Final report

DTS: Diathermic Syncope®: Revision of dossier for regulatory assessment in the EU

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Abstract

In response to a submission on DTS: Diathermic Syncope ® made to the European Food Safety Authority (EFSA) (MLA Project V.SRP.0002), the Animal Health and Welfare (AHAW) panel responded with a request for reanalysis of electroencephalograms (EEGs) and a number of questions requiring additional information and analysis to be provided.

The DTS: Diathermic Syncope® Technical dossier has been revised in response to these questions and with additional data that has been collected since the dossier was first submitted. This report is a public version of the dossier that has been submitted to EFSA. The dossier is necessary in order to seek approval for export to this market, for export of meat processed using DTS technology from Australia to this market, and as an indication of acceptance of the technology by an influential international regulator.

Executive summary

Background

DTS: Diathermic Syncope ® technology (DTS) for stunning of cattle has been developed and provides consistent induction of insensibility at the time of slaughter. In order gain regulatory approval in Europe, an application needs to be made addressing the criteria important to this market. A previous submission raised questions and a request from the European Food Safety Authority (EFSA) Animal Health and Welfare (AHAW) Panel to re-analyse some electroencephalogram (EEG) data. The technical dossier has now been revised and resubmitted to the European Food Safety Authority, so consideration of the application may continue.

Objectives

1. Re-analysis of existing EEG data according to the requirements of the EFSA AHAW panel

2. Revision of the dossier provided to EFSA according to the request of the EFSA AHAW panel

3. Submit the revised dossier for EFSA AHAW consideration

All three objectives have been achieved.

Methodology

EEG data in the submitted dossier were examined and selected data were reanalysed according to EFSA's preferred method. Additional data were submitted.

The questions raised by the EFSA AHAW Panel were analysed and answered in the context of the legislative requirements and prior publications of the Panel.

Answers to the questions raised and a revised technical dossier have been submitted to EFSA.

Results/key findings

The resubmitted technical dossier makes better use of the data collected to support the acceptance of the DTS method of stunning. The considerations of the Panel and the eventual outcome cannot be anticipated, but resubmission has increased the likelihood of a successful outcome. Alternatively, the Panel would be in a better position to specify the further evidence required before acceptance in Europe. This version of the technical dossier is the most complete and comprehensive exposition of DTS: Diathermic Syncope®.

Benefits to industry

Acceptance of DTS in Europe would allow the technology to be used in this market, and also meat processed from animals stunned using this technology could be sold in Europe. Europe is one of the few markets with prescriptive animal stunning requirements, so its acceptance of the technology would be influential in markets with less prescriptive requirements.

Future research and recommendations

The dossier prepared for EFSA is valuable as a technical reference for applications in other markets. Regulatory acceptance is a prerequisite for acceptance by religious authorities.

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1. Background

The DTS: Diathermic Syncope ® technology (DTS) for stunning of cattle has been developed and provides consistent induction of insensibility at the time of slaughter. A number of previous reports and peer-reviewed articles provide validation of the technology's ability to provide for animal welfare and humane slaughter. For DTS to be commercialised, it must be approved for use by the Competent Authority in the countries in which the technology is to be used, and in some countries to which Australian product, stunned by this method, is to be exported. Approval has been gained for use of DTS in Australia, and further approval is being sought internationally.

As part of past MDC project P.PIP.0587 and V.SRP.0002., a technical dossier was prepared for the European Food Safety Authority (EFSA), the risk assessors for the European Competent Authority - 'DTS: Diathermic Syncope®: preparation of dossier for regulatory approval'. The EFSA Animal Health and Animal Welfare (AHAW) panel reviewing the application has raised a large number of questions (99) requiring responses in specific sections of the application format and required that electroencephalogram (EEG) data be analysed according to their preferred method.

The dossier needs to be revised according to the EFSA panel's requirements in order to gain approval for export to this market, for export of meat processed using DTS technology from Australia, and as an indication of acceptance of the technology by an influential international regulator.

2. Objectives

1. Re-analysis of existing EEG data according to the requirements of the EFSA AHAW panel
2. Revision of the dossier provided to EFSA according to the request of the EFSA AHAW panel
3. Submit the revised dossier for EFSA AHAW consideration

3. Methodology

3.1 EEG

Available EEG data was assessed for quality: technically complete with minimal artefacts confounding interpretation when DTS was applied at energy and power levels recommended for use in stunning.

EEGs were analysed according to the method used for the analysis on EEGs of broiler chickens submitted to LAPS (EFSA Panel on Animal Health Welfare et al., 2017) based on that of Vyssotski et al. (2009).

3.2 Responding to questions

Questions needed to be understood with respect to EU regulations, EFSA opinions, previous decisions of the AHAW Panel. It appeared that there was an expectation for all information to be in the dossier rather than in the reports or peer reviewed publications that were submitted and referenced in the dossier. Additional data analysis was required. Formulations of standardised measures of animal welfare was required and applied across all live animal studies. Questions were answered and the dossier modified as necessary.

3.3 Additions to the technical dossier

Additional work had been performed after the submission of the dossier to EFSA, and these were incorporated into the dossier as Steps 13 and 14.

4. Results

4.1 Revised dossier

The revised technical dossier, without the administrative details required by EFSA, and with redactions in accordance with EFSA's procedures (confidential data is redacted), appears in Appendix 1.

4.2 Submission to EFSA

The dossier and response to questions raised by the EFSA AHAW Panel have been submitted to EFSA, and acknowledgement of receipt has been received.

5. Conclusion

The process of providing analysis of EEGs according to EFSA AHAW Panel expectations and answering a large number of questions from the panel, provided new insights into the expectations of the panel; since only one previous application has been made to EFSA for approval of a new stunning method, the panel's expectations were not clear, and perhaps were not even well-formed prior to their consideration of this application.

The dossier now contains more analysis and consideration of previous, as well as new, studies of DTS. Animal-based measures (ABMs) of welfare have now been consistently applied across all studies to provide a consistent measure of success of maintenance of animal welfare during stunning which will be valuable for verification of welfare in a slaughter establishment.

The AHAW Panel will continue to consider the application on DTS; their role is to assess the animal welfare implications of the method. If their conclusion is positive, then processes within the European Commission consider changes to the animal welfare directive, which approves methods of stunning during the slaughter process. It is possible that the AHAW Panel may require further information and even new data to further their assessment.

5.1 Key findings

- More comprehensive understanding of the approaches and criteria of EFSA and its AHAW Panel would have resulted in an initial dossier that was closer to their expectations, but almost certainly, questions would still be asked, and not all of their requests could have been anticipated.
- Using the recommended parameters for DTS, animal welfare is maintained through restraint, induction of unconsciousness, through to death by exsanguination. It delivers an animal welfare outcome that is at least equivalent to that observed with other stunning methods approved for cattle

- ABMs assessed during restraint and application of DTS indicated only minor discomfort or distress in a small number of animals, not unexpected in any form of restraint or stunning.
- In the absence of technical problems leading to interrupted delivery of energy, all animals were rendered unconscious using DTS when the recommended parameters for administration of DTS are used.
- Animals are unconscious for at least 60s following the induction of unconsciousness. EEG data indicate a at least 60 s unconsciousness with long transition to return of consciousness
- The period of unconsciousness is sufficiently long to ensure death by exsanguination without return of consciousness when a rotary box is employed to ensure effective removal of the animal from the box during the tonic phase.
- The equipment configuration allowed consistent repeatable induction of insensibility, which was sustained during Halal bleeding, without the need to apply an additional ventral cut.
- There was no requirement to use an immobiliser during application of the exsanguination cuts, and the animal bled rapidly to brain death using the Halal cut alone. The blood flow on exsanguination was strong and rapid.
- Using the recommended parameters for DTS there are no significant physical effects on the brain and it therefore normal recovery can occur in animals that are not exsanguinated.

5.2 Benefits to industry

Acceptance of DTS in Europe would allow for the MLA/MDC-funded and owned technology to be used in this market for the stunning of cattle. Additionally, meat processed from animals stunned using this technology (in Australia) would be acceptable in this market. Acceptance in Europe, with high animal welfare standards and prescriptive requirements would provide validation of the animal welfare claims and encourage acceptance in other markets with less prescriptive requirements.

Regulatory approval is a necessary practical prerequisite for religious acceptance; technological and welfare assessments and approval, provide religious authorities the opportunity to make an assessment against religious criteria, knowing that, if they accept the method then it can be implemented without hindrance.

6. Future research and recommendations

It is possible that the EFSA AHAW Panel may request additional demonstration and validation of the claims of DTS: Diathermic Syncope® prior to assessing its suitability for maintaining animal welfare during the slaughter process.

The revised dossier provides a basis for making applications in other jurisdictions that require prior approval of stunning methods, and should be used for that purpose.

The revised dossier provides information that would be valuable to religious authorities making an assessment of the suitability of DTS for religious purposes, though additional information may be required to address all religious criteria.

7. References

EFSA Panel on Animal Health Welfare, More, S., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, B., Good, M., Gortazar Schmidt, C., Miranda, M.A., Nielsen, S.S., Sihvonen, L., Spoolder, H., Willeberg, P., Raj, M., Thulke, H.-H., Velarde, A., Vyssotski, A., Winckler, C., Cortiñas Abrahantes, J., Garcia, A., Muñoz Guajardo, I., Zancanaro, G., Michel, V., 2017. Low atmospheric pressure system for stunning broiler chickens. EFSA Journal 15, e05056.

Vyssotski, A.L., Dell'Omo, G., Dell'Ariccia, G., Abramchuk, A.N., Serkov, A.N., Latanov, A.V., Loizzo, A., Wolfer, D.P., Lipp, H.P., 2009. EEG responses to visual landmarks in flying pigeons. Curr Biol 19, 1159-1166.

8. Appendix

8.1 Revised DTS: Diathermic Syncope ® Technical dossier

DTS: Diathermic Syncope® Technical dossier

Article 4 (2) of Council Regulation (EC) No 1099/20094 on the protection of animals at the time of killing allows the Commission to amend stunning parameters laid down in Annex I to this Regulation to take into account scientific and technical progress on the basis of an EFSA opinion. Any such amendments shall ensure a level of animal welfare at least equivalent to that ensured by the existing methods. This technical dossier summary presents a comprehensive overview of the use of electro-magnetic energy applied directly to the brain to induce volumetric heating (DTS: Diathermic Syncope®, hereafter referred to as 'DTS'), and presents the supporting scientific studies to address the criteria outlined in the EFSA Guidance on the assessment criteria for applications for new or modified stunning methods regarding animal protection at the time of killing (EFSA Journal 2018;16(7):534, hereafter referred to as 'EFSA guidance').

Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing defines "stunning" in Article 2 (f) as "any intentionally induced process which causes the loss of consciousness and sensibility without pain including any process resulting in instantaneous death". Article 4 on stunning interventions states that "animals shall only be killed after stunning in accordance with the methods and specific requirements related to the application of those methods set out in Annex I of the Regulation" and "that loss of consciousness and sensibility shall be maintained until the death of the animal".

As the use of DTS is a novel stunning system, no parameters for its use are currently defined by Council Regulation (EC) No 1099/2009.

3.1 Description of the stunning method

3.1.1 Name of the method

DTS: Diathermic Syncope® hereafter referred to as 'DTS'.

3.1.2 Description of the method including potential sources of pain, distress and suffering

DTS: Diathermic Syncope® applies electromagnetic energy consisting of both a magnetic and electrical field, moving as sine waves, perpendicular to one another. The electrical field induces an epileptiform seizure in the brain, while the changing electromagnetic field induces heat in the brain tissue through a process called 'diathermy'. This heat leads to cessation of neurotransmitter function (Ikarashi et al 1984), maintaining unconsciousness until such time as the brain temperature returns to with 1 degree of normal (Guy and Chou 1982). The onset of the epileptiform seizure is calculated to occur prior to the onset of unconsciousness as a result of hyperthermia, and before the onset of any physical sensation of overheating of the skin.

3.1.2.1 Technical description of apparatus

The stunning system apparatus includes six critical components which are shown in Figure 1 and are listed below:

1. DTS Generator
2. User interface panel
3. Waveguide system
4. Applicator
5. Animal Restraint (not shown in Figure 1)
6. Faraday cage

1. **DTS generator**. This water-cooled generator contains a magnetron (an evacuated tube for generating microwaves, with the flow of electrons controlled by an external magnetic field), high power components, automation hardware and the electronics required for generating electromagnetic energy at a frequency of 922 MHz (or within the region of 890-925 MHz). Generator output is a minimum of 10 kW and maximum of 40 kW.
2. **User interface panel**. A software-based user control panel which allows pre-selection of the total amount of energy to be delivered to the animal. Energy calculations are based on variations of power (kW) and length of applied energy (seconds). Each dose of energy delivered to the animal is carefully monitored and recorded against a specific animal. The interface unit includes emergency stop capability; indicators of magnetron status (standby, warming, on); fault alerts and reset. Fault diagnostics and permanent storage of energy transmission data are included in the software.
3. **Waveguide system**. These are used to transport the energy from the DTS generator to the Applicator. Electromagnetic energy leaves the DTS generator via rectangular aluminium tubing (waveguide). The cross-sectional dimensions of which are sized according to the frequency of the electromagnetic energy being produced. This rectangular waveguide is rigid and as such requires the energy to be transitioned to a more flexible apparatus, e.g., waveguide rotary and telescopic joints and/or coaxial cable. This flexibility allows for accurate positioning of the Applicator on the animal's head (Section 3.7.2). There is an auto tuner located on the generator side of the coaxial cable in the waveguide section, this ensures we optimise the energy transfer to the animal's head for the wide variety of head sizes and shapes.

4. **Applicator**. This provides the direct interface between the DTS system and the animal's head. It is a unique design specific to the electromagnetic energy frequency and the type of animal being processed (e.g., cattle, calves, sheep). The waveguide energy is focused to a region considered the target area so that the penetrative energy is directed to the desired region of the brain in an efficient manner. Fixed tuning stubs are located just prior to the applicator ensuring that is well tuned into the animal's head for an acceptable range of animals. The autotuner corrects any small mismatch in tuning that may arise due to animal head variations. A quartz window protects the coaxial to waveguide transition piece from water, hair and foreign material.

NB: Poor tuning results in in-efficient energy transfer, which could lead to failure to induce unconsciousness, or inappropriate surface heating.

5. **Animal restraint**. (Section 3.6) The animal is restrained in an upright position in a mechanism that will allow animal rotation, e.g., in a tipping or rotating box (to allow rotation and ejection after unconsciousness has been confirmed). A rear pusher may be used to ensure that the animal is positioned with its head protruding through the neck yoke aperture. A neck capture with chin lift (based on the ASPCA Pen design) is applied, the chin lift is used to position the head such that the frontal bones are between 0 and 30 degrees from horizontal. The head capture unit is engineered with hydraulic rams, which are more rigid than the pneumatic rams, reducing the chance of compression during application.
6. **Faraday cage**. The entire rotary box is enclosed in a Faraday cage (an enclosure created by conducting materials that blocks static and non-static electric fields) to protect operators in the case of energy leakage. The Faraday cage consists of a steel mesh with holes less than 12mm in diameter. Personnel doors for access to the restraint unit are fitted at the level of the animal's head, from one side when in the upright position, and from the front when in the rotated position. These doors seal using electromagnets when the energy application is underway. The rear door that allows animal access is also sealed using electromagnets. Safety switches are installed at the doors such that energy application cannot proceed if the doors are not properly sealed (these safety interlocks cannot be manually over-ridden). An emergency stop button is fitted inside the Faraday cage in case of the unlikely event of personnel being trapped within. DTS Active warning lights are fitted in prominent positions on the DTS generator and the Faraday Cage.

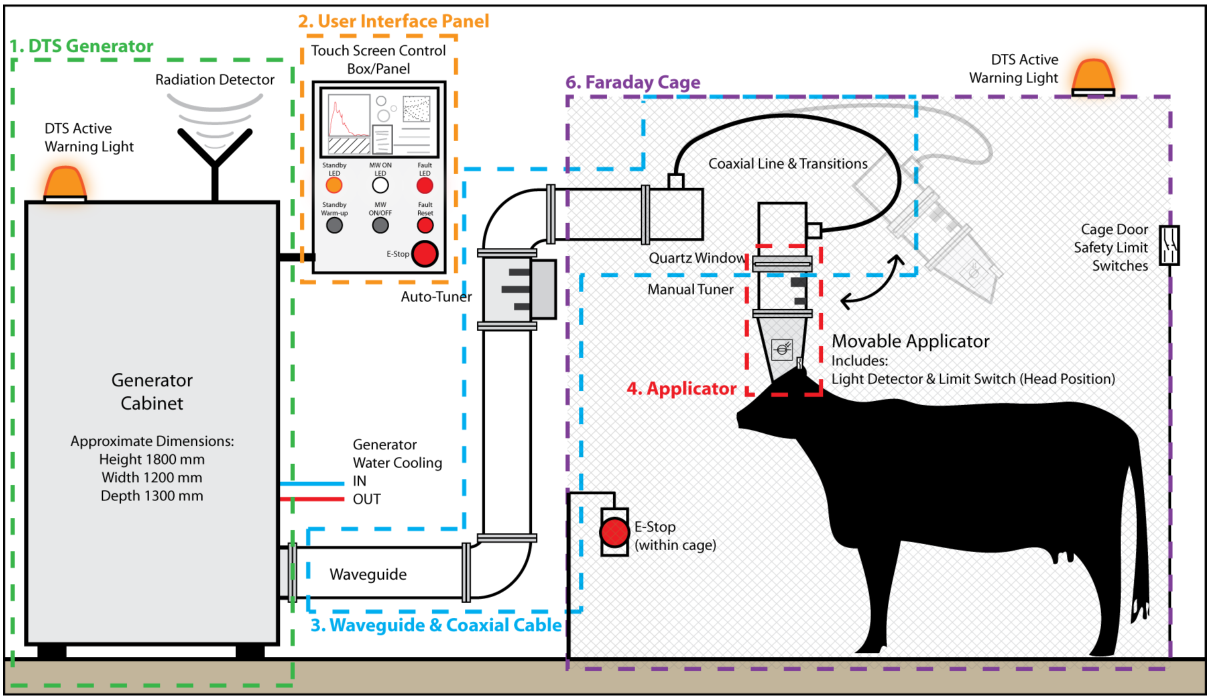

Figure 1: Apparatus Overview (Animal Restraint Not Shown)

3.1.2.2 Stunning system operation sequence

Start-up sequence:

1. Turn on Cooling Water.
2. Switch on Mains Power.
3. Use the key to turn 'Key-Switch' O

4. Wait for the computer to start up and the DTS control program to initialise.
5. Press 'Continue' on the 'Open Com Port' box.
6. Press 'STANDBY' to begin the 3 min Warm Up Sequence.

Warm up is complete when STANDBY LED stays illuminated and Generator Status Reads "Generator Ready (Standby)".

Once the Start-Up Sequence is completed, DTS Energy can be applied and reapplied continuously without the need to go through the Start-Up Sequence again, until the system is shut down, either manually, as a result of an electrical fault or in the event of a power outage.

Applying Energy:

1. Before each energy application, select the correct DTS Profile (Power & Time Combination) on the Main Operations Page.
2. Applicator is manually put in place (fitted against animal's head) by the operator.
3. When the animal is ready, one of the 'APPLY ENERGY' buttons is pushed to initiate energy application. To cancel the energy application, push the 'CANCEL' button.

Note: Energy application is only possible when there are no generator faults, the animal is in place and all safety interlocks (E-Stop, gates, and gen doors) read OK. Some faults may result in the need to restart the warm-up sequence. If the animal is not in place and the applicator positioned on its forehead, energy escaping from the applicator will trigger the automatic safety cut-out and energy delivery will immediately cease.

Shut-down Procedure:

1. Exit Standby mode by pressing the STANDBY push button.
2. Press the touch screen Shutdown button to close the program.
3. Remove the key from the key switch.
4. Shut down the computer via the Windows start menu.
5. Allow 10 minutes to elapse for cooling
6. Turn off mains power.
7. Turn off cooling water.

3.1.2.3 Stunning apparatus cleaning and maintenance

Generator Care

Daily:

- Remove any build-up of rubbish from within and around the generator.
- Ensure that all air intakes and outlets are clear.

Weekly:

- Check water flow and arc detection devices are functional.
- Check for water leaks inside the generator.
- Check and replace (if required) air filters on generator side walls.
- Check waveguide for loose fittings and bolts.

Monthly**:**

- Check generator doors, limit switches and adjust to suit door stroke.
- Replace/wash air filters on generator side walls.

Applicator Care

Daily:

- Wipe out any moisture, dust or hair.
- Visually inspect for cracks or other damage.

Weekly:

- Check waveguide and applicator for loose fittings and bolts.

Restraint Unit Care

Daily:

- Wash out any faecal matter, blood, hair or excreta.
- Visually inspect for cracks or other damage.
- Grease moving parts.
- Visually inspect unit and faraday cage for damage.

Microwave safety

Primary microwave safety lies in the design of the Faraday cage which prevents any microwaves from escaping into the surrounding environment. All doors on the Faraday cage are fitted with specially designed microwave chokes which reflect energy back into the cage. The legal requirements pertaining to this screening differ in different countries, as do the requirements around the specific frequencies generated by the magnetron. Alternative frequencies should be validated prior to commercial use. In the case of a cage or choke failure, a radiation interlock system is placed on the outside of the cage and will instantly shut the DTS system down and prevent further operation, if an unsafe level of radiation is detected. All Faraday cage doors are to be fitted with double interlocks to prevent operation if a door is not correctly closed/sealed. An optional interlock may be fitted on the applicator which checks head placement is correct before energy can be applied.

Calibration of the apparatus

With the exception of the auto-tuner for which calibration is required every 2 years (manufacturer's recommendation), the stunning apparatus does not need regular calibration. However, care should be taken to inspect the waveguide, applicator and Faraday cage each day for damage and ensure everything is in good condition. Assessment of tuning should be carried out by a specialist technician annually, or in the event of a change in size of the applicator (e.g., for use on another species).

3.1.2.4 Technical description of restraining system construction and operation

The restraining system does not deviate substantially from that associated with conventional stunning and slaughter processes for cattle, e.g., using captive bolt or electric stun. The restraint unit is fed via a crowd pen and race, as per existing commercial restraint boxes used for mechanical or electrical stunning. The animal is restrained in an upright position in a mechanism that will allow rotation or inversion of the unconscious animal. A rear pusher may be used to ensure that the animal is positioned with its head protruding through the neck yoke aperture. A neck capture with chin lift is applied, the chin lift is used to position the head such that the frontal bones are between 0 and 30 degrees from horizontal. The head capture unit is engineered with hydraulic rams, which are more rigid than the pneumatic rams, reducing the chance of compression during application. The design of the restraining system allows for prompt ejection and immediate slaughter. Schematic diagrams of a rotating box restraining system are shown in Figures 2 and 3. Additional photographic images of a rotating box restraining system under construction are presented in Figures 4 to 7, and 3D representations of the rotating restraint box within the Faraday cage are shown in Figures 8 and 9.

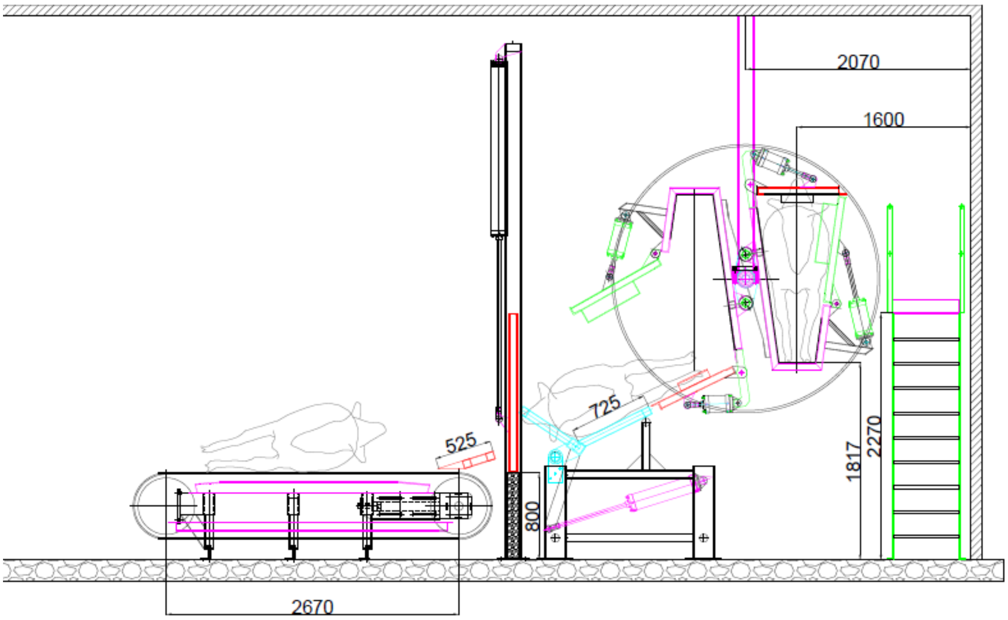

Figure 2: Schematic diagram of restraint and ejection system onto bleed conveyor, front elevation. One animal is upright prior to DTS application (right), one is in the catching cradle post exsanguination (middle), and a third is on the bleed conveyor (left).

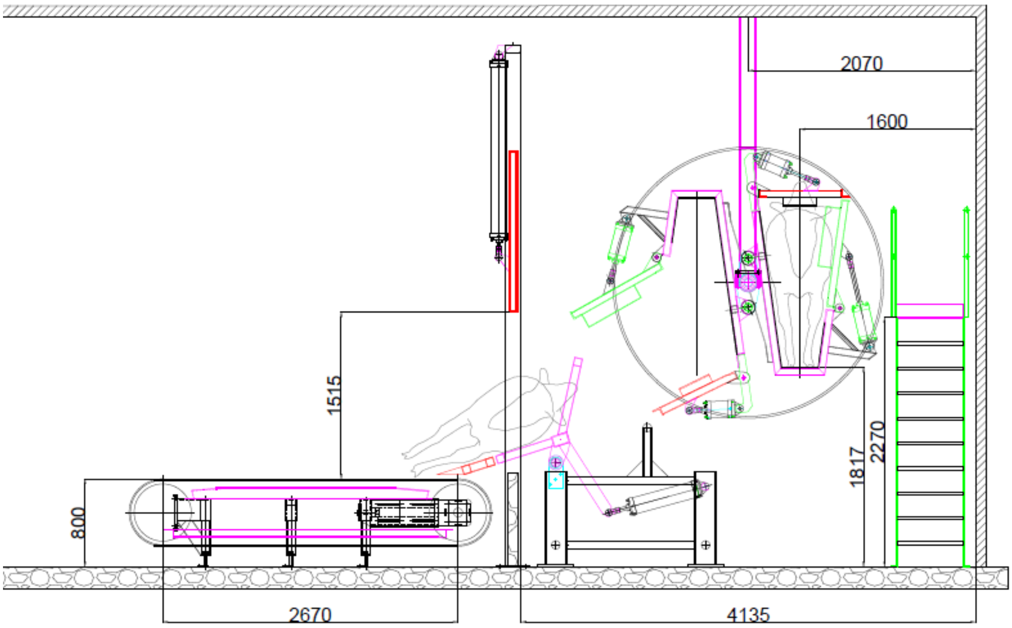

Figure 3: Schematic diagram of restraint and ejection system onto bleed conveyor, front elevation. One animal is upright prior to DTS application (right), one is in the tipped catching cradle post exsanguination (middle), in the process of being ejected onto the bleed conveyor (left).

Figure 4: View of animal access to rotating restraint box, from rear. Image taken during workshop construction. In this image, the upright side is to the left, and the inverted side to the right.

Figure 5: View of the neck capture, side and 'top' doors opened to allow ejection of the animals when inverted. Image taken during workshop construction. In this image, the upright side is to the right, and the inverted side to the left.

Figure 6: Side view of the upright section of the rotating restraint box. Image taken during workshop construction. In this image, the animal would be facing to the left, with the head protruding through the 'U'-shaped aperture left of middle, upper portion.

Figure 7: View of the inverted part of the rotating restraint box, showing the side and 'top' doors opened to allow ejection of the animals when inverted. Image taken during workshop construction.

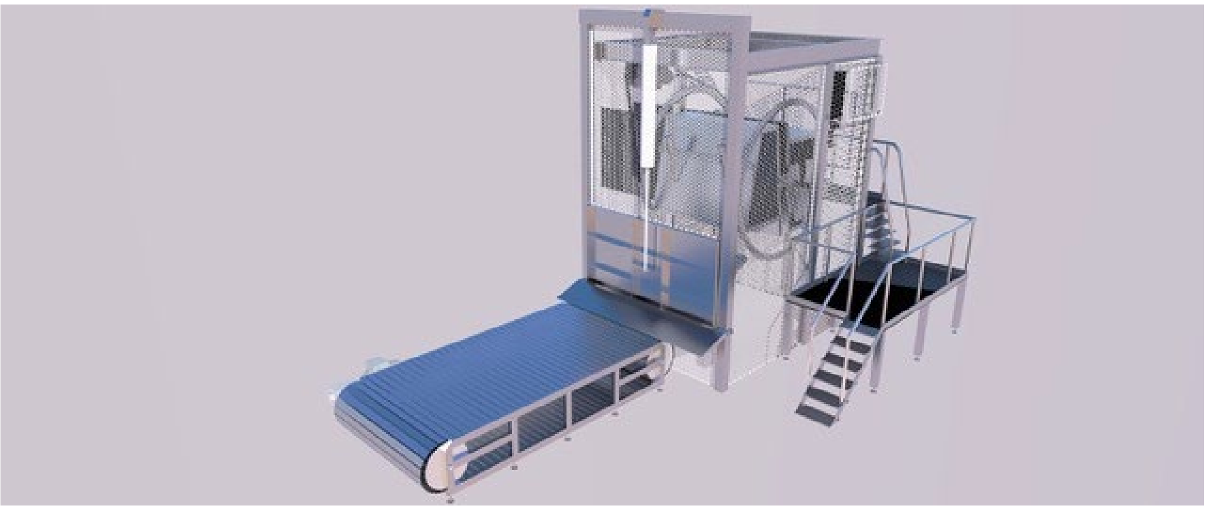

Figure 8: 3D representation of the rotating restraint box within the Faraday cage. Personnel access steps are on the right side of the image, with the bleed conveyor to the left.

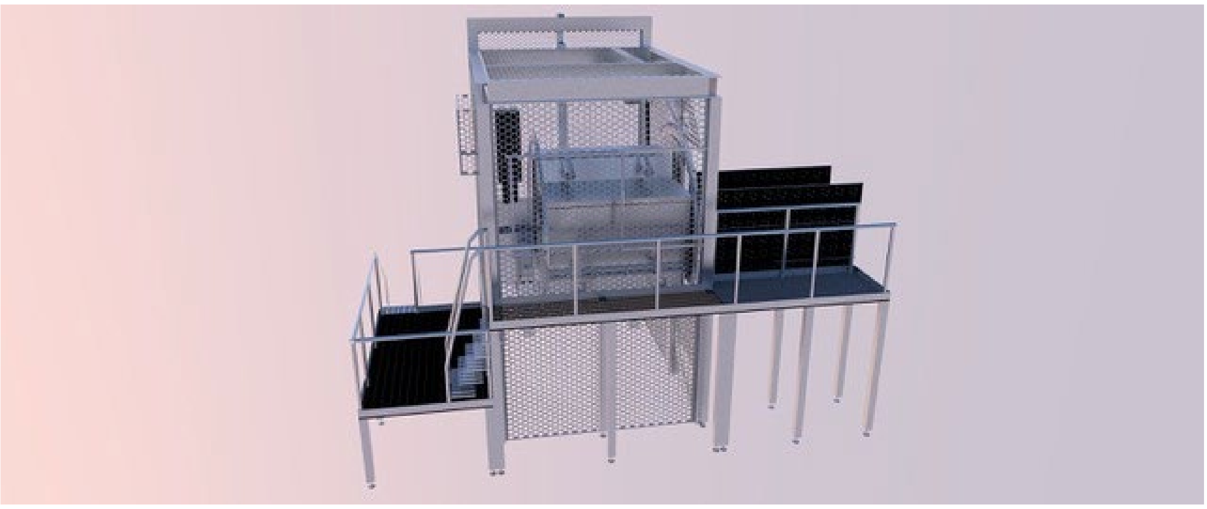

Figure 9: 3D representation of the rotating restraint box within the Faraday cage. Rear view showing personnel access.

Prolonged restraint in any system is stressful to animals (Ewbank 1992). The restraint unit used with the DTS: Diathermic Syncope® system is a standard commercial restraint, with a neck capture and chin-lift system based on the ASPCA Pen design described by Grandin (1992) and Marshall (1963). Quiet handling and smooth application of the neck capture and chin lift apparatus is well tolerated by cattle (Dunn 1990, Grandin 1992) and the head position achieved allows accurate placement of the applicator onto the forehead of the animal. The animal is held in the chin lift for a maximum of 30 s while the applicator is positioned and DTS energy delivery initiated.

3.1.2.5 Technical description of waveguide position and application of energy

The applicator is manually positioned directly over the frontal lobes of the brain by the operator. Energy application is prevented if the animal's head is not pressed firmly against the applicator and if any of the access door limit switches are open or faults are triggered.

Contact between the Applicator and the animal's head

The Applicator is attached to a flexible waveguide system, allowing it to be applied at different heights and angles to match the various head shapes. Contouring within the Applicator head interface end also enables a uniform and continuous contact.

Position of the Applicator on the animal's head

The applicator is placed high on the forehead of the animal, on the frontal bones in front of the poll, such that the energy is directed into the brain mass (Figures 10 and 11).

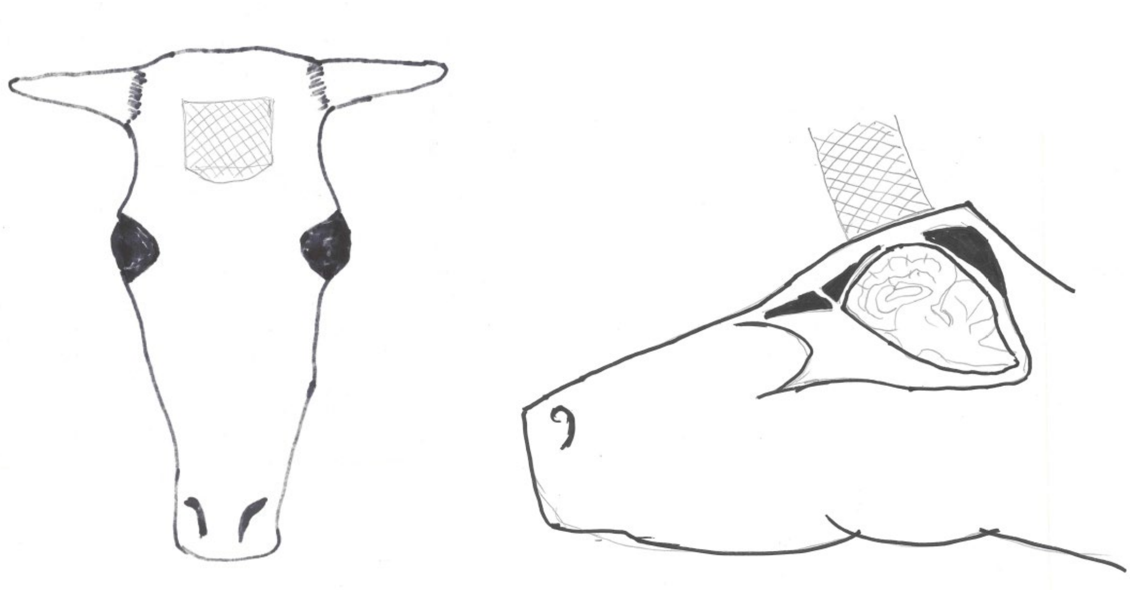

Figure 10: Diagram of placement of applicator on the bovine head. Left: frontal view, hatched area shows applicator position; Right: sagittal view, hatched area represents the applicator, showing positioning relative to the brain and frontal sinuses (black).

Figure 11: Animal in restraint with chin lift activated and applicator in position, immediately prior to DTS energy application.

Minimum energy (kJ) application

Although energy levels of 85 kJ have been shown to result in unconsciousness in some animals, the duration of unconsciousness is short. For commercial processing, energy levels of 160 kJ or above are recommended. Energy levels above 220 kJ can lead to damage to brain tissue, while energy levels of 300 kJ and above will result in death.

Duration of energy application

The duration of energy application is related to the incident power and the desired total energy 'package' to be delivered. The units of power (kW) is shorthand for 'kJ per second', so a higher power setting will reduce the time required to reach an energy delivery of 200 kJ than a lower power setting. However, the higher power setting may predispose to focal overheating, while a very low incident power may be insufficient to raise the temperature of the brain sufficiently rapidly to overcome the natural cooling provided by the blood circulation.

Our current data set indicates that a power setting of 18-20 kW is suitable. 18 kW will deliver 180 kJ in a period of 10 s, while 20 kW will deliver 180 kJ in a period of 9 s.

DTS, delivering 160-220 kJ at an incident power of 18-20 kW induces loss of posture within 13 seconds of onset of energy application, and the duration of unconsciousness recorded during validation was between 100 and 240 seconds, based on absence of corneal reflex, and duration of EEG changes.

For humane slaughter purposes, the insensibility should be of a duration that allows death through exsanguination prior to recovery. In cattle, death through exsanguination occurs within 4 min or less (Newhook & Blackmore 1982; Gregory et al 2010).

3.1.2.6 Additional commercial considerations

Animal characteristics

The DTS: Diathermic Syncope® system has been validated on over 300 cattle ranging from 160 to 413 kg liveweight **(PR12).** The cattle were predominantly *Bos taurus*, beef, dairy and cross-bred types. Heifers, steers, cows and bulls were represented; some were horned, some naturally polled, and some dehorned. Five Brahman (*Bos indicus*) animals were included in the validation. Animal type does not influence efficacy of induction of unconsciousness, providing that the applicator could be placed in contact with the forehead with no air gaps. The applicator design has been refined during the course of development to maximise the range of forehead shapes that can be presented to the applicator.

Animals with very wide horns may not fit onto the restraint unit: this is also the case with existing restraint units for captive bolt or electrical stun application.

While the DTS can be used for any medium to large animal, applicators are designed to suit specific animal head types. As of December 2021, there are applicators that have been developed for various types of bovine. Custom applicators will need to be designed and validated for use with other animals, such as sheep or calves.

Penetration depth can be predicted based on the dielectric properties of the tissues involved (**SS04 – McLean et al., 2017**). For the theoretical scenario where a bovine's head consists of 5 mm of skin and 12 mm of bone, brain penetration depth is approximately 38 mm. Penetration depth can vary depending on the size and shape of the bovine head. Penetration depth also changes with frequency of the microwave energy applied. Frequency is fixed by the magnetron used. Heat is distributed through the brain mass via direct electromagnetic heating, conduction and via circulating cerebrospinal fluid and blood.

All animals processed to date have been lairaged, so the skin of the forehead has been dry. Some animals have had a layer of dew in the hair coat. This moisture rapidly evaporates when energy application begins and observations as well as calculations based on theory indicate that the presence of moisture has no influence on efficacy of induction of unconsciousness (**PR14**). There has been no evidence of differences related to thickness of hair coat.

Commercial throughput

Using a rotating box for animal restraint allows for a second animal to be loaded while the first (unconscious) animal is being exsanguinated. Under these conditions, animals have been processed at a rate of one each minute (60 per hr). The engineering team are working on an alternative design of box to reduce the footprint of the restraint unit and increase the speed of ejection of the unconscious animal from the restraint, with a view to achieving a processing rate of 2 per minute (120 per hr). This is in line with current processing rates in commercial beef abattoirs in Australia (60-120 per hr per restraint box).

3.1.2.7 Assessment of animal welfare when DTS is used

Table 1 outlines key definitions related to the stunning procedure and the animal welfare outcomes. The source of each definition is also provided.

Table 1: Definition of the relevant terminology having an impact on animal welfare assessment.

Term	Definition	Source
Pain	An unpleasant sensory and emotional experience associated with actual ot potential tissue damage, or described in terms of such damage. Pain may be caused by tissue lesions or by mechanical, chemical or thermal stimulation.	The International Association for the Study of Pain (IASP).
Distress	An aversive, negative state in which coping and adaptation processes fail to return an organism to physiological and/or psychological homeostasis (Carstens and Moberg, 2000; Moberg, 1987; NRC, 1992)	EFSA AHAW Panel 2020 – Welfare of cattle at slaughter
Loss of consciousness	Unconsciousness is a state of unawareness (loss of consciousness) in which there is temporary or permanent damage to brain function and the individual is unable to perceive external stimuli (which is referred to as insensibility) and control its voluntary mobility and, therefore, respond to normal stimuli, including pain (EFSA, 2004)	EFSA AHAW Panel, 2013 – Scientific Opinion on monitoring procedures at slaughterhouses for bovines
Loss of sensibility	See definition of unconsciousness: 'inability to perceive external stimuli' - According to Regulation 1099/2009, the **sensibility** of an animal is essentially its ability to feel pain. In general, an animal can be presumed to be	EFSA AHAW Panel, 2013 – Scientific Opinion on monitoring procedures at

Term	Definition	Source
	insensible when it does not show any reflexes or reactions to stimuli such as sound, odour, light or physical contact. In the context of this dossier, consciousness includes sensibility and unconsciousness includes insensibility.	slaughterhouses for bovines

Table 2: ABMs used to detect pain and fear during restraint and application of the DTS stun – Relevant to submitted studies

ABM used to detect pain and fear	Outcome of pain and fear during restraint and application of stun
Vocalisation	Vocalising response in terms of mooing, bellowing or roaring. Frequently referred to as distress vocalisation in the literature.
Voluntary movement	Kicking, tail swishing
Escape attempts	Attempts to go through, under, or over gates and other barriers. Head and neck stretched forward and either slightly raised above back, slightly lowered, or level with back (modified after Lanier et al., 2001)
Injuries	Tissue damage (bruises, scratches, broken bones, dislocations) (EFSA AHAW Panel, 2012)

Table 3: ABMs used to detect unconsciousness when DTS used (post-stun assessment) – Relevant to submitted studies

ABM used to detect unconsciousness	Outcome of unconsciousness
Posture	Permanent loss of posture. When animals are restrained in neck bails with chin capture, the forequarter is held in place and loss of posture is evidenced as a dropping of the hindquarters towards the floor of the box (hindquarter collapse).
Breathing	Absence of breathing or slower deep breathing. No change in breathing in response to slaughter
Tonic seizure	Stiff, rigidly extended legs; stiff extended neck.
Corneal reflex	Absence of corneal reflex
Ocular tracking	Eye does not follow movement of a hand passed in front of the eye.
Spontaneous blinking	No voluntary opening and closing of eyelids. Note: Involuntary flicking of the eyelids and third eyelid may be seen in the unconscious animal. Nystagmus (spontaneous rapid side-to-side movements of the eyeball) may rarely occur during application of DTS energy.
Body movement	No intentional or purposeful kicking or body or head movements as a response to incision of the skin and/or insertion of the knife.
Vocalization	No deliberate / effortful vocalisation. Note: Passive, low frequency sounds, coincidental with the exhalations of the breath may occur when the animal is in the inverted position, as air passes through the larynx.

3.1.3 Key parameters of the effective use of the method

DTS: Diathermic Syncope® uses electromagnetic energy to induce volumetric heating of the brain to a level that results in loss of consciousness (hyperthermic syncope, or 'fainting'). Hyperthermic loss of consciousness, or 'fainting' occurs in people when core body temperatures are in the range of 40-45 °C (Ohshima et al., 1992), while rats have been rendered unconscious (based on behavioural observations) after the brain temperature was elevated by 8 °C (Guy and Chou, 1982). Ikarashi et al. (1984) demonstrated that neurotransmission (based on Acetylcholinesterase activity) abruptly diminished in rodent brain tissue when the brain was heated to temperatures above 45 °C. Bench-top cadaver work conducted as part of the development of the DTS: Diathermic Syncope® system indicated that the temperature of cattle (**SS04 - McLean et al., 2017**) and sheep (**SS02 - Small et al., 2013**) brains could be raised by the required amount (5-12 °C) to achieve induction of unconsciousness. The microwaves are delivered at a frequency selected to maximise penetration into the substance of the brain and can be imagined as a column of energy entering via the applicator. Heat generated within that column is transferred throughout the entire brain via direct conduction, and we infer also through the circulation of warmed blood and cerebrospinal fluid. We expect that the skull acts as an insulator, reducing radiant heat loss and maintaining the increased brain temperature (**PR02**: **Report on Microwave Technology Development Stage 1**). The effect of heating was then confirmed using electroencephalography (EEG) in anaesthetised sheep (**SS02 - Small et al., 2013**) and anaesthetised cattle (**SS03 - Rault et al., 2014**); and subsequently in conscious cattle (**SS05 - Small et al., 2019**).

According to (EC) Regulation 1099/2009, key parameters are defined as the critical factors for ensuring proper stunning of all animals subjected to the stunning process. Table 4 provides a summary of the key parameters associated with the use of DTS and associated values of the key parameters where appropriate. The key parameters related to various existing methods are provided in Annex A of the EFSA guidance (EFSA, 2018). As DTS is a completely novel stunning method, key parameters have been established using a similar approach, using research to-date. References are provided to sections of this dossier, peer-reviewed publications, and project reports that contain further information and justification for the parameters. Further to the references to scientific studies (Steps), Table 6 provides a summary of the steps involving conscious animals stunned with DTS and some of the key parameters investigated and outcomes obtained.

Table 4: Parameters considered relevant for DTS stunning method.

Parameter	Component	Description	Reference
Position and application of the Applicator	Restraining system	Commercial rotating or tipping knocking box; Head restraint with chin lift is used which fixes the head and holds the neck in extension; Rear pusher may be used to help push smaller animals into the neck yoke;	Section 3.1.2.4
	Applicator	Purpose built device that interfaces directly onto the animal's head and directs DTS energy to the correct region.	Section 3.1.2.5 SS04

Parameter	Component	Description	Reference
	Applicator positioning	Applicator positioned directly over the frontal lobes of the brain. Operation prevented if the animal's head is not pressed firmly against the applicator.	Section 3.1.2.5
	Energy penetration	Energy penetration through the skin and bone into frontal lobes.	Section 3.1.2.5 SS04
Appropriate energy according to animal size and species	Microwave generator energy output	Subject to operator set-up of power level and time duration.	Section 3.1.2.5 SS05, PR08, PR12
	Safety mechanism	Safety interlocks throughout include: microwave leakage monitors in appropriate areas, screened animal stunning room (faraday cage), interlocks on all doors and openings. Warning lights fitted on the cage and generator. Auto cut-off mechanism which operates if the applicator is not in contact with the animal's head.	Section 3.1.2 Section 3.1.2.4
	Animal Type (e.g., beef or dairy cattle), size of animal, horned or polled	Validated on cattle ranging from 160 to 413 kg liveweight. Bos taurus, beef, dairy and cross-bred types. Heifers, steers, cows and bulls were represented; some were horned, some naturally polled, and some dehorned. Five Brahman (Bos indicus) animals	Step 11, 3.2.3.1 PR12
	Minimum energy (KJ)	160 kJ for simple stun	SS05; PR08, PR12 Section 3.1.2.5 Step 11, step 3.2.3.1 Part 2 Step 13, 3.2.3.1 SS05; PR08, PR12
	Animal skin condition	Wet, dry or damp	Section 3.1.2.5 Step 14
	Depth of penetration	A function of the animal's skin, bone and brain thickness and ability to absorb the applied energy. Energy frequency will impact the penetration depth. Energy frequency is fixed by the magnetron used.	Step 2 SS04
Duration of intervention	Minimum time of exposure	Related to incident power. Recommend 18-20 kW, so approximately 9 seconds to achieve 160 kJ energy applied.	Section 3.1.2.5 Step 7, 3.2.3.1 Step 9, 3.2.3.1 Step 11, 3.2.3.1 Part 2 Step 13, 3.2.3.1 PR12

Parameter	Component	Description	Reference
Equipment maintenance, cleaning and storage conditions		**Generator Care** *Daily:* Remove any build-up of rubbish from within and around the generator. Ensure that all air intakes and outlets are clear. *Weekly:* Check water flow and arc detection devices are functional. Check for water leaks inside the generator. Check and replace (if required) air filters on generator side walls. *Monthly:* Check generator doors, limit switches and adjust to suit door stroke. Replace/wash air filters on generator side walls. **Applicator Care** *Daily:* Wipe out any moisture, dust or hair Visually inspect for cracks or other damage *Weekly:* Check waveguide and applicator for loose bolts and connections **Restraint Unit Care** *Daily:* Wash out any faecal matter, blood, hair or excreta Visually inspect for cracks or other damage Grease moving parts Visually inspect for damage Visually inspect Faraday Cage for damage	Section 3.1.2.3
Frequency of calibration of the equipment		Calibration of the auto-tuner is required every 2 years (manufacturer's recommendation). Assessment of tuning should be carried out by a specialist technician annually, or in the event of a change in size of the applicator (e.g., for use on another species)	Section 3.1.2.3
Maximum stun to stick interval(s)		Maximum stun to stick interval of 60s.	Section 3.1.4 Step 7, 3.2.3.1 Step 9, 3.2.3.1 Step 11, 3.2.3.1 Parts 1 and 2 Step 13, 3.2.3.1

Parameter	Component	Description	Reference
			SS05; PR08, PR12

3.1.3.1 List of related specifications (as per Annex I of EC 1099/2009)

Name	Description	Conditions of use	Key Parameters
DTS: Diathermic Syncope®	Exposure of the brain to electromagnetic energy generating an increase in brain temperature and a generalised epileptic form on the electro-encephalogram (EEG). Simple stunning.	Cattle, sheep. Slaughter	Minimum energy (kJ). Minimum power (kW). Maximum stun-to stick interval(s) in the case of simple stunning. Position and contact of applicator with animal's forehead.

Recommended minimum energy for simple stunning of cattle is 160 kJ for simple stun.

Recommended minimum power for cattle is 16 kW.

Recommended maximum stun-to-stick interval(s) in the case of simple stunning is 60 seconds.

The applicator shall be positioned on the frontal bones, directly above the brain .

On installation, the applicator shall be selected to suit the size of the animal processed in that facility.

3.1.4 Scientific basis of induction and maintenance of unconsciousness

The purpose of the EU Slaughter Regulation 1099/2009 is to avoid pain and suffering during stunning and slaughter. Regulation 1099/2009 requires that stunning method should induce immediate loss of consciousness. If the loss of consciousness is not immediate, then the onset of unconsciousness needs to occur without causing avoidable pain and suffering. Council Regulation (EC) No 1099/2009 also states that unconsciousness and insensibility induced by stunning should last until the animal is dead. The submitted studies characterize the animals' responses (unconsciousness, absence of pain) using the most sensitive and specific methods available (e.g., electroencephalography (EEG), blood samples) and establishes the correlations between these measurements and non-invasive parameters (e.g., Behavioural observations) that can be applied in abattoirs (EFSA, 2018).

For DTS, the aim is to induce insensibility through selective heating of the brain tissue to a temperature above which neurotransmission cannot occur. Thermal unconsciousness, such as that induced by exercise heat stress or fever, is reported to occur when core body temperatures reach between 40 and 45 °C (McDaniel et al., 1991; Ohshima et al., 1992; Mohanty et al., 1997; Roccatto et al., 2010; Lerman et al., 2014; Yoshizawa et al., 2016; Hjeresen et al., 1983). Under hyperthermic conditions, the extensive circulatory network of the brain assists in maintaining brain temperatures 1-2 °C below core body temperature (Hjeresen et al., 1983), suggesting that thermal unconsciousness is achieved when the brain temperature reaches between 39-44 °C. Where destruction of neurological tissue is intended (e.g., for ablation of neoplastic material), temperatures above 43 °C for a duration of one hour are utilized (Ryan et al., 1994), and it appears that the maximum thermal tolerance of nervous tissue lies in the range of 40–60 min at 42 °C, or 10–30 min at 43 °C (Sminia et al., 1994; Fike et al., 1991; Lyons et al., 1984). Brain metabolism and neurotransmitter activity is affected by temperature. In rats, at a brain temperature of 41 °C (5.4 °C above control brain temperature of 35.6 °C), adenosine triphosphate (ATP) concentration was reduced by 29.2 % and creatine phosphate (CP) concentration was reduced by 44 % (Sanders and Joines, 1984). In the same study, nicotinamide adenine dinucleotide (NADH) fluorescence increased during microwave application and returned to baseline levels within one minute after cessation of energy application. Another study (Ikarashi et al., 1984) demonstrated that there was a rapid reduction in acetylcholinesterase activity in rat brains once a temperature of 50 °C was reached.

Experimental work has shown that, using 2 to 10 kW of 915 MHz microwave radiation, rats could be stunned (Guy & Chou, 1982). It was demonstrated that the animals would be unconscious after a brain temperature rise of 8 °C and would remain unconscious for 4 to 5 min post exposure. Consciousness was regained when brain temperature returned to within 1 °C of normal values. Also, Lambooy et al. (1989) successfully induced unconsciousness in rats using a low-power microwave (2 kW): application for 1.5–2.0 s resulted in unconsciousness in 100 % of the rats tested. In cattle, the core body temperature tends to be 1-2 degrees higher than in humans (38.6 °C in resting cattle, 37 °C in humans), so we expect that in cattle thermal unconsciousness would occur in the range 42-47 °C. Thus, the required rise in temperature to achieve insensibility is in the range of 3.4 to 8.4 degrees. During development and testing of the DTS equipment utilised in this study, the temperature profiles of cadaver heads was tested, and a predictive equation describing the heating rate within the brain was generated (0.124 °C/kW.sec). From this equation, we can infer that for a 30 kW application, the time taken to achieve an 8 degree rise in temperature would be 2.15 sec (8/30/0.124); and for a 20 kW application, the time taken would be 3.23 sec (8/20/0.124). Both these time intervals are less than the time taken (4-8 sec) to observe physical changes through the camera installed within the faraday cage. In a commercial situation, the real-time monitoring system incorporated within the control panel will allow an objective record of energy delivered and rate of energy delivery to be maintained, and an alarm feature could be incorporated to use in conjunction with animal-based indicators (e.g., response to reflexes) when confirming insensibility prior to exsanguination.

Microwave irradiation is an accepted method of euthanasia of laboratory rodents (AVMA, 2013), and microwave heating is commonly used to inactivate enzymes and fix brain tissue for histological purposes (Moroji et al., 1977). Suggested microwave energy inputs for this application in mice are 3.2 kW for 1.1 s (Cosi and Marien, 1998) and 2.5 kW for 0.68 s (Nordgren et al., 1985), resulting in a brain temperature of 75–90 °C. In rats, Delaney and Geiger (1996) used 10 kW for 1.25 s to achieve a brain temperature of 85 °C, while Ikarashi et al. (1984) used 10 kW irradiation at 2,450 MHz to achieve a brain temperature of 90 °C in less than 900 ms in rats, and Zeller et al. (1989) also euthanized chickens in less than 1 s using microwave irradiation at 2,450 MHz. During Development of the DTS: Diathermic Syncope® system, sheep and cattle have been successfully rendered unconscious though application of microwave energy at 922 MHz (**SS03-**Rault et al., 2014; **SS02-**Small et al., 2013; **SS05-**Small et al., 2019).

In the early stages of development, the risk of overheating of the skin of the forehead prior to induction of insensibility was identified. The current apparatus, when properly tuned and incorporating the auto-tuner system, directs the majority of the energy deep into the brain tissue, so that insensibility occurs rapidly (within 1-8 s of energy application), and heating of the skin is minimised. In rats, a transient skin and muscle necrosis was observed when the tissues were maintained at 44-45 °C for one hour; while maintaining the spinal cord at a temperature of 42.6 °C for one hour led to no neurological effects, and some ataxia was noted after the spinal cord was held for one hour at 43.0 °C (Franken et al., 1992).

3.1.5 Potential causes of system failure and chances of occurrence

Potential causes of system failure were discovered during the simultaneous development of the system with animal applications (3.2 introduction to Description of the individual scientific studies). In general terms, potential causes of system failure could only be solved sequentially, starting with the generation of microwaves, the transfer of these to the applicator, and from the applicator to the animal's head, for sufficient time to deliver the required energy to heat the brain. The potential causes of system failure and the chance of occurrence are summarised in Table 5.

The gradual gentle induction of stunning effect with DTS: Diathermic Syncope(R), as evidenced by absence of ABMs related to the experience of pain and distress (in restraint or during induction), means that, even though the system may fail during application, the welfare consequences are likely to be minimal.

Table 5: Potential causes of system failure, likelihood of occurrence and correction

Problem	Cause	Likelihood	Action
Power outage	Infrastructure failure	Rare	Wait till power returns, then run the Start-Up-Sequence.
Door interlocks not activated	Operator error	Possible	Check that doors are fully closed, then re-start application.
Energy not delivered	Failure of coaxial cable	Rare	Replacement of cable
Incorrect waveguide positioning	Operator error	Possible	Reposition waveguide, then re-start application
Electrical fault in generator cabinet	Component failure	Rare	Detailed diagnostics through user control panel. Work with tech support and electrician to fix.
Component failure	Plant failure	Rare	Detailed diagnostics through user control panel. Work with tech support to fix.

3.1.6 Section 3.1 References

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3.2 Description of the individual scientific studies submitted

DTS: Diathermic Syncope® was developed over several years, through multiple steps (**figure 12**) as the technology was explored, refined, and data collected to validate it as a method for simple stunning at the time of slaughter. Giving consideration to the ethical treatment of animals in research, development of the system of intervention and measurement of outcomes occurred simultaneously. The outcomes determined included:

- onset of unconsciousness and insensibility
- absence of pain, distress and suffering
- duration of unconsciousness and insensibility

The description of each step and provision of scientific studies and supplementary materials, provides transparency of the development process, but not every step applying DTS to cattle provided data to reliably set parameters for its operation, since the intervention system was not operating under the final operating parameters. In the steps of the development process conducting trials on conscious animals key operating parameters (listed in 3.1.3.1) were being determined, against these animal welfare outcomes and the causes of equipment failure were being assessed (Table 6). Only in later studies were intervention parameters reliably delivered and therefore useful for measuring and contributing to the determination of key operating parameters. Through the technical development process, assessment was made of the first stun effectiveness and reasons for ineffective stun or compromised animal welfare (Table 8).

Table 6: Determination of key operating parameters (refer to Figure 12 for identification of the steps) This table indicates whether input parameters were reliably delivered and therefore whether output parameters measured contribute to the recommendations for inclusion in regulation and acceptability of the stunning method

Step	Equipment development	Input parameters		Method of measuring welfare	Outcome parameters for ≤ 200kJ and ≤ 20kW		
		Energy (indicative) kJ	Power (indicative) kW		Time to unconscious/ insensibility	Duration unconscious	First stun success rate
7	L	200-300	20-30	EEG	N=2 Immediately following application	N=2	
9	L-M	200-375	20-30	ABM skin temp	N=2 4-6s for reaction, 8s for LoP	N=2 >200s	
11.1	M-H U	160-240	20-35	ABM	N=6 13s to LoP	N=10 mean 114s	N=187 96.8%
11.2	H R	140-200	18-25	ABM	N=30, 2s to first reaction	N=29 mean 81s	n=35 94.3%
13	C R	140-180	18-20	EEG ABM Brain histology	n=27 5s to tonic/clonic	n=27 mean 71s	N=27 92.6%

Key:
Low, Medium, High, Commercial operation of the DTS generation/delivery system, U=upright box, R=rotary box, LoP loss of posture

Table 7: Ethogram of behaviours and physical activity

Behavioral observation	Aspects recorded	Parameter derived for analysis
Initial physical response (tensing muscles, eye either opens wide or shut tight)	Time of occurrence	Used as the reference data point
Eyelid flutter/involuntary blinking	Onset and offset	Latency from initial physical response Duration
Ear flicking/involuntary movements	Onset and offset	Latency from initial physical response Duration
Nose twitching	Onset and offset	Latency from initial physical response Duration
Convulsive movements	Onset and offset	Latency from initial physical response Duration
Return of deliberate/voluntary blink	Time of occurrence	Latency from initial physical response
Assumption of rigid tonic posture, with all four legs rigidly extended	Time of occurrence	Latency from initial physical response
Initial awareness of movement in surroundings evident	Time of occurrence	Latency from initial physical response
Drowsing with eyelids drooping, leaning heavily into neck yoke of restraint	Onset and offset	Latency from initial physical response Duration
Return of corneal reflex	Time of occurrence	Latency from initial physical response
Standing unsupported	Time of occurrence	Latency from initial physical response
Return of full alertness	Time of occurrence	Latency from initial physical response

Table 8: First stun effectiveness and reasons for ineffective stun or compromised animal welfare

Step	DTS delivered	DTS stun application	% first stun***	Reason for ineffective stun or compromised animal welfare (%)*							
				a	**b**	**c**	**d**	**e**	**f**	**g**	**h**
7**	11	10	**90.9**		63.7				**9.1**		18.2 [1]
9**	20	17	**85**		10 [2]				**15**+20		70 [1]
11.1**	203	187	**96.8**		19.5 [4]	**3.2**	2.1		2.1	2.7 [3]	0
11.2**	35	33	**94.3**		0	0	**2.85**[5]		**0**	**2.85**	0
13	27	25	92.6		0	0	**7.45**[5]		0	0	0

% in BOLD – stun or failure to stun – add up to 100% ; other % may contribute to adverse animal welfare without contributing to a failure to stun

* Reasons: a) Lack of skilled operators; b) Overheating of the skin (blistering) of the forehead prior to the induction of unconsciousness.; c) Incorrect key parameters: The key parameters fail to effectively stun and render animals unconscious within a reasonable time.; d) Wrong placement of the applicator: The position of the applicator does not span the brain to induce immediate unconsciousness or death. This could lead to pain in conscious animals.; e) Overheating of the applicator: Repeated use in quick succession will lead to overheating of the applicator and, as consequence, it will be difficult to apply correctly, or will cease to function properly.; f) Energy leakage is reported to be a problem; g) Too short exposure time: the duration is too short to cause unconsciousness. ; h) Prolonged stun-to-kill interval: The interval between stunning and bleeding is too long so animals could recover consciousness and experience pain and fear

** * Noting the limitations of equipment development discussed in 3.2 (Table 6)

*** achieving unconsciousness through DTS treatment in the head restraint prior to release

[1] more than 82 seconds after stunning

[2] the average surface temperature on the forehead at the point of loss of posture was estimated to be between 26 and 49 °C, although 2 animals treated with 250-275kJ @ 30kW and showed a delayed loss of posture also demonstrated hot-spots of up to 109 °C

[3] low Power (kW) settings, which were used during the study to determine the lower critical limits of expected operational parameters

[4] When power levels were 25kW and above

[5] Lack of experience of the operator may contribute to poor placement of the applicator

Year	Step	Nature of step: Laboratory / development	Nature of step: Science	Nature of step: Practice
2004-05	STEP 1: Literature review		✓	
2006-09	STEP 2: Laboratory benchtop cadaver evaluations	✓		
2009-10	STEP 3: Laboratory scale: Anaesthetised sheep study (Prototype 1)		✓	
2011-12	STEP 4: Laboratory engineering: Development of applicator and auto-tuner	✓		
2012-13	STEP 5: Laboratory scale: Anaesthetised cattle study (Prototype 2)		✓	
2013-14	STEP 6: Laboratory engineering: Refinement of delivery apparatus and development of user interface	✓		
2015	STEP 7: Laboratory scale: Pilot study on conscious cattle (Prototype 3)		✓	✓
2015-19	STEP 8: Laboratory engineering: Refinements to delivery system: applicators and coaxial cabling	✓		
2018	STEP 9: Laboratory scale: Validation study on conscious cattle (Prototype 4)		✓	✓
2018-21	STEP 10: Laboratory engineering: Refinement to delivery systems: applicators, coaxial cabling and tuning	✓		
2019-21	STEP 11: Commercial validation			✓
2021	STEP 12: Regulatory Authority review	✓		
2021-22	STEP 13: Further validation of stunning and recovery			✓
2023	STEP 14: Wet scalp and energy transfer	✓		

Figure 12 DTS: Diathermic Syncope®: Flowchart representation of development timeline, identifying the nature of each step

The **scientific studies submitted** are outlined in Tables 9 and 10. A list of supplementary information is also included. Figure 13 indicates which Steps contributed to each of the submitted peer-reviewed publications.

Table 9: Peer reviewed publications

ID	Author	Title	Journal	Status
SS01	Small, A., McLean, D., Owen., J. S., Ralph, J.	Electromagnetic induction of insensibility in animals: a review	Animal Welfare 22: 287-290	Published 2013
SS02	Small, A., McLean, D., Keates, H., Owen., J. S., Ralph, J.	Preliminary investigations into the use of microwave energy for reversible stunning of sheep	Animal Welfare 22: 291-296	Published 2013
SS03	Rault, J.-L., Hemsworth, P., Cakebread, P., Mellor, D., Johnson, C.	Evaluation of microwave application as a humane stunning technique based on electroencephalography (EEG) of anaesthetised cattle	Animal Welfare 23: 391-400	Published 2014
SS04	McLean, D., Meers, L., Ralph, J., Owen, J. S., Small, A.	Development of a microwave energy delivery system for reversible stunning of cattle	Research in Veterinary Science 112: 13-17	Published 2017
SS05	Small, A., Lea, J., Niemeyer, D., Hughes, J., McLean, D., Ralph, J.	Development of a microwave stunning system for cattle 2: Preliminary observations on behavioural responses and EEG	Research in Veterinary Science 122: 72-80	Published 2019
SS06	Hughes, J., Small, A.	A comparison of beef carcases stunned using DTS: Diathermic Syncope® or captive bolt in terms of selected meat quality attributes and plasma biomarker concentrations	Animal Review 9(1): 13-23	Published 2022

Table 10: Project reports - not peer reviewed - CONFIDENTIAL

ID	Title	Date	Status
PR01	SRP.003 Investigate the Use of Microwave Technology for Stunning Beef.	2005	CONFIDENTIAL
PR02	Report on Microwave Technology Development Stage 1.	2009	CONFIDENTIAL
PR03	Microwave induced insensibility for animals Stage 2.	2010	CONFIDENTIAL
PR04	Development of applicator and autotuner	2011 - 2012	CONFIDENTIAL
PR05	Evaluation of microwave application as a humane stunning technique based on electroencephalography (EEG) of anaesthetised cattle.	2013	CONFIDENTIAL
PR06	Microwave results from the live testing conducted [redacted] Interim project report	2012	CONFIDENTIAL
PR07	Refinement of delivery apparatus and development of user interface software.	2014	CONFIDENTIAL
PR08	Dielectric induction of temporary insensibility in cattle - animal trials.	2015	CONFIDENTIAL
PR09	Meat quality from microwave stunned cattle.	2015	CONFIDENTIAL
PR10	Refinements to delivery system: applicators and coaxial cabling. Interim project report.	2016 - 2019	CONFIDENTIAL
PR11	DTS: Diathermic Syncope - commercial validation trials.	2018	CONFIDENTIAL
PR12	DTS: Diathermic Syncope® controlled trials.	2021	CONFIDENTIAL
PR13	Refinements to delivery system: applicators, coaxial cabling and tuning.	2018 - 2021	CONFIDENTIAL
PR15	Repeatability of induction of insensibility in cattle using DTS: Diathermic Syncope®; and recovery of consciousness from DTS-induced insensibility	2023	CONFIDENTIAL
PR16	Wet scalp impact on energy transfer to the head	2023	CONFIDENTIAL

Supplementary material

SUP1: Conference material. Evaluation of microwave application as a humane stunning technique based on electroencephalography. International Society for Applied Ethology 2013. Peer-reviewed. PUBLIC.

SUP2: Conference material. DTS®: a novel method for stunning cattle. Impacts on cortisol response and post slaughter meat quality attributes. Pan-Commonwealth Veterinary Conference 2015. Peer-reviewed. PUBLIC.

SUP3: Conference material. Meat quality attributes of beef carcases slaughtered using DTS: Diathermic Syncope®. International Congress of meat Science and Technology 2015. Peer-reviewed. PUBLIC.

SUP4: Conference material. DTS®: Diathermic Syncope for cattle stunning. Humane Slaughter Association 2015. Peer-reviewed. PUBLIC.

SUP5: Conference material. DTS: Diathermic Syncope® - induction of insensibility in cattle. Presentation Slides used for a number of stakeholder meetings. Not Peer-reviewed. PUBLIC.

SUP6: Conference material. DTS: Diathermic Syncope® - a new technology for pre-slaughter induction of insensibility. Royal Society for the Prevention of Cruelty to Animals Australia Conference 2018. Peer-reviewed. PUBLIC.

SUP7: Conference material. Applicator development for animal stunning. AMPERE Conference 2017. Peer-reviewed. PUBLIC.

SUP8: Factsheet. DTS: Diathermic Syncope®. Not peer-reviewed. PUBLIC.

SUP9: Conference material. DTS: Diathermic Syncope® - A new technology for pre-slaughter induction of reversible unconsciousness. North American Meat Industry Animal Care Conference 2021. Peer-reviewed. PUBLIC.

SUP10: Conference presentation recording. DTS: Diathermic Syncope® - A new technology for pre-slaughter induction of reversible unconsciousness. North American Meat Industry Animal Care Conference 2021. Peer-reviewed. PUBLIC.

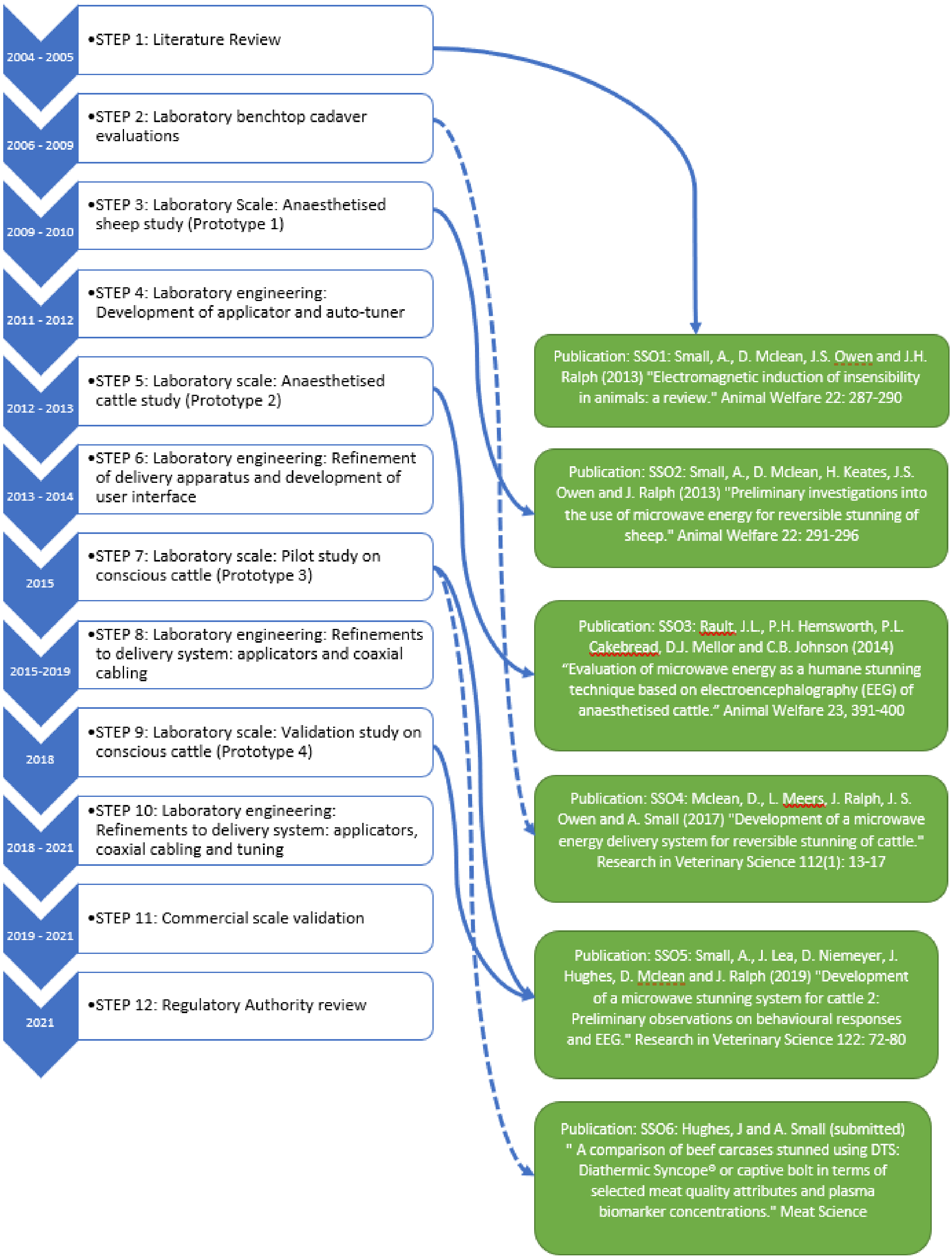

Figure 13DTS: Diathermic Syncope®: Flowchart representation of development timeline and scientific publications

STEP 1: Literature review

Submitted study: **SS01** Electromagnetic induction of insensibility in animals: a review.

Other reference documents: **PR01**: SRP.003 Investigate the Use of Microwave Technology for Stunning Beef. Project Report. Not peer-reviewed. CONFIDENTIAL. The Literature review component was revised and updated in 2013, for publication in a peer-reviewed journal (**SS01**).

3.2.1 Introduction

3.2.1.1 Background and rationale

Some communities require that animals processed for human consumption are healthy, uninjured, and normal at the moment of carrying out the slaughter cut. As a result, many methods of stunning used in modern commercial slaughter are not acceptable, because the animals could be considered injured, or because a proportion of animals would not recover from the stun. Head-only electrical stunning is used in sheep but is thought to be unsuitable for use in cattle because of concerns over the duration of insensibility being insufficient to allow death through total blood loss to occur prior to recovery from the stun (Blackmore & Newhook, 1982; Lambooy & Spanjaard, 1982; Anil et al., 1995a; Anil et al., 1995b), and also because of problems with blood splash in the meat (Gregory, 2005). The possibility of using microwave energy to heat the brain and thereby induce unconsciousness was suggested, leading to a scoping review being conducted.

3.2.1.2 Objective

To undertake a scoping review of scientific literature and patents and to consult with microwave engineers on the potential for using microwave energy for inducing unconsciousness in cattle.

3.2.2 Materials and methods

3.2.2.1 Method

Literature searching was carried out using the CSIRO library service databases, e.g., Web of Science® Science Collection and Derwent Innovations Index. Consultation with Microwave engineers at the University of Melbourne was carried out.

The review was neither a systematic review nor a meta-analysis. As such the reporting quality was not according to the PRISMA Statement (Moher et al., 2009). Neither are the criteria listed in the ARRIVE guidelines (Kilkenny et al., 2010) applicable to this study:

- Study population,
- Sampling strategy,
- Ethical considerations,
- Experimental design,
- Randomisation and blinding,
- Reporting data quality (If applicant uses external data),
- Reporting the methods of analysis.

3.2.2.2 Measurement of the outcome

Not relevant to this step:

- Onset and duration of unconsciousness and time to death,
- Magnitude of pain, distress and suffering.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

Not applicable to a non-systematic literature review.

3.2.3.2 Reporting uncertainty

Not applicable to a non-systematic literature review. Potential sources of bias lie in the literature databases used (not reported) and inclusion and exclusion criteria for the search (not reported). Based on the authorship of PR01, it is likely that articles in a language other than English were excluded; in SS01 articles in English, German and French would have been included.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

PR01: From extensive studies with laboratory rodents there appears no doubt that microwave irradiation can provide a humane stun with unconsciousness (caused by a brain temperature increase of 8 – 10 °C) being induced within milliseconds rather than seconds. Microwave irradiation can produce a reversible stun in rodents with the heart remaining beating and subsequent return of normal consciousness. This form of stun would appear to satisfy the principles of Halal slaughter. As with all reversible stunning methods, the stunning parameters and the subsequent sticking (exsanguination) procedures would need to be finely tuned to ensure welfare issues were addressed.

The incidence of convulsions and seizures following microwave irradiation of rodents is variable. It does seem possible for a successful stun to occur without convulsions. Similarly, the incidence of haemorrhages is variable. Microwave irradiation does not appear to invariably cause the intense generalized muscle body contractions which occur during electrical stunning and which are believed to be involved in the production of blood splash. While microwave radiation can cause thermal damage to the brain, it seems highly unlikely that there would be mechanical damage sufficient to cause release and subsequent transport of any BSE material which might be present in the brain.

Based on extrapolations of very limited data for microwave stunning of sheep and pigs available at the time of the review, it would appear that a ballpark figure for the energy requirements of a microwave generator for stunning cattle might be 100 kW. This is not a totally unrealistic proposition as presently 100 kW and 300 kW units are being trialled for use in the Australian timber industry.

SS01: Early attempts to induce insensibility and death in laboratory species were successful, but the technology to apply the technique to larger animals was not available at that time. More recently, however, technological advances have led to new work in the areas of transcranial magnetic stimulation and microwave irradiation, both of which are potential methods of inducing a recoverable stun in larger species.

3.2.5 Conflicts of interest

No conflicts of interest were reported. The authors of PR01 have no commercial interest in the DTS: Diathermic Syncope® technology. The authors of SS01 are listed on the patent (WO2011137497), registered in 2011. [redacted] is the licensee of the technology, all other authors have no commercial interest in the technology.

STEP 2: Laboratory benchtop cadaver evaluations:

Submitted studies:

SS02 Preliminary investigations into the use of microwave energy for reversible stunning of sheep.

SS04 Development of a microwave energy delivery system for reversible stunning of cattle.

Other reference documents: **PR02**: Report on Microwave Technology Development Stage 1. Project report. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

The scoping study described in **SS01** indicated that induction of unconsciousness in large animals may be feasible. Previous literature indicated that rats could be stunned successfully using 2–10 kW of 915 MHz microwave radiation (Guy and Chou, 1982). It was demonstrated that the animals would be stunned after brain temperature rise of 8 °C and would remain unconscious for 4-5 mins post exposure. The regaining of consciousness occurred when brain temp dropped to within 1 °C of normal values. In other studies, the figures of required brain temperature rise vary. Temperature increases required range from 4 °C (Schwartz, 1974) to 10.3 °C (Lambooy et al., 1989).

The next stage in development of the DTS: Diathermic Syncope® system was to understand in greater detail the dielectric properties of the tissues to be irradiated (brain, bone and skin), and then to conduct benchtop evaluations of the ability of microwave irradiation to selectively raise brain temperature. Two key parameters for evaluation were the temperature rise in the brain required to induce unconsciousness in the animal, and the length of time that unconsciousness could maintain effect.

There were three stages to this study: A literature review; an initial comparison of two microwave frequencies in terms of depth of penetration and heating in different parts of the head; and an evaluation of the heating profiles in different parts of the head.

3.2.1.2 Objective

Literature review objective: to outline any major findings or achievements in the field of biological microwave heating and from the literature highlight likely challenges for the development of a stunning system for cattle and sheep in an abattoir.

Stage 1 experimental work objective: to determine the optimum microwave frequency for future processing and determine the extent of heating at a range of locations around the head.

Stage 2 experimental work objective: to gain initial estimates into the depth of microwave penetration into the brain and magnitude of subsequent heating occurring through the brain and at skin level.

3.2.2 Materials and methods

3.2.2.1 Method

The research in this step was engineering and design work and did not include in vivo animal experiments. As such, the criteria listed in the ARRIVE guidelines (Kilkenny et al., 2010) are not relevant to this step:

- Study population,
- Sampling strategy,
- Ethical considerations,
- Experimental design,
- Randomisation and blinding,
- Reporting data quality (If applicant uses external data),
- Reporting the methods of analysis.

Literature searching was carried out using databases such as Web of Science®, Scopus® and Google Scholar®.

<u>Stage 1 experimental work</u>

A test apparatus was built, which delivered energy from the generator to an applicator point, via hollow rectangular tubing called waveguide, through a series of components designed to optimize energy transmission. The generator contained the power supply and magnetron and could provide microwave power output levels of between 0–30 kW at a frequency of 922 MHz and a separate generator could supply 0–5 kW at 2.45 GHz. From the generator, energy was passed through a circulator, which eliminates problems associated with reflected energy. If microwave energy is allowed back into the launcher the reflected energy can be reabsorbed by the magnetron causing premature ageing and hence device failure. The circulator acts as a one-way valve allowing energy to pass from the magnetron to the rest of the circuit but prevents any reflected energy from being returned to the magnetron. The reflected energy is directed into the dummy load and is dissipated into a constant flow of water. The circulator was followed by a directional coupler, which enables the measurement of the forward and reflected powers at that point. The energy then passed through a tuner, which allows a wide range of varying load/applicator impedances to be matched to the microwave network. Reflected power is due to impedance mismatches and the tuner acts as a matching element allowing an impedance transformation to take place, thus reducing the reflected power and improving the net power transfer to the applicator/load. From the tuner, the energy is directed through the applicator into the test load. For this study, the test loads were cadaver cattle and sheep heads, sourced from a local abattoir, stored frozen until required for testing and defrosted overnight prior to testing.

Fiberoptic temperature probes (Neoptix, Canada) were inserted into the frontal skin immediately below the waveguide; nose; neck skin behind the jaw, ear (under the skin), eye (subconjunctival); and five locations within the brain to allow measurement of temperature immediately before and immediately after energy application. Two sheep heads were irradiated at 2.45 GHz, two sheep heads at 922 MHz, and eight cattle heads at 922 MHz.

Stage 2 experimental work

The same test apparatus as in stage 1 was used, applying energy at 922 MHz. Temperatures were continuously monitored using fiberoptic probes placed on and under the skin of the frontal area and in the brain at superficial, middle and deep locations. Four sheep heads and 12 cattle heads were irradiated, varying power applied and exposure time. For sheep, power levels varied from 3 to 6 kW and exposure times from approximately 10 to 20 seconds. For cattle heads, power levels were varied from 2.5 to 9kW and exposure times from approximately 15 to 60 seconds.

3.2.2.2 Measurement of the outcome

Not relevant to this step:

- Onset and duration of unconsciousness and time to death,
- Magnitude of pain, distress and suffering.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

Literature review

Two key parameters for development of a system for stunning animals will be the temperature rise in the brain required to stun the animal, and the length of time that stunning maintains effect. Experimental work has shown that using 2–10 kW of 915 MHz microwave radiation, that rats could be stunned successfully (Guy and Chou, 1982). It was demonstrated that the animals would be stunned after brain temperature rise of 8 °C and would remain unconscious for 4-5 mins post exposure. The regaining of consciousness occurred when brain temp dropped to within 1 °C of normal values. Figures of required brain temperature rise vary within literature. Temperatures required range from 4 °C (Schwartz, 1974) to 10.3 °C (Lambooy et al., 1989).

A wide range of microwave delivery apparatus were used in the literature studied. Applicator types include single antenna arrangements for poultry (Takamura and Ishida, 1997), multiple antennae for pigs (Lambooy et al., 1989), singular waveguide (Maruyama et al., 1986), and semi-circular waveguide arrays (Schwartz, 1992). Merritt et al. (1977) found that ensuring the electric field element of the radiation is parallel to the centre line of the head created much higher coupling rates. The microwave delivery system developed for stunning animals will be critical to the success of the technology, as it will affect energy coupling efficiency and focusing of the energy into the brain, without excessive external skin heating.

Basic theory regarding dielectric properties as relevant to stunning of animals was also collated and summarized. The dielectric properties of any material are the main parameters that influence how microwave energy is coupled into the material and how efficiently this energy is then converted into thermal energy. These properties are described by the following complex equation:

$$\varepsilon^* = \varepsilon' + j\varepsilon'' \text{ (Equation 1)}$$

The real part of the equation (ε'), the dielectric constant, represents the ability of the material to store energy when a microwave field is applied. The imaginary part of the equation (ε''), the loss factor, determines how readily the microwave energy is converted into heat within the material. The penetration depth refers to a wave passing into a dielectric material, whose amplitude is diminishing owing to power absorption as heat into the material. The wave's field intensity and power flux density fall exponentially with distance from the surface. The rate of decay is a function of the complex dielectric constant ε, which comprises the real component ε' and the loss factor ε'' as shown in Equation 1.

The penetration depth is defined as the depth into the material at which the power flux has fallen to 37 % (1/e) of its value on the surface. penetration depth is proportional to the wavelength of the incident microwave energy, thus higher frequencies will achieve lower penetration into the workload given the dielectric properties are similar at both frequencies. In commercial applications in Australia the two frequencies available are 2.45 GHz and 922 MHz.

<u>Stage 1 experimental work</u>

Sheep heads irradiated at 2.45 GHz: little heating occurred in the five brain probe locations compared to the skin, and that the nose, eye, ear and neck temperature rose by similar amounts to the brain. The minimal microwave penetration into the brain caused only slight heating; confirming the dielectric theory was accurate. These results indicated that the 2.45 GHz frequency would not be suitable for this application.

Sheep heads irradiated at 922 MHz: there was far greater temperature rise within the brain compared with irradiation at 2.45 GHz. The brain temperatures rose by similar amounts to that of the skin. This increased heating of the brain is an effect of the higher penetration achieved at this lower frequency as was predicted by the dielectric theory. No significant heating of the neck, eyes or nose regions occurred.

Cattle heads irradiated at 922 MHz: similar results were obtained to those achieved with the sheep heads. Almost all the heating occurred in the brain and on the head directly above it. The cow head displayed slightly less penetration (compared with sheep) into the lower parts of the brain due to larger brain and skull size.

<u>Stage 2 experimental work</u>

Sheep heads: Heating rates of each area were linear for all test runs. The skin experienced greater heating than the brain and heating rate decreased with depth into brain. This correlates to what the dielectric data predicted. Upper brain temperature change was 40 % of skin temperature change and that small reduction in heating was observed in the lower parts of the brain. These results show significant potential for 922 MHz microwave energy to penetrate and heat the brain effectively and consistently.

Cattle heads: The cattle temperature profiles largely showed linear temperature changes. The brain heated slower than the skin on top of the head and the upper part of the brain heated by more than the lower parts as was predicted from dielectric data. Upper brain temperature change was 20 % of skin temperature change and that significant reduction in heating was observed in the lower parts of the brain; 12 % and 4 % of skin temperature change for the middle and lower brain respectively.

The results for both sheep and cattle irradiation provided data from which approximate power-time curves could be formulated. These curves plot predicted power required versus desired process time for a given brain temperature rise.

The curves illustrate that cattle treatment requires much more power than sheep for equivalent brain temperature rise. This was largely caused by the higher degree of absorbed power into the thicker skin and bone layers of the cattle heads coupled with larger brain size. The power curves are asymptotic towards a process time of 0 seconds. The curves are estimates only and are based on a relatively small sample of trials, but do, however, give good indication of likely powers required. The curves also illustrate the significant reduction in required power if lower brain temperature change is needed or longer process times are acceptable.

During trials the weight of the whole cow corresponding to each test head was noted. This allowed further analysis of the temperature versus power relationship. The relationship between cow weight and temperature change gradient was established and used to plot new curves taking into account cow weight. This increases the accuracy of power level prediction and allows for estimation based on cattle weight. The relationship between weight and power required was found to be non-linear.

3.2.3.2 Reporting uncertainty

Sources of bias and confounding include:

- Small numbers of cadaver runs (Phase 1: 4 sheep runs; 8 cattle runs, Phase 2: 4 sheep runs, 12 cattle runs);
- Cadaver heads were frozen and thawed.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

One of the main concerns in any microwave heating system is the penetration depth of the power flux into the workload. It gives a direct understanding to the heat distribution within the material and hence to heating uniformity. It is important to remember that the penetration depth only refers to the distance into the material where the power flux density has decreased to 37 % of its surface value and that the corresponding temperature will be directly related to the exponential decay in power the deeper into the animal head.

The following are some general conclusions that may be derived from the published dielectric values of brain, bone and skin:

- These data represent a generalised set of loss factors ε'' that may be encountered. For a given electric field E the absorption of energy depends on ε''. The effectiveness of heating will depend as much on the design of the applicator as on the loss factor of the material.
- Penetration depths calculated from both dielectric data sources match quite closely at 922 MHz but differ considerably at 2.45 GHz. Generally, penetration depth at this frequency is approximately 30 % of that at 922 MHz due mainly to frequency effect.

The choice of frequency is basically between using multiple magnetrons operating at either 2.45 GHz maximum rated power 30kW or larger power magnetrons operating at 922 MHz rated maximum power 100kW. Some facts about each approach are listed below:

- Magnetron efficiencies are between 50-70 % for 2.45 GHz compared to 80-87 % for high power 922 MHz,
- Applicator dimensions and complexity increases as the number of individual magnetron sources increases,
- In multiple magnetron installations, magnetron life is reduced due to cross coupling of power,
- Multiple magnetron systems provide a high degree of redundancy,
- Penetration depths are significantly larger at 922 MHz than at 2.45 GHz.

One key to success in the development of a system for animal stunning is to localise the heating to the brain as much as is possible. This preliminary experimental work suggests that this may be possible. The tests performed, particularly at 922 MHz, demonstrated that little heating took place in the regions of the eyes, ears, neck and nose when compared to the area around the brain. With further applicator design and development, it should be possible to reduce external heating even further. The trials also clearly demonstrated that 922 MHz is the preferred frequency. It offers higher penetration into the brain and is commercially available with single generators up to 100 kW.

While a large degree of attenuation was shown in the trial results, it should be stated that the applicator used was not designed to reduce this. Specific designs in later stages of work would endeavour to further focus the heating into the brain. These trials were undertaken to gain understanding into the nature of the temperature rise within the head. The trials founds that largely the temperature rise was highly linear within the brain and the skin. Linear temperature rise leads to easier control and repeatability in later development.

The experimental results clearly show that both cattle and sheep brains can be heated very quickly using microwave energy and that no excess heating of eyes, ears, nose and neck areas occur. The realisation of a commercial system appears to be very promising given the results from this initial study.

Literature suggests that there are many advantages to reversibly stunning livestock with microwave radiation; however previous attempts have been discontinued due to assumed risk of radiation exposure to operators or equipment cost. These issues may no longer be relevant due to information now available within this report.

Dielectric data predicted higher penetration into the brain at 922 MHz. This was confirmed with early trials. The first phase of trials demonstrated that 922 MHz energy penetrates further into the brain and also reduces heating to the other areas of the subject head. Based on this; 922 MHz is the recommended frequency for future work.

The second phase of trials found that heating rates within the head were largely linear and that skin temperatures rose at higher rates than brain temperatures. This effect was less pronounced in the sheep head trials. This skin heating effect may require the design of an applicator which promotes high penetration into the head and focuses microwave coupling into the brain.

Likely power levels were predicted based on the phase 2 trial data. This revealed likely power levels of 4 kW to 40 kW to process sheep in 10 and 1 second respectively at 10 °C temperature rise and 12 kW to 100 kW to process 300 kg cattle in 10 to 1.5 seconds respectively at 10 ° C temperature rise.

A conceptual design was included in the phase 2 study report.

3.2.5 Conflicts of interest

No conflicts of interest were reported. The authors of PR02 have no commercial interest in the DTS: Diathermic Syncope® technology. The authors of SS02 and SS03 are listed on the patent (WO2011137497), registered in 2011. [redacted] is the licensee of the technology, all other authors have no commercial interest in the technology.

STEP 3: Laboratory scale: Anaesthetised sheep study (Prototype 1)

Submitted study: **SS02** Preliminary investigations into the use of microwave energy for reversible stunning of sheep

Other reference documents: **PR03**: Microwave induced insensibility for animals Stage 2. Project Report. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

In recent years, microwave technology has developed to the point that high power equipment is available that can focus the energy into the animal's brain to produce a rapid rise in temperature. It is expected that controlling the brain temperature will result in insensibility and allow the animal to regain consciousness without any adverse effects when the temperature of the brain has returned to normal. This will give a recoverable insensibility that should be acceptable for religious slaughter. Benchtop cadaver work (Step 2) has demonstrated that both cattle and sheep brains can be heated very quickly using microwave energy and that no overheating of eyes, ears, nose and neck areas occur.

This project was intended to provide a proof-of-concept outcome, aiming to demonstrate whether or not induction of reversible insensibility, of sufficient duration to allow exsanguination during insensibility (or death before recovery) is indeed attainable, and thus to advise on the merit of pursuing development of this technology.

3.2.1.2 Objective

- To determine whether there is a significant effect on the heating effects of microwave energy application as a result of an active blood circulation in the brain. It is possible that the physiological thermoregulatory response of the animal will interfere with the heating effect;
- To adjust the power calculations to take account of this effect, if present;
- To ascertain that rapid heating of the brain can induce insensibility in sheep;
- To ascertain that this insensibility is maintained for a sufficient period to allow the animal to die, as a result of bleeding, prior to the onset of recovery.

3.2.2 Materials and methods

3.2.2.1 Method

Ethical considerations: The study protocol was reviewed and approved by the CSIRO Animal Ethics Committee, reference 2-09, according to the Australian code for the care and use of animals for scientific purposes (NHMRC, 2013).

Study population: Four cross-breed ewes aged 3–4 years were used for the study. The study was carried out on a single day, with one sheep being anaesthetised, treated and euthanased prior to commencement of procedures on of a subsequent animal. **Randomisation** was in the form of a pre-assigned treatment schedule (table 1 in SS02) and sheep being brought to the restraint unit in the random order in which they were captured from the holding pen. Each sheep underwent general anaesthesia (premedicated with diazepam, induced with thiopentone and maintained on isoflurane in oxygen), and was placed in a custom-built v-restraint crate with chin support, presenting the forehead to the waveguide applicator. Respiratory rate and rhythm were observed and monitored by the anaesthesiologist, while pulse and haemoglobin oxygen saturation were monitored using a pulse oximeter (Nellcor N-595, Covidien, USA).

Sampling strategy: A single channel, 2-needle montage was used to collect electroencephalogram (EEG) traces on paper (Neocardiotrace, Australia). In two sheep, fiberoptic thermoprobes (Neoptix, Canada) were inserted into the brain at superficial, middle and deep levels, via a single trephine hole in the frontal bone.

Microwave energy at 922 MHz, 4 kW power was applied to each sheep for between 5 and 20 s. The two sheep from which brain temperature was measured were immediately euthanased (pentobarbitone overdose) after irradiation; while the two others were disconnected from inhalational anaesthesia, removed from the restraint unit and monitored until the swallowing, chewing and corneal reflexed had returned, at which point they were euthanased.

Heads were inspected and skinned to assess the physical appearance of the energy application site. Brains were removed after 3 days storage and underwent histological examination.

The study was a controlled observational pilot study, so there was no **blinding** and no statistical **analysis.**

3.2.2.2 Measurement of the outcome

The aim of the study was to confirm that unconsciousness could be induced based on EEG data. No attempt was made to estimate latency to onset or duration of unconsciousness.

The sheep were anaesthetized, so no estimation of pain, distress or suffering could be made.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

Temperature profiles (sheep 1 and 2): In sheep 1, the energy was applied for 10 seconds, resulting in a temperature rise of 4.4 °C at the top of the brain, and 2.15 °C at the bottom of the brain. In sheep 2, the energy was applied for 20 seconds, resulting in a rise of 9.65 °C (peak temperature 48.5 °C) at the top and middle of the brain and 2.5 °C to 40.95 °C at the bottom of the brain. Again, by 10 seconds duration of application, the required temperature of 43 °C (expected point of induction of hyperthermic unconsciousness, based in literature review, Step 1) was achieved. During and after this period, breathing remained rhythmic, the pulse regular, and haemoglobin oxygen saturation level at around 98 %.

Electroencephalogram (sheep 1, 2 and 3): On the electroencephalogram (EEG) traces, it could be seen that brain activity in all three sheep changed from low amplitude before application of energy, to high amplitude after application of energy. This high amplitude activity is similar to the epileptiform activity induced by the current practice of electrical stunning, and suggests that the sheep, if they had not been anaesthetised, would have noticeably lapsed into unconsciousness. EEG was not recorded from sheep 4 due to failure of the battery pack in the unit.

The progress of recovery from deep to light anaesthesia in sheep 3 and 4 was uneventful; return of jaw tone and chewing movements were observed in both animals prior to euthanasia.

On dissection, there was no visible effect on the skin or skull where the energy had been applied, apart from one sheep (sheep 4) where excess heating of the skin had been observed, as a result of the prolonged application of energy (20 s compared with 10 s in sheep 3). In this sheep, the surface of the skull showed a light, tan-coloured scorch mark, and the overlying skin was noticeably detached and crisp. The brains were also grossly normal, apart from sheep 4, which showed an area of hyperaemia on the surface of the brain at the point of energy application. Histologically, tissues from sheep 1, 2 and 3 were generally normal, although signs of autolysis were evident. The exception was sheep 4, which had received overheating; two sections of brain were normal, but one section (taken from the hyperaemic area) showed malacia with loss of nuclear detail and fragmentation of the neuropil. The histopathologist concluded that “the malacia is similar to what would be expected with complete and sudden ischaemia and possibly caused by damage to and within associated blood vessels, as well as thermal effects on the parenchymal cells”.

3.2.3.2 Reporting uncertainty

As an observational pilot study, there was no statistical analysis performed. Sources of bias or confounding could include: small numbers of sheep involved in the study (n = 4) and the fact that they were all from a similar age and source cohort.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

Hyperthermic insensibility, is predicted to occur when brain temperatures reach around 43-44 °C (Ohshima et al., 1992; Guy and Chou, 1982). A sheep's core body temperature is around 38 °C, so the temperature change desired is in the order of 6 °C or more. The cadaver work described in Step 2 demonstrated that microwave energy at a frequency of 922 MHz could induce this temperature rise. However, the living brain does have an inherent cooling mechanism via the blood circulation (Niemark et al., 2007), and to induce unconsciousness without distress, it is imperative that this cooling mechanism is rapidly overcome by the energy applied.

The trials on anaesthetised sheep were highly successful. Application of microwave energy caused rapid increases in brain temperature to a point above which insensibility would be expected to occur (43 °C), and below that which protein denaturation and damage would be expected to occur (50 °C). There were no visible signs of damage to the surrounding tissues, or to the brain itself, except in one sheep in which the surface temperature was known to have been raised too high. In one sheep, there was a single convulsion on application of the energy, and she stopped breathing momentarily. Rhythmic breathing returned after about 10 seconds, and there was little effect on pulse rate and haemoglobin oxygen saturation.

3.2.5 Conflicts of interest

No conflicts of interest were reported. The authors of PR03 have no commercial interest in the DTS: Diathermic Syncope® technology. Many of the authors of SS02 are listed on the patent (WO2011137497), registered in 2011[redacted] is the licensee of the technology, all other authors have no commercial interest in the technology.

STEP 4: Laboratory engineering: Development of applicator and auto-tuner

Submitted study: **PR04**: Development of applicator and auto-tuner. Interim project reports. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

Previous work (Sections 4.2 and 4.3) indicated that the induction of a suitable rise in brain temperature to lead to insensibility was feasible. The next stage in development of the DTS: Diathermic Syncope® system was do develop suitable applicators and tuning systems to generate a 5-10 °C increase in brain temperature with minimal heating of skin and bone.

3.2.1.2 Objective

- To design three applicators that may be suited to induction of insensibility in animals (**PR04** part 1);

- To test these applicators on cadaver cattle heads (**PR04** part 3);
- To investigate the suitability of an auto-tuner system to be incorporated into the proposed system (**PR04** part 2).

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.2.2.2 Measurement of the outcome

Not relevant to this step:

- Onset and duration of unconsciousness and time to death,
- Magnitude of pain, distress and suffering.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

[redacted]

[redacted]

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[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

- [redacted]
- [redacted]
- [redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

[redacted]

[redacted]

[redacted]

3.2.5 Conflicts of interest

[redacted]

STEP 5: Laboratory scale: Anaesthetised cattle study (Prototype 2)

Submitted study: **SS03**: Evaluation of microwave application as a humane stunning technique based on electroencephalography (EEG) of anaesthetised cattle.

Other reference documents: **PR05**: Evaluation of microwave application as a humane stunning technique based on electroencephalography (EEG) of anaesthetised cattle. Project Report. Not peer-reviewed. CONFIDENTIAL.

PR06: Microwave results from the live testing conducted [redacted] April 2012. Interim project report. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

This study was the first on live cattle with a limited number of animals (limited by the University of Melbourne Animal Ethics Committee). The aim was to determine whether the temperatures predicted by microwave application to cadaver heads (Step2, PR02), could be reproduced in a live animal and investigate suitable power and duration of application on anaesthetised cows to induce insensibility.

This limited observational study, performed in 2013 used electroencephalography (EEG) and electrocardiography (ECG) to understand the animal's response to microwave application, primarily to determine whether the technique had potential to be further developed as a method of stunning meeting animal welfare expectations.

At this stage the system for delivery (autotuner etc.) and application were at an early stage of development, so parameters measuring the achievement of unconsciousness were not applicable to the final operating system.

The animal was anaesthetised and supported in a specifically designed V-restrainer rolling crate for energy application and EEG recording.

Input variables (power, energy and time) were only investigated superficially to determine parameters for future work on conscious cattle. It was not appropriate to estimate output parameters such as the time to insensibility in this study on anaesthetised animals.

3.2.1.1 Background and rationale

Microwave application has been reported to induce loss of consciousness when applied to conscious rats, causing petit or grand mal seizures for 1 min after exposure and an unconscious state for the following 4 to 5 min with the animal ultimately recovering (Guy and Chou, 1982; Lambooy et al., 1989). However, Lambooy et al. (1989) deemed this technique unsuitable for pigs at that time, partly because of the capacity of the microwave generator being too low to deliver sufficient power. In recent years, microwave technology has developed to the point that high power equipment is available that can focus the energy to produce a rapid rise in temperature in cattle brains (Ralph et al., 2011. Patent number: PCT/AU2011/000527). It is expected that raising the brain temperature will stop brain function and result in insensibility, in other words eliminate the ability for the cows to feel pain, whilst still allowing the animal to regain consciousness after a period of time (reviewed by **SS01**-Small et al., 2013a). This is supported by preliminary evidence in sheep (**SS02**-Small et al., 2013b). Consequently, this may allow for a recoverable sensibility acceptable for religious slaughter since it would not have 'physically injured' the animal.

3.2.1.2 Objective

The aim of this observational project was to investigate the effectiveness of different settings of the microwave technique, power and duration of application, on anesthetized cows to induce insensibility, in other words eliminate the ability for the cows to feel pain, based on electroencephalography (EEG).

3.2.2 Materials and methods

3.2.2.1 Method

Ethical considerations: The project was approved by the University of Melbourne Animal Ethics Committee (approval number 1212620.1) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013).

Study population: Ten *Bos taurus* Hereford x Angus crossbred female cows, estimated liveweight 180 kg, were used over a total of 6 days. The animals were sourced from the daily intake of the abattoir at the convenience of the researchers. There was no *a priori* knowledge that variation in breed, animal type or weight may affect response to microwave application. Each animal was processed individually. The animal was moved from the lairage area through a single chute race and into a restraining box. The head of the animal was restrained and anaesthesia was induced using intravenous ketamine and propofol. Once the animal was anaesthetised the animal was rolled out of the restraint box and intubated. The animal was then placed in dorsal recumbency onto a specifically designed V-restrainer rolling crate, and the endotracheal tube connected to the anaesthetic machine delivering halothane in oxygen via a circle breathing system using standard clinical flow rates and vapouriser settings. End-tidal halothane tension was maintained at 0.9 %. Patient stability and depth of anaesthesia was monitored throughout the procedure using an anaesthetic agent monitor (Cardell® Veterinary Monitor Max-12HDim multiparameter monitor), recording the end-tidal carbon dioxide tension, end-tidal halothane tension, respiratory rate and heart rate every 5 minutes throughout the anaesthetic procedure.

Subdermal 27-G stainless steel needle electrodes were placed in a four-electrode montage to record two channels of EEG. For each channel, the common non-inverting electrode was placed midline between the medial canthi of the eyes, the inverting electrodes placed bilaterally over the mastoid processes, and the ground electrode placed caudal to the poll. The EEG was amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA), with a gain of 1,000 and pass-band of 0.1 to 500 Hz and digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia). Data were analysed off-line after completion of the experiment. Insensibility was assessed by the appearance of seizure-like complexes in the EEG. Four variables were derived from combinations of the raw data and/or the variables derived from the frequency spectra: time to onset of EEG suppression (raw data), time to nadir of EEG suppression (95% spectral edge), duration of EEG suppression (combination of raw data, 95% spectral edge and total EEG power), and maximum effect (95% spectral edge) . Time to onset of EEG suppression was measured from the start of the microwave application until the first appearance of seizure-like complexes in the EEG, hence including the duration of microwave application. Time to nadir of EEG suppression was measured from the start of the microwave application until the maximum depression of 95% spectral edge. Duration of EEG suppression was measured from the time of onset until the re-emergence of a normal EEG pattern similar to that seen prior to the application of the microwaves. The maximum effect was determined by the maximum reduction of the 95% spectral edge frequency.

Electrocardiogram (ECG) data were recorded by placing three ECG electrodes on the animal's body, for the last 4 animals. The electrodes were placed in a three-electrode montage. Adhesive electrode pads were adhered to the skin; the positive electrode was placed on the chest wall 5 cm behind the left point of the olecranon; the negative electrode was situated and 10cm out from midline of the thoracic back on the right-hand side; the ground electrode was placed in the same position as the negative electrode but on the left han2d side. Electrocardiogram recordings were acquired using Powerlab 4/25T (ADInstruments, Castle Hill, Australia) and Chart 5® software (version 5.5.5) (ADInstruments, Castle Hill, Australia). The ECG tracings were analysed using Chart 5 software to produce continuous heart rate recordings.

The microwave applicator was applied in contact with the top of the front head, on a mid-line half-way between the medial canthi of the eyes and the poll. The exact location varied slightly depending on the head shape. The microwave application was imposed at different powers and for different durations. It was repeated 35 min later at an identical dose to give a total of 2 applications per animal. This second application was used in an attempt to deliver multiple applications to a single animal maintained under anaesthesia, hence allowing the collection of additional data while reducing the number of animals needed for the experiment. However, the second application resulted in more efficient microwave delivery and more profound EEG effects than the first. This does not accurately reflect field conditions (each animal would be subjected to a single application). Therefore, the data from the second applications for each animal were not included in the analysis. The animal was maintained under anaesthesia throughout and was euthanized 10 min after the last microwave application by administrating a lethal dose of barbital into the jugular vein. One animal did not respond to the anaesthetic agents and was euthanized before any treatment could be applied. Thus, data were collected from nine animals. Energy deliveries were 20 kW for 15 s (n = 2); 20 kW, 10 s (n = 2), 30 kW, 10 s (n = 3), 30 kW, 5 s (n = 1) and 12 kW, 25 s (n = 1).

The external head temperature was recorded after microwave delivery by using a digital electric probe placed on the front head of the animal approximately 5 cm below the application point on the surface of the skin, for the first 3 animals. The temperature was monitored continuously from 3 min after the first microwave delivery until the second microwave delivery.

Post-mortem autopsies were performed by a veterinary pathologist on the heads of the last four animals to determine histological changes in the skin and brain tissues. Of these animals, two animals were given two microwave applications whereas two other animals were only given one microwave application in order to reliably observe the effects of a single microwave application as its intended use in the field. The skin was examined after a haematoxylin and eosin staining. The parts of the brains that were examined consisted of the frontal and parietal lobes (meninges, cortex, white matter), the basal nuclei, the thalamus and hypothalamus, and the caudal colliculi. These tissues were assessed for different parameters: tissue necrosis, vascular necrosis, cavitation or rarefaction, vascular haemorrhage, vascular congestion or oedema, and thrombosis using a grading scale from none to mild, moderate or severe.

Study design: this was an observational study, with no statistical analysis performed.

Randomisation and blinding: Treatments were pre-determined, so randomization was related to the order in which animals presented themselves to the restraint unit. Observers were not blinded to treatment.

3.2.2.2 Measurement of the outcome

Onset and duration of unconsciousness were measured using EEG.

Subdermal 27-G stainless steel needle electrodes were placed in a four-electrode montage to record two channels of EEG. For each channel, the common non-inverting electrode was placed midline between the medial canthi of the eyes, the inverting electrodes placed bilaterally over the mastoid processes, and the ground electrode placed caudal to the poll.

The EEG was amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA), with a gain of 1,000 and pass-band of 0.1 to 500 Hz and digitised at a rate of 1 kHz (Powerlab/4sp; ADInstruments Ltd, Sydney, Australia).

Data were analysed off-line after completion of the experiment. Insensibility was assessed by the appearance of seizure-like complexes in the EEG. Four variables were derived from combinations of the raw data and/or the variables derived from the frequency spectra:

- time to onset of EEG suppression (raw data),
- time to nadir of EEG suppression (95% spectral edge),
- duration of EEG suppression (combination of raw data, 95% spectral edge and total EEG power), and
- maximum effect (95% spectral edge).

Time to onset of EEG suppression was measured from the start of the microwave application until the first appearance of seizure-like complexes in the EEG, hence including the duration of microwave application. Time to nadir of EEG suppression was measured from the start of the microwave application until the maximum depression of 95% spectral edge. Duration of EEG suppression was measured from the time of onset until the re-emergence of a normal EEG pattern similar to that seen prior to the application of the microwaves. The maximum effect was determined by the maximum reduction of the 95% spectral edge frequency.

EEG data were inspected for pain-related complexes.

Magnitude of distress and suffering were not assessed as the animals were anaesthetized.

3.2.3 Reporting the results

One animal did not respond to the anaesthetic agents and had to be euthanised before any treatment could be applied. Hence, data from nine animals was obtained. One animal received low power treatment (12kW) and the effects were considered outliers and excluded from summary statistical analysis. One animal received only a short application (5s) and was excluded from summary statistical analysis.

3.2.3.1 Reporting outcomes and estimations

EEG

Interference from the electromagnetic field prevented collection of EEG data during application and for approximately 3 s after. Data presented are based on time to visible EEG changes, which may have occurred before the onset of recording. All of the applications resulted in changes in the EEG pattern indicative of seizure-like activity. Animal 5 was an outlier and was the animal that had received only 12 kW incident power. When data for animal 5 are excluded, time to onset of EEG changes ranged from 12 – 50 s from the start of energy application (mean 21 s), and time to nadir effect was 20 – 65 s (mean 35 s). Animal 9 only received 5 s application and was also an outlier in terms of duration of EEG changes and maximum reduction in Spectral Edge (SE). When animals 5 and 9 are excluded, duration of EEG change was 81 – 215 s (mean 118.9 s) and maximum reduction in SE was 11 – 59 % (mean 194 %). When data were pooled by treatment (excluding animal 9, and one other in which the EEG was heavily contaminated with artefact), the data are summarized in Table 11.

Pain-related complexes were not evident in the EEG.

Table 11: EEG results pooled by treatment: time to onset of EEG suppression, time to nadir of EEG suppression, duration of EEG suppression, and maximum effect (average and (value1, value2)). Time is counted from the start of the microwave application

Treatment	**1** (n = 2)	**2** (n = 2)	**3** (n = 2)	**4** (n = 1)
Power (kW)	20	20	30	12
Duration (s)	15	10	10	25
Energy delivered (kW x s = kJ)	300	200	300	300
Time to onset of EEG changes (s)	37 *(24, 50)*	13 *(12, 14)*	15 *(14, 16)*	138
Time to nadir effect (s)	59 *(52, 65)*	25 *(22, 28)*	31 *(24, 37)*	142
Duration of EEG changes (s)	105 *(81, 129)*	109 *(78, 140)*	162 *(109, 215)*	37
Maximum effect (% reduction in SE)	22 *(18, 25)*	25 *(21, 29)*	45 *(31, 59)*	6

ECG

Baseline heart rate prior to microwave application was 92.2 (± 8.4) bpm. After the start of microwave application, heart rate dropped within 5.0 (± 2.4) sec to 65.8 (± 24.0) bpm, and then rebounded after 23.75 (± 1.9) sec from the start to 82.3 (± 10.3) bpm. It stabilised within 160 (± 37.4) sec to 73.2 (±12.1) bpm.

Frontal skin temperature

Temperature of the skin surface 5 cm below the point of microwave application was recorded from the first three animals, using an electric probe placed on the surface of the skin. Skin temperature increased quickly after microwave application and returned to baseline within 35 min (Table 12). Skin temperature increased less in Animal 3 (20 kW, 10 s), in comparison to Animals 1 and 2 (20 kW, 15 s).

Table 12: Skin temperature (°C) after microwave application, on the frontal head skin surface approximately 5 cm below the application point.

Time post microwave application (min)	**5**	**10**	**15**	**20**	**25**	**30**	**35**
Animal 1 (20 kW, 15 s)	44.3	39.7	37.0	35.6	34.8		
Animal 2 (20 kW, 15 s)	44.8	43.2	40.0	37.6	35.9	34.6	33.4
Animal 3 (20 kW 10 s)	33.1						

Post-mortem examinations

At the point of application there was an area of complete skin loss, surrounded by a larger region displaying grey-tan discolouration of the subcutaneous tissue. This skin within this area displayed full-thickness coagulative necrosis extending down to the skull. Beyond this area, there was rapid progressive decrease in the severity of necrosis, with normal unaffected skin present within 0.5cm of the margin. In the brain, Animal 8 (single application, 30 kW, 10 s) had medium to severe lesions in the frontal and parietal lobes and Animal 9 (single application, 30 kW, 5 s) had relatively minor to no lesions in the same regions. For both animals, the basal nuclei, thalamus, hypothalamus, and the caudal colliculus were relatively unaltered except for mild to moderate vascular congestion. Animal that had had two microwave applications showed severe lesions at various levels (meninges, cortex and white matter) in the frontal and parietal lobes, with severe tissue and vascular necrosis, cavitation, haemorrhage and vascular congestion. Minor or no changes were apparent in the basal nuclei, thalamus, hypothalamus, and the caudal colliculus.

Performance of applicator system (**PR06**)

Data from the control software indicated that the auto-tuner reduced the reflected power from 80 % to less than 30 %, leading to an increase in efficiency.

3.2.3.2 Reporting uncertainty

A potential source of bias is the small group size (n=10), animals being sourced from a single mob presented at the abattoir.

Unfortunately, the microwave application caused artefacts that rendered the EEG unreadable until after the end of treatment. This is an inherent limitation of using electroencephalographic technique to assess the microwave technique since both techniques interact with electric activity. This leaves a window of uncertainty regarding the aversiveness of the microwave technique during its application and the experience of the animal during that short period of time.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

Microwave application induced a pattern of seizure-like activity in the EEG, a pattern that is not considered to be compatible with continued awareness or the ability to feel pain (Devine et al., 1986; Coenen, 1998; Velarde et al., 2002). Hence, this pattern of seizure-like activity in the EEG is interpreted as an assessment of insensibility in this study. The interval between the time when effects started appearing ('Time to onset') and the maximum effects were seen ('Time to nadir') was within 4 to 22 sec depending on the treatments. These results confirm findings on other species (rodents: AVMA, 2013; sheep: Small et al., 2013b) that microwave irradiation is a relatively quick process in comparison to other reversible stunning procedures such as cattle electrical stunning, for which applications of up to 15 sec can be performed in order to depolarize the spine and reduce kicking (NAMI, 2017), or carbon dioxide stunning in pigs, with latency of about 25 sec to the loss of posture (Velarde et al., 2007). In comparison, non-penetrative captive-bolt stunning induced EEG changes in about 8 sec in calves, in a study which used a similar anaesthesia model (Gibson et al., 2009). Furthermore, the current results indicated that a shorter duration of application induced more rapidly developing EEG changes, in the range of duration tested (10-25 sec), as evidenced by shorter 'time to onset' and 'time to nadir'.

Another consideration for reversible stunning techniques is the duration of insensibility, or the time before the animal regains consciousness. Insensibility should last until death ensues through exsanguination. Following microwave application, EEG suppression lasted for at least 37 sec and up to more than 2 min. Our results also indicated that applying higher power extended the duration of insensibility. The search for a long period of insensibility is useful for cattle because consciousness can last from 1 to 2 min after exsanguination (Newhook and Blackmore, 1982; Gregory et al., 2010).

Unfortunately, the microwave application caused artefacts that rendered the EEG unreadable until after the end of treatment. This is an inherent limitation of using electroencephalographic technique to assess the microwave technique since both techniques interact with electric activity. This leaves a window of uncertainty regarding the aversiveness of the microwave technique during its application and the experience of the animal during that short period of time. Pain-related complexes were not evident in the post-DTS EEG. The animal's perception of the procedure up to the induction of insensibility (in the order of 10-15 sec) should be investigated with alternative scientific methods that allow for data collection during microwave application.

The abrupt bradycardia observed following microwave application is in agreement with the literature on the heart rate response to noxious stimuli (Woodbury et al., 2005; Johnson et al., 2005; Gibson et al., 2007). The magnitude of that drop differed between animals, but the heart rate rebounded within 24 sec, irrespective of the treatment applied. Interestingly, the heart rate stabilised to a different, lower level, following microwave application. The most plausible explanation is that this may be the result of temporary and longer-persisting effects on the brain-stem or thalamus (Benarroch, 2001).

Based on the post-mortem autopsies, most histological changes appeared in the upper regions of the brain, with the frontal lobe, adjacent to the zone of application of the microwave, being the most affected, closely followed by the parietal lobes which are located on the sides of the animal's brain. However, the regions of highest interest in regards to consciousness, the deeper regions of the brain, namely the basal nuclei, thalamus, hypothalamus, and the caudal colliculus, appeared to be relatively unaffected by microwave application, even following two microwave applications. Further research is warranted regarding the dissipation of energy throughout the brain and whether this is a homogeneous process or not. The lesions observed would suggest that animals may be able to regain consciousness following microwave application, although the frontal regions of the brain would unlikely be intact.

This experiment provided novel and crucial knowledge regarding the effects of different power and duration of microwave applications on anesthetized cattle. However, only a small number of animals, hence a small number of settings, could be tested. A possibility for future research is to perform further anaesthesia trials to refine the duration of application (< 10 sec), the potential for higher power to lengthen the duration of insensibility, and the variation between animals.

3.2.5 Conflicts of interest

This work was funded by Meat & Livestock Australia, the Australian Meat Processor Corporation (both statutory research and development bodies), and [redacted]. The work was performed by staff from the University of Melbourne and Massey University with no involvement from the founder.

No conflicts of interest were reported. The authors of SS03, PR05 and PRO6 have no commercial interest in the DTS: Diathermic Syncope® technology.

STEP 6: Laboratory engineering: Refinement of delivery apparatus and development of user interface software

Submitted study: **PR07**: Refinement of delivery apparatus and development of user interface software. Interim project reports. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

Subsequent to the anaesthetized cattle study, refinements to the delivery apparatus, auto-tuner and user interface software were required to bring the next prototype closer to a configuration that may be used in a commercial setting, on conscious animals.

3.2.1.2 Objective

- To design and fabricate a waveguide reticulation system suitable to application on standing animals in a commercial restraint unit.
- To integrate a high-speed auto-tuner into the system.

- To commission the system into the [redacted] abattoir and test the system on cadaver heads and dummy loads (water bodies).

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

[redacted]

3.2.2.2 Measurement of the outcome

[redacted]

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

[redacted]

Table 13: Digital components developed

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	

Figure 14: Example of test run showing near zero reflected power (green line) as a result of the auto-tuner.

Table 14: temperature rise recorded from three cadaver head tests post installation at Wagstaff Garfield abattoir

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

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[redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

[redacted]

[redacted]

3.2.5 Conflicts of interest

[redacted]

STEP 7: Laboratory scale: Pilot study on conscious cattle (Prototype 3)

Submitted studies:

SS05: Development of a microwave energy delivery system for reversible stunning of cattle 2: Preliminary observations on behavioural responses and EEG.

SS06: A comparison of beef carcases stunned using DTS: Diathermic Syncope® or captive bolt in terms of selected meat quality attributes and plasma biomarker concentrations.

Other reference documents: **PR08**: Dielectric induction of temporary insensibility in cattle - animal trials. Project Report. Not peer-reviewed. CONFIDENTIAL. **PR09**: Meat quality from microwave stunned cattle Project Report. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

This study was the first on conscious cattle with the number of animals limited by the Victorian State Government Wildlife and Small Institutions Animal Ethics Committee. The aim was to translate what was learned from the anaesthetised cattle trial (Step 3, SS03, PR05, PR06), incorporating improvements to the microwave delivery system and user interface (Step 6, PR07) to conscious cattle: to confirm that insensibility could be achieved and investigate behavioural and endocrine responses, and impact on carcase and meat quality.

The improvements in the delivery system resulted in a 50 % increase in the rate of heating, when tested on cadaver heads (Step 6), and therefore it was expected that the energy required to induce unconsciousness would be less than previously predicted.

This limited observational study, performed in September 2014 used electroencephalography (EEG) and endocrine (hormone) responses to understand the animal's response to microwave application, and meat quality assessment to determine whether meat quality was compromised. A comparison of endocrine, carcase and meat quality parameters against penetrative captive bolt (as the recognised highest-standard commercial stunning method) was conducted.

At this stage the electronics of delivery (autotuner etc.) was performing satisfactorily, but the energy was delivered via a rigid waveguide, such that the animal's head had to be lifted to the applicator, rather than the applicator being moved to the animal. This meant that the applicator was not always in good contact with the animal's forehead, so a portion of the energy could escape rather than entering the brain, and if sufficient energy leaked, the generator automatically switched off. All work was performed in a standard box with neck bails and a chin lift based on the ASPCA design for ritual slaughter.

This study demonstrated that satisfactory animal welfare outcomes could be achieved on animals receiving microwave treatment. Only limited values of input variables (power, energy and time) could be investigated within the number of cattle approved for use. It was possible to estimate output parameters such as the times to achieve unconscious and return of consciousness but due to poor energy delivery, these times were not used for estimation of operating parameters for simple stunning.

3.2.1.1 Background and rationale

Some communities require that animals processed for human consumption are healthy, uninjured and normal at the moment of carrying out the slaughter cut. As a result, many methods of stunning used in modern commercial slaughter are not acceptable, because the animals could be considered injured, or because a proportion of animals would not recover from the stun. Head-only electrical stunning is used in sheep but is not suitable for use in cattle because of concerns over the duration of insensibility being insufficient to allow death through total blood loss to occur prior to recovery from the stun, and also because of problems with blood splash in the meat. Preliminary research has shown that electromagnetic energy technology is likely to induce recoverable insensibility in animals and could result in an effective reversible stunning method that is suitable for religious slaughter.

Background research has shown that electromagnetic energy can lead to unconsciousness in animals. In chickens, Lambooij et al. (2011) applied an electromagnetic field, and produced electroencephalogram (EEG) traces that indicated insensibility and the duration of unconsciousness was calculated to be in the range of 15 to 20 s. Guy and Chou (1982) similarly induced unconsciousness in rats that lasted for 4-5 minutes. The mechanism of action is essentially selectively increasing the temperature slightly in the brain, by only 7 °C, to the point that hyperthermic syncope (fainting) occurs, but below the point at which irreversible brain damage and death occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45 °C.

[redacted] have designed a system for delivery of electromagnetic energy to sheep, goats, cattle, buffalo and camel (Ralph et al., 2011:

Patent PCT/AU2011/000527), DTS: Diathermic Syncope®. To date, trails have been carried out on anaesthetised sheep and anaesthetised cattle.

The trials on four anaesthetised sheep were highly successful. Application of microwave energy caused rapid increases in brain temperature to a point above which insensibility would be expected to occur (43 °C), and below that which damage would be expected to occur (50 °C). The sheep breathed regularly and normally throughout the application of energy, and afterward, in contrast to electrically stunned sheep, in which breathing stops until the animal begins to recover from the stun. Two animals were allowed to partially recover from anaesthesia, to the point at which neck tone, jaw movements and swallowing reflexes had returned, indicating that the animals were likely to recover. Full recovery was not permitted under this trial, as a condition of the Australian Animal Ethics Approval.

In the anaesthetised cattle trial, nine cattle were lightly anaesthetised, and electroencephalogram (EEG) traces collected before and after microwave energy application. All applications resulted in EEG traces that indicated unconsciousness (seizure-like activity, similar to that seen when electrical stunning is used). There was a slight slowing of the heart rate during and after application, but the rhythm remained regular. In comparison, when electrical stunning is used, the heart rate drops while the stun is applied, but rises to above normal rates after application, during the unconscious period. Five combinations of microwave power and durations were tested. Higher power led to a longer period of EEG disruption; while shorter application led to more rapid onset of EEG disruption. The EEG traces were analysed for evidence of pain according to the method of Gibson et al. (2007), and no evidence of pain was found. These results indicated that microwave application to conscious animals would lead to rapid onset of insensibility.

3.2.1.2 Objective

- To provide a proof of concept demonstration of induction of unconsciousness in cattle;
- To determine what signs, comparative to other validated stunning processes, might eventually be examined experimentally for future assessment on the effectiveness of the device in rendering the animal unconscious; and
- To compare DTS against the industry reference standard (penetrative captive bolt stunning), in terms of:
    - physiological variables (Cortisol, ACTH, β-endorphin and catecholamines) and
    - meat quality (carcase characteristics, pH, Colour, shear force, drip loss and lipid oxidation) at slaughter, one week and ten weeks post slaughter (vacuum packaged primals).

3.2.2 Materials and methods

3.2.2.1 Method

Ethical considerations: The studies were carried out under the authority of the Victorian State Government Wildlife and Small Institutions Animal Ethics Committee, Authority 29.13.

Study population: Eighteen *Bos taurus* Aberdeen Angus cross bred heifers (weight range 350-400 kg) with a quiet temperament were selected from the normal commercial intake [redacted]

[redacted]. They were fed and rested in lairage for 4 days prior to the trial, cared for by an experienced stockperson. On the day of the trial, each animal was individually brought to the restraint box, by the same familiar stockperson, using low-stress animal handling techniques. A baseline blood sample was taken from the tail vein, and the animal was restrained using a head restraint unit, lifting the forehead to be in contact with the DTS energy applicator. After head capture, baseline EEG measurements were recorded. The assigned treatment was then applied.

Experimental design: This study was the first observational study on live non-anaesthetised cattle with numbers limited by the Victorian State Government Wildlife and Small Institutions Animal Ethics Committee. Since the major objective was to provide proof of concept demonstration of induction of unconsciousness, an observational study was conducted. Eighteen *Bos taurus* Aberdeen Angus cross bred heifers (weight range 350-400 kg) with a quiet temperament (for animal welfare and occupational safety reasons) were selected from the normal commercial intake.

Animals were randomly assigned to the treatments based on the order in which they entered the restraint box, the treatments being conducted as 2x captive bolt to start the trial and ensure all team members were correctly positioned for data collection; followed by two DTS; and then randomization of treatments by selection of a card from a pre-prepared stack.

The initial plan had been to balance animal numbers across treatments to allow statistical analysis utilizing captive bolt as a control group, but problems with energy delivery meant that the energy applied to each animal differed, and when the final two cards remaining in the pack turned out to both be captive bolt treatments, the decision was taken to again ‘reduce’ animal numbers according to the 3R's of animal experimentation and not process these final two animals as part of the trial.

The treatments were:

- Control (captive bolt): animals 1, 2, 8, 9, 12, 13, 15
- High energy DTS, receiving greater than 290 kJ: animals 3, 4, 11
- Low energy DTS, receiving less than 200 kJ: animals 7, 10, 14, 16
- Intermediate and double-application DTS: animals 5, 6 and 18.

Animal 17 was excluded from analysis: it received a very low dose of energy (35.55 kJ), which did not render the animal insensible, and was euthanased by captive bolt stun and exsanguination.

Randomization and blinding: the order of treatment application was pre-determined, randomization was based on the order in which the animals presented themselves to the lead-in race to the restraint unit.

Personnel carrying out offline video behaviour recording, EEG, blood and meat quality sample analysis were blinded to treatment applied.

Sampling strategy: Following treatment, insensibility was assessed by corneal reflex, assessment of visual function and response to a painful stimulus of the nose, EEG measurements were repeated, the animal removed from the restraint box, terminal EEG measurements recorded and the animal exsanguinated. A back-up captive bolt stun was delivered in cases where a risk of recovery during exsanguination was perceived, the time elapsed between application of the treatment and exsanguination being prolonged by the need to capture post-treatment EEG recordings and behavioural measures. A second blood sample was collected from the free-flowing exsanguinate. Blood samples were centrifuged and plasma extracted. The entire process was video recorded using six cameras, capturing animal movements and behaviours from above, at the head, and on the roll-out table, and these videos were subsequently annotated against an ethogram designed for the trial.

The carcase was then dressed as normal practice, chilled overnight, and de-boned the following day. pH measurements were taken from the carcases every hour from slaughter till below pH6, and again at 24 hours post slaughter, prior to de-boning. Heads were sections for inspection, and brain samples collected for histological analysis. Colour was measured on the cut surface of the m. longissimus lumborum 30 minutes after quartering. At de-boning, two samples each of loin (m. longissimus lumborum) and round (m. semitendinosus) was removed, vacuum packed and refrigerated. These samples were transported to the laboratory by refrigerated vehicle, within the first week post slaughter. At each of 1 and 10 weeks post slaughter, muscle samples were unpacked, and sectioned into subsamples for colour, pH, shear force, lipid oxidation and drip loss evaluation.

Meat Colour was measured using a MINOLTA CR300® colorimeter under light source D65; pH was measured using a WP-80 digital pH meter (TPS instruments, Springwood, QLD), with a combination electrode for temperature compensation; Warner-Bratzler (WB) shear force was measured according to the protocols outlined by Bouton et al. (1971) and Bouton and Harris (1972). Drip loss was measured using the method outlined by Honikel et al. (1986); Lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method of Witte et al. (1970). Plasma samples were tested for cortisol (RIA), ACTH (EIA), β-endorphin (EIA) and catecholamines (ELISA) concentrations.

Method of analysis: Blood sample and meat quality data were analysed using R Studio (R Core Team, 2014). The EEG data were analysed offline using LabChart 8 (ADInstruments, Sydney, Australia). The Spectral Analysis Package within LabChart 8 was used to apply Fast Fourier Transformation (FFT), with multiplication using a Hann window in 1-second epochs with a 25 % overlap. Total power (Ptot), median frequency (F50) and 95% Spectral Edge frequency (F95) were extracted. Epochs containing artefacts were identified and rejected manually, with reference to video footage to identify event-related artefact (e.g., animal movements, eye/ ear movements, personnel movement or movement of leads), and the first and last 2 s of each recording were removed to eliminate edge artefacts. Heavily contaminated recordings, and recordings in which poor electrode contact was present were discarded in entirety. For each animal the median value of Ptot during T1 was calculated and this was used as the baseline value. Baseline normalization was then carried out by transforming data for each 1-s epoch into decibel change from baseline according to the formula: dB=10*log10(value/ baseline), to bring all data sets into a comparable format. These data, and data for F95 and F50 were charted and inspected for EEG suppression and epileptiform activity, and where possible time to resolution of EEG suppression was recorded.

3.2.2.2 Measurement of the outcome

Live and video behavioural monitoring was utilized to assess the magnitude of pain, suffering and distress; latency to return of corneal reflex and duration of EEG suppression were recorded as indicators of onset and duration of unconsciousness and time to death.

Once the head was captured, but before applying the waveguide, a baseline (T1) EEG was recorded using PowerLab and LabChart (ADInstruments, Sydney, Australia), applying a low-pass filter of 50 Hz, for a period of up to 2 min. The electrode montage was prepared using low impedance (< 5 kΩ) electrode pads (RedDotMini, 3M Australia, North Ryde, NSW). Good contact with skin was achieved by fixing the pad to the skin using cyanoacrylate (Loctite 454, Loctite Australia, Caringbah, NSW). Hair was not shaved, to minimize stress to the animal, and electrode gel was not applied, the research team having previously found that close adhesion using cyanoacrylate allowed adequate collection of EEG data.

A single channel, semi-hemispheric, three-electrode EEG montage (ground electrode on the bony protruberance of the atlas behind the ear; reference electrode midline on the nose, midway between the nostrils and the eyes; and the inverting electrode on the left frontal bone, between the eye and the poll) was used. A second channel was not used, as access to the right frontal bone was restricted due to the head capture and waveguide apparatus, and it was felt that attempting to apply an electrode here would add to the stress for the captured animal.

Once the T1 EEG recording was complete, the EEG leads were then removed, leaving the electrodes in situ, and the waveguide applied to the forehead. EEG cannot be recorded during, and for at least 3 s after the end of, DTS application, as the high power microwave energy interferes with the recording (Rault et al., 2014), and the leads were removed to protect the equipment from damage either physically from the faraday cage apparatus or electronically from a high energy microwave burst. The mean time to start of T2 EEG recording was 72.82 s (range 38–124 s).

The EEG data were analysed offline using LabChart 8 (ADInstruments, Sydney, Australia). The Spectral Analysis Package within LabChart 8 was used to apply Fast Fourier Transformation (FFT) with multiplication using a Hann window in 1-second epochs with a 25% overlap. Total power (Ptot), median frequency (F50) and 95% Spectral Edge frequency (F95) were extracted. Epochs containing artefacts were identified and rejected manually, with reference to video footage to identify event-related artefact (e.g. animal movements, eye/ear movements, personnel movement or movement of leads), and the first and last 2 s of each recording were removed to eliminate edge artefacts. Heavily contaminated recordings, and recordings in which poor electrode contact was present were discarded in entirety. T2 EEG recordings were heavily contaminated with movement artefact.

For each animal the median value of Ptot during T1 was calculated and this was used as the baseline value. Baseline normalization was then carried out by transforming data for each 1-s epoch into decibel change from baseline according to the formula: dB = 10*log10(value/baseline), to bring all data sets into a comparable format. These data, and data for F95 and F50 were charted and inspected for EEG suppression and epileptiform activity, and where possible time to resolution of EEG suppression was recorded.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

The number of animals receiving a DTS treatment and the response was:

Number of animals receiving DTS treatment	11
less Low dose not achieving unconsciousness	1
Available for measurement of ABMs relating to achieving unconsciousness	10
less Animals with low dose and transient unconsciousness	2
less Animals that did not recover consciousness	3
Available for measurement of ABMs relating to duration of unconsciousness	5

Under live observation of the animals, the clearest indicators of effective induction of insensibility were:

- Loss of corneal responses (n=10)
- Loss of withdrawal response (pinprick) (n=10)
- Eye staring, not following movement (n=10)

Less consistent indicators are:

- Loss of posture (n=9): there is an initial loss of posture, but by the time the DTS cycle is complete, the animal is entering a rigid tonic state: the legs have re-extended, and paddling or walking movements of the hind limbs in particular, are seen. This pushes the shoulders of the animal hard into the neck bail, jamming it into an upright position. When the animal is removed from this jammed position, it is in lateral recumbency, entering a convulsive or clonic phase, after which reflexes do appear to begin to return.
- Breathing characteristics (: breathing and heart function is maintained – the posterior parts of the brain stem appear to be unaffected by the DTS application. In the unconscious animal breathing is deep and slow. When reflexes return, breathing tends to be fast and shallow.
- Vocalisation: it appears that some animals do make sounds, even when there are no responses from the eye (following movement, corneal response, palpebral response) nor withdrawal reflexes. This 'vocalization', when it occurs, is continuous, occurring on every exhalation, and is not associated with any external stimulus.
- Blinking: as in electric stun, random movements of the facial muscles and eyelids can occur, particularly as the animal enters the clonic (convulsive) phase. Therefore, it is difficult to interpret a blink as to whether it is a response, or a random movement.

From the video footage it was confirmed that on application of a captive bolt, all four limbs immediately tucked into the body, and the animal fell to the floor of the restraint box. As it was rolled out of the box, the forelegs first became rigidly extended, followed by the hind limbs. No rhythmic breathing was observed, and the animal's eye was fixed and staring. There was no corneal response, no pupillary response to light, and no response to nose prick. No vocalization and no righting reflex was observed.

During application of energy the animals showed rapid blinking and flickering of the third eyelid, with uncontrolled involuntary eye movements, and in some animals the back arched and the muscles of the neck contracted, pulling the chin down into the chin lift. In the low energy applications, this was followed by a period of convulsive movements, lasting 10-20 seconds, then ataxia and loss of posture, particularly in the hindquarters. Following this ataxic phase, the body became tense and tetanic, with the four limbs slightly splayed. There was no response to efforts to topple the animal: the tail hung limp and flaccid, and the limbs remained motionless. The animal then progressed into slow walking and paddling movements, pushing forward into the head restraint unit. There was no vocalisation, and rhythmic breathing continued throughout. The eyes became fixed and staring, with no ocular following of movement, and no pupillary response to light. There was no withdrawal response to nose prick.

All DTS treated animals, with the exception of one animal receiving only 38.55 kJ, were assessed as unconscious immediately following energy application, based on the indicators loss of corneal reflex, loss of withdrawal reflex, and fixed, staring eye.

The duration of ABMs relating to unconsciousness were measured and related to the energy treatment received (Table 15).

Table 15: Duration of absence of various ABMs when receiving DTS treatment

Energy applied (kJ)	Number of animals	Minimum duration of absence of ABM (seconds)			
		eye movement	posture	Corneal response	Withdrawal response
150-200	2	137.5	5	204	12
200-250	3	161.5	375	98	168

Head inspection of captive bolt animals revealed one or two (in the instances where the animal was re-stunned) circular penetrations of the frontal bone. Haemorrhage was evident within the skull cavity, with clotted blood pooling around the lower parts and brainstem. This contusive damage was also noticeable in histological sections from deeper regions of the brain. Heads from high-energy DTS showed varying degrees of heating damage. On the forehead, an extensive area of scorched, dried skin was easily sloughed, showing brown discolouration of the bone below.

On splitting the skull, the mucous lining of the sinus cavity and nostrils was noted to be dried and discoloured. The brain was discoloured and firm immediately below the application site, with the appearance of cooked brain tissue. On histological examination, marked evidence of vascular congestion and thrombosis, with loss of neuropil structure and malacia was found in upper sections, and less markedly in middle sections. Low energy DTS heads similarly showed some degree of heating damage to the forehead, but this was much reduced compared to those of High energy DTS. The skin at centre point of waveguide application was dry and leathery, with loss of hair. Brain tissues were essentially normal, although there was some malacia and hyperaemia of the upper 2mm of the surface of the cerebral cortex.

The EEG responses of both Captive bolt and DTS animals were similar. Qualitative assessment of the EEG traces indicated that immediate post treatment EEG traces showed a reduction in amplitude with intermittent activity (trace not compatible with sensibility), and post-rollout traces tended towards the isoelectric state (flat-line, trace not compatible with sensibility). There were no significant treatment differences between baseline, post-treatment and terminal values of Mean Power; Root Mean Square power (RMS); Amplitude; Median Power Frequency (F50); or 95% Spectral Edge Frequency (F95).

There were no significant differences between DTS and captive bolt animals in terms of cortisol, ACTH, β-endorphin and catecholamine responses. Both treatments resulted in an increase in cortisol from baseline (DTS 33.19 ±16.89 nmol/L; Captive bolt 61.43 ±12.59 nmol/L) to post-stun levels (DTS 150.38 ±17.79 nmol/L; Captive bolt 160.64 ±13.26 nmol/L), indicating physiological stress. However, it is unclear if this stress is due to the stunning methods; or to the head capture and restraint, which was longer than in a commercial situation due to the need to take pre-stun EEG recordings, or to a combination of both restraint and stun.

There were no significant differences between DTS and captive bolt carcases in terms of pHu (24 h post slaughter); pH, Warner Bratzler Shear Force and Drip loss at 1or 10 weeks post slaughter. DTS carcases were slightly yellower at quartering (MINOLTA b* 2.71 ± 0.59 DTS; 1.06 ± 0.44 control); DTS loins were slightly redder (MINOLTA a* 23.22 ± 0.92 DTS; 20.89 ± 0.69 control) and slightly yellower (MINOLTA b* 2.79 ± 0.93 DTS; 0.77 ± 0.70 control); and DTS rounds were slightly lighter (MINOLTA L* 43.32 ± 1.05 DTS; 40.94 ± 0.78 control) at week 1, than control samples ($P<0.05$). There were no differences between DTS and captive bolt meat colour measurements at 10 weeks post slaughter.

3.2.3.2 Reporting uncertainty

A potential source of bias is the small group size (n=18), animals being sourced from a single mob presented at the abattoir. All animals were female Australian Angus cattle.

Good contact between the microwave applicator and the forehead of the animal is required for transfer of the microwave energy into the brain without leakage. Some variation to expected treatments was observed.

The microwave application caused artefacts that rendered the EEG unreadable, leaving a period in which discomfort or pain could only be measured by ABMs.

Overheating of the skin at the point of application was observed. At this stage in development it is unclear whether the animal experienced discomfort as a result of this overheating, or if the overheating occurred after loss of consciousness.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

Based on live observations at the time of stun application, the research team was satisfied that DTS induced a state of insensibility, of a sufficient duration to allow humane slaughter through exsanguination. Live observations indicated that the process was painless, and evidence of distress was not observed.

EEG data indicated that DTS induced insensibility. DTS animals in the current study remained unresponsive to stimuli and showed evidence of EEG suppression for 3-4 minutes post energy application. In a commercial situation, it would be expected that exsanguination would be carried out within 30-60 seconds of energy application.

Video footage demonstrated a convulsive phase during low energy DTS application, but it is unclear as to whether this is involuntary convulsion, or an attempt to escape. Facial expression seems to indicate that it is involuntary. A further convulsive or clonic phase occurred between 60 and 90 seconds post energy application.

Endocrine data indicated no differences between DTS and captive bolt. Meat quality parameters in DTS carcases did not differ from captive bolt carcases. pH declines suggested that there may be a potential for increased metabolic rate, which would be predicted to result in a "PSE-like" or "heat toughening" condition, but this was not corroborated by the meat quality analyses.

Brain lesions suggest that 300 kJ would result in a non-recoverable state, while behavioural observations suggest that less than 100 kJ gives a short-duration insensibility.

DTS animals maintained rhythmic breathing and a strong heart beat throughout the period of insensibility, which lasted for at least 3-4 minutes post application of energy. During this time, there was no corneal reflex, no response to a painful stimulus of the nose, and no evidence of the eye beginning to focus and follow movement. There is a temporary cessation of breathing during energy application, while the animal undergoes the epileptiform phase. This quickly passes, and breathing recommences, but slow and deep. The respiratory and cardiac centres are affected but not obliterated. As per chemical general anaesthesia, loss of reflexes (and subsequent return of reflexes) occurs in a specific order, related to the depth of anaesthesia, and also related to the order in which cranial nerves leave the brainstem. The cranial nerves, in descending order are:

I Olfactory nerve

II Optic nerve

III Oculomotor nerve

IV Trochlear nerve

V Trigeminal nerve

VI Abducent nerve

VII Facial nerve

VIII Vestibulocochlear nerve

IX Glossopharyngeal nerve

X Vagus nerve

XI Spinal division of accessory nerve

XII Hypoglossal nerve

Thus, under general anaesthesia, the earliest signs of returning consciousness, are voluntary tongue movements (Cranial nerve XII) and swallowing or gag reflex (Cranial nerves IX and X); while loss of spontaneous breathing is an early indicator that anaesthetic depth is too great. Cranial nerve X also modulates cardiac and respiratory rate – lesions in cranial nerve X can result in tachycardia and hyperventilation, while stimulation of cranial nerve X can result in bradycardia and deeper/slower breathing.

Following the return of the gag reflex, the next sense to return is hearing (cranial nerve VIII), which is challenging to assess in an abattoir situation. Following this, reflexes associated with the eye return. Cranial nerves III to VII control the muscular responses to stimuli detected by cranial nerves I and II, but until cranial nerves I and II return to function, our ability to assess function of cranial nerves III to VII is limited.

Cranial nerve II is the afferent, or sensory nerve associated with the eye responses: pupillary reflex, menace reflex, corneal reflex and fixating response (ability to focus and follow movement with the eyes). Thus, as soon as corneal response is confirmed, we can be confident that full consciousness is close.

Two animals showed evidence of return to consciousness:

> Animal 16 received 184.68 kJ. Return of blink and corneal reflexes were noted prior to captive bolt application, 228 seconds after DTS application.
>
> Animal 18 received 217.62 kJ. It was unresponsive to stimuli for the first 90 seconds post treatment, and then appeared to go into a clonic or convulsive phase. Following this, it lay quietly for a further 90 seconds, showing no response to stimuli. Towards the end of this period the eye was beginning to regain focus, followed closely by corneal reflex, and within 15 seconds, return of righting reflex.

It is evident that the duration of insensibility achieved in DTS animals is sufficient to allow exsanguination prior to recovery, which can be a problem for some other simple stunning methods. Like other simple stunning methods, the return from focusing of the eye, through corneal reflex to return of righting reflex is rapid so in a commercial situation it would be strongly recommended to maintain a back-up captive bolt instrument on the bleed rail.

Overheating of the skin at the point of application was observed. At this stage in development it is unclear whether the animal experienced discomfort as a result of this overheating, or if the overheating occurred after loss of consciousness.

3.2.4.2 Conclusions

The application of DTS with a dose above 45 kJ resulted in signs of unconsciousness immediately following energy application.

The duration of insensibility was at least 3 to 4 minutes after energy application.

No signs of pain, distress and suffering were observed during the application of the intervention, EEG confirmed the observations of unconsciousness. Endocrine data indicated no differences between DTS and captive bolt stunning.

3.2.5 Conflicts of interest

This work was funded by Meat & Livestock Australia (MLA), the Australian Meat Processor Corporation (both statutory research and development bodies), and [redacted] The work was performed by staff from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) under contract to MLA. [redacted] is the licensee of the technology and applicant;, all other authors have no commercial interest in the technology.

STEP 8: Laboratory engineering: Refinements to delivery system: applicators and coaxial cabling

Submitted study: **PR10**: Refinements to delivery system: applicators and coaxial cabling. Interim project report. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

The pilot study described in Step 7 drew some recommendations for further development:

Aspect 1. Modifications to the technology

If recoverability is desired, and the animal to return to herd life, as opposed to being immediately slaughtered, the desiccation and skin changes at the application point should be minimised. Modifications to the technology should be accompanied by measurement of rates of heating within the brain and in superficial tissue.

Aspect 2. Engineering aspects:

Development to improve:

- Ease of capturing and positioning head;
- Maintenance of contact between the animal's head and the applicator;
    - Consideration of potential for contouring or flexibility within the waveguide tip;
- Ease of extraction of body from box;
    - Consideration of side clamp and tipping technologies.

Note: designs must ensure compliance with OH&S regulatory standards and guidelines.

Aspect 3. Research aspects

Subsequent to development of Prototype 4, addressing particularly the surface heating aspect, commercial pilot trials are required to understand and define the critical limits of energy application that allow induction of insensibility, and reduce brain tissue damage to a negligible level, such that the animal may recover and function in a normal manner.

This phase of work aimed to address aspect 1 and item 2 of aspect 2 of these recommendations. Items 1 and 3 of aspect 2 were addressed by an engineering company specializing in livestock handling systems in abattoirs, and a description of that design is provided in PR11 (Step 9) and Section 3.6.

3.2.1.2 Objective

- To investigate alternative applicator designs to reduce the footprint on the bovine head, and to minimize surface skin and hair heating.
- To investigate potential system designs that improve maneuverability of the applicator.

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Figure 15: Test set-up for coaxial cable evaluation

3.2.2.2 Measurement of the outcome

[redacted]

- [redacted]
- [redacted]

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

[redacted]

3.2.5 Conflicts of interest

[redacted]

STEP 9: Laboratory scale: Validation study on conscious cattle (Prototype 4)

Submitted study: **SS05**: Development of a microwave energy delivery system for reversible stunning of cattle 2: Preliminary observations on behavioural responses and EEG.

Other reference documents: **PR11**: DTS: Diathermic Syncope - commercial validation trials. Interim project report. Not peer-reviewed. CONFIDENTIAL. **PR12**: DTS: Diathermic Syncope® controlled trials. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

This study was conducted with conscious cattle with the number of animals limited by the Victorian State Government Wildlife and Small Institutions Animal Ethics Committee. The aim was to progress the knowledge generated during the first study on conscious cattle trial (Step 7, SS05, PR08, PR09), incorporating improvements to the microwave applicator (Step 8, PR10) and investigate ABMs and EEGs for insensibility over a range of operating parameters.

Prior to this study, improvements to the delivery system included redesign of the restraint box to improve the ease of capturing and positioning the animal's head; replacement of the rigid waveguide with a flexible coaxial cable so that the applicator could be brought to the forehead, rather than attempting to lift the head to the applicator, and contouring of the applicator to better fit the shape of the animal's head: thereby reducing the risk of energy leakage and generator shut-down. These alterations to the delivery system led to an increase of 69.5 % in the rate of heating, when tested on cadaver heads (Step 8), and therefore it was expected that the energy required to induce unconsciousness would be less than previously required.

This limited observational study, performed in October 2017 used electroencephalography (EEG) responses to understand the animal's response to microwave application, thermal imaging to evaluate surface heating at the point of energy application, and recorded behavioural responses to energy application. EEG is not further mentioned for this step because the energy application was higher than recommended for use, there was interference from machinery that makes interpretation difficult, and the method of analysis did not conform to EFSA's preferences. Analyses of these data can be found in the reports of this step.

At this stage the electronics of delivery were performing satisfactorily, and the rigid waveguide had been replaced with a co-axial cable, such that the applicator was maneuverable to the animal's head, and as such contact with the head was more reliable. All work was performed in a standard box with improved head restraint to maintain contact between the applicator and the animal's forehead.

This study continued to collect data on animal welfare outcomes. It was possible to estimate output parameters such as the times to achieve unconscious and return of consciousness but due to some instances of energy leakage resulting in generator cut-out, three animals received limited energy applications, were immediately stunned using penetrative captive bolt, and their results were discarded as not representative of a full energy delivery cycle.

3.2.1.1 Background and rationale

[redacted] have designed a system for rendering an animal unconscious prior to slaughter, using radiofrequency electromagnetic energy (Ralph et al., 2011: PCT/AU2011/000527). The mechanism of action is essentially selectively increasing the temperature slightly in the brain to the point that hyperthermic syncope (fainting) occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45 °C (Ohshima et al., 1992). A pilot study carried out in 2014 indicated that DTS provides a humane method of inducing insensibility prior to exsanguination, of sufficient duration to allow exsanguination prior to recovery, and produces comparable post slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt (**PR08**, **PR09**). However, energy leakage occurred when the animal convulsed on entering the insensible state, leading to activation of the generator safety cut-out, and incomplete delivery of energy to the brain. Subsequent to the pilot study, the restraint, head capture and waveguide set-up have been re-engineered to improve animal handling and restraint, and to limit energy leakage and automatic cut-out of the generator. Prior to the conduct of more extensive commercial scale studies and statistically robust assessments, the latest version of the system (Prototype 4) should be validated in terms of ability to induce unconsciousness in cattle.

3.2.1.2 Objective

To validate the efficacy of the re-engineered system, termed 'Prototype 4', in terms of its ability to induce unconsciousness in cattle.

3.2.2 Materials and methods

3.2.2.1 Method

Ethical considerations: The protocol and conduct of the experiment was approved by the DEDJTR Wildlife and Small Institutions Animal Ethics Committee under the VIC Prevention of Cruelty to Animals Act, 1986 (Animal Research Authority 30.16).

A total of 20 cattle were processed during the period 4th – 6th October 2017.

Study population: Animals were *Bos taurus* mixed breed, predominantly dairy crosses, some were aged cull cows, and others were poor-quality dairy cross steers, randomly drafted from the normal intake at the abattoir. Age and gender of individuals was not recorded.

Sampling strategy: Cattle were brought individually to the restraint unit. For application of DTS, the cattle were individually restrained in a stun box with neck yoke, head capture and chin lift (Figure 16), all of which was fully enclosed in a faraday cage.

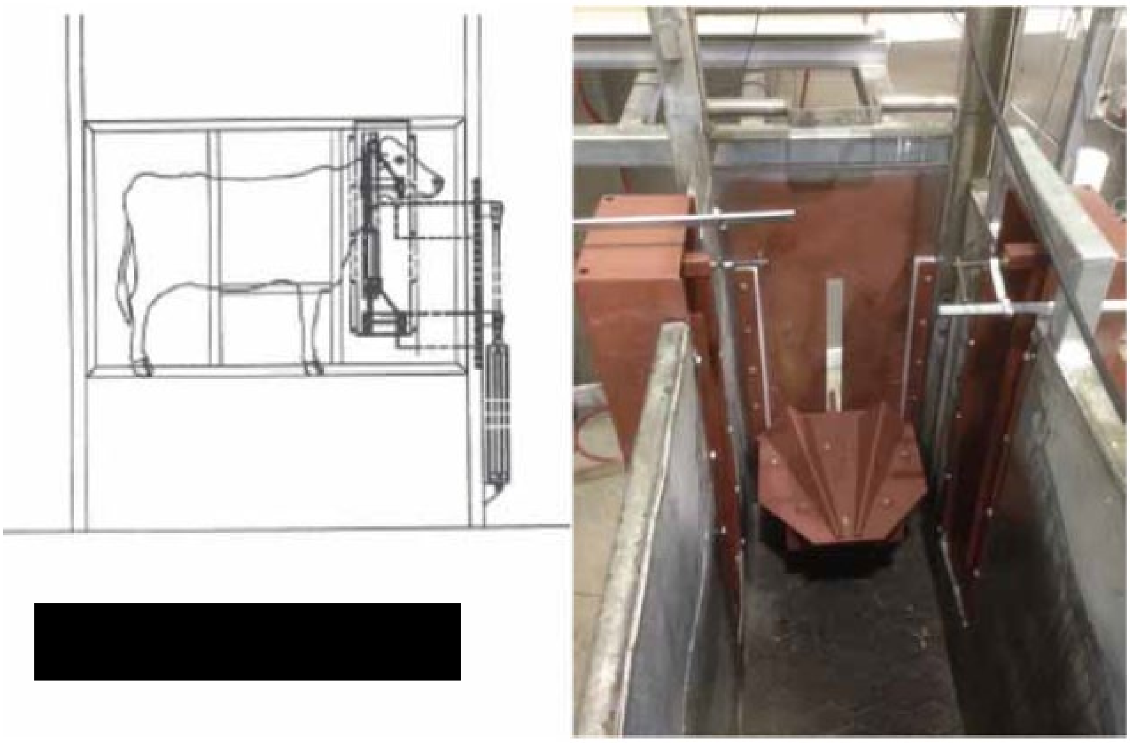

Figure 16: Left: Engineering concept diagram of restraint unit; Right: photograph of chin lift unit, taken during installation, prior to enclosure with faraday cage shielding.

A baseline thermal image was taken using an infrared thermography (IRT) camera (ThermaCam T640, FLIR Systems AB, Danderyd, Sweden). The waveguide was then positioned on the forehead at the application point, checked for fit, the faraday cage shielding fastened, and personnel evacuated the immediate area in case of shielding failure.

Experimental design: DTS was then applied to a pre-determined total energy delivery, at a defined power setting, which varied for each individual. Energy application began at 360 kJ, delivered using a power setting of 30 kW, and when each energy setting was confirmed to induce insensibility, the energy setting was sequentially reduced by increments of 25 kJ throughout the 20 animals, with a return to higher energy levels at the beginning of each processing day such that the settings applied were: one animal at 360 kJ, five at 300 kJ, five at 275 kJ, two at 250 kJ, five at 225 kJ, and two at 200 kJ. Towards the end of the study, power settings of 20 kW were attempted, with the total energy delivery set at levels (275 kJ and 200 kJ) that had produced insensibility when delivered using a power setting of 30 kW and finally one animal was processed using an energy delivery setting of 200 kJ and power setting of 15 kW.

Sampling strategy: Immediately the generator deactivated following energy application, the faraday cage was opened, the waveguide was removed, and the animal visually assessed for signs of distress (and in the event that distress was evident, which did not occur, the instructions were to immediately apply captive bolt). Corneal reflex was tested, and a post-application thermal image taken. The body was released from the restraint unit onto the bleed conveyor, where the oesophagus was sealed, and exsanguination was performed using the 'thoracic stick method' which severs the common carotid artery close to the thoracic inlet. If signs of returning consciousness were detected, namely the return of corneal reflex or eye focus and following movements, the animal was stunned using captive bolt, regardless of where in the process it was – i.e., if it was still in the restraint unit, it was stunned, then rolled out before exsanguination; if it was on the bleed conveyor, it was stunned there and exsanguinated immediately if this had not previously begun. Once the body was exsanguinated, it was processed and inspected for human consumption according to the normal practices at this abattoir.

During application of DTS, the animals were monitored using real-time video capture through a security camera system (Dahua HCVR4108HS-S3/8, Zhejiang Dahua Technology Co. Ltd, China) with one camera positioned over the animal's head within the head capture unit, one over the body within the restraint box unit, one over the control panel, and two over the bleed conveyor. Observations on animal reaction were recorded from the video footage in real time, then subsequently footage was played back at reduced speed (up to 16 times reduction) in order to prepare a detailed event log for each individual.

Randomization and blinding: the order of treatment application was pre-determined, randomization was based on the order in which the animals presented themselves to the lead-in race to the restraint unit.

Personnel carrying out offline video behaviour recording, EEG and thermal imaging analysis were blinded to treatment applied.

Data analysis: Thermal image data were imported into ResearchIR (FLIR Systems AB, Danderyd, Sweden) for analysis. On each image, the forehead area was delineated to exclude the ears, eyes and muzzle area, the minimum, maximum and average temperatures within this area and the number of pixels represented by the delineated area was returned from the software. For post-DTS images, the image was then manipulated to show only areas where the surface temperature was greater than 45 °C ; greater than 50 °C or greater than 60 °C and the number of pixels represented by each of these subsets returned from the software, and these were expressed as a proportion of the number of pixels in the delineated forehead area. Pre- and post-DTS minimum, maximum and mean temperatures were used along with the total energy delivered as recorded by the DTS control software to generate a 'temperature change by energy delivered' chart for each individual. Comparing 'total energy delivered by time' from the DTS control software with video annotation of behaviours allowed an estimation of the energy delivered at the point of loss of posture, and the associated surface temperature at that point could then be estimated from the 'temperature change by energy delivered' chart.

3.2.2.2 Measurement of the outcome

Live and video behavioural monitoring were utilized to assess the magnitude of pain, suffering and distress; latency to return of corneal reflex was recorded as indicators of onset and duration of unconsciousness and time to death.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

The number of animals receiving a DTS treatment and the response was:

Number of animals receiving DTS treatment	20

less not achieving unconsciousness (loss of posture and loss of corneal and somatic withdrawal reflexes)	3
Available for measurement of ABMs relating to achieving unconsciousness	17
less animals that did not recover consciousness	4
Available for measurement of ABMs relating to duration of unconsciousness	13

For each of the three animals that were not deemed insensible, there had been problems with maintaining contact between the waveguide and the forehead, resulting in leakage of energy into the environment, rather than penetration into the brain. All three appeared partially conscious, with loss of eye focus and no visual following of movement, and a slow response to a touch on the cornea. All three were stunned using captive bolt immediately following assessment of consciousness. The unconscious animals demonstrated behavioural signs consistent with an electrical stun – rapid blinking or flicking of the eyelids including the membrane nictitans (third eyelid), loss of posture, tonic (stiff) and clonic (convulsive) phases.

Table 16: Time (seconds) after commencing treatment to reaction

Reaction	**Number of animals**	**Mean time, (range without outliers, outlier/s,) (seconds)**
First reaction	20	2 (1-6)
Blinking	14	3.5 (1-5, 19)
Eyes closed	4	3.25 (1-6)
Eyes fixed	4	1.25 (1-2)
Body movement	5	2.6 (1-4)
Loss of posture	20	5.1 (1-9,15,19)

The 5/20 animals with evidence of body movement (front legs paddling, hind limb movement, loss posture and regain, pull back and unspecified body movement) were the only ABMs that might indicate pain, or distress during induction of insensibility but the one animal recorded as pulling back might be the only one that made a voluntary movement.

Loss of posture occurred between 1 and 8 seconds after onset of energy delivery. In 4 animals, which received 300-360 kJ, this insensibility progressed to death. Two animals (19 and 20) received a dose of 200 kJ at 15-20 kW and demonstrated a response to energy application in 4-6s and loss of posture after 8s.

In the remaining 13 animals, absence of corneal reflex persisted for between 100 and seconds, at which point captive bolt was administered in case of returning consciousness. From video footage, loss of posture, which is considered to be a definitive indicator of unconsciousness, occurred between less than 1 second from DTS application to 19 seconds after the onset of DTS application (Table 16). It is unclear from the current data set whether leakage of energy contributed to the more prolonged intervals from onset of DTS application to loss of posture, but it could be expected that leakage of energy resulted in slower heating of the brain, and thus a delay in loss of posture. A number of animals (12 in total, all within the first 13 animals) returned to an upright, rigid tonic position after initial loss of posture. This occurred between 5 and 144 seconds after loss of posture (mean 50.75; median 25.5 seconds), often prior to roll-out as the animals remained in the restraint for post-DTS EEG capture. The animals were considered unconscious due to lack of reflex responses..

A summary of the surface temperatures recorded on the delineated forehead are of cattle pre- and post-DTS, and the percentage area post-DTS in which surface temperature was greater than 45, 50 and 60 °C is presented in Table 18. However, it must be borne in mind that the post DTS images were taken after the entire energy load had been delivered. From live and video observations, loss of posture usually occurred before the entire energy load had been delivered, so the remaining energy can be considered to be excess energy, not required for inducing insensibility. The continued application of energy would continue to heat tissues, resulting in the thermal maps produced. By comparing the data prior to energy application against the post-application data, it was possible to estimate a heating rate by energy delivered, and utilising this heating rate, estimate the surface temperature at the point of loss of posture. These data for each animal are shown in Table 19 but to summarise, the average surface temperature on the forehead at the point of loss of posture was estimated to be between 26 and 49 °C, although some animals (animals 6 and 7) that showed a delayed loss of posture also demonstrated hot-spots of up to 109 °C.

Of the 13 animals successfully stunned and beginning to return to consciousness the time from DTS application to return of reflexes (after which captive bolt was applied) was a mean of 175 seconds (range 102-225). Two animals (19 and 20) received a dose of 200 kJ and were unconscious for at least 200s.

Table 17: Time intervals from onset of DTS application to behavioural changes and interval from loss of posture to return of reflexes.

Animal number	*Time from onset of energy application to onset of physical response (and response character)*	*Time from onset of energy application to loss of posture*	*Time from onset of physical response to loss of posture*	*Time from loss of posture to return of reflexes (last reflex tested before (or) captive bolt applied)*
1	< 1 sec (rapid blinking)	7 sec	<7 sec	Indefinite, animal died
2	< 1 sec (eye fixed open) 5 sec (rapid blinking)	5 sec	< 5 sec	2 min 5 sec
3	1 sec (loss of posture) 4 sec (rapid blinking)	1 sec	<1 sec	Indefinite, animal died
4	<1 sec (rapid blinking, loss of posture)	1 sec, regained feet then fell again at 6 sec	<1 sec	3 min 4 sec
5	2 sec (rapid blinking)	6 sec	4 sec	Indefinite, animal died
6	2 sec (hind limb movement) 19 sec (rapid blinking, loss of posture)	19 sec	17 sec	Indefinite, animal died
7	1 sec (eyes open wide) 2 sec (body movements)	15 sec	14 sec	3 min 36 sec
8	1 sec (eyes open wide)	9 sec	8 sec	3 min 37 sec
9	4 sec (eyes close tight then rapid blinking)	5 sec	1 sec	2 min 47 sec

Animal number	*Time from onset of energy application to onset of physical response (and response character)*	*Time from onset of energy application to loss of posture*	*Time from onset of physical response to loss of posture*	*Time from loss of posture to return of reflexes (last reflex tested before (or) captive bolt applied)*
10	2 sec (eyes closed) 5 sec (loss of posture)	5 sec	3 sec	2 min 52 sec
11	1 sec (eyes close tight then open wide) 6 sec (body movements and blinking)	9 sec	8 sec	2 min 52 sec
12	1 sec (rapid blinking, loss of posture)	<1 sec	1 sec	2 min 12 sec
13	<1 sec (blinks hard)	4 sec	3 sec	2 min 25 sec
14	2 sec (rapid blinking) 4 sec (front legs paddling)	8 sec	6 sec	>3 min 45 sec (exsanguinated without application of bolt)
15	1 sec (blinks)	5 sec	4 sec	3 min 35 sec (back-up stun applied during exsanguination)
16	5 sec (blinks, loss of posture)	5 sec	<1 sec	2 min 14 sec
17	1 sec (blinks) 2 sec (loss of posture)	2 sec	1 sec	>2 min 34 sec (captive bolt applied early for personnel safety reasons as body was trapped in crate)
18	2 sec (blinks, loss of posture)	2 sec	<1 sec	>1 min 22sec (captive bolt applied early for personnel safety reasons as body was trapped in crate)

Animal number	*Time from onset of energy application to onset of physical response (and response character)*	*Time from onset of energy application to loss of posture*	*Time from onset of physical response to loss of posture*	*Time from loss of posture to return of reflexes (last reflex tested before (or) captive bolt applied)*
19	6 sec (eyes closed)	8 sec	2 sec	Estimated 3 min (visibility obscured by person carrying out reflex testing)
20	4 sec (pull back)	8 sec	4 sec	1 min 42 sec

Table 18: Surface temperatures recorded on the forehead of cattle pre- and post-DTS, and the percentage area post-DTS in which surface temperature was greater than 45, 50 and 60 °C.

Animal number	Minimum temperature (°C)	Maximum temperature (°C)	Average temperature (°C)	Area > 45 °C (%)	Area > 50 °C (%)	Area > 60 °C (%)
1 pre-DTS	20.4	35.7	27.8			
1 post-DTS	23.9	83.1	47.8	54	45	18
2 pre-DTS	21.8	38.1	29.6			
2 post-DTS	24.0	106.8	49.9	58	49	27
3 pre-DTS	19.8	35.5	25.0			
3 post-DTS	22.1	95.5	43.6	45	38	18
4 pre-DTS	17.1	34.4	24.4			
4 post-DTS	20.9	82.8	39.4	25	18	7
5 pre-DTS	18.2	34.6	24.1			
5 post-DTS	20.5	91.8	45.5	49	38	10
6 pre-DTS	20.8	36.4	27.9			
6 post-DTS	24.0	99.6	49.7	57	44	17
7 pre-DTS	20.0	37.5	26.9			
7 post-DTS	23.1	109.2	46.6	46	41	27
8 pre-DTS	23.2	34.4	29.4			

Animal number	*Minimum temperature (°C)*	*Maximum temperature (°C)*	*Average temperature (°C)*	*Area > 45 °C (%)*	*Area > 50 °C (%)*	*Area > 60 °C (%)*
8 post-DTS	24.0	95.5	51.4	60	50	32
9 pre-DTS	21.9	35.5	28.5			
9 post-DTS	24.0	92.3	49.3	52	44	26
10 pre-DTS	21.9	34.9	28.1			
10 post-DTS	21.8	98.5	46.4	51	43	21
11 pre-DTS	20.4	39.4	27.9			
11 post-DTS	23.8	89.2	51.9	62	53	37
12 pre-DTS	18.4	34.3	24.7			
12 post-DTS	21.4	77.6	31.6	10	7	3
14 pre-DTS	12.6	34.4	20.2			
14 post-DTS	15.2	91.1	41.4	32	23	13
15 pre-DTS	16.1	33.4	25.3			
15 post-DTS	17.4	77.3	44.2	46	27	9

Animal number	*Minimum temperature (°C)*	*Maximum temperature (°C)*	*Average temperature (°C)*	*Area > 45 °C (%)*	*Area > 50 °C (%)*	*Area > 60 °C (%)*
16 pre-DTS	14.8	31.6	21.8			
16 post-DTS	17.0	90.1	43.1	41	33	11
18 pre-DTS	17.1	35.1	25.2			
18 post-DTS	15.5	62.9	39.1	23	08	0
19 pre-DTS	17.2	36.2	26.1			
19 post-DTS	16.6	80.3	43.3	45	32	7
20 pre-DTS	15.9	36.2	24.7			
20 post-DTS	17.7	79.9	42.9	36	25	13

Table 19: Summary of data collected from each animal.

Animal	*setting (kJ, kW)*	*energy delivered (kJ)*	*latency to react (s)*	*latency to loss of posture (s)*	*loss of posture to stand (s)*	*loss of posture to reflex return (s)*	*energy at loss of posture (kJ)*	*mean surface temp at loss of posture (°C)*	*min (°C)*	*max (°C)*	*unconscious*	*leakage*
1	360, 30	421	1	7	never	never	170	35.88	21.81	54.83	yes, then death	not observed
2	300, 30	350	1	5	88	125	104	35.63	22.46	58.5	yes	evident on live observation
3	300, 30	351	1	1	69	never	135	32.16	20.69	58.57	yes, then death	evident on live observation
4	300, 30	171	1	6	80	184	143	36.88	20.26	74.7	yes	evident on live observation
5	300, 30	338	2	6	17	never	138	32.82	19.13	57.9	yes, then death	visible on thermal image
6	275, 30	317	2	19	5	never	317	49.71	24	99.64	yes, then death	visible on thermal image
7	250, 30	292	1	15	144	216	292	46.55	23.1	109.07	yes	not observed
8	250, 30	284	1	9	17	217	233	47.39	23.85	84.38	yes	visible on thermal image
9	225, 30	267	4	5	5	167	114	37.37	22.8	59.7	yes	evident on live observation
10	225, 30	258	2	5	14	172	112	36.04	21.94	62.51	yes	evident on live

Animal	*setting (kJ, kW)*	*energy delivered (kJ)*	*latency to react (s)*	*latency to loss of posture (s)*	*loss of posture to stand (s)*	*loss of posture to reflex return (s)*	*energy at loss of posture (kJ)*	*mean surface temp at loss of posture (°C)*	*min (°C)*	*max (°C)*	*unconscious*	*leakage*
												observation
11	225, 30	261	1	9	10	172	215	47.59	23.2	80.27	yes	evident on live observation
12	225, 30	125	1	1	34	132	6	25.02	18.54	36.29	partial	evident on live observation
13	225, 20	247	1	4	126	145	58	not recorded			partial	evident on live observation
14	300, 30	351	2	8	never	> 225	196	32.04	14.05	66.03	yes	not observed
15	275, 30	310	1	5	never	215	110	32	16.23	48.95	yes	visible on thermal image
16	275, 20	310	5	5	never	134	80	27.28	15.37	46.66	yes	evident on live observation
17	275, 20	312	1	2	never	> 154	23	not recorded			yes	evident on live observation
18	275, 20	297	2	2	never	> 82	23	26.27	16.98	37.24	partial	evident on live observation
19	200, 20	226	6	8	never	180	135	36.36	17.56	62.51	yes	evident on live observation

Animal	*setting (kJ, kW)*	*energy delivered (kJ)*	*latency to react (s)*	*latency to loss of posture (s)*	*loss of posture to stand (s)*	*loss of posture to reflex return (s)*	*energy at loss of posture (kJ)*	*mean surface temp at loss of posture (°C)*	*min (°C)*	*max (°C)*	*unconscious*	*leakage*
20	200, 15	223	4	8	43	102	101	32.92	16.71	55.94	yes	visible on thermal image

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3.2.3.2 Reporting uncertainty

A potential source of bias is the small group size (n=20), animals being sourced from a single mob presented at the abattoir. A variety of breed, sex and age were presented.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The findings of this study phase were as follows:

Seventeen of 20 cattle were assessed as insensible following DTS application. These animals demonstrated behavioural signs consistent with an electrical stun, loss of posture occurring between 1 and 8 seconds after onset of energy delivery. In 4 animals, which received 300-360 kJ, this insensibility progressed to death. In the remaining 13 animals, absence of corneal reflex persisted for between 100 and 170 seconds, at which point captive bolt was administered in case of returning consciousness. When these timeframes are compared with those reported for head-only electrical stunning in cattle (application of 4 to >20 sec, duration 31 to 90 seconds), it is evident that DTS provides a better welfare outcome.

For each of the three animals that were not deemed insensible, there had been problems with maintaining contact between the waveguide and the forehead, resulting in leakage of energy into the environment, rather than penetration into the brain. All three appeared partially conscious, with loss of eye focus and no visual following of movement, and a slow response to a touch on the cornea. All three were stunned using captive bolt immediately following assessment of consciousness.

Thermal imaging indicated that the average surface temperature on the forehead at the point of loss of posture would be between 26 and 49 °C, although some animals (animals 6 and 7) that showed a delayed loss of posture also demonstrated hot-spots of up to 109 °C. In context, Australian cattle may experience ambient air temperatures of 45 °C in summer and could experience higher surface temperatures when standing in in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition, while temperatures above 60 °C will result in skin tissue damage, as seen in hot branding which is commonly used for identification of cattle.

The results gathered were confounded by problems with waveguide to forehead contact, resulting in loss of energy to the environment instead of being transmitted into the brain. This variability in energy delivery may account for the variability in latency to loss of consciousness, using loss of posture as the proxy indicator, and problems with waveguide to forehead contact may have contributed to the variability in surface temperatures and the presence of hot spots on the forehead.

3.2.4.2 Conclusions

- The application of DTS (all doses 200 kJ or above) resulted in signs of unconsciousness immediately following energy application.
- The duration of insensibility was between 100 and 225 seconds based on duration from loss of posture to return of corneal reflex.

- No signs of pain, distress and suffering were observed during the application of the intervention.

3.2.5 Conflicts of interest

This work was funded by Meat & Livestock Australia (MLA, a statutory research funding body), [redacted] And the Australian Government. The work was performed under contract to MLA. [redacted] is the licensee of the technology and the applicant, all other authors have no commercial interest in the technology.

STEP 10: Laboratory engineering: Refinements to delivery system: applicators, coaxial cabling and tuning

Submitted study: **PR13**: Refinements to delivery system: applicators, coaxial cabling and tuning. Interim project reports. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

The validation study (Step 9) identified some issues with energy leakage from the applicator, maintenance of contact between head and applicator, surface heating, and robustness of the coaxial cabling. Further engineering modifications were required to minimize or correct these issues. Subsequent to these initial modifications (**PR13** part1 and part 2), a series of further modifications were developed and tested during the course of the commercial-scale validation (Step 11).

3.2.1.2 Objective

Phase 1 (April-May 2018). To confirm functionality of coaxial cable components, bench-test a variety of alternative applicator designs and to optimize robust energy coupling to the animal's head and maximise the heating spread (**PR13** part 1 and part 2).

[redacted]

Phase 3 (December 2020 – March 2021). To investigate reasons for failure of coaxial cable components (**PR13** part 5) To improve shielding around the restraint unit (**PR13** part 6); to investigate the potential for using metallic ducting as a flexible waveguide (**PR13** part 6); and to bench-test the latest applicator design (**PR13** part 7).

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

3.2.2.2 Measurement of the outcome

[redacted]

- [redacted]
- [redacted]

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

[redacted]

[redacted]

[redacted]

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[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

- [redacted]
- [redacted]
- [redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.2.5 Conflicts of interest

No conflicts of interest were reported. The authors of PR13 have no commercial interest in the DTS: Diathermic Syncope® technology.

STEP 11: Commercial scale validation

Submitted study: **PR12**: DTS: Diathermic Syncope® controlled trials. Project report. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

This study was conducted with conscious cattle with a larger number of animals since acceptable animal welfare outcomes had been demonstrated in earlier stages (stages 7 (SS05, PR08, PR09) and 9 (SS05, PR11, PR12). The aim was to continue to collect data from the conscious cattle trial (Step 9) over a range of cattle types and operating parameters, while also undertaking iterative improvements to the microwave applicator, cabling and tuning (Step 10, PR13), observing behavioural and physiological ABMs and EEGs for insensibility.

This observational study was performed in two parts. The first was performed over a series of small groups of animals between October 2017 and October 2019 and the second over a series of small groups of animals in December 2020; in the intervening period a rotary box was installed to facilitate extraction of the unconscious, tonic animal from the restraint unit.

At this stage the electronics of delivery were performing satisfactorily, and the applicator was reliable. Some component failures (e.g., co-axial cabling, electrical relays, electrical earthing) occurred during the validation period: these resulted in failure to deliver full energy cycles, so data from animals involved were removed from analysis, while possible impact on animal welfare (use of behavioural and physiological ABMs) are reported. These technical issues were corrected iteratively during the course of this study (see Step 10 for detail). All work was performed in a box with improved head restraint to maintain contact between the applicator and the animal's forehead.

It was possible to estimate output parameters such as the time to achieve unconscious and return of consciousness and associate these with microwave input parameters. It was also possible to estimate stunning effectiveness. Confirmation of the estimated parameters to provide unconsciousness from which animals can recover, and repeatability of induction of insensibility using these parameters, with component failure issues corrected, is addressed in Step 13.

3.2.1.1 Background and rationale

A dielectric (electromagnetic) stunning system has been developed by [redacted]. This system, trademarked DTS: Diathermic Syncope®, has the potential to address the requirements of the Halal and Kosher markets, without current disadvantages of existing stunning methods (e.g., the potential for cracked skulls associated with percussive stunning, the need to use an immobiliser to improve operator safety in electrical head-only stunning, the need for a second exsanguination cut following the neck cut to remove the risk of the animal regaining consciousness during bleed-out). Pilot trials on live animals have been successful in inducing electroencephalogram traces consistent with unconsciousness, of sufficient duration to allow exsanguination prior to recovery. DTS produced comparable post-slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt. This phase of development comprised trials on live animals in a controlled working environment, validating the previous outcomes in a larger number of animals, demonstrating repeatability. Ultimately the outcomes of this work will be used to gain industry stakeholder agreement on full commercialisation of the technology. The progress of research and development of this technology is evidence of the Australian Industry's commitment to continual improvement in Animal Welfare at processing, which in turn supports the continued social license to operate.

3.2.1.2 Objective

- Confirmation of previous science works in a commercial environment.
- Collection of data to support approval of the technology as a commercial means of inducing insensibility on cattle for the production of meat for human consumption.

3.2.2 Materials and methods

3.2.2.1 Method

There were two parts to this phase of work – the first was a pre-commercial-scale evaluation in which cattle were processed using the restraint unit described in Step 9. Variations in input power and energy parameters and a variety of applicator head designs were tested. Finally, an evaluation of a proposed commercial set-up including a rotary box for handling of the stunned body was carried out.

Ethical considerations: The study was approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Wildlife and Large Animal Animal Ethics Committee (CWLA AEC), reference 2019-17.

For the first part of the study, a total of 235 cattle was processed in a series of small batches of between 8 and 50 cattle between October 2017 and October 2019. Between batches, modifications were made to the waveguide apparatus (Step 10) and/or restraint unit in order to optimise stun quality and ease of processing. These modifications led to reductions in the required input power (kW) and elimination of the surface overheating that had been observed during the previous study (Step 9). Data were collected on animal type, energy delivery parameters, presence/absence of indicators of unconsciousness, time of back-up stunning and behavioural observations.

Study population: The animals included steers, heifers, cows and bulls, of varying ages from milk teeth only to full mouth cull animals. The animals were predominantly *Bos taurus*, with 5 *Bos indicus* animals, and 13 had horns. Carcase weights ranged from 160 to 413 kg. Power settings for DTS application ranged from 15 to 40 kW, and energy settings from 100 to 371 kJ.

For Part 2, A rotary box was installed [redacted], and fully enclosed in a Faraday cage (Section 3.6). Over a three-day period from 1st to 3rd December 2020, 35 *Bos taurus* slaughter generation cattle of mixed breeding were processed using DTS: Diathermic Syncope® to induce insensibility, inverted using the rotary box and bled in the inverted position using the Halal cut delivered by a registered Halal slaughterman. The animals were processed to give carcase weights ranging from 242 to 413 kg (10 steers and 25 heifers). Breeds were crosses of Angus, Hereford, and Holstein/Friesian and 13 had horns. Energy applications between 140 and 230 kJ were used in 33 animals, all of which were rendered insensible using DTS. Two animals received energy levels below 100 kJ as a result of equipment failure, these were immediately stunned using a non-penetrating device. An attempt was made to collect Electroencephalogram (EEG) data from 19 DTS animals using the protocol outlined in Step 7, while maintaining a near-commercial rate of processing.

Study design and methods of analysis: this was an observational study, with no statistical analysis performed.

Randomisation and blinding: Treatments were pre-determined, so randomization was related to the order in which animals presented themselves to the restraint unit. Observers were not blinded to treatment.

3.2.2.2 Measurement of the outcome

Live and video behavioural monitoring were utilized to assess the magnitude of pain, suffering and distress. Absence of corneal reflex was used as the key indicator of unconsciousness.

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

<u>Part 1</u>

Of 235 animals processed, 6 were assigned to penetrative captive bolt only as a result of their being collapsed in the restraint or overly agitated to securely restrain; and a further 6 were stunned and slaughtered at the end of the final batch using penetrative captive bolt because the current phase of DTS trials was complete and they were 6 remaining animals in the lairage when the plant was being decommissioned for refurbishment in preparation for the rotary box evaluation. These 12 animals were excluded from the evaluation of DTS, leaving 223 animals in the current evaluation.

Of the 223 animals included in the evaluation, 20 were animals that are reported on at Step 9 and a further 16 were excluded from analysis as a result of equipment failure resulting in generator shutdown before the required energy could be applied. These animals were also stunned using a penetrative captive bolt prior to exsanguination.

Of the remaining 187 animals, 181 were rendered unconscious (insensible) on first application of DTS (96.8%). This exceeds the expected minimum standard of 96 % described in appendix 7 of the National Animal Welfare Standards for Livestock Processing Establishments (AMIC, 2009) stipulated for non-penetrative captive bolt stunning. The 6 other animals appeared sedated. Analysis of the data indicated that several of these cases were related to low Power (kW) settings, which were used during the study to determine the lower critical limits of expected operational parameters. Applicator positioning was the apparent cause of some failures and adjustments were made during the course of the work. In the 181 fully insensible animals, live observations suggested that there was rapid induction of insensibility, which lasted for between 2 and 4 minutes in the majority of cases.

In summary:

number of animals at Step 11	215	
number of animals not treated with DTS (ie captive bolt only)	6	
number of animals where DTS failed to operate and not stunned	6	
number of animals treated with DTS	203	
Subject to system failures	16	
Successfully receiving DTS treatment in step 11	187	
Rendered unconscious	181	(96.8%)

Live observations indicated that low energy levels resulted in shorter duration of insensibility, while energy levels above 220 kJ appeared to result in early onset of intense convulsions. Surface overheating and visible blistering of the skin (assessed after application completed) was evident when power settings were 25 kW and above.

Three entire Angus young bulls, three mature bulls and five Brahman animals (three of which were horned) were included in the data set. Energy applied to bulls ranged from 200 to 250 kJ, and all were rendered insensible using DTS. Forehead skin thickness in the bulls ranged from 5 mm (Brahman) to 24 mm (Angus).

Visual observations were used to assess the welfare of animals during restraint and induction of unconsciousness. Behavioural ABMs used to evaluate animal welfare during restraint and application are detailed in Table 20.

Of the animals receiving a dose of energy of 200 kJ, the average time to loss of posture was 13s (5-26s) and 15s (0-29s) until an indication of a tonic/clonic response) and 3/10 animals displayed some head movement during induction.

Table 20: Observed animal based measures (ABMs) of pain, fear or distress during stunning using upright box

Phase	ABM	Description	Number (%)
Restraint (prior to application)			
	Voluntary Movement	agitated	10 (4.5%)
	Escape attempts		0

	Injuries		0
During application of DTS			
	Voluntary Movement	Agitated	2 (0.9%)
		Kicking	5 (2.3%)
	Escape attempts		0
	Injuries		0

In the absence of technical problems leading to interrupted or intentional low delivery of energy, all animals were rendered unconscious using DTS. For those animals in which the time to change in consciousness was measured the time from the beginning of the stun application to the loss of posture was 13s (n=6) the first sign of twitching was an average of 16s (n=8) when 200kJ was applied at a rate of 20kW. For those animals in which the period of unconsciousness was estimated (minimum of time when head tensed until reflex returned or captive bolt application) (n=10) the mean minimum duration of unconsciousness was 114 seconds (range 62-176 seconds, all with energy application of 200 kJ).

On exsanguination, blood flow was noted to be strong, and the rhythmic pumping of the heart was evident in the first few seconds of blood flow. Both these characteristics are desired by the Halal and Kosher markets.

Visual inspection of a sample of brains, removed from the skull cavity immediately following head inspection, indicated that when energy delivery was 220 kJ or less, there was no visible damage to the brain.

The main challenge during this phase was extracting the stunned bodies from the restraint unit, which was a standard abattoir knocking box designed for use with captive bolt. Cattle that are stunned using DTS (or for that matter, electrical stunning) can quickly enter a stiff, tonic 'rocking-horse' position, with all four legs rigidly extended. If this happens before the body is rolled out from the box, the body is obstructed by the side door, which cannot open sufficiently to allow ejection from the box. After this 'rocking horse' phase, the stunned animal develops a convulsive or kicking phase (similar to that seen in an epileptic episode), followed by a recovery phase. In commercial processing it is important to exsanguinate the animal before the kicking phase begins – both to ensure that the animal does not recover during bleed-out, and because the size of the animal makes handling during that kicking phase very dangerous for the operator. The difficulties in extracting the body from the upright restraint box prevented prompt exsanguination during the tonic phase.

As such, installation of a rotary box was undertaken, so that the stunned animal could be tipped to allow rapid exsanguination.

<u>Part 2</u>

The number of animals receiving a DTS treatment and the response was:

Number of animals receiving DTS treatment	35
less not achieving ≥ 100 kJ and therefore risking short unconsciousness	2

Successful DTS First stun	33
less animals that did not have all video recorded	3
Available for measurement of ABMs relating to duration of unconsciousness	30

The first stun success rate was therefore, 94.%

Lower energy application was more frequently used during this part of the work. Energy application in the range of 140-200 kJ (18-20 kw) resulted in unconsciousness in 2 s (until tonic/clonic response). Energy levels of 140-160 kJ appeared to result in shorter durations of insensibility, while energy levels above 200 kJ appeared to result in early onset of intense convulsions.

Visual observations were used to assess the welfare of animals during restraint and induction of unconsciousness (Table 21).

Table 21*: Observed animal based measures (ABMs) of pain, fear or distress during stunning using rotary box*

Phase	**Description**	**N=35**
Restraint	Vocalise	0
	Voluntary movement	2
	Escape attempt	0
Induction	Vocalise	0
	Voluntary movement	2
	Escape attempt	0

The first sign of a change in the level of consciousness (eyelid flutter, ear flick, nose twitch) often occurred immediately on commencement of DTS application (mean 2 seconds, range 0-7 seconds).

The study reconfirmed consistent induction of insensibility, based on live observations of the animal and assessment of corneal and palpebral reflexes. For those animals in which the period of unconsciousness was estimated (n=29) the mean duration from the end of induction to the last negative corneal reflex was 71 seconds (range 43-245 seconds). The duration of unconsciousness was not particularly sensitive to the energy applied (see Figure 18)

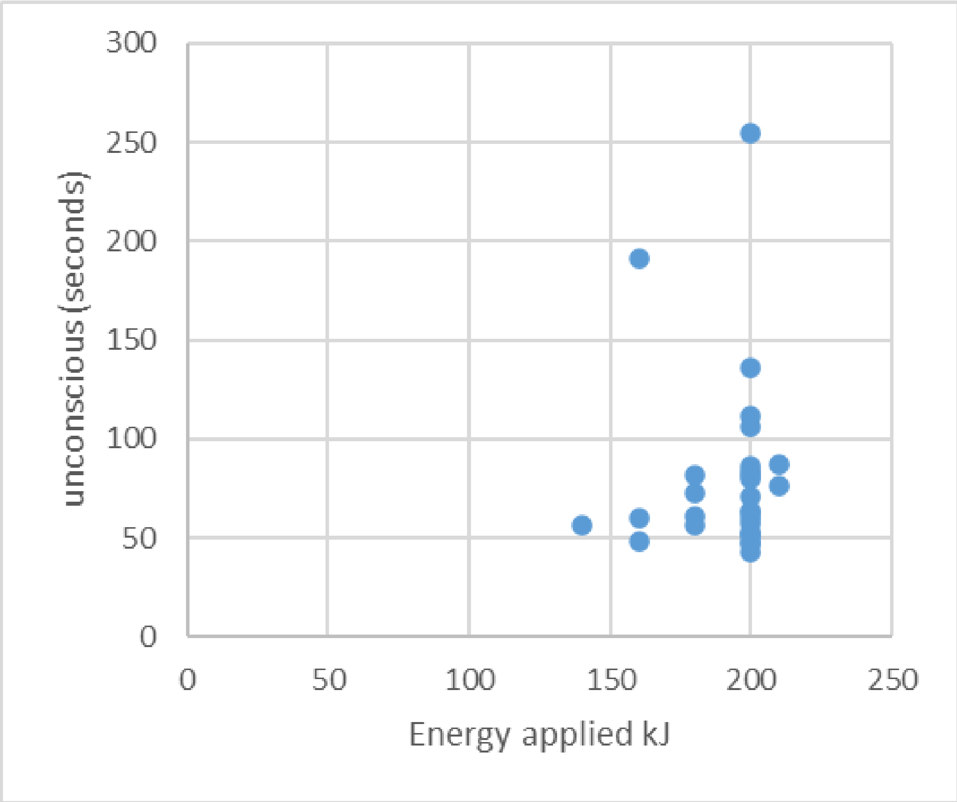

Figure 18: Minimum period of unconsciousness (from tending of head to either sticking or captive bolt.

Insensibility was sustained during bleeding using the Halal neck cut. . All EEG traces were heavily contaminated with artefact originating from muscle movements during the tonic-clonic phase, rolling of the rotary box and operation of other machinery (bleed conveyor) in the immediate surroundings.

Use of the rotary box improved handling of the stunned body, and the updated applicator configuration allowed consistent repeatable induction of insensibility, which was sustained during Halal bleeding. When delays associated with EEG data collection were not present, sticking was carried out within 30 seconds of DTS application.

3.2.3.2 Reporting uncertainty

Although attempts were made to include a wide range of animal type, sex, weight and age, numbers are still limited in a commercial sense), and the full range of cattle weights and feedbase origins that are processed commercially are not represented.

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

The latency to onset of physical signs of insensibility was related to power setting. A higher power (kW), providing a more rapid delivery of the energy 'package', led to a shorter time to onset of physical signs of insensibility. The work on anesthetised cattle, carried out by Rault et al. (2014:

SS03), indicated that shorter durations of applications resulted in more rapid onset of EEG suppression, while greater power settings resulted in a longer duration of insensibility. This is difficult to interpret fully, based on the small number of animals processed in their study and the fact that the animals in their study each received two applications of energy; however, there is a relationship between power setting and rate of heating. The units of power (kW) is shorthand for 'kJ per second', so a higher power setting will reduce the time required to reach an energy delivery of 200 kJ than a lower power setting. However, the higher power setting may predispose to focal overheating, as seen in the first phase of the current study.

Based on the outcomes of this project, we recommend that operational guidelines define the desired range of energy application to be between 160 and 200 kJ.

The stunned animals demonstrated behavioural signs consistent with an electrical stun – rapid blinking or flicking of the eyelids including the membrana nictitans (third eyelid), loss of posture, tonic (stiff) and clonic (convulsive) phases, so it is of value to compare the outcome of DTS against those reported for electrical stunning. In cattle, electrical stunning can be applied as head-only, which affects the brain, resulting in an epileptiform seizure, from which the animal can regain consciousness; or as head-to-chest, which incorporates a current passed through the heart, resulting in cardiac arrest, and the death of the animal. As DTS does not directly affect cardiac function, the comparison should be against head-only electrical stunning.

The biggest problem with head-only stunning of cattle is a very short duration of epilepsy, followed by strong convulsions (EFSA, 2004). The electrical current must be applied across the brain for sufficient duration to overcome the inherent impedance (resistance) across the head, and application of electrical stun ranges from 4 to greater than 20 seconds (Devine et al., 1986; Vonmickwitz et al., 1989; Schatzmann and Jaggin-Schmucker, 2000). The duration of unconsciousness in those studies was reported as 31 - 90 seconds. In contrast, DTS induces loss of posture within 8 seconds of onset of energy application, and the duration of unconsciousness recorded during the current study was between 100 and 240 seconds, based on absence of corneal reflex. Return of reflexes are considered to be the earliest indication that consciousness is returning and was the point at which penetrative captive bolt was applied during the current study for animal welfare reasons. In the current study this occurred between 2 and 4 minutes, 30 seconds from loss of posture, which would allow ample time to exsanguinate the animal prior to recovery. Using the rotary box, when a delay to capture EEG data was not required, sticking was performed within 30 seconds of DTS application.

In the early stages of the project, overheating of the skin surface was identified. This was associated with incident power levels of > 25 kW. This was eliminated in the later stages of the project, through optimisation of the auto-tuning device and applicator apparatus such that incident power levels ranged from 18-20 kW. Australian cattle may experience ambient air temperatures of 45 °C in summer and could experience higher surface temperatures when standing in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition.

The animal stunned using DTS can also return to consciousness, so this method of stunning is likely to be acceptable to the Halal and Kosher markets. Furthermore, blood flow was observed to be strong, and the rhythmic pumping of the heart was evident in the first few seconds of blood flow. Both these characteristics are desired by the Halal and Kosher markets.

Use of the rotary box optimised handling of the stunned body, allowing a short stun-to-stick interval without the use of an immobiliser. The updated applicator configuration allowed consistent repeatable induction of insensibility, which was sustained during Halal bleeding, without the need to apply an additional ventral cut.

Key messages:

In the absence of technical problems leading to interrupted delivery of energy, all animals were rendered unconscious using DTS. At no time was Animal Welfare compromised by technical faults.

Power settings of 20 kW provided a rapid onset of insensibility, while a power setting of 18 kW led to a slower onset of insensibility. Power settings of 25 kW and above led to overheating at the skin surface.

Energy deliveries in the range of 160 to 200 kJ achieved insensibility of sufficient duration to allow exsanguination using a neck cut alone, with operator safety during exsanguination optimised. Energy deliveries of 160 kJ and less resulted in shorter duration of insensibility with a risk of early return to consciousness, while energy deliveries of 220 kJ and above resulted in early onset of intense convulsive activity, reducing operator safety.

Live observations indicated that DTS consistently induces insensibility, with no vocalisation, no evidence of pain or distress.

There was no requirement to use an immobiliser during application of the exsanguination cuts, and the animal bled rapidly to brain death using the Halal cut alone. The blood flow on exsanguination was strong and rapid.

Visual inspection of the brains of carcases indicated no visible damage when energy deliveries of less than 220 kJ were applied.

3.2.4.2 Conclusion

DTS applied to cattle at 160-200 kJ results in rapid loss of consciousness, with minimal indication of adverse animal welfare outcomes during restraint, application and post-stun. Unconsciousness is maintained for a sufficient duration to ensure that death results from exsanguination without a return of consciousness. The use of a rotary box facilitates sticking, a short stun-stick interval and ensures welfare outcomes are achieved.

3.2.5 Conflicts of interest

The work described here was funded by Meat & Livestock Australia, Australian Meat Processor Corporation (both statutory research and development bodies) with some funds coming from the Australian Government and [redacted]. The report was submitted under contract to Meat & Livestock Australia. The authors of PR12 have no commercial interest in the DTS: Diathermic Syncope® technology.

STEP 12: Regulatory Authority review

Submitted study: **PR14**: Independent review of the new technology DTS: Diathermic Syncope. Report - With Australian Regulatory Authority Approval (letter). Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

3.2.1.1 Background and rationale

[redacted]

3.2.1.2 Objective

[redacted]

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

3.2.2.2 Measurement of the outcome

[redacted]

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

[redacted]

[redacted]

- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.2.5 Conflicts of interest

No conflicts of interest were reported. The authors of PR14 have no commercial interest in the DTS: Diathermic Syncope® technology.

STEP 13: Commercial scale verification of ABMs, insensibility, and recovery

Other reference documents: **PR15**: Repeatability of induction of insensibility in cattle using DTS: Diathermic Syncope®; and recovery of consciousness from DTS-induced insensibility. Draft manuscript. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

This study was conducted under commercial conditions and DTS operating parameters that are recommended for simple stunning. It was designed to confirm, or otherwise, the validation of ABMs (STEP 7 (SS05, PR08, PR09), the welfare of animals during induction of unconsciousness and the duration of unconsciousness (insensibility) (STEP 11 (PR12)), and the reversibility of stun (STEP 7 (SS05, PR08, PR09).

The animal welfare outcomes of operating DTS under essentially commercial conditions were investigated. The suitability of the previously identified ABMs under these conditions were reconfirmed. The consistent induction of unconsciousness was verified on a large number and variety of cattle. The reversibility of stun was investigated through undertaking brain histology and allowing full recovery and delayed restunning of cattle.

Power levels were varied and both ABMs (behavioural and physiological) and EEGs were measured.

3.2.1.1 Background and rationale

[redacted]

3.2.1.2 Objective

- To verify the relationship between ABMs (behavioural and physiological) and neurophysiological status at recommended operating parameters for simple stunning
- To verify animal welfare outcomes at the recommended operating parameters.
- To demonstrate minimal permanent effects on the brain (that would prevent recovery), and animal recovery under recommended conditions of simple stunning.

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Table 22: Behavioural animal-based measures recorded from video footage during and after energy application, and analysis parameters derived.

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

[redacted]

3.2.2.2 Measurement of the outcome

[redacted]

[redacted]

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Table 23: Behavioural animal-based measures in the animals: Mean (range) latencies of onset from the initial physical response to energy application and mean (range) duration.

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

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[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

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[redacted]

[redacted]

[redacted]

3.2.4.2 Conclusions

[redacted]

3.2.5 Conflicts of interest

[redacted]

[redacted]

STEP 14: DTS of animals with wet hides

Other reference documents: **PR16**: Impact of Wet Scalp on Energy Impacting the Head. Advanced Microwave Technologies. September 2023. Not peer-reviewed. CONFIDENTIAL.

3.2.1 Introduction

[redacted]

3.2.1.1 Background and rationale

[redacted]

3.2.1.2 Objective

[redacted]

3.2.2 Materials and methods

3.2.2.1 Method

[redacted]

[redacted]

3.2.3 Reporting the results

3.2.3.1 Reporting outcomes and estimations

[redacted]

3.2.3.2 Reporting uncertainty

[redacted]

3.2.4 Discussion and conclusions

3.2.4.1 Reporting interpretation of results

[redacted]

3.2.4.2 Conclusions

[redacted]

3.2.5 Conflicts of interest

[redacted]

3.3 Overall integration of findings from all studies

3.3.1 Demonstration of equivalence with existing methods

3.3.1.1 Biomarkers of stress

The endocrine stress responses measured indicate activation of two physiological response systems. One is the 'fight – or – flight' response initiated by all animals when a threatening or 'emergency' situation is perceived: activation of this response leads to increases in catecholamines (adrenaline and noradrenaline). The catecholamines speed blood circulation and divert blood, and therefore oxygen, from internal organs to the brain and muscle, readying the animal for action. The second response is the HPA axis (Hypothalamic-Pituitary-Adrenal axis): activation of this response leads to the release of Adrenocorticotrophic hormone (ACTH) which leads to increased cortisol levels. This in turn increases the rate of production of glucose from glycogen reserves, so that the energy required for action is available. This speeding up of glycogen breakdown, circulation, and metabolism generally can have detrimental effects on meat quality: very fast metabolism at the point of slaughter can lead to pale meat colour or heat toughening; prolonged raised metabolism leads to reductions in glycogen stores, which in turn lead to dark cutting.

In a commercial slaughterhouse situation, the stresses associated with movement of the animal along the race into the knocking box and positioning the head into the head capture unit are common to all cattle, whether subjected to DTS, electrical stunning or captive bolt stun. Comparison against published reference ranges for various biomarkers, based on resting animals, is inappropriate, as there will always be some underlying effect of the pre-slaughter transport and handling phase. Even differences between abattoirs can strongly influence the stress response (Hemsworth and Barnett, 2001). For example, Zulkifli et al. (2014), working in a large commercial slaughterhouse, report higher, and a wider range in, cortisol concentrations than do Tume and Shaw (1992), from a small experimental facility. This is why baseline samples are taken prior to treatment, against which the post-treatment samples are compared. Examples of published data are presented in Table 24 alongside the ranges found in the detailed evaluation of 18 cattle subjected to either DTS or penetrative captive bolt (Step 7), which align with the published values. The large range in values makes it difficult to draw firm statistical conclusions from small numbers of animals.

For the DTS pilot study, group mean and range (converted to SI units) for each sample point are summarised in Table 25. Baseline cortisol in the DTS group is significantly lower than post-treatment cortisol in that group, however, it is not significantly different from baseline captive bolt, and post-treatment cortisol in either group is not significantly different from the aggregate of all baseline cortisol results. Therefore, the fact that post-treatment cortisol is significantly different from baseline cortisol in the DTS group, and not in the control (captive bolt) group, is likely to be an artefact of small sample size.

Table 24: Published data on endocrine parameters measured in the DTS pilot study (Step 7)

	Rulofson et al. (1988)	Mitchell et al. (1988)	Dunn (1990)	Shaw & Tume (1990)	Tume & Shaw (1992)	Zulkifli et al. (2014)	DTS pilot study
Cortisol (nmol/L)		*25.0 ± 13.7 to 176.7 ± 65.5*	*124.8 ± 10.7 to 259.6 ± 104.0*	*44.9 ± 6.9 to 88.3 ± 10.8*	*41.0 ± 3.7 to 123.2 ± 5.3*	*175.11 to 274.08*	*14.36 to 212.46*
ACTH (pmol/L)		*20.6 ± 7.9 to 301.8 ± 142.9*				*922.1 to 4092.9*	*3.7 to 125.4*
Adrenaline (nmol/L)	*1.2 ± 0.1 to 81.3 ± 20.7*	*9.28 ± 0.44 to 49.12 ± 89.51*				*102.28 to 519.66*	*22.33 to 420.12*
Noradrenaline (nmol/L)	*1.9 ± 0.3 to 135.8 ± 31.6*	*0 to 37.82 ± 54.37*				*271.21 to 754.35*	*<0.12 to 503.64*
B-Endorphin (pmol/L)				*14.6 ± 1.8 to 30.7 ± 4.3*	*19.2 ± 1.5 to 20.9 ± 1.2*	*259.9 to 441.8*	*4.5 to 1559.8*

Table 25: Summary endocrine data

		DTS		Captive Bolt	
		Baseline	**Post-treatment**	**Baseline**	**Post-treatment**
Cortisol (nmol/L)	*Mean*	33.20	150.38	61.43	160.64
	Range	14.36 – 65.20	94.09 – 198.42	21.45 – 169.01	85.82 – 212.46
ACTH (pmol/L)	*Mean*	25.69	11.25	42.21	26.6
	Range	14.4 – 47.2	3.7 – 33.1	11.9 – 125.4	6.6 – 55.7
Adrenaline (nmol/L)	*Mean*	67.76	78.23	50.37	151.35
	Range	22.33 – 70.40	45.67 – 420.12	25.48 – 161.33	25.08 – 248.16
Noradrenaline (nmol/L)	*Mean*	17.84*	138.10	24.14*	80.32**
	Range	<0.12 – 56.39	19.18 – 503.64	<0.12 – 110.79	<0.12 – 262.88
b-endorphin (pmol/L)	*Mean*	794.8	646.1	686.8	614.1
	Range	11.8 – 1334.0	313.5 – 1059.9	177.8 – 1100.2	4.5 – 1559.8

* five of seven samples were below detection limit (0.12 nmol/L)

** one of seven samples was below detection limit (0.12 nmol/L)

Both stunning methods resulted in an increase in cortisol from baseline (DTS 33.19 ±16.89 nmol/L; Captive bolt 61.43 ±12.59 nmol/L) to post-treatment levels (DTS 150.38 ±17.79 nmol/L; Captive bolt 160.64 ±13.26 nmol/L), indicating a physiological response. However, it is unclear if this response is due to the methods; or to the head capture and restraint, which was longer than in a commercial situation due to the need to take baseline EEG recordings; or to a combination of both restraint and treatment. Shaw and Tume (1990) and Zulkifli et al. (2014) both report that cortisol levels in cattle are not affected by stunning (captive bolt), however, the former carried out their study in a highly controlled research abattoir environment, in which external stimuli are likely to have been minimised, while the latter carried out their study in a commercial slaughterhouse, and baseline cortisol levels prior to slaughter were already high, as a result of the preslaughter handling and environment. Both Dunn (1990) and Zulkifli et al. (2014) report post stun cortisol levels greater than those measured in the DTS pilot study, but it is important to note that these both relate to the case of unstunned slaughter. Indeed, the latter authors demonstrated an increase in cortisol levels associated with unstunned slaughter, but not in the case of captive bolt stunned slaughter. Mitchell et al. (1988) did find that slaughter (captive bolt) resulted in an increase in cortisol levels (+61.2 nmol/L), but this increase was less than in cattle that were handled through a race (+151.7 nmol/L). By inference, it is likely that the cortisol response seen in the DTS pilot study is predominantly due to the head capture and restraint. A tightly clamped head is required ensure delivery of the DTS energy; however, prolonged tight head capture is likely to be very stressful to the animal and struggling while restrained is considered to be an indication of excessive pressure (Grandin and Regenstein, 1994). The European Food Safety Authority recommends that “all restraining devices should use the concept of optimal pressure” (EFSA, 2004), however, the parameters that constitute ‘optimal pressure’ have not been determined.

In the DTS pilot study, there was neither a difference in ACTH levels between baseline and post-treatment samples, nor was there a treatment effect. The values generated in the DTS pilot study were lower than those reported by Zulkifli et al. (2014), but similar to those reported by Mitchell et al. (1988), who found that ACTH was, like cortisol, affected more by transportation and handling than by stunning alone.

Catecholamines (adrenaline and/or noradrenaline) have been reported to increase as a result of stunning (Mitchell et al., 1988; Rulofson et al., 1988; Zulkifli et al., 2014). Zulkifli et al. (2014) report no significant changes in adrenaline levels as a result of penetrative captive bolt stunning, or high powered percussive stunning, but an increase as a result of low powered percussive stunning and unstunned slaughter, while noradrenaline levels increased in all treatment groups; findings that concur with the DTS pilot study. However, Mitchell et al. (1988) and Rulofson et al. (1988) both report an increase in both adrenaline and noradrenaline associated with captive bolt slaughter. In the DTS pilot study, adrenaline levels tended to decrease between the pre-slaughter and post-slaughter samples, while noradrenaline levels tended to increase. There were no significant differences between treatments.

β-endorphins have a role in modulating the physiological stress response, and increases are associated with painful stimuli, fear and excitement. In the DTS pilot study, there was no significant effect of treatment on β-endorphin concentrations in either group, although the mean value appeared to decrease slightly. This finding concurs with that of Zulkifli et al. (2014), who demonstrated no significant change in concentrations as a result of penetrative captive bolt, percussive captive bolt, and unstunned slaughter at a commercial abattoir. The slight decline in β-endorphin concentrations also reflects the findings of Shaw and Tume (1990), who demonstrated a significantly lower concentration in captive bolt slaughtered cattle than in the live animals. Those authors however, carried out their study in a small research abattoir, and the live animal blood samples were collected on a separate occasion within a month prior to slaughter. Thus, the live animal values they report may not relate well to the baseline samples collected in the DTS pilot study and that of Zulkifli et al. (2014), which were collected immediately prior to stunning, in the restraint box.

3.3.1.2 Behavioural indicators of insensibility

In research, the gold standard of assessing consciousness is electroencephalography (EEG, in which electrodes are place on or into the skin of the scalp), or electrocorticography (ECoG, in which the electrodes are implanted into the superficial parts of the brain. However, in a commercial situation, it is impossible to utilise techniques such as EEG or ECoG to monitor the effectiveness of stunning, and behavioural indicators and responses to certain stimuli are used as surrogates. For example, captive bolt stunning is considered effective if the observer notes:

- Immediate collapse, hind legs tucked in then slowly extend, forelegs rigidly extended,
- Immediate and sustained absence of rhythmic breathing
- Fixed, staring eye with no corneal of palpebral reflex
- No righting reflex, no response to ear or nose pinch, no vocalisation

An effective electrical stun results in:

- Immediate collapse, hind legs tucked in, forelimbs rigidly extended
- Immediate onset of tonic (stiff) seizure that lasts for several seconds, followed by clonic (convulsing) seizure
- No rhythmic breathing
- Eyes rotated upwards, dilated pupils
- No response to nose prick

A summary of the indicators suggested by various international standards and guidelines, and indication of whether these are evident in DTS animals, is presented in Table 26 (mechanical stun) and Table 27 (electrical stun).

Table 26: Indicators of an effective mechanical stun

Behavioural sign	***DTS***	***OIE Terrestrial Animal Health Code***	***EFSA Scientific Opinion 2004***	***AMIC animal welfare standard***	***USDA Humane Handling Guidebook*****
Immediate collapse		☑	☑	☑	
No attempt to stand up*		☑			☑
Body and muscles immediately rigid (tonic)		☑	☑	☑	
Lack of normal rhythmic breathing		☑	☑	☑	
Eye open and staring straight ahead / glazed expression	☑	☑		☑	
No attempt to raise head				☑	
Ears relaxed and drooping				☑	
Tongue loose and flapping / hanging out				☑	☑
Corneal reflex absent	☑		☑	☑	
No spontaneous eye blinking	☑			☑	
Straight back and floppy head					☑
No vocalisation of any kind					☑
No vocalisation in response to stimulus	☑				
No response to painful stimulus	☑		☑		
Gradual pupillary dilation			☑		

*The USDA Humane Handling Guidebook describes: “Absence of righting reflex, including an arched back”.

** The USDA Humane Handling Guidebook also lists indications that an animal is NOT properly stunned: Vocalisation, eye blinks, eye reflexes in response to touch, rhythmic breathing, curled tongue.

Table 27: Indicators of an effective electrical stun

Behavioural sign	***DTS***	***EFSA scientific opinion 2004***	***AMIC animal welfare standard***
Immediate collapse	☑*	☑*	☑*
Epileptiform seizure (described in detail)	☑†	☑	☑
Lack of normal rhythmic breathing	☑#	☑	☑
No spontaneous eye blinking	☑		☑
Gasping (breathing in without breathing out) sometimes occurs	☑		☑
Upward rotation of eyes		☑	
Dilated pupils		☑	
No response to nose prick (painful stimulus)	☑	☑	

*: Row 1: With regards to induction of insensibility prior to slaughter, there is much discussion over the 'immediacy' of the lapse into unconsciousness. in electrical stunning, the current must flow for a period of at least 2 (sheep) or 5 (cattle) seconds in order to ensure a sustained loss of consciousness (Warrington, 1974, Von Holleben et al., 2010).

†: Row 2, Column 1: Due to the fixed head restraint, full body collapse was not evident. However, the haunches initially dropped, followed by a tonic phase, and then convulsive movements or paddling of the limbs.

#: Row 3, Column1: In the DTS validation study, a short suspension of respiration was evident in some animals, but not all. However, when respiration was present it was much slower and deeper than in the conscious animal.

For mechanical stun, Gracey and Collins (1995) cite 'no rhythmic breathing' as the cardinal sign, while Grandin (2002) advises observers to focus on "limp head, extended tongue and blank stare". Vocalisation during application of the stun is considered to be indicative of pain or distress (EFSA, 2004), but lack of vocalisation does not guarantee absence of pain or distress, some animals are naturally stoic, and disinclined to vocalise. Indeed, in the DTS pilot study, two animals vocalised during application of the head restraint and chin lift, but once in position, and not being handled by people, the animals fell silent, and remained so throughout the stun process. Similarly, the corneal reflex does not distinguish accurately between consciousness and unconsciousness, but if there is no corneal reflex, it is likely that the animal is unconscious (Anil and McKinstry, 1991; Gregory and Grandin, 2007). In the DTS pilot study, corneal reflex was present in animals prior to treatment, and absent following treatment, in both captive bolt and DTS groups.

The response to a painful event, such as a nose prick, is, however, is a good indicator of consciousness. Anil and McKinstry (1991) demonstrated that an animal that perceives pain will draw back from the nose prick, and during recovery from electrical head-only stunning, sheep that demonstrated this withdrawal reflex soon regained the righting reflex. In the DTS pilot study, prior to treatment, animals visibly flinched in response to the nose prick, but this response was absent post treatment in both captive bolt and DTS animals.

Other authors have reversed the approach and considered utilising indicators of ineffective stunning. For example, Gouveia et al. (2009), studying 2800 cattle stunned using captive bolt in a commercial Portuguese slaughterhouse, found that the most common signs of ineffective stunning were muscle tone of the ears (17.8 %), absence of muscle spasms in the back and legs (11.5 %), presence of rhythmic breathing (9.4 %), and vocalisation (7.9 %). They also observed animals that showed signs of recovery (e.g., corneal response and righting reflex returning), and suggested that this might be predicted by lack of immediate collapse (100 %), eyes rotated rather than fixed (91.3 %), rhythmic breathing (91 %) and response to nose or ear pinch (84.6 %).

For DTS animals, rhythmic breathing continued throughout, so this cannot be considered an indicator of effective induction of insensibility for this treatment. The eyes were fixed and staring, with no ocular following of movement, and there was no withdrawal response to nose prick. Initially, rapid twitching of ears and the third eyelid (nictitating membrane) was evident, with ataxia and loss of posture, particularly in the hindquarters. This was noted as the hind limbs slipping out from behind the animal and attempts to replace the foot showed incomplete stride length, such that the foot remained behind the centre of balance. The limbs did not suddenly tuck in tightly to the body as seen in mechanical and electrical stun (the main reason for 'immediate collapse', as there is no longer any support for the body): the effect was more similar to a heavily sedated animal attempting to maintain posture, similar to that seen in pigs slaughtered using gas inhalation. It is probable that, had the animal not been supported by the head restraint unit and the close confines of the restraint box, the animals would indeed have collapsed.

In the low energy applications, the animals at first arched its back and tensed the muscles in the neck, pushing the chin down into the chin lift, and then demonstrated a period of convulsive movements. This convulsive activity progressed to ataxia as described above. It is not clear whether the convulsive movements are deliberate escape attempts or involuntary convulsions, but the facial expression at the time is of rapid flickering of the third eyelid, with the eyeball partially drooping (a half-moon of white sclera visible in the medial corner of the eye), as opposed to eyes open, fearful and vigilant; and the back is arched rather than flat; and the hind limbs seem to kick forward and higher as compared with a pre-stun animal resisting the head restraint. Animal 4 resisted head capture strongly, and its struggles were characterised by a flat back, and limb movements pushing the body backwards from the neck bails.

Following this ataxic phase, the body became tense and tetanic, with the four limbs slightly splayed. During this phase, it was impossible to push or pull the animal onto its side, as the limbs were locked into place. There was no response to efforts to topple the animal: the tail hung limp and flaccid, and the limbs remained motionless. The animal then progressed into slow walking and paddling movements, pushing forward into the head restraint unit. At this point, the animal could be overbalanced and rolled out, but again there was no response in terms of tail flicking or vocalisation. Muscle tone in the neck persisted, with the head arching up and back, similar to an electrically stunned sheep or chicken. When rolled onto its side, the animal's limbs remained extended, performing slow walking or paddling movements, progressing into more rapid paddling and then convulsive motions. Animals 14, 16 and 18 progressed from convulsions to a quiescent phase, lying still on the roll-out table, while the EEG was recorded, towards the end of which, return of corneal reflex was observed.

This progression is very similar to that seen in electrical stunning – first there is the tonic (stiff) phase; followed by the clonic (convulsive or kicking phase). The convulsions lapse, and the animal enters a quiescent or quiet phase, in which the muscles relax, rhythmic breathing recovers, and then the reflexes return – first corneal, palpebral (blink) and withdrawal; and then righting.

The stunned animals demonstrated behavioural ABMs consistent with an electrical stun – rapid blinking or flicking of the eyelids including the membrana nictitans (third eyelid) during application, loss of posture, tonic (stiff) and clonic (convulsive) phases (Table 28), so it is of value to compare the outcome of DTS against those reported for electrical stunning. In cattle, electrical stunning can be applied as head-only, which affects the brain, resulting in an epileptiform seizure, from which the animal can regain consciousness; or as head-to-chest, which incorporates a current passed through the heart, resulting in cardiac arrest, and the death of the animal. As DTS does not directly affect cardiac function, the comparison should be against head-only electrical stunning.

Table 28: Behavioural indicators of unconsciousness following DTS application, as compared with electrical stun.

Behavioural sign	***DTS***	***EFSA scientific opinion 2004***	***AMIC animal welfare standard***
Immediate collapse	☑*	☑*	☑*
Epileptiform seizure (described in detail)	☑†	☑	☑
Lack of normal rhythmic breathing	☑#	☑	☑
No spontaneous eye blinking	☑		☑
Gasping (breathing in without breathing out) sometimes occurs	☑		☑
Upward rotation of eyes		☑	
Dilated pupils		☑	
No response to nose prick (painful stimulus)	☑	☑	

*: Row 1: With regards to induction of insensibility prior to slaughter, there is much discussion over the 'immediacy' of the lapse into unconsciousness. in electrical stunning, the current must flow for a period of at least 2 (sheep) or 5 (cattle) seconds in order to ensure a sustained loss of consciousness (Warrington, 1974, Von Holleben et al., 2010).

†: Row 2, Column 1: Due to the fixed head restraint, full body collapse was not evident. However, the haunches initially dropped, followed by a tonic phase, and then convulsive movements or paddling of the limbs.

#: Row 3, Column1: In the DTS validation study, a short suspension of respiration was evident in some animals, but not all. However, when respiration was present it was much slower and deeper than in the conscious animal.

The biggest problem with head-only electrical stunning of cattle is a very short duration of epilepsy, followed by strong convulsions (EFSA, 2004). The electrical current must be applied across the brain for sufficient duration to overcome the inherent impedance (resistance) across the head, and application of electrical stun ranges from 4 to greater than 20 seconds (Devine et al., 1986; Vonmickwitz et al., 1989; Schatzmann and Jaggin-Schmucker, 2000). The duration of unconsciousness in those studies was reported as 31 - 90 seconds. In contrast, DTS induces loss of posture within 8 seconds of onset of energy application, and the duration of unconsciousness recorded during the DTS validation study was between 100 and 240 seconds, based on absence of corneal reflex, and duration of EEG changes.

Return of reflexes are considered to be the earliest indication that consciousness is returning and was the point at which penetrative captive bolt was applied during the DTS validation study for animal welfare reasons.

In the early stages of the project, overheating of the skin surface was identified. This was associated with incident power levels of > 25 kW. This was eliminated in the later stages of the project, through optimisation of the auto-tuning device and applicator apparatus such that incident power levels ranged from 18-20 kW. Australian cattle may experience ambient air temperatures of 45 °C in summer and could experience higher surface temperatures when standing in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition.

3.3.1.3 Latency to insensibility

With regards to induction of insensibility prior to slaughter, there is much discussion over the ‘immediacy’ of the lapse into unconsciousness. Indeed, for both mechanical and electrical stun, normal neurological function is disrupted almost instantaneously following application of the equipment (Daly et al., 1987; Daly and Whittington, 1989). Estimates of ‘immediacy’ in these situations suggest loss of sensibility within less than one second following application, although in electrical stunning, the current must flow for a period of at least 2 (sheep) or 5 (cattle) seconds in order to ensure a sustained loss of consciousness (Von Holleben et al., 2010; Warrington, 1974). Even using EEG assessment, in those situations in which EEG can be measured, we can identify traces that are consistent with awareness and traces that are inconsistent with awareness, but these are separated by a transition trace (McKeegan et al., 2007), during which the ability of the animal to perceive external stimuli is unknown. At present the best option available is to calculate or infer the time taken to reach a point at which EEG changes indicative of insensibility are present. For example, in electrical stunning, Wotton et al. (2000) demonstrated that a minimum current of 1.15 Amps was required to induce EEG changes indicative of insensibility in cattle. Subsequently, Weaver and Wotton (2009) applied a current of 3.28 Amps or more to 287 cattle in a commercial situation, measuring the current flow between the electrodes, across the animal’s brain. In all animals, a current flow of 1.15 Amps was achieved within 100 msec, and this figure has been accepted as the ‘time to unconsciousness’ for electrical stunning of cattle. In practice, the current flows for longer than 100 msec, to ensure that the insensibility is maintained.

In contrast, it is well known that gaseous inhalation (CO_2 and CO_2/N_2 mixtures) is not as dramatically 'immediate': the induction of unconsciousness is a gradual process as the inhaled CO_2 is absorbed into the circulation, progressively lowering the pH of the cerebrospinal fluid (CSF) to a threshold level at which neurotransmission fails (Woodbury and Karler, 1960), and the animal loses consciousness. At this threshold, the lapse from consciousness to unconsciousness is considered to be 'immediate'. In comparison, DTS utilises an increase in brain temperature to achieve the point at which neurotransmission fails (Small et al. 2013b: **SS02**). In cattle, the core body temperature tends to be 1-2 degrees higher than in humans (38.6 °C in resting cattle, 37 °C in humans), so we expect that in cattle thermal unconsciousness would occur in the range 42-47 °C. Thus, the required rise in temperature to achieve insensibility is in the range of 3.4 to 8.4 degrees. During development and testing of the DTS equipment utilised in this study, the temperature profiles of cadaver heads was tested, and a predictive equation describing the heating rate within the brain was generated (0.124 °C/kW.sec). From this equation, we can infer that for a 30 kW application, the time taken to achieve an 8 degree rise in temperature would be 2.15 sec (8/30/0.124); and for a 20 kW application, the time taken would be 3.23 sec (8/20/0.124). In a commercial situation, the real-time monitoring system incorporated within the control panel will allow an objective record of energy delivered and rate of energy delivery to be maintained, and an alarm feature could be incorporated to use in conjunction with animal-based indicators (e.g., response to reflexes) when confirming insensibility prior to exsanguination.

It is useful to compare the application of DTS with the use of inhalational agents used for stunning, as both approaches do not produce unconsciousness 'immediately'. The gradual induction of insensibility by gaseous inhalation has been demonstrated to expose animals to a period of discomfort of 20 seconds or more prior to loss of consciousness. Llonch et al. (2012) described 'retreat attempts' in pigs 10 seconds after entry into a commercial Dip-Lift gas chamber mixtures; followed by 'escape attempts' and 'gasping' at 25 seconds exposure, with loss of balance at 30-32 seconds, muscular excitation at 33 seconds, and vocalisations at 35 seconds. Raj and Gregory (1996) described loss of posture occurring at 15-18 seconds of exposure to gas mixtures, vocalisations at 26-30 seconds and convulsions at 21-22 seconds (persisting for 17-33 s); while Raj et al. (1997) measured ECoG suppression beginning between 15 and 25 seconds of exposure, and an isoelectric trace (maximum effect) between 23 and 45 seconds. In comparison, for DTS energy exposures of 140-210 kJ (based on prototype 2 delivering only 70% energy as compared with prototype 3), Rault et al. (2014: **SS03**) estimated EEG changes to occur within 3 seconds of the end of each application, and the nadir (maximum effects) occurring within 4-22 s post application. In the DTS pilot study, by the time the Faraday cage was removed (approximately 20-30 s post energy application), DTS animals were unresponsive to stimuli, and the EEG recorded was suppressed. Furthermore, predictions of the time to threshold temperature in the DTS pilot study are 2.15 sec for a 30 kW application, and 3.23 sec for a 20 kW application. Thus, although DTS can also be considered a 'gradual' induction of insensibility, as the brain temperature is raised to the threshold level at which the 'immediate' lapse into unconsciousness occurs, the duration of this induction is substantially less than that experienced by animals undergoing gas inhalation.

3.3.1.4 Impacts on meat quality attributes

Carcase and quality assessments were carried out on 18 animals (Step 7). Visual inspection of the carcases showed no abnormality. Bruises were present on the bony tip of the pelvis on some animals, from both DTS and captive bolt treatment groups, and were deemed to be caused pre-slaughter during handling and restraint. No abnormalities of colour were noted. In general, there were no differences in pH declines between treatments, although five carcases showed a very rapid initial drop in pH post slaughter, reaching values below pH 6, while the carcase temperature was still above 35 °C: Animals 1 (captive bolt), 5 (double application – total energy delivered 239.68 kJ), 7 (low energy DTS), 14 (very low energy DTS) and 16 (low energy DTS). This may be indicative of a raised metabolic rate and may suggest a tendency towards development of heat toughening, although this was not borne out by the meat quality analyses undertaken. There were also four animals for which the slope of the pH decline differed from others. These were animals: 1 (captive bolt); 14 (45.87 kJ); 16 (184.68 kJ) and 18 (217.62 kJ). Animal 14 received a very low amount of energy – there was an initial loss of posture, and convulsive movements began. There were no responses to stimuli, and the EEG trace was visibly suppressed, with intermittent bursts of activity. A risk of recovery was considered to be present after 5 minutes post energy application, and captive bolt was applied. Animal 16 spent a prolonged period in the head restraint prior to energy application (box entry to stun period 14 min 5 s) and return of blink and corneal reflexes were also noted prior to captive bolt application at 5 min 23 s post DTS application. Animal 18 similarly demonstrated return of reflexes, including the righting reflex, over 3 minutes post DTS application. It is possible that the return to consciousness process indicated by returning reflexes induces an increase in metabolic rate in these animals, when may contribute to alterations in the rate of pH decline.

After death, energy stored (as glycogen) in the animal's muscles is converted into lactic acid, making those muscles slightly acidic, i.e., lowering the muscle pH. Well-fed and well-rested animals normally have sufficient muscle energy reserves at processing to yield enough lactic acid to reduce the pH of muscles to near to 5.5. If an animal has lowered glycogen (usually as a result of tiredness, that could be due to 'exercise events' in the last few days before slaughter (e.g., long-distance transport, fighting, mustering) and has had insufficient time to re-establish the muscle energy reserves, the pH will not fall to below 5.8, and will yield what is known as 'dark-cutting' meat. When meat has an ultimate pH above 5.8:

- meat toughness increases;
- the colour of the meat is darker, and therefore less attractive to the consumer;
- the raw meat holds water, and feels dry or tacky to touch
- the meat becomes dry and chewy in texture when cooked;
- the meat oxidises (the muscle goes brown; and the fat becomes rancid) more rapidly

The converse quality problem encountered in meat is PSE – Pale, Soft, Exudative meat. It is more commonly associated with pigs and poultry, but has been reported in beef, particularly in young bulls, associated with slow chilling of the large muscles of the hindquarters while the metabolic rate is high. PSE occurs when the animal is well rested and has plenty of stored muscle energy at the time of slaughter – but something occurs that raises the metabolic rate. This results in a very rapid fall in pH, and sometimes to levels closer to pH 5.3. Events that could trigger this include acute stress immediately prior to slaughter (it has been demonstrated in pigs that have been pushed into 'fight or flight' mode through dreadful handling practices) and also activation of the nervous system by the stun process (it has been associated with electrical stun where the current has flowed for too long). PSE meat is characterised by:

- decreased meat toughness;
- pale colour;

- the meat does not hold water – it feels wet to touch, and a lot of fluid is seen in the packs, and when it is cooked, lots of water is released and the meat becomes dry and tough;
- the meat oxidises (the muscle goes brown; and the fat becomes rancid) more rapidly.

With regard to DTS, the concern is that either the electromagnetic field induced by the microwave energy, or the heating effect on the brain, or both, may induce an increase in metabolic rate, resulting in PSE-like changes in the meat. Therefore, the analyses carried out aimed to provide some information on toughness (shear force); colour; water holding capacity (drip loss); and oxidation (fat rancidity; TBARs) of DTS animals as compared with captive-bolt stunned animals.

In general, there were no significant differences between meat from DTS animals and meat from control (captive bolt stun) animals. Ultimate pH (pHu) in both groups was below pH 5.8, indicating that DFD was not induced, and pH values of loin and round at weeks 1 and 10 of storage lay within normal ranges (pH4 – pH 6).

There were some slight differences in meat colour at quartering, and in the first week after slaughter. DTS quarters were slightly yellower (greater MINOLTA b*) at quartering; and at 1 week post slaughter DTS loins were slightly redder (greater MINOLTA a*) and slightly yellower (greater MINOLTA b*); and DTS rounds were slightly lighter (greater MINOLTA L*) than control samples. However, these differences were marginal, and the values align with published data on MINOLTA colour attributes of loin (m. longissimus lumborum) and round (m. semitendinosus) (Onenc and Kaya, 2004; Sazili et al., 2013; Warner et al., 2007). In light of the small sample size (n=9 in each treatment group) these results should be interpreted with caution.

There was also a trend that DTS samples were more tender than control samples (Warner Bratzler Shear force measurement), but similarly, this trend should be interpreted with caution in light of the small sample size. Shear force values for loins at one week post slaughter in the DTS pilot study were 4.62 ± 0.34 kg and 4.01 ± 0.45 kg in control and DTS respectively, while at 10 weeks post slaughter these values were 3.45 ± 0.29 kg and 3.28 ± 0.39 respectively. These results lie within normal ranges: Warner et al. (2007) report values of 7.0 kg at 6 days post slaughter, and 4.8 kg at 21 days post slaughter; Gruber et al. (2010) report a range of 3.5 to 5.11 kg measured over a range of ageing periods from 3 to 28 days; while Sazili et al. (2013) report 9.19 ± 0.97 to 9.96 ± 0.72 kg at one week post slaughter. For rounds, the DTS pilot study measured 5.54 ± 0.25 kg for control and 4.84 ± 0.33 kg for DTS at one week post slaughter, and 5.46 ± 0.26 and 4.71 ± 0.36 kg respectively at week 10. These values again align with previously published ranges, for example 4 – 18 kg (Odusanya and Okubanjo, 1983), 4.12 ± 0.16– 6.63 ± 0.2 kg (Otremba et al., 1999) and 4.6 – 9.5 kg (Hwang et al., 2004).

The TBARs results are higher than would be expected from fresh meat. This is not reflective of the product or treatment but is due to a loss of temperature control during sample storage, after sample collection. The samples in week 1 underwent an unforeseen delay between collection and storage at -80°C, which will have resulted in excess lipid oxidation. For consistency, at week 10, we replicated the storage conditions encountered at week 1. Nevertheless, there were no significant differences in TBARs levels between the DTS and the captive bolt muscles.

3.3.1.5 Aversiveness of the system

The overall aversiveness of any stunning system can be considered to be made up of three main components:

- Pre-restraint handling;

- Restraint;
- The stunning method itself.

To minimize the adverse impacts of pre-restraint handling, low-stress stock handling by trained and competent personnel are recommended, according to normal commercial practice. Similarly, to minimize the adverse impacts of restraint, the units utilized of DTS: Diathermic Syncope are designed based on current best practice designs, specifically the ASPCA Pen design described by Grandin (1992) and Marshall (1963). Quiet handling and smooth application of the neck capture and chin lift apparatus is well tolerated by cattle (Dunn 1990, Grandin 1992) and the head position achieved allows accurate placement of the applicator onto the forehead of the animal. For DTS application, the is held in the chin lift for a maximum of 30 s while the applicator is positioned and DTS energy delivery initiated, as it is known that prolonged restraint in any system is stressful to animals (Ewbank, 1992).

Assessment of aversiveness of the final component, the stunning method itself, separate from the impacts of restraint and handling, is challenging, However, the fact that pain-related complexes were not evident in the EEG traces of minimally-anaesthetised animals (STEP 5; Rault, *et al.* 2014); and that endocrine and meat quality parameters from DTS cattle and carcases were not significantly different from captive bolt cattle and carcases when handled using the same pre-restraint handling and restraint unit (STEP 7), serve to indicate that the DTS:Diathermic Syncope system is no different in terms of overall aversiveness than captive-bolt stunning.

3.3.2 Overall discussion and conclusion

Fig 17*3.3.2.1 Benefits to the industry*

DTS is an effective means of inducing insensibility in cattle, with a duration of insensibility suited to exsanguination using the Halal cut. The project was planned and executed under end-user-centred design principles, with the Halal and Kosher markets in mind. Key parameters that are likely to be viewed favourably when compared against the current stunning method are:

- There was no requirement to perform a back-up ventral cut (thoracic stick), as the blood flow was strong and exsanguination was rapid;
- Blood flow was visibly strong, and the pulsations associated with heart function were visible in the early stages of bleed-out;
- Exsanguination could be performed safely, without the need for an immobiliser;
- Visual inspection of the brains of carcases indicated no visible damage when energy deliveries of less than 220 kJ were applied, and the stun did not cause cracks in the skull, so eliminates a source of rejection against Halal requirements, and eliminates a potential source of breaches noted on audit when the auditors' interpretation of 'cracked' differs from the regular inspector;
- The stun is effective in heavy animals and bulls, which currently pose challenges to processors using percussive stunning;
- To date, no evidence of blood splash or ecchymosis has been encountered in any of the carcases processed.

- The animal stunned using DTS can also return to consciousness, so this method of stunning is likely to be acceptable to the Halal and Kosher markets.

3.3.2.2 Conclusion

1) Using the recommended parameters for DTS, animal welfare is maintained through restraint, induction of unconsciousness, through to death by exsanguination. It delivers an animal welfare outcome that is at least equivalent to that observed with other stunning methods approved for cattle (Table 29)

2) ABMs assessed during restraint and application of DTS indicated only minor discomfort or distress in a small number of animals, not unexpected in any form of restraint or stunning.

3) In the absence of technical problems leading to interrupted delivery of energy, all animals were rendered unconscious using DTS when the recommended parameters for administration of DTS are used.

4) Administration of between 160 and 200 kJ energy at 18kW ensures rapid loss of consciousness without overheating the skin of the forehead (prior to loss of consciousness) or inducing extreme convulsions.

5) Animals are unconscious for at least 60s following the induction of unconsciousness. EEG data indicate a at least 60 s unconsciousness with long transition to return of consciousness (as indicated by behavioural ABMs).

6) ABMs used to detect consciousness and unconsciousness are confirmed to correlate with EEG. ABMs to monitor the state of consciousness are suggested in Table 30, to be used at three key stages. For each key stage, three or four ABMs that are reliable in monitoring consciousness are suggested

6) The period of unconsciousness is sufficiently long to ensure death by exsanguination without return of consciousness when a rotary box is employed to ensure effective removal of the animal from the box during the tonic phase.

7) Use of the rotary box optimised handling of the stunned body, allowing a short stun-to-stick interval without the use of an immobiliser. The updated applicator configuration allowed consistent repeatable induction of insensibility, which was sustained during Halal bleeding, without the need to apply an additional ventral cut.

8) There was no requirement to use an immobiliser during application of the exsanguination cuts, and the animal bled rapidly to brain death using the Halal cut alone. The blood flow on exsanguination was strong and rapid.

9) Using the recommended parameters for DTS there are no significant physical effects on the brain and it therefore normal recovery can occur in animals that are not exsanguinated.

Table 29: Equivalence of DTS: Diathermic Syncope® with existing stunning methods for cattle

	DTS	**Mechanical stunning (penetrative)**	**Electrical head-only Stunning**	**Electrical head-to chest stunning**
Per-restraint handling	Forcing pen and single-file race feed to restraint	Forcing pen and single-file race feed to restraint	Forcing pen and single-file race feed to restraint	Forcing pen and single-file race feed to restraint
Restraint type	Upright individual restraint with neck capture and chin lift	Upright individual restraint. Neck capture and chin lift improve first-shot efficacy	Upright individual restraint with neck capture and chin lift	Upright individual restraint with neck capture and chin lift
Minimum restraint duration (prior to onset of application)	10 s	10 s	10 s	10 s
Duration of application	9 – 10 s (simple stun)	< 1 s	4 - >20 s	4 - >20 s
Latency to behavioural response	3 s (eye and ear twitching) 8 s (loss of posture)	< 1 s	4 - >20 s	4 - >20 s
Latency to changes in EEG	12 – 50 s from onset of energy application	0 – 14 s post application	Data not located	Data not located
Duration of insensibility	120 – >240 s (simple stun)	indefinite	31 – 90 s	indefinite