

Policy on the Care and Use of Sheep for Scientific Purposes Based on Good Practice

**From a Workshop held on the 20-21 April 2005
at the Bayview Conference Centre, Clayton Victoria**

Organised by Monash University and the Animal Welfare Science Centre

Sponsored by the Monash University Department of Physiology



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Preface

These principles and guidelines have been developed to assist both researchers and Animal Ethics Committees (AECs) when designing and evaluating protocols using sheep as experimental subjects. They originated from a two-day workshop held at Monash University on April 20-21, 2005 and attended by delegates from all the Australian States and New Zealand. Included among the attendees were research scientists, members of regulatory bodies and members of AECs.

The workshop was conducted by moderated discussion of introductory presentations given by invited expert speakers. The discussion was minuted and edited to develop this document, which was circulated to all attendees for commentary before publication.

Characterisation of Good Practice

“Good practice” may be taken to mean a standard of care that has a general level of acceptance among knowledgeable practitioners and experts in the field, is based on good sense and sound judgement, is practical and thorough, has robust experiential or scientific foundations, and prevents unreasonable or unnecessary harm to, or promotes the interests of, the animals to which it is applied. Good practice exceeds the requirement to observe minimum standards and changes with the evolution of attitudes about animals and their care.

Context and Purpose

In using animals for experimental purposes it is necessary to keep the scientific purpose clearly in mind and recognise there may be animal welfare and other ‘trade-offs’ to achieve the purpose. The magnitude of the ‘trade-offs’ that will be allowable fall within the remit of AECs, and will depend on the magnitude of the anticipated benefit(s). There needs to be an ongoing focus on the principles of replacement, reduction and refinement to minimise both the ‘trade-offs’ and the risks to animal welfare.

It is acknowledged that sheep are used for a range of experimental procedures from completely non-invasive to highly invasive, including behavioural, surgical and pathological studies. These studies can involve indoor and outdoor housing, both short and long term.

Policy on the Care and Use of Sheep for Scientific Purposes Based on Good Practice

All research involving animals must comply with the Australian code of practice for the care and use of animals for scientific purposes.

Key Principles:

1. Teamwork and training

- Good animal welfare is everyone's responsibility and requires commitment to a cooperative team approach to maximise research and welfare outcomes. The team includes the researchers, the animal carers and the AEC.
- The successful conduct of research using sheep requires a wide range of skills and knowledge specific to the species. This includes a good knowledge of their biology, behaviour and husbandry as well as the special requirements pertaining to experimental techniques and procedures
- It is necessary for all personnel to be appropriately skilled and to regularly practise and maintain currency of their skills and knowledge. This may involve seeking expert advice from within the organisation or elsewhere.
- All team members should acknowledge the need for ongoing review of experimental and husbandry practices and implement changes that will improve animal welfare and research outcomes.

2. Applying the Code of Practice

- Researchers must provide clear and detailed protocols that fully justify the use of animals and the specified procedures. AECs should take into consideration any peer review of the scientific integrity of the project. Both parties need to effectively communicate with each other and be clear about their areas of responsibility.

3. Appropriateness of animals for the purpose, including their selection, acclimatisation and training

- Standard operating and selection procedures should be developed to ensure animals are fit for the purpose and procedures are in place to ensure this.
- All aspects of the use and management of sheep must suit the scientific purpose. This requires appropriate selection of sheep to minimise risks to animal welfare. Consideration should also be given to all aspects of acclimatising sheep to experimental conditions.

4. Minimising Stress

- Those who use sheep for experimental purposes need to be aware of the potential sources of stress for their animals and take action to minimise the risks to their welfare.

5. Pain relief

- Every invasive procedure should be examined for the necessity for, and the application of, pain relief.

6. Facilities

- All facilities should provide a safe environment that will depend on the nature of the procedure and the age, condition and breed of sheep.

7. Confinement

- The level of confinement must be appropriate for the scientific purpose and be justified in terms of degree and duration of confinement.

8. Movement of animals

- Animals must be handled and transported in a safe environment in facilities and vehicles appropriate for the class of sheep.
- The standards for transport of fit and healthy sheep should at the very least conform to industry standards and relevant codes of practice and recognise the high degree of expertise required of handlers and drivers.
- Animals whose health, fitness, strength or ability to cope in any other way has been lessened or affected by experimental procedures or their preparation must be handled and transported according to the needs of their condition, and expert advice sought relative to this if necessary.

9. Monitoring

- Monitoring of animals is an essential task for both good animal welfare and good research. Standard operating procedures should be developed to include the frequency of inspection, hazard analysis, record keeping and utilisation of records to review procedures/management.

Appendix 1.

Notes for Investigators and AECs on the Management, Housing and Care of Sheep used for Scientific Purposes.

Principle 1 – Teamwork and training

Good welfare is everyone's responsibility and requires commitment to a cooperative team approach to maximise research and welfare outcomes. The team includes the researchers, the animal carers and the AEC.

The successful conduct of research using sheep requires a wide range of skills and knowledge specific to the species. This includes a good knowledge of their biology, behaviour and husbandry as well as the special requirements pertaining to experimental techniques and procedures.

It is necessary for all personnel to be appropriately skilled and to regularly practise and maintain currency of their skills and knowledge. This may involve seeking expert advice from within the organisation or elsewhere.

All team members should acknowledge the need for ongoing review of experimental and husbandry practices and implement changes that will improve animal welfare and research outcomes.

- The expertise of all those involved, including the research team, field/animal care staff and AEC members is fully utilised to contribute to the development and review of standard operating procedures (SOPs), the conduct of research and the care of animals.
- There needs to be respectful and constructive engagement between researchers and animal care staff.
- Selection and training of personnel should be appropriate to achieve both the research outcomes and high standards of animal welfare.
- Training must ensure that personnel are competent in their tasks. Such training may be on-the-job, via courses or working with an experienced practitioner and should encompass the needs of both new and experienced staff.
 - Institutions should facilitate and maintain constructive working relationships within and across teams to maximise teamwork and achieve high standards of research and animal welfare.
 - Where there is doubt about a practice and local expertise is insufficient, advice should be sought from an appropriate expert. AECs may wish to consider the use of animals for the acquisition of skills including developing surgical techniques on cadavers, non-recovery surgery and small-scale pilot studies.

Principle 2 – Applying the Code of Practice

Researchers must provide clear and detailed protocols that fully justify the use of animals and the specified procedures. AECs should take into consideration any peer review of the scientific integrity of the project. Both parties need to effectively communicate with each other and be clear about their areas of responsibility.

- All parties should inform themselves of their responsibilities under the Australian Code of Practice, other codes of practice and relevant legislation.
- If there is some disquiet within an institution, either by researchers or AEC members, communication between parties should be improved.
 - Suggestions include, having researchers present at AEC meetings, one-on-one discussions between a nominated AEC member and the researcher to clarify the protocol and any areas of concern and, if necessary, inputs from a mutually respected third party.
 - Good communication between researchers and community members is essential, and this aim may be assisted by public seminars and presentations to the AEC made by researchers.
- If there is uncertainty concerning the scientific value of a novel experimental approach, the appropriate number of animals per group required for statistical analysis, the impact of a procedure upon animal welfare, or the correct dose or duration of a particular experimental treatment, it is recommended that a pilot study be performed to obtain the necessary scientific information upon which to base further decisions.
- Pilot studies, where proposed, should be regarded as integral to the overall project, especially to enable assessment of the feasibility of the project and the potential for Refinement and Reduction. They should be assessed by the AEC according to the usual criteria applied to project approval. *Australian Code 2.2.19*

Principle 3 – Appropriateness of animals for the purpose including their selection, acclimatisation and training.

Standard operating and selection procedures should be developed to ensure animals are fit for the purpose and procedures are in place to ensure this.

All aspects of the use and management of sheep must suit the scientific purpose. This requires appropriate selection of sheep to minimise risks to animal welfare. Consideration should also be given to all aspects of acclimatising sheep to experimental conditions

- Researchers should consider and justify the choice of sheep as the most appropriate species for the purpose.
- There must be appropriate selection of sheep to minimise risks to animal welfare:
 - Physical and clinical examination should be performed to a veterinary standard.
 - Evaluation of source farm worming and vaccination practices.
 - The breed and class of sheep should be considered in relation to the purpose including age, physiological status, body condition, amount of wool and condition of feet.
 - To meet the event that some animals are rejected as unsuitable for experimentation and to provide for companion sheep (eg. at the ends of rows of individually-housed sheep), allowance should be made for additional sheep to be selected.
 - Criteria for rejection would include both behavioural (eg. will not settle within a specified period, will not feed within 2 days) and health (eg. become sick) reasons.

- Humane procedures must be developed to manage rejected animals.
- Each animal should be individually identified to facilitate accurate record keeping. Temporary identification (eg. stock spray marker, electronic tagging) or more permanent methods (eg. double ear tagging, implanted microchip or electronic tag) should be selected as appropriate.
- Procedures should be in place to manage acclimatisation of sheep to assist transition to experimental facilities and husbandry, such as pre-feeding of the laboratory diet in the field, some training for confinement (eg. in sheep shed, outdoor pens, indoor pens) and providing animals with experience of new social groups prior to moving,
- To both assist transition from field to laboratory and to maintain condition, appropriate roughage should be included in the diet (eg. feeding chaff or hay) which will maintain digestive function and normal levels of oral activity to reduce oral stereotypies and risks to welfare such as wool biting and mouthing of cage bars.
- An appropriate period of acclimatisation should be planned bearing in mind the nature of the experiment. For example, sheep can be acclimatised to laboratory conditions within 2-3 weeks with appropriate positive handling; previous experience of laboratory conditions may assist.
- Recognise there can be a conflict in the selection criterion of lack of variation in body weight (for experimental purposes) and the time taken to establish a social hierarchy (acclimatisation).
- Recognise that positive handling of sheep early in life can improve their subsequent behaviour and minimise stress in response to novelty.
 - Sheep can be adapted to humans by early human contact, habituation (regular positive human contact eg. to encourage touching of sheep by humans) and conditioning (associating humans with rewarding experiences eg. molasses). Trained sheep seem to like having their neck/chin stroked and head to head contact with humans. They seem to dislike stroking on the ventral abdomen and touching on top of the head. Because sheep appear to be able to discriminate between people, aversive and positive procedures should be conducted by different personnel, if possible, and exposure to strangers should also be minimised.
- It is a matter of judgement by individual AECs whether it is appropriate to reuse sheep that have had previous experience of laboratory conditions versus using naïve sheep. Previous experience may assist in reducing the number of animals used (including rejected animals) and the time to acclimatise. This benefit in reduction of numbers used and refinement of procedure needs to be balanced against the cumulative impact on individual animals. Not all procedures are perceived by the sheep as unpleasant and the nature of procedures, previous and envisaged, will be relevant to the decision.

Principle 4- Minimising Stress

Those who use sheep for experimental purposes need to be aware of the potential sources of stress for their animals and take action to minimise the risks to their welfare.

- Stress is the physiological response to an alteration of homeostasis, whether actual, threatened or perceived. As an acute response to a limited challenge it can be adaptive but prolonged stress can adversely impact on animal welfare and may compromise the results of scientific studies. Those who use sheep should be aware of the responses of sheep to specific stressors and act to minimise the risks to animal welfare.

- Withdrawal of food and water prior to general anaesthesia should be kept to a minimum period to minimise the effects on energy metabolism and rumen microflora. While sheep may be kept off feed for a limited period to lessen the risk of rumen tympany (bloat), the rumen contents of sheep are more liquid than normal after a period of fasting and may constitute an increased risk of aspiration pneumonia.
- The need for and choice of premedication for surgical procedures should be carefully considered. While the reduction of anxiety is a principal objective of premedication, the drugs used may potentiate the effects of the anaesthetic agents and induce ataxia which can interfere with handling procedures and may be stressful in itself.
- As sheep find unfamiliar environments stressful, animals recovering from surgery should be returned to their home cage with familiar sheep nearby. During this period, animals should be protected from injury that may arise from unco-ordinated movements by removal of unnecessary objects (food and water containers) and solid cage sides may be necessary until motor co-ordination has returned. Disturbance should be minimised during recovery from surgery but, where human presence is necessary, familiar humans are preferred.
- As social isolation from other sheep is a potent stressor, animals should not be transported alone or held out of sight of other sheep. Whenever possible the minimum group size should be three animals so that removal of one will not leave a single individual. Where the holding of a single animal cannot be avoided, a large mirror or a surrogate object (eg. a cardboard box covered with a sheep skin) may lessen the stress of isolation.

Principle 5 – Pain relief

Every invasive procedure should be examined for the necessity for, and the application of, pain relief.

- Some invasive procedures, such as venipuncture and ear tagging require no pain relief.
- The possibility that sedation and local or regional anaesthesia can replace the routine use of general anaesthesia should be considered.
- Longer term surgery requires use of appropriate general anaesthesia. Choice of induction and maintenance agents should be considered in each case.
- Pain relief is a sophisticated and advanced area of management and expert advice should be sought to obtain sufficient, appropriate information and skills. Consideration should be given to the use of agents that act at several levels in the pain pathway and the timing of analgesic treatments. Reference should be made to suitable sources, eg. Flecknell 1996.
- Sheep need to be closely observed for signs of pain as they are relatively undemonstrative when in pain. Indices of pain are predominantly not specific to pain alone and can include:
 - jaw-grinding,
 - posture (i.e. head-up or head-down, alertness, avoidance, stance with back arched and grunting respiration suggestive of peritoneal pain, tucked-up vs. upright, head turned back along side, stretched back legs, changes in individual and social behaviours, position of ears, ‘staring’ into space, reluctance to move, guarding of affected areas, limping or carrying a limb).
 - vocalisations

- reduced water/food intake, indices of catabolic state (eg. low plasma glucose, increased ketones), increased heart rate, increased stress hormones (i.e. ACTH, cortisol and prolactin), loss of appetite.
- indices of sickness behaviours such as increased time spent lying down or sleeping.
- Monitoring should be carried out for an appropriate period after a particular challenge, using purpose-designed checklists.
- When choosing an analgesic regimen for use in pregnant ewes, its safety for the foetus should be considered.
- The sheep foetus responds differently from the adult in response to pain stimuli and analgesics. There is evidence that the foetus is unconscious throughout pregnancy and birth, however foetal surgery should be performed under general anaesthesia of both the ewe and the foetus.
- The reduction of anxiety as an adjunct to pain relief, based on limited evidence in humans should be considered.
- Further research is required to define safe and efficacious analgesic regimes for sheep, as there is insufficient evidence to guide the choice of analgesics.

Principle 6 - Facilities

All facilities should provide a safe environment that will depend on the nature of the procedure and the age, condition and breed of sheep.

- All facilities used by sheep should be well designed, constructed and maintained. They should be designed and managed to ensure that competition for resources (eg. food and water) does not disadvantage individuals within a group and that low status animals can avoid bullying (eg. by provision of dividers within a pen). They should present as overall comfortable living quarters, suitable for the amount of time they will be occupied.
- Other than newly shorn sheep and young lambs requiring particular attention to their thermal requirements, sheep are relatively tolerant of a wide range of environmental temperatures although this tolerance should not be taken for granted or to extreme. However, the breed of sheep, purpose of the work, presence and nature of bedding, facility ventilation rate, experimental requirements and demands and their interactions need to be considered in the provision of facilities.
- Housing requirements:
 - From the choice of housing options available, those that best meet the requirements of the experiment and minimise risks to welfare should be chosen. This involves balancing experimental needs, duration and degree of confinement and risks to animal welfare. While there is increased concern for welfare as the level of confinement increases, and taking into account the balances involved, it is recognised, that a range of housing environments can meet the welfare requirements of sheep.
 - While there are few studies on space allowance for sheep and it is commonly recommended that a space allowance for singly and group housed adult sheep of 1-1.5 m² per animal is provided (eg. SCARM Report 29), factors that need to be considered are the size of sheep, group size and how the space is utilised for various activities.
 - Social contact with other sheep must be provided, unless the experiment requires isolation and AEC approval is obtained. The important aspects of social contact are visual, auditory, olfactory and tactile, in decreasing order of importance.

- Consideration should be given to the provision of environmental elements that meet the needs of sheep, eg. subdivision of space to allow avoidance of dominant sheep by subordinate animals, provision of choices such as salt licks, molasses blocks and stalky hay. Sheep do not appear as interested in “play” objects that are provided with the intention of enriching their environment (eg. balls, bottles).
- As sheep find both transport and adaptation to new environments stressful, experimental protocols should be well planned to minimise the number of movements.
- Appropriate shelter from weather extremes should be available for outdoor sheep.
- Sheep housed indoors need to experience periods of both light and dark in a 24 hour cycle.
- Experimental crates/metabolism cages – see principle 7.
- Flooring and bedding:
 - The type of flooring and use of bedding should balance the needs of the sheep and the experiment.
 - Wooden slats, woven wire and welded wire mesh flooring may all be suitable provided they support the animal and minimise pressure points on the sole of the foot and are maintained in a good state of hygiene. Experience indicates that heavy sheep (eg. > 100 kg) require impact absorbing mats to maintain foot health.
 - Bedding (eg. straw, rice hulls, rubber mats) can improve comfort, especially where wool cover is short. This factor needs to be balanced with the need for increased hygiene in the peri-operative stages. If bedding is provided, dirty bedding must be regularly replaced to avoid injuries (eg. brisket burns).
 - For sheep held for long periods on hard floors, bedding/mats should be provided to reduce the risk of arthritis.
- Air quality should be monitored in all indoor facilities and waste managed accordingly to prevent the build-up of noxious gases, especially ammonia. N.B. Human occupational health & safety exposure standard for atmospheric ammonia is 25 ppm (Time Weighted Average over 8 hours) (NOHSC:1003, 1995).

Principle 7 - Confinement

The level of confinement must be appropriate for the scientific purpose and be justified in terms of degree and duration of confinement.

- For each experiment, the level and duration of close confinement must be justified. Experimental cages/metabolism crates were originally used to collect urine and faeces for energy metabolism studies, but are now used for a range of procedures that require all round access to animals and to prevent damage of instruments attached to animals eg. catheters and probes.
- When deciding on individual housing for sheep, options that should be considered include double cages and pens.
- A sufficient number of animals should be housed together so that the removal of one (eg. for surgery) does not leave a single animal alone.
- When using experimental cages, consideration must be given to the need for housing sheep in the cages for the entire period of the experimental protocol or only for specific procedures.
- Duration of housing in experimental cages is a matter for determination by individual AECs. Experience suggests that sheep can be successfully held in experimental cages for several months with no demonstrable risks to their welfare provided that there has been

appropriate selection and acclimatisation, and caring staff who have the time to both physically interact with the sheep and provide ongoing monitoring to a high standard are in place.

- Recommendations on duration of use and dimensions of experimental cages should be the subject of research.
- The use of tethers in cages is undesirable and should be limited to contingencies described in an appropriate SOP for the animal facility. Such use should be reported to and reviewed annually by the AEC.

Principle 8 – Movement of animals

Animals must be handled and transported in a safe environment in facilities and vehicles appropriate for the class of sheep.

The standards for transport should at the very least conform to industry standards and relevant codes of practice and recognise the high degree of expertise required.

Animals whose health, fitness, strength or ability to cope in any other way has been lessened or affected by experimental procedures or their preparation must be handled and transported according to the needs of their condition, and expert advice sought relative to this if necessary.

- Animals should be transported in a safe environment that minimises injuries and stress.
- Transport, particularly loading and unloading, are stressful for sheep and therefore multiple transports should be planned and minimised.
- Sheep of different size and class should be separated. Extra care needs to be taken when transporting ewes with their lambs so that smaller animals are not trampled.
- Sheep should be placed at an appropriate density in the transport vehicle to minimise slipping and falling and to allow animals that fall to recover a standing posture.
 - If the transport vehicle is too large for the number and size of sheep, options such as the use of secured straw bales or panels should be considered to reduce the floor area.
- Sheep should not be transported individually. Non-experimental sheep may be used as companion animals if necessary.
- Sheep should not be transported with their legs tied.
- The transport vehicle should provide shelter from extremes of weather and buffeting from the wind, while providing adequate ventilation.
- Use of a mesh and/or tarpaulin cover can provide shelter and discourage animals from jumping out of the crate.
- The floor should provide grip for the sheep's feet and separation or absorption of waste.
- There should be no need to starve sheep before transport of short duration (eg. less than two hours), and they may be provided with feed until being readied for transport. For sheep on pasture, depending on its quality, there may be a need to withhold feed prior to transport (if diarrhoea is a problem).
- Water should be available to sheep until transport commences.
- Ewes should not be transported in the last month of pregnancy except for short distances/duration. Risks to welfare can be minimised by using a glucogenic drench before transport to avoid pregnancy toxæmia.
- Animals should be loaded and unloaded in a safe environment that includes the ability to walk in and out of the transport vehicle thereby avoiding the need for lifting devices.
- The use of dogs to load and unload sheep can speed these operations, however exposure to dogs is stressful to sheep and should therefore be avoided unless specifically justified.

- Personnel transporting sheep should be trained and competent. In particular they should be capable of assessing the welfare of animals prior to and during transport and able to deal with emergencies. Commercial transporters should be accredited eg. Truckcare accreditation.

Table 1. Recommended loading densities for half-wool sheep

Mean liveweight (kg)	Space allowance (m ² /head)
20	0.17
30	0.19
40	0.22
50	0.25
60	0.29

Principle 9 - Monitoring

Monitoring is an essential task for both good animal welfare and good research. Standard operating procedures should be developed to include the frequency of inspection, hazard analysis, record keeping and utilisation of records to review procedures/management.

- Monitoring should commence at selection and continue until the end of use.
- For animals used periodically over time, monitoring should occur in any non-experimental period and animal health and husbandry records should be maintained throughout the entire period of use.
- The parameters that should be monitored include food and water intake, body condition, body weight, general bearing and alertness. These basic parameters may be supplemented for specific purposes to include body temperature, heart rate, parasite status and blood pressure, depending on the nature and duration of the study.
- Frequency of monitoring will depend on the preparation, but should be at least daily.
- The results of monitoring should be recorded and the form that the monitoring takes and the data collected should enable the detection of adverse trends.
- If facilities (or procedures) are changed, the animals' behavioural responses should be observed to provide an indication of any adverse effects of the change.

Appendix 2.

References for further details

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