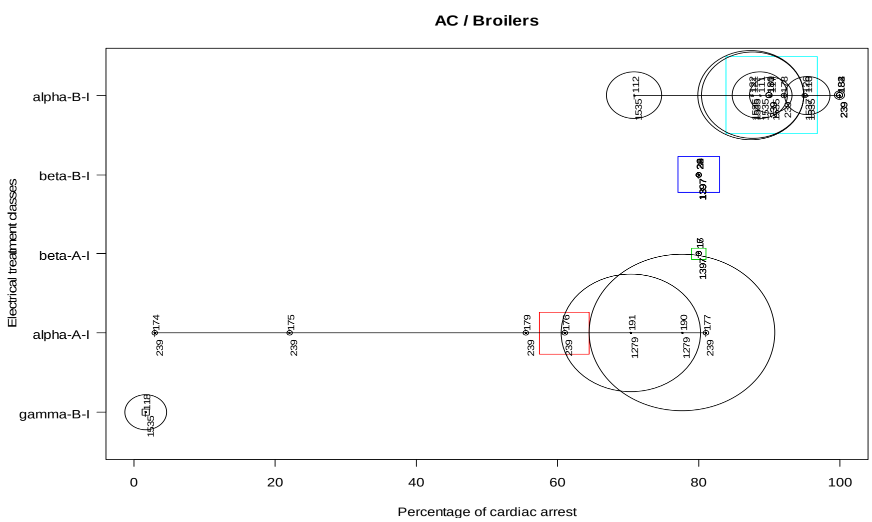


Figure 16. Percentage of treated animals reported with cardiac arrest - Individual experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

Current Type: AC / Species: Laying Hens

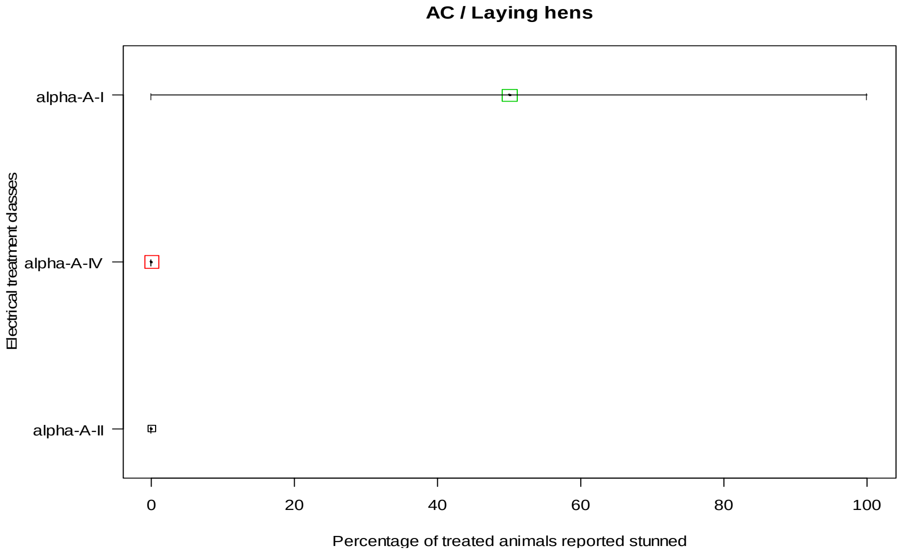


Figure 17. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

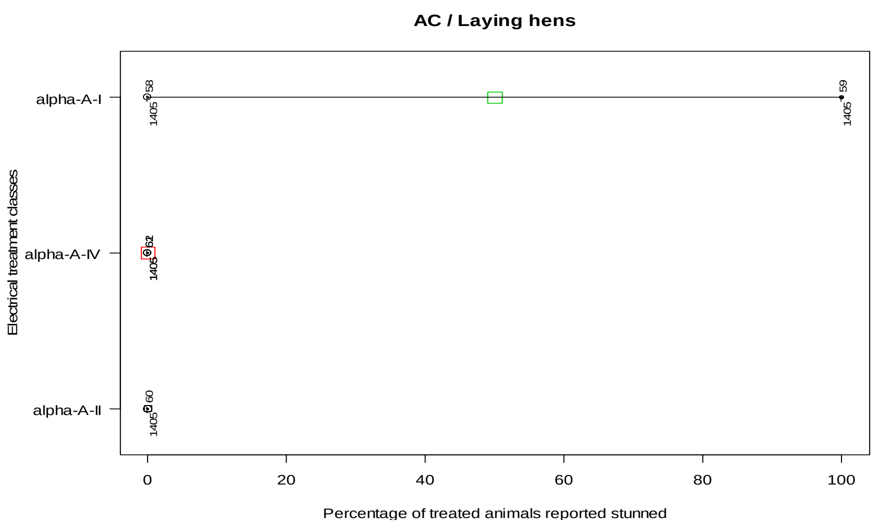


Figure 18. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

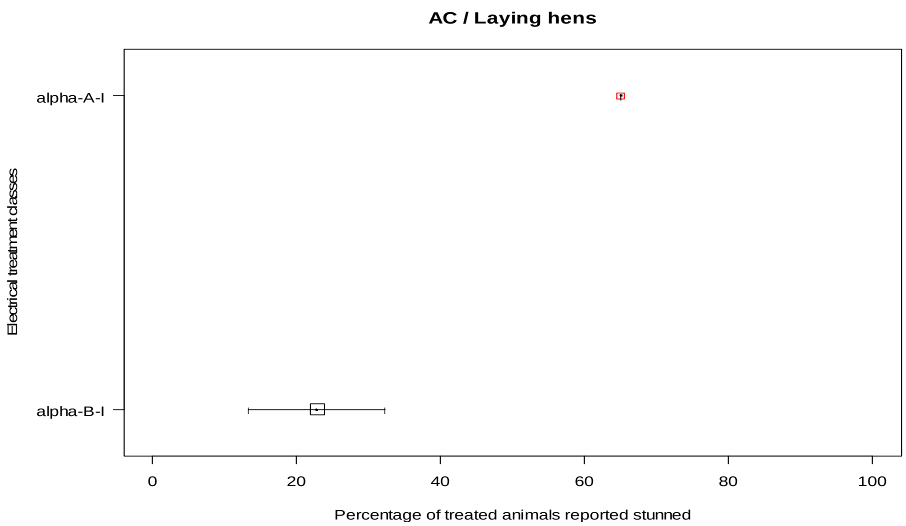


Figure 19. Percentage of treated animals reported stunned, using non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

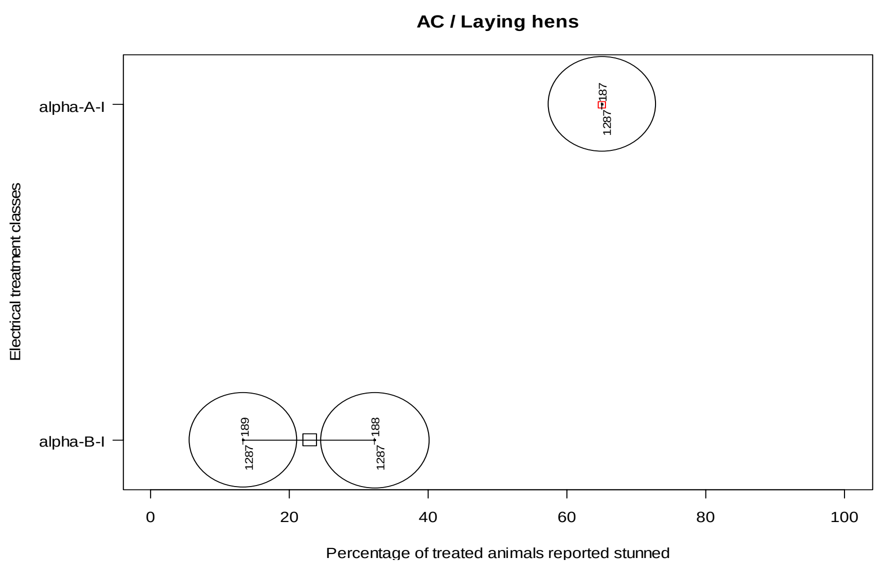


Figure 20. Percentage of treated animals reported stunned, using Non-EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

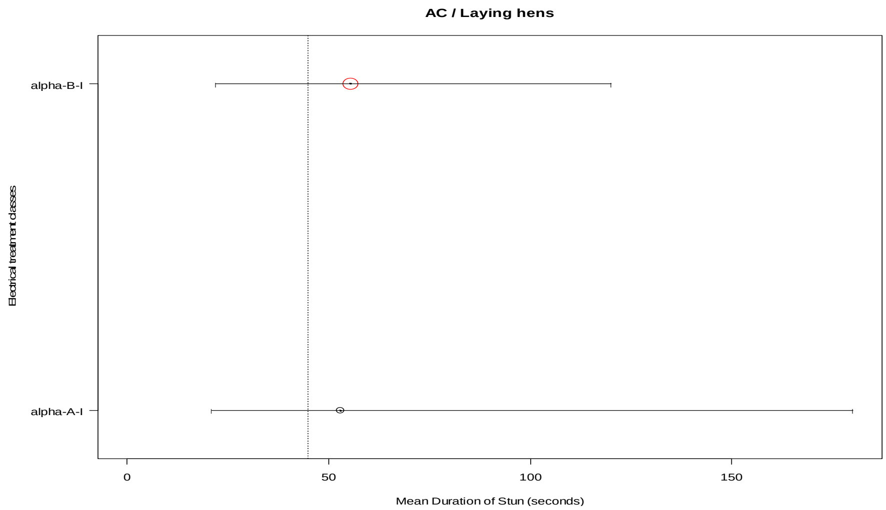


Figure 21. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Circles' centre = median of the mean values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Dotted vertical line = 45 seconds.

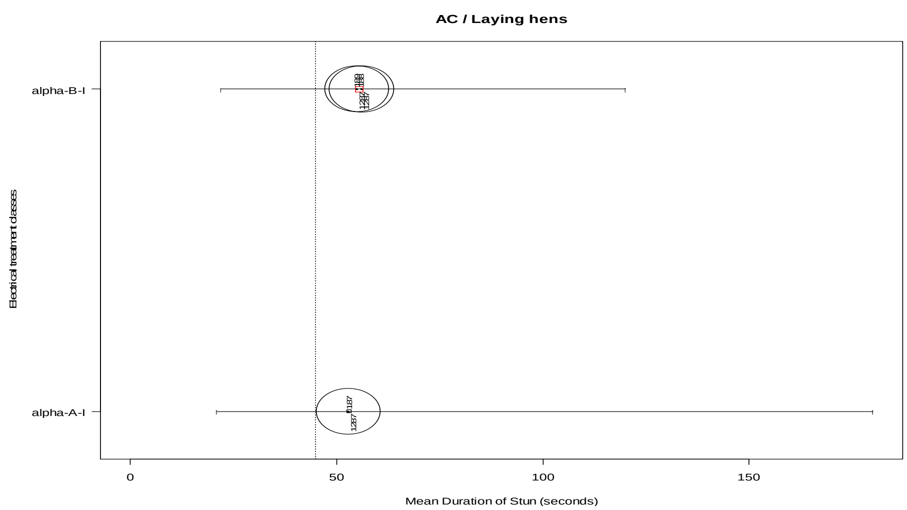


Figure 22. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment - Individual experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the mean values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Circles = individual studies/records. Circles' centre = observed mean value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record). Dotted vertical line = 45 seconds.

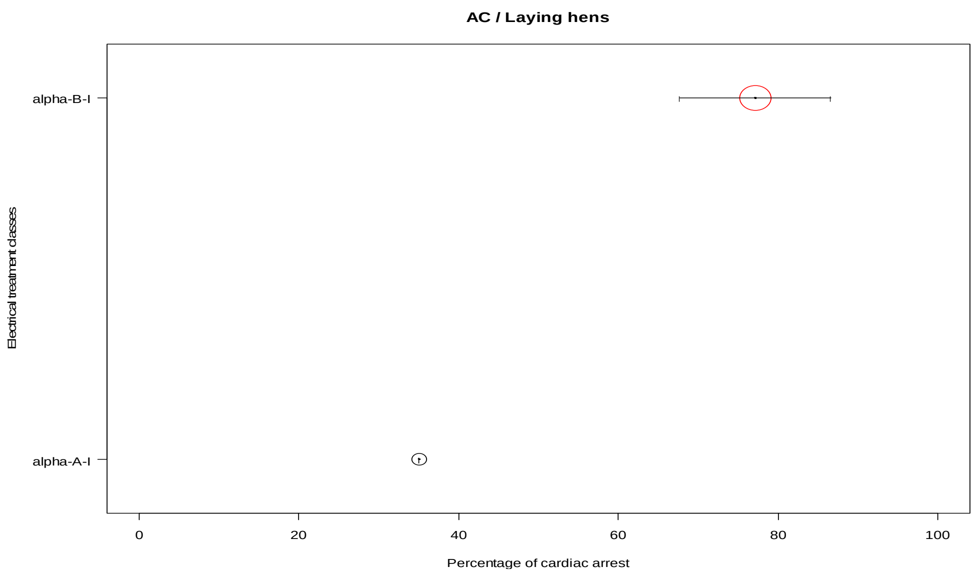


Figure 23. Percentage of treated animals reported with cardiac arrest - Summary of experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Circles' centre = median of the percentage values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies.

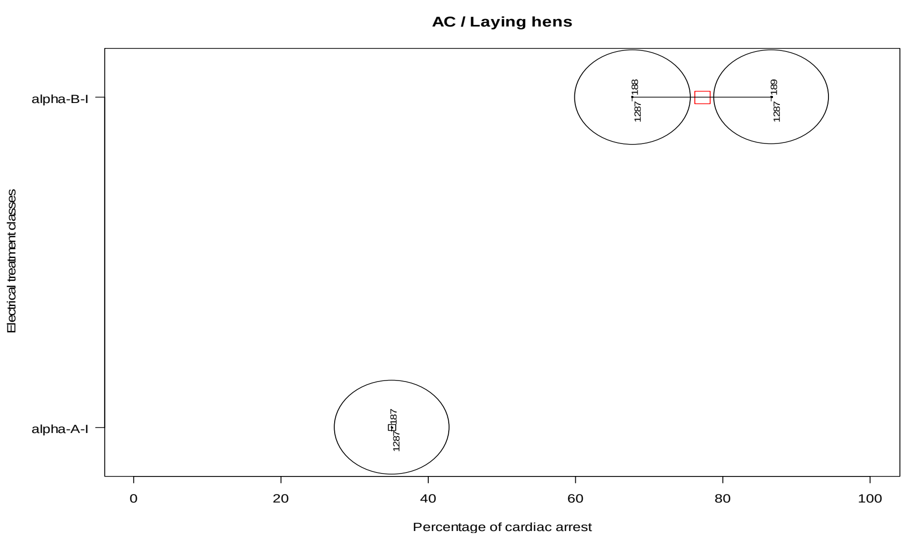


Figure 24. Percentage of treated animals reported with cardiac arrest - Individual experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 1). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

Current Type: DC / Species: Turkeys

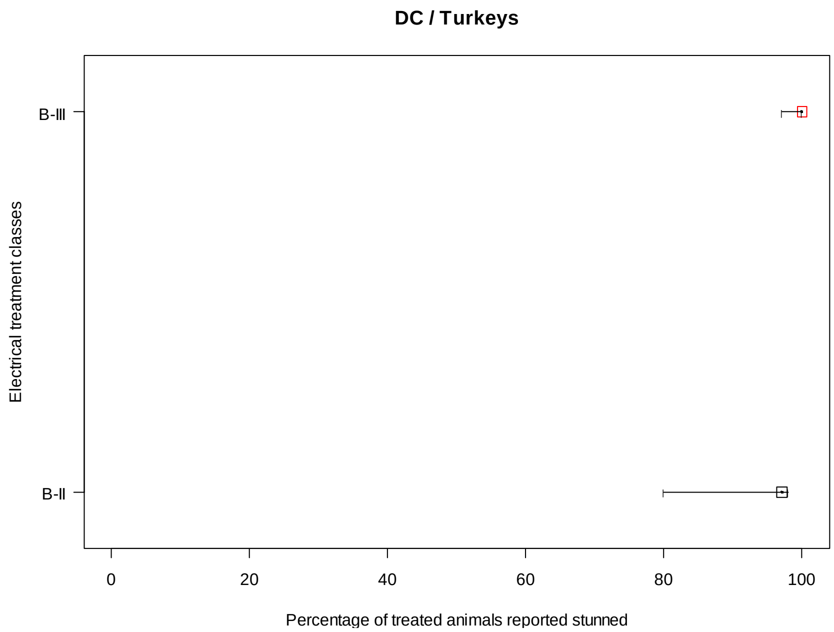


Figure 25. Percentage of treated animals reported stunned, using non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

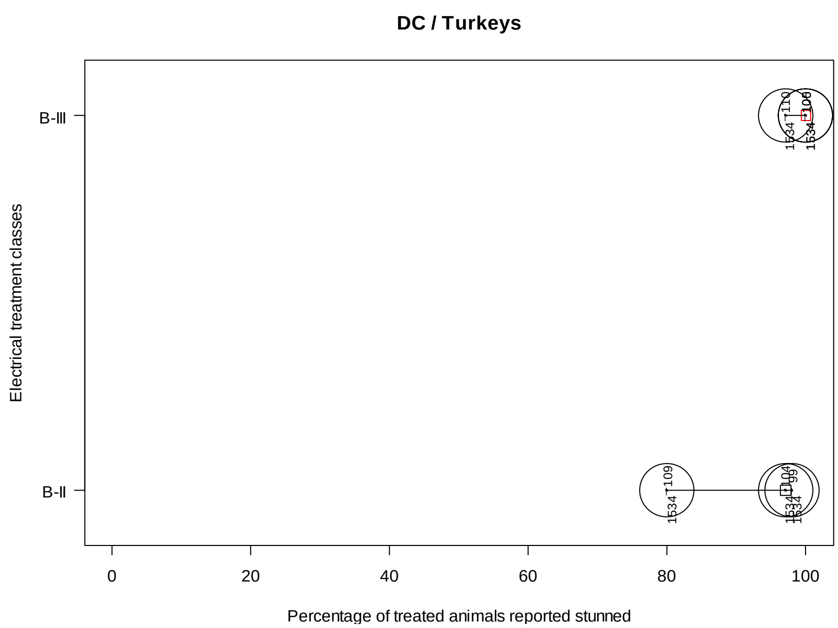


Figure 26. Percentage of treated animals reported stunned, using Non-EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the percentage values for each treatment class. Squares' dimension =

number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

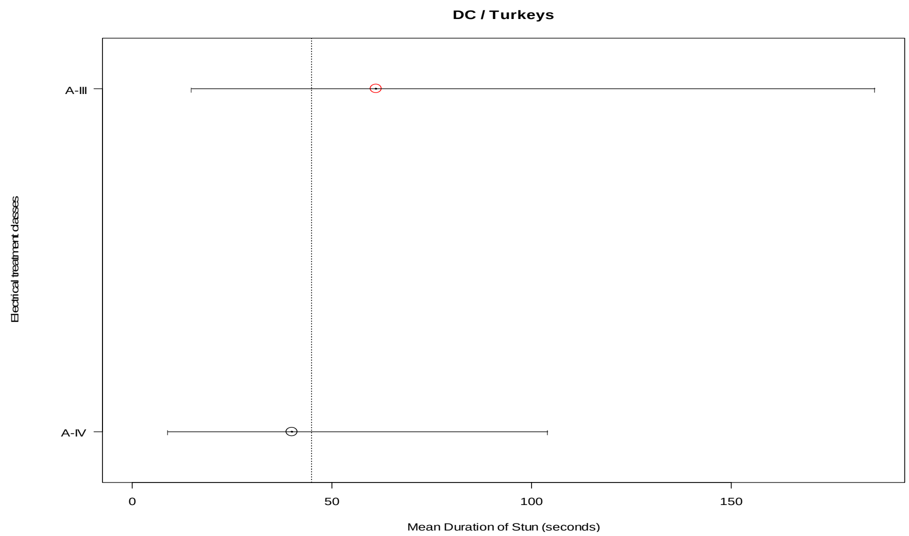


Figure 27. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Circles' centre = median of the mean values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Dotted vertical line = 45 seconds.

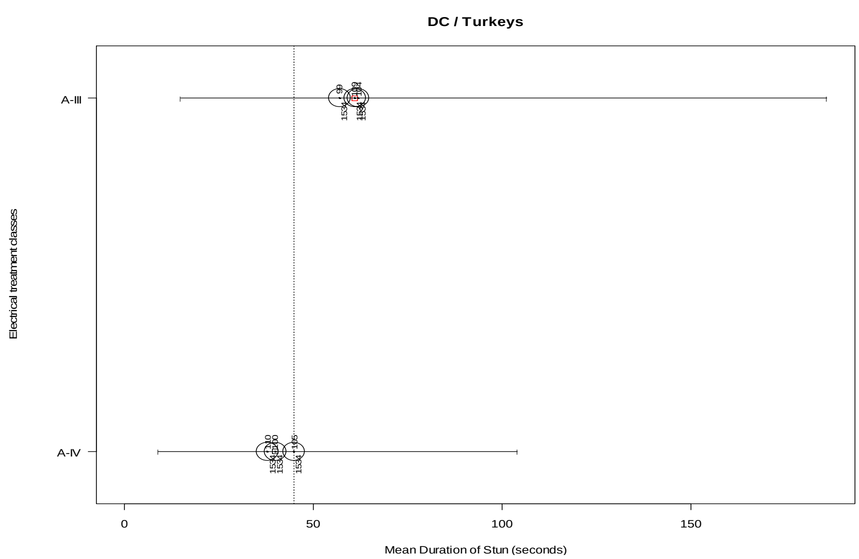


Figure 28. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment - Individual experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the mean values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of

stun) observed in the studies within each treatment class. Circles = individual studies/records. Circles' centre = observed mean value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record). Dotted vertical line = 45 seconds.

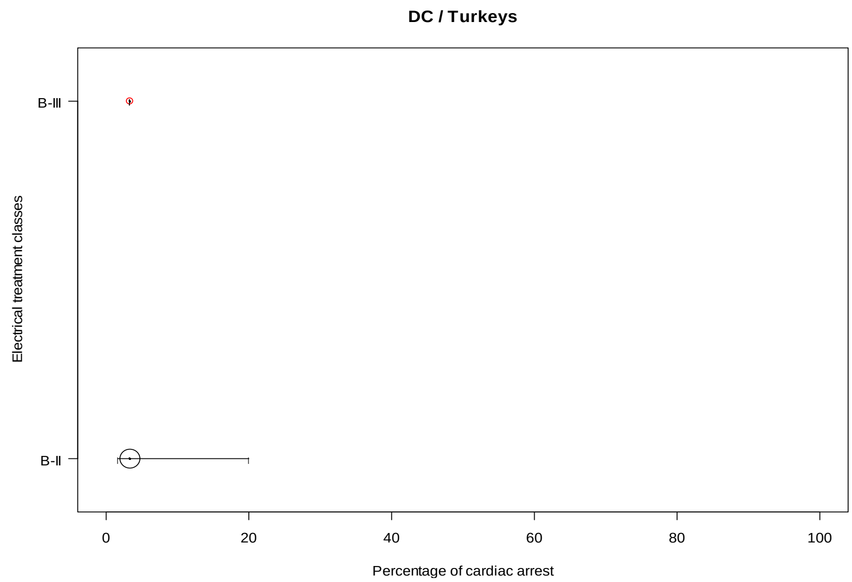


Figure 29. Percentage of treated animals reported with cardiac arrest - Summary of experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Circles' centre = median of the percentage values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies.

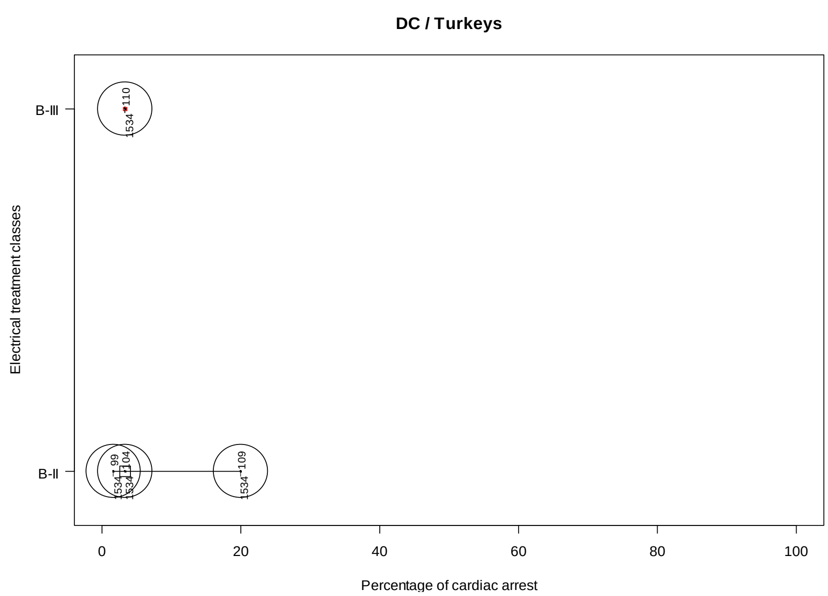


Figure 30. Percentage of treated animals reported with cardiac arrest - Individual experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal

stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

Current Type: DC / Species: Broilers

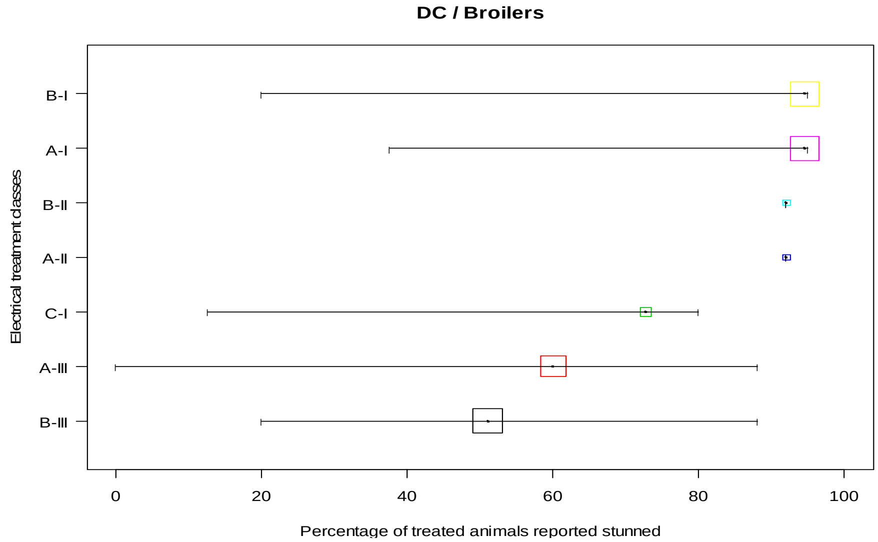


Figure 31. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

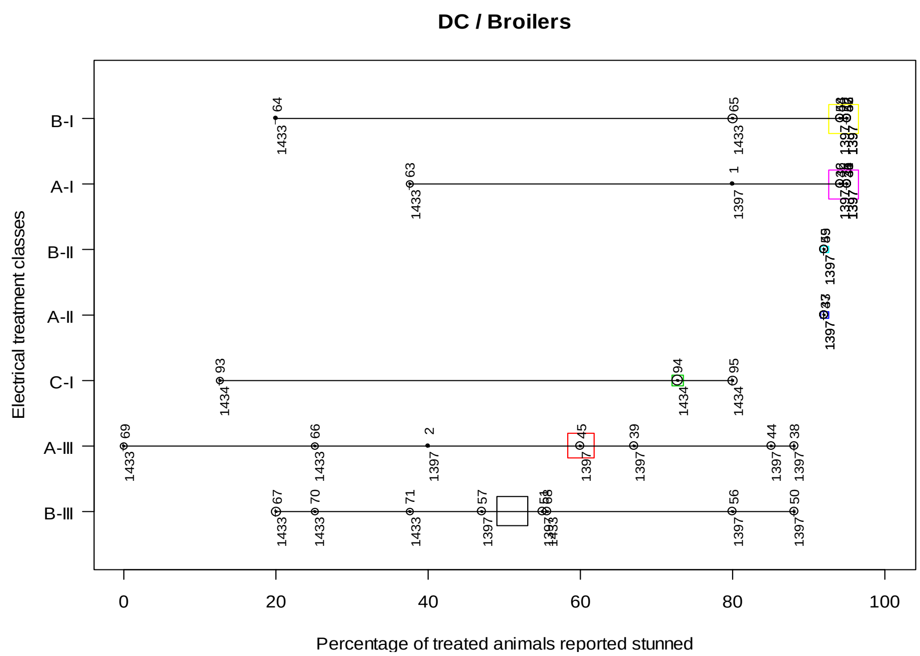


Figure 32. Percentage of treated animals reported stunned, using EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the percentage values for each treatment class. Squares' dimension =

number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

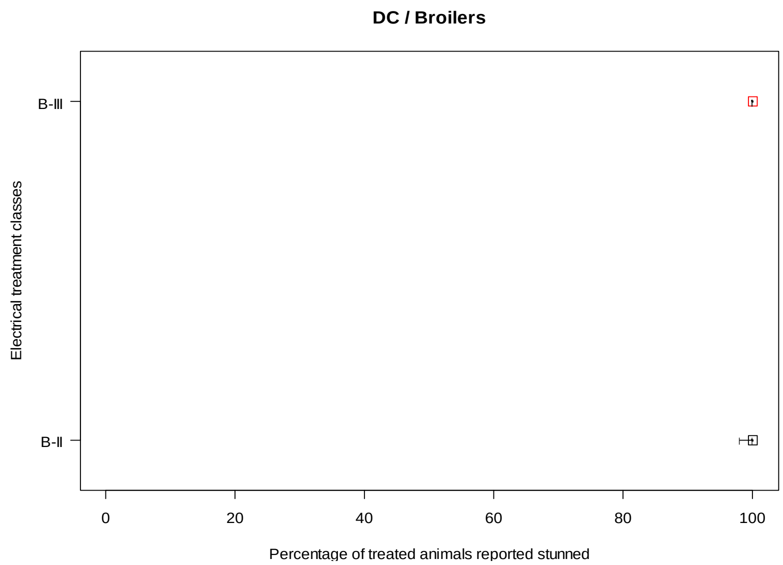


Figure 33. Percentage of treated animals reported stunned, using non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). The centre of the boxes = median of the percentage values for each treatment class. The size of the boxes = number of studies (individual records) that are contributing to the calculation of the median. Lines extending from the boxes = the range from the minimum value to the maximum value (as a percentage of animals stunned) observed in the studies.

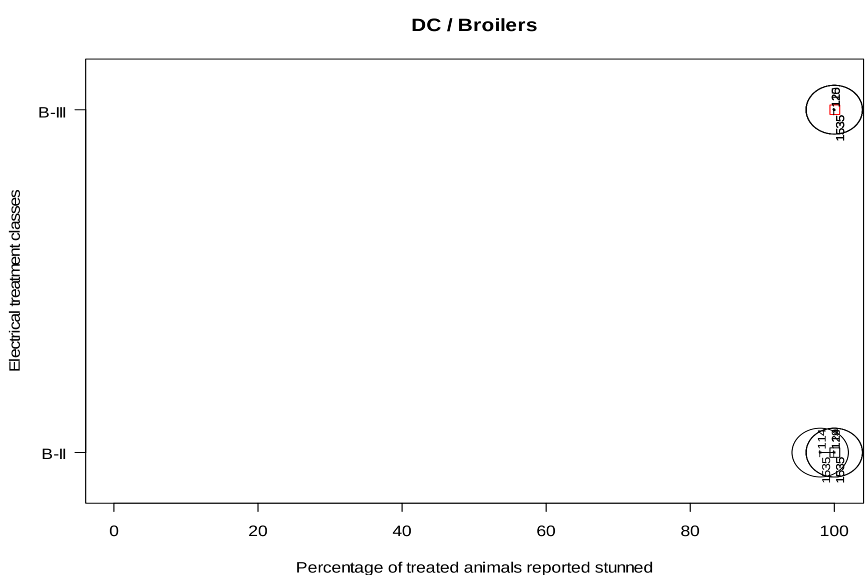


Figure 34. Percentage of treated animals reported stunned, using Non-EEG methods for ascertainment of unconsciousness – Individual experiments in each electrical treatment class. X-axis = percentage of animals stunned (unconscious). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the

minimum value to the maximum value (percentage of animal stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

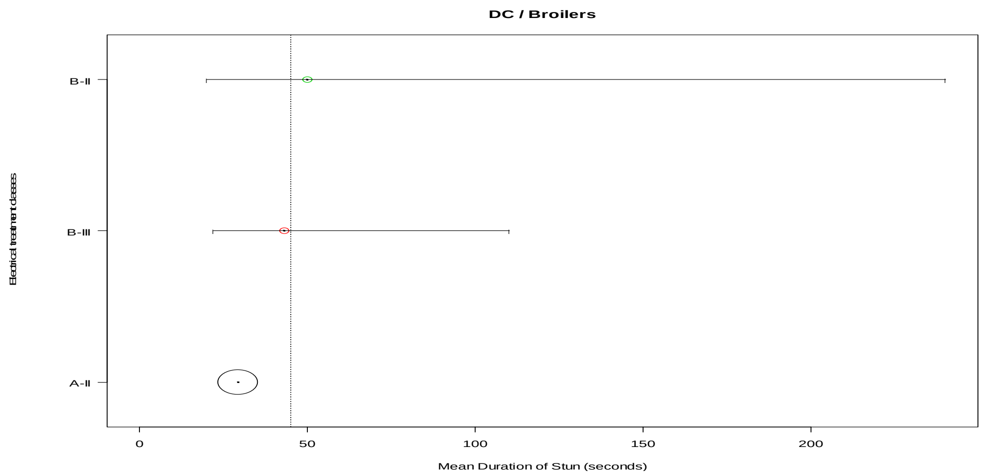


Figure 35. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment of unconsciousness - Summary of experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Circles' centre = median of the mean values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Dotted vertical line = 45 seconds.

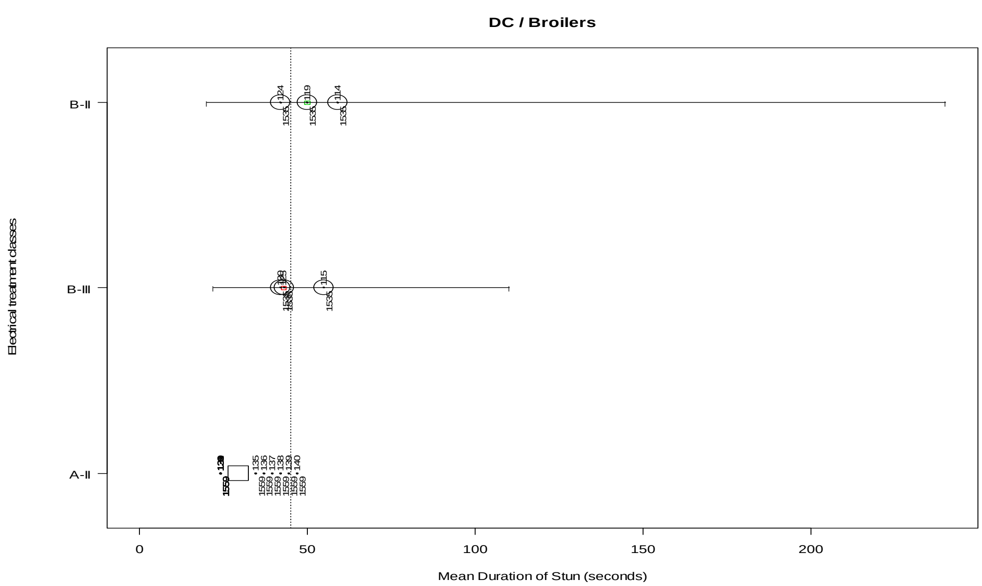


Figure 36. Mean duration of unconsciousness for treated animals reported stunned, using both EEG and Non-EEG methods for ascertainment - Individual experiments in each electrical treatment class. X-axis = mean duration of stun (seconds). Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the mean values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum of the minimum observed value to the maximum of the maximum observed value (mean duration of stun) observed in the studies within each treatment class. Circles = individual studies/records. Circles' centre =

observed mean value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record). Dotted vertical line = 45 seconds.

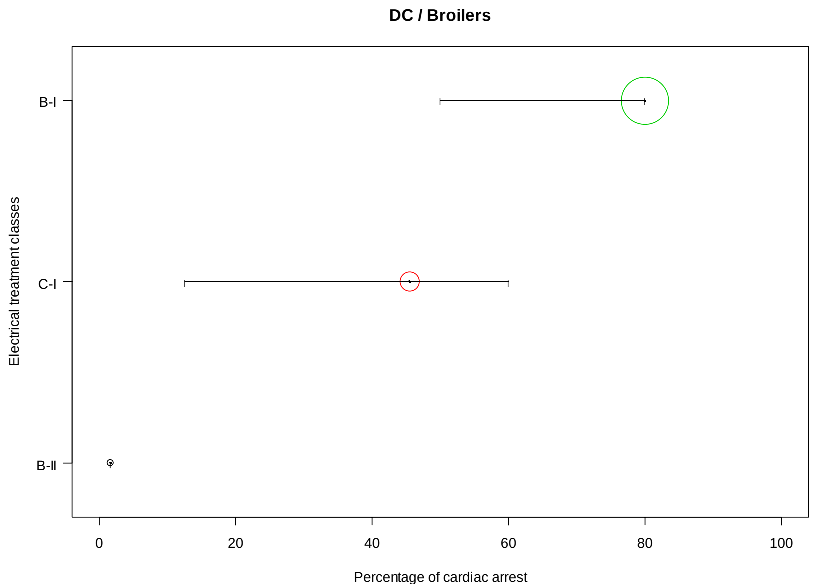


Figure 37. Percentage of treated animals reported with cardiac arrest - Summary of experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Circles' centre = median of the percentage values for each treatment class. Circles' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal stunned) observed in the studies.

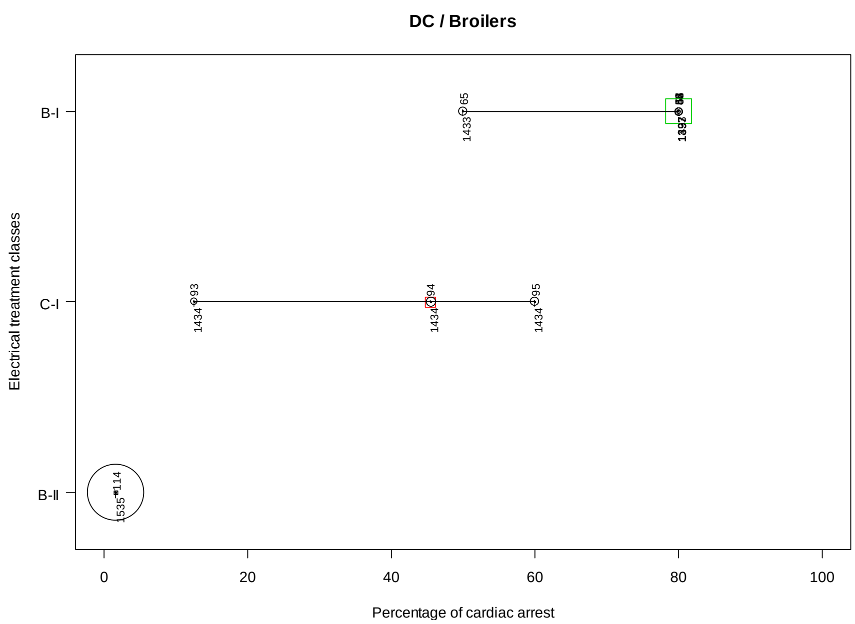


Figure 38. Percentage of treated animals reported with cardiac arrest - Individual experiments in each electrical treatment class. X-axis = percentage of animals with cardiac arrest. Y-axis = treatment classes: combination of waveform, current class and frequency class (see Table 3). Squares' centre = median of the percentage values for each treatment class. Squares' dimension = number of studies (individual records) that are contributing to the calculation of the median. Lines = from the minimum value to the maximum value (percentage of animal

stunned) observed in the studies. Circles = individual studies/records. Circles' centre = observed percentage value for that study/record. Circles' dimension = sample size (number of animals involved in the study/record).

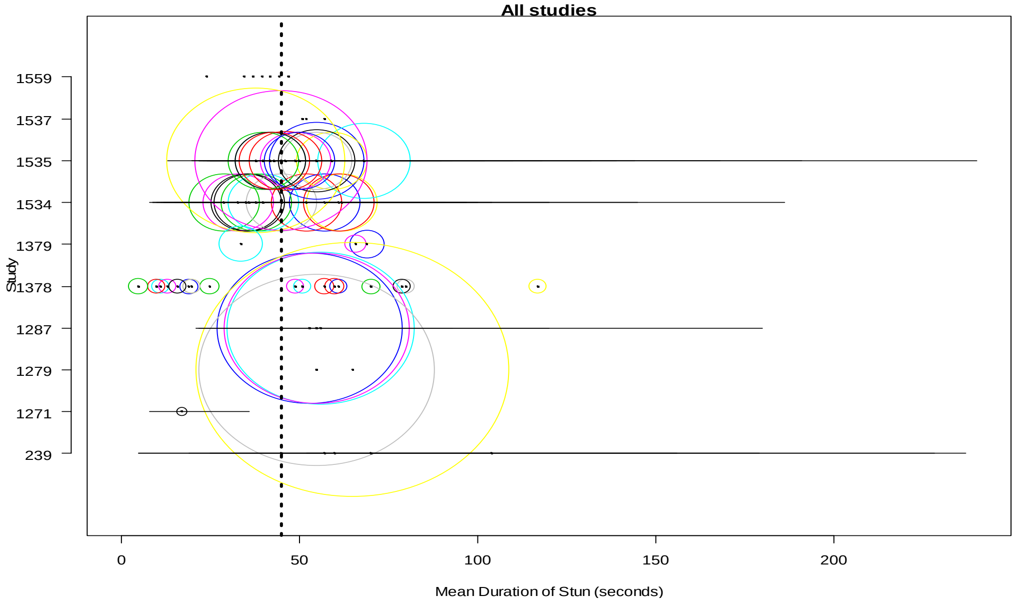


Figure 39. Summary of Mean Duration: All Studies and All Species (Turkeys Broilers and Laying hens). X-axis = mean duration of stun (seconds). Y-axis = scientific paper ID (pool of studies). Circles = individual studies/records. Circles' centre = mean value for each experiment (within the scientific paper), i.e. single record. Circles' dimension = sample size (number of animals involved in the study/record). Lines = from the minimum value to the maximum value (mean duration of stun) observed in the studies. Dotted vertical line = 45 seconds.

C. APPENDIX 3: INDIVIDUAL DATA LISTINGS

Individual Data Listing: Percentage of birds stunned using EEG methods of ascertainment.

Turkey AC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Unconscious**	**No. of birds in experiment**
689	194	alpha-A-I	88	17
689	195	alpha-A-I	88	8
689	196	alpha-A-I	92	12
689	197	alpha-A-I	100	8
689	198	alpha-A-I	100	11

Broiler AC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Unconscious**	**No. of birds in experiment**
1397	3	beta-A-IV	0	4
1397	4	beta-A-I	84	NA
1397	5	beta-A-I	83	NA
1397	6	beta-A-I	81	NA
1397	7	beta-A-II	75	NA
1397	8	beta-A-IV	60	NA
1397	9	beta-A-IV	30	NA
1397	10	beta-A-I	90	NA
1397	11	beta-A-I	89	NA
1397	12	beta-A-I	87	NA
1397	13	beta-A-II	83	NA
1397	14	beta-A-IV	70	NA
1397	15	beta-A-IV	40	NA
1397	16	beta-A-I	93	NA
1397	17	beta-A-I	92	NA
1397	18	beta-A-I	91	NA
1397	19	beta-A-II	88	NA
1397	20	beta-A-IV	79	NA
1397	21	beta-A-IV	50	NA
1397	22	beta-B-I	95	NA
1397	23	beta-B-I	94	NA
1397	24	beta-B-I	92	NA
1397	25	beta-B-II	91	NA
1397	26	beta-B-IV	85	NA
1397	27	beta-B-IV	62	NA
1397	28	beta-B-I	98	NA
1397	29	beta-B-I	98	NA
1397	30	beta-B-I	97	NA
1397	31	beta-B-II	96	NA
1397	32	beta-B-IV	92	NA
1397	33	beta-B-IV	77	NA
1435	72	alpha-A-I	89	9
1435	73	alpha-B-I	100	7
1435	74	alpha-C-I	88	8
1435	75	alpha-A-II	67	9
1435	76	alpha-B-II	75	8
1435	77	alpha-C-II	88	8

1435	78	alpha-A-III	33	9
1435	79	alpha-B-III	86	7
1435	80	alpha-C-III	88	8
1435	81	alpha-A-IV	0	6
1435	82	alpha-B-IV	44	9
1435	83	alpha-C-IV	88	8
1435	84	alpha-A-IV	25	8
1435	85	alpha-B-IV	0	8
1435	86	alpha-C-IV	63	8
1435	87	alpha-A-IV	9	11
1435	88	alpha-B-IV	13	8
1435	89	alpha-C-IV	38	8
1435	90	alpha-A-IV	11	9
1435	91	alpha-B-IV	0	8
1435	92	alpha-C-IV	25	8
239	179	alpha-A-I	78	9
239	180	alpha-B-I	90	10
239	181	alpha-B-I	40	10
239	182	alpha-B-I	90	10
239	183	alpha-B-I	100	10
239	184	alpha-B-I	100	15
1271	199	alpha-A-I	100	9

NA = Data not available

Laying Hens AC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Unconscious**	**No. of birds in experiment**
1405	58	alpha-A-I	0	12
1405	59	alpha-A-I	100	8
1405	60	alpha-A-II	0	14
1405	61	alpha-A-IV	0	12
1405	62	alpha-A-IV	0	12

Broilers DC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Unconscious**	**No. of birds in experiment**
1397	1	A-I	80	5
1397	2	A-III	40	5
1397	34	A-I	95	NA
1397	35	A-I	95	NA
1397	36	A-I	94	NA
1397	37	A-II	92	NA
1397	38	A-III	88	NA
1397	39	A-III	67	NA
1397	40	A-I	95	NA
1397	41	A-I	95	NA
1397	42	A-I	94	NA
1397	43	A-II	92	NA
1397	44	A-III	85	NA
1397	45	A-III	60	NA
1397	46	B-I	95	NA
1397	47	B-I	95	NA
1397	48	B-I	94	NA
1397	49	B-II	92	NA
1397	50	B-III	88	NA
1397	51	B-III	55	NA
1397	52	B-I	95	NA
1397	53	B-I	95	NA
1397	54	B-I	94	NA
1397	55	B-II	92	NA
1397	56	B-III	80	NA
1397	57	B-III	47	NA
1433	63	A-I	38	8
1433	64	B-I	20	5
1433	65	B-I	80	10
1433	66	A-III	25	8
1433	67	B-III	20	10
1433	68	B-III	56	9
1433	69	A-III	0	8
1433	70	B-III	25	8
1433	71	B-III	38	8
1434	93	C-I	13	8
1434	94	C-I	73	11
1434	95	C-I	80	10

NA = Data not available

Individual Data Listing: Percentage of birds stunned using non-EEG methods of ascertainment.

Turkey AC

Reference Identification (RefID)	Unique Experiment No.	Electrical Treatment Class	% birds Unconscious	No. of birds in experiment
1534	96	alpha-A-I	3	60
1534	97	alpha-A-I	10	60
1534	98	gamma-A-I	92	60
1534	103	gamma-A-I	67	60
1534	108	gamma-A-I	20	60
1378	141	alpha-A-I	53	17
1378	142	alpha-A-II	63	16
1378	143	alpha-A-III	100	15
1378	144	alpha-A-III	100	15
1378	145	alpha-A-III	100	15
1378	147	alpha-A-II	40	15
1378	148	alpha-A-III	67	15
1378	149	alpha-A-III	67	15
1378	150	alpha-A-III	100	17
1378	151	alpha-A-I	53	17
1378	152	alpha-A-II	63	16
1378	153	alpha-A-III	100	15
1378	154	alpha-A-III	100	15
1378	155	alpha-A-III	100	15
1378	157	alpha-A-II	40	15
1378	158	alpha-A-III	67	15
1378	159	alpha-A-III	67	15
1378	160	alpha-A-III	100	17

Broiler AC

Reference Identification (RefID)	Unique Experiment No.	Electrical Treatment Class	% birds Unconscious	No. of birds in experiment
1535	111	alpha-B-I	11	80
1535	112	alpha-B-I	29	79
1535	113	gamma-B-I	100	60
1535	116	alpha-B-I	5	65
1535	117	alpha-B-I	10	62
1535	118	gamma-B-I	98	60
1535	121	alpha-B-I	12	146
1535	122	alpha-B-I	12	151
1535	123	gamma-B-I	100	60
1279	190	alpha-A-I	22	264
1279	191	alpha-A-I	30	199

Laying AC

Reference Identification (RefID)	Unique Experiment No.	Electrical Treatment Class	% birds Unconscious	No. of birds in experiment
1287	187	alpha-A-I	65	157
1287	188	alpha-B-I	32	158
1287	189	alpha-B-I	13	157

Turkey DC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Unconscious**	**No. of birds in experiment**
1534	99	B-II	98	60
1534	100	B-III	100	60
1534	104	B-II	97	60
1534	105	B-III	100	60
1534	109	B-II	80	60
1534	110	B-III	97	60

Broiler DC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Unconscious**	**No. of birds in experiment**
1535	114	B-II	98	60
1535	115	B-III	100	60
1535	119	B-II	100	60
1535	120	B-III	100	60
1535	124	B-II	100	60
1535	125	B-III	100	60

Individual Data Listing: Unconsciousness Duration (seconds).

Turkey AC

Reference Identification (RefID)	Unique Experiment No.	Electrical Treatment Class	Mean duration	Min Duration	Max Duration	No. of birds in experiment
1534	96	alpha-A-I	35	26	44	60
1534	97	alpha-A-I	52	20	86	60
1534	98	gamma-A-I	29	10	89	60
1534	103	gamma-A-I	33	9	97	60
1534	108	gamma-A-I	36	8	78	60
1378	141	alpha-A-I	57	NA	NA	17
1378	142	alpha-A-II	70	NA	NA	16
1378	143	alpha-A-III	61	NA	NA	15
1378	144	alpha-A-III	51	NA	NA	15
1378	145	alpha-A-III	49	NA	NA	15
1378	147	alpha-A-II	117	NA	NA	15
1378	148	alpha-A-III	80	NA	NA	15
1378	149	alpha-A-III	79	NA	NA	15
1378	150	alpha-A-III	60	NA	NA	17
1378	151	alpha-A-I	25	NA	NA	17
1378	152	alpha-A-II	19	NA	NA	16
1378	153	alpha-A-III	11	NA	NA	15
1378	154	alpha-A-III	13	NA	NA	15
1378	155	alpha-A-III	20	NA	NA	15
1378	157	alpha-A-II	20	NA	NA	15
1378	158	alpha-A-III	16	NA	NA	15
1378	159	alpha-A-III	10	NA	NA	15
1378	160	alpha-A-III	5	NA	NA	17

NA = Data not available

Broiler AC

Reference Identification (RefID)	Unique Experiment No.	Electrical Treatment Class	Mean duration	Min Duration	Max Duration	No. of birds in experiment
1535	111	alpha-B-I	55	30	73	80
1535	112	alpha-B-I	68	25	144	79
1535	113	gamma-B-I	49	13	191	60
1535	116	alpha-B-I	55	45	66	65
1535	117	alpha-B-I	46	22	90	62
1535	118	gamma-B-I	40	22	139	60
1535	121	alpha-B-I	45	24	102	146
1535	122	alpha-B-I	38	24	93	151
1535	123	gamma-B-I	42	20	168	60
1537	126	alpha-B-I	51	NA	NA	NA
1537	127	alpha-B-III	52	NA	NA	NA
1537	128	alpha-B-IV	57	NA	NA	NA
239	174	alpha-A-I	45	5	179	NA
239	175	alpha-A-I	70	5	228	NA
239	176	alpha-A-I	57	19	156	NA
239	177	alpha-A-I	60	25	104	NA
239	178	alpha-B-I	104	52	237	NA
1279	190	alpha-A-I	65	NA	NA	264
1279	191	alpha-A-I	55	NA	NA	199

1271	199	alpha-A-I	17	8	36	9

NA = Data not available

Laying Hens AC						
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**Mean duration**	**Min Duration**	**Max Duration**	**No. of birds in experiment**
1287	187	alpha-A-I	53	21	180	157
1287	188	alpha-B-I	56	22	120	158
1287	189	alpha-B-I	55	27	93	157

Turkey DC						
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**Mean duration**	**Min Duration**	**Max Duration**	**No. of birds in experiment**
1534	99	B-II	57	15	120	60
1534	100	B-III	40	12	104	60
1534	104	B-II	62	15	186	60
1534	105	B-III	45	11	100	60
1534	109	B-II	61	26	145	60
1534	110	B-III	38	9	91	60

Broiler DC						
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**Mean duration**	**Min Duration**	**Max Duration**	**No. of birds in experiment**
1535	114	B-II	59	22	132	60
1535	115	B-III	55	25	NA	60
1535	119	B-II	50	25	240	60
1535	120	B-III	42	22	110	60
1535	124	B-II	42	20	118	60
1535	125	B-III	43	23	84	60
1559	129	A-II	24.2	NA	NA	NA
1559	130	A-II	24.2	NA	NA	NA
1559	131	A-II	24.2	NA	NA	NA
1559	132	A-II	24.2	NA	NA	NA
1559	133	A-II	24.2	NA	NA	NA
1559	134	A-II	24.2	NA	NA	NA
1559	135	A-II	34.7	NA	NA	NA
1559	136	A-II	37.1	NA	NA	NA
1559	137	A-II	39.6	NA	NA	NA
1559	138	A-II	42	NA	NA	NA
1559	139	A-II	44.5	NA	NA	NA
1559	140	A-II	46.9	NA	NA	NA

NA = Data not available

Individual Data Listing: Percentage of birds with cardiac arrest

Turkey AC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Cardiac arrest**	**No. of birds in experiment**
1534	96	alpha-A-I	97	60
1534	97	alpha-A-I	90	60
1534	98	gamma-A-I	8	60
1534	101	alpha-A-I	100	60
1534	102	alpha-A-I	100	60
1534	103	gamma-A-I	33	60
1534	106	alpha-A-I	100	60
1534	107	alpha-A-I	100	60
1534	108	gamma-A-I	80	60
1378	141	alpha-A-I	47	17
1378	142	alpha-A-II	38	16
1378	146	alpha-A-I	100	10
1378	147	alpha-A-II	60	15
1378	148	alpha-A-III	33	15
1378	149	alpha-A-III	33	15
1378	151	alpha-A-I	47	17
1378	152	alpha-A-II	38	16
1378	156	alpha-A-I	100	10
1378	157	alpha-A-II	60	15
1378	158	alpha-A-III	33	15
1378	159	alpha-A-III	33	15
689	194	alpha-A-I	76	17
689	195	alpha-A-I	75	8
689	196	alpha-A-I	83	12
689	197	alpha-A-I	88	8
689	198	alpha-A-I	100	11

Broiler AC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Cardiac arrest**	**No. of birds in experiment**
1397	16	beta-A-I	80	NA
1397	17	beta-A-I	80	NA
1397	22	beta-B-I	80	NA
1397	23	beta-B-I	80	NA
1397	24	beta-B-I	80	NA
1397	28	beta-B-I	80	NA
1397	29	beta-B-I	80	NA
1397	30	beta-B-I	80	NA
1535	111	alpha-B-I	89	80
1535	112	alpha-B-I	71	79
1535	116	alpha-B-I	95	65
1535	117	alpha-B-I	90	62
1535	118	gamma-B-I	2	60
1535	121	alpha-B-I	88	146
1535	122	alpha-B-I	87	151
1537	126	alpha-B-I	95	NA
239	174	alpha-A-I	3	NA

239	175	alpha-A-I	22	NA
239	176	alpha-A-I	61	NA
239	177	alpha-A-I	81	NA
239	178	alpha-B-I	92	NA
239	179	alpha-A-I	56	9
239	180	alpha-B-I	90	10
239	181	alpha-B-I	90	10
239	182	alpha-B-I	100	10
239	183	alpha-B-I	100	10
239	184	alpha-B-I	100	15
1279	190	alpha-A-I	78	264
1279	191	alpha-A-I	70	199

NA = Data not available

Laying Hens AC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Cardiac arrest**	**No. of birds in experiment**
1287	187	alpha-A-I	35	157
1287	188	alpha-B-I	68	158
1287	189	alpha-B-I	87	157

Turkey DC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Cardiac arrest**	**No. of birds in experiment**
1534	99	B-II	2	60
1534	104	B-II	3	60
1534	109	B-II	20	60
1534	110	B-III	3	60

Broiler DC				
Reference Identification (RefID)	**Unique Experiment No.**	**Electrical Treatment Class**	**% birds Cardiac arrest**	**No. of birds in experiment**
1397	46	B-I	80	NA
1397	47	B-I	80	NA
1397	52	B-I	80	NA
1397	53	B-I	80	NA
1397	54	B-I	80	NA
1433	64	B-I	80	5
1433	65	B-I	50	10
1434	93	C-I	13	8
1434	94	C-I	45	11
1434	95	C-I	60	10
1535	114	B-II	2	60

NA = Data not available

Median Values **Percentage of birds stunned using EEG methods of ascertainment.**

Turkey AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-A-I	92	88	100

Broiler AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-B-IV	6	0	44
alpha-A-IV	10	0	25
alpha-A-III	33	33	33
alpha-C-IV	50	25	88
beta-A-IV	50	0	79
alpha-A-II	67	67	67
alpha-B-II	75	75	75
beta-B-IV	81	62	92
beta-A-II	83	75	88
alpha-B-III	86	86	86
alpha-C-I	88	88	88
alpha-C-II	88	88	88
alpha-C-III	88	88	88
alpha-A-I	89	78	100
beta-A-I	89	81	93
beta-B-II	94	91	96
alpha-B-I	95	40	100
beta-B-I	96	92	98

Laying Hens AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-A-II	0	0	0
alpha-A-IV	0	0	0
alpha-A-I	50	0	100

Broiler DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
B-III	51	20	88
A-III	60	0	88
C-I	73	13	80
A-II	92	92	92
B-II	92	92	92
A-I	95	38	95
B-I	95	20	95

Median Values: Percentage of birds stunned using non-EEG methods of ascertainment.

Turkey AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-A-I	31	3	53
alpha-A-II	51	40	63
gamma-A-I	67	20	92
alpha-A-III	100	67	100

Broiler AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-B-I	12	5	29
alpha-A-I	26	22	30
gamma-B-I	100	98	100

Laying Hens AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-B-I	23	13	32
alpha-A-I	65	65	65

Turkey DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
B-II	97	80	98
B-III	100	97	100

Broiler DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
B-II	100	98	100
B-III	100	100	100

Median Values: Unconsciousness Duration (seconds)

Turkey AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
gamma-A-I	33	8	97
alpha-A-III	34.5	NA	NA
alpha-A-I	43.5	20	86
alpha-A-II	45	NA	NA

NA = Data not available

Broiler AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
gamma-B-I	42	13	191
alpha-B-III	52	NA	NA
alpha-B-I	53	22	237
alpha-A-I	57	5	228
alpha-B-IV	57	NA	NA

NA = Data not available

Laying Hens AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-A-I	53	21	180
alpha-B-I	55.5	22	120

Turkey DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
B-III	40	9	104
B-II	61	15	186

Broiler DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
A-II	29.45	NA	NA
B-III	43	22	110
B-II	50	20	240

NA = Data not available

Median Values: Percentage of birds with cardiac arrest

Turkey AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-A-III	33	33	33
gamma-A-I	33	8	80
alpha-A-II	49	38	60
alpha-A-I	97	47	100

NA = Data not available

Broiler AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
gamma-B-I	2	2	2
alpha-A-I	61	3	81
beta-A-I	80	80	80
beta-B-I	80	80	80
alpha-B-I	90	71	100

Laying Hens AC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
alpha-A-I	35	35	35
alpha-B-I	77	68	87

Turkey DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
B-II	3	2	20
B-III	3	3	3

Broiler DC			
Electrical Treatment Class	**Median Value**	**Minimum Value**	**Maximum Value**
B-II	2	2	2
C-I	45	13	60
B-I	80	50	80