Appendix H: Examples of methods used to implement the '3Rs'

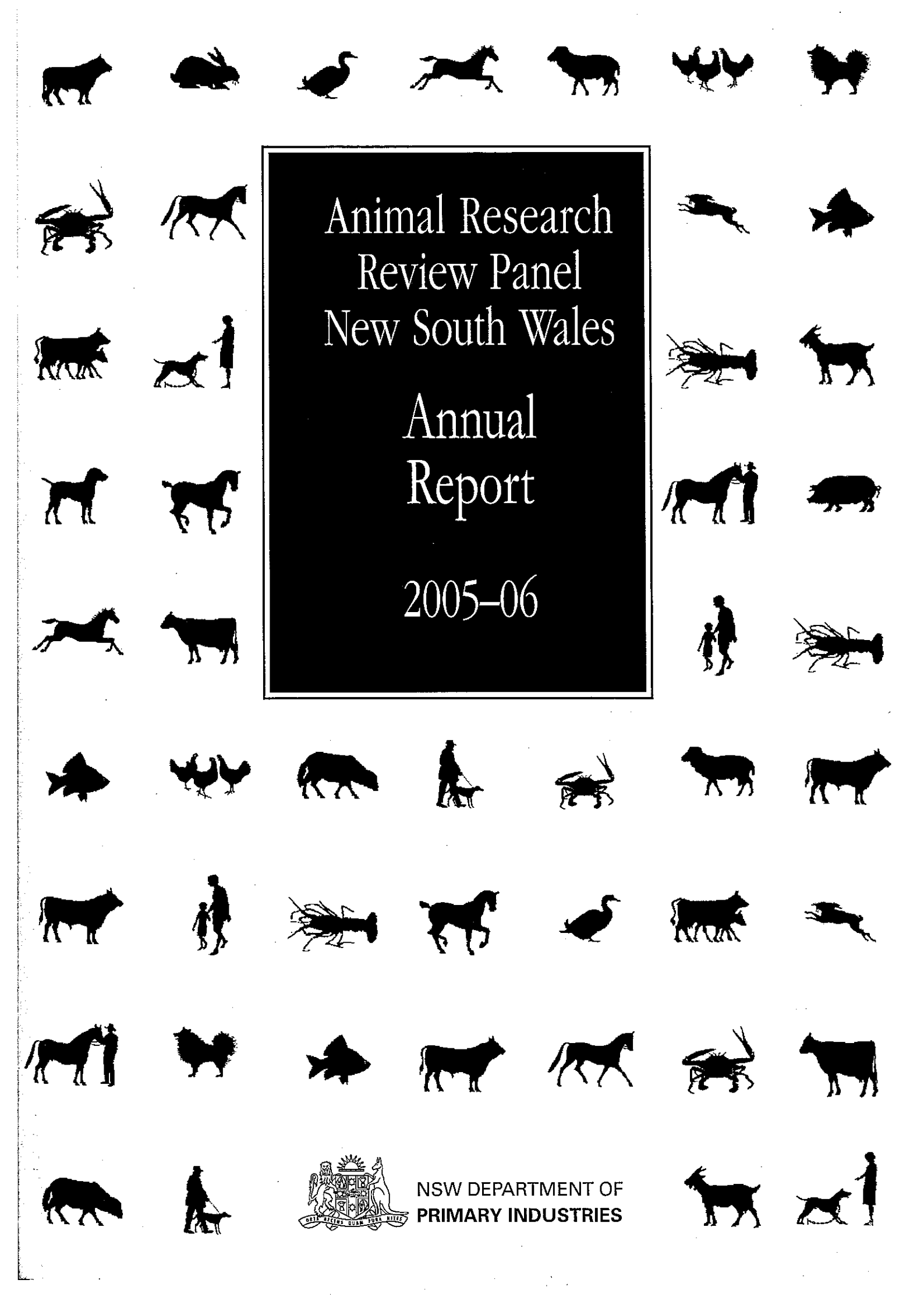
The following are practical examples of strategies used to implement the '3Rs' (Replacement, Reduction and Refinement in animal use). These examples have all been reported by accredited establishments. They are under the headings of 'Replacement' (of animals with other methods), 'Reduction' (in the number of animals used in specific protocols) and 'Refinement' (of techniques used to reduce the impact on animals).

Category	Comments
Replacement	• Education of Committee members of available alternatives to the use of animals. • Promotion of alternatives to animals in teaching (e.g. 'Muscle Physiology' CD-ROM). • Testing of an *in vitro* assay in parallel with the *in vivo* assay. Although the *in vitro* assay gives similar results in some areas, further optimisation and testing of the *in vitro* assay is required before it can replace the *in vivo* assay. • Mathematical modelling experiments that will augment physiology experiments (just commenced). • Rather than measure adherence in mice, tissue culture models are used and the antisera used to measure adhesion *in vitro*. The tissue-culture models are currently being established and will be more representative of the *in vivo* conditions. • Use of audiovisual material such as videos, slides and interactive computer programs. • Use of plant tissue as a replacement for animal tissue for certain enzymatic assays. • Development of a computer model to predict potential worm burdens in sheep. • In applications submitted to the AEC, researchers are increasingly adopting *in vitro* techniques or using the results of *in vitro* studies by others to identify the elements involved in the physiological and pathophysiological states under study. This has the benefit of reducing subsequent experiments on animals.
Reduction in numbers	• Sharing of animal tissues between members of research groups and centres. • Use of new analytical methods such that fewer animals are required as sources of tissues. • Close scrutiny of the number of animals requested in applications to the Committee. • Number of animals always determined by statistical analysis or minimum number required to satisfy regulatory authority for new drug products. • Use of biometricians' comments before approval by AEC. • Limiting the number of birds used to the minimum required for statistical outcomes at defined confidence and power levels (reduced in one experiment from a proposed 200 birds down to 180). • In-vitro testing efficacy of the test product before its use of animals. These techniques allowed us to reduce the number of animals used. • Implementation of new oral toxicity guidelines by the Organisation for Economic Cooperation and Development that use fewer animals. • Application of compounds to skin explants rather than mice. This reduced the number of animals required and removed the pain induced from UV exposure, as the UV was applied to skin explants instead. • Testing program was reviewed, resulting in a reduction in overall testing requirements per unit of production output. Certain tests have been refined to reduce the animal numbers used per test. • Use of abattoir specimens and cadavers. • Development of new computer software that can simulate breeding strategies for any species to improve commercial livestock breeding and reduce experimentation on animals.

Category	Comments
	• Animal use has been minimised by careful scrutiny of numbers of animals requested; approval of new techniques for embryo freezing rather than continuous breeding to maintain lines' reuse of animals, where appropriate, after an extended recovery interval; making surplus tissue available through a tissue availability database; and seeking prior agreement from investigators to make surplus tissue available. The Committee has instigated the consolidation of breeding protocols to ensure that there is no overbreeding; this, in turn, reduces the need for culling.
Refinement of techniques	• Promotion of adequate training of researchers in research methods and procedures. • AEC is now requiring video evidence of the processes used in undeveloped techniques. • Development of endpoints in protocols, so that animals are euthanased before they are exposed to unnecessary pain and suffering. • All personnel have been going through training in techniques such as blood sampling, faecal sampling and ear tagging. • Use of the saphenous vein method as the standard technique for blood collection in rodents. • Use of monitoring checklists developed specifically for each project, as well as documented welfare intervention points and endpoints. • Requirement for the use of analgesia in all recovery surgical procedures. • Group housing of animals wherever possible, with separation of animals only when required and for the minimal period of time. • Refinement of surgical techniques to achieve a reduction in the incidence of post-operative problems. • Refinement of anaesthetic regimes to methods that cause less stress in the animals, with fewer mortalities and improved recovery. • Improved peri-and-post operative analgesia to reduce pain from surgery. • In polyclonal antibody production, the use of adjuvants that are less likely to result in granulomas, and the use of chick eggs whenever possible. • Use of adjuvants known not to produce adverse reactions. • In the case of infection models, the use of low infective doses and short infection times to minimise the impact on animals. • The use of visible implant elastomer (VIE) tags for the identification of amphibians < 50 mm long. Microchips are used for larger amphibians. • Use of a material bag (pillow slip) when weighing animals after removal from the Elliott trap. • Placement of a heat source in a box taken to the trapping site for use on animals suffering hypothermia. • Attempt to establish paracetamol in jelly cubes as an analgesic protocol for rats. Although these were consumed preoperatively they were rarely consumed postoperatively. Paracetamol in drinking water appeared more effective, although no measures of analgesic effect were made. • Use of xylazine and ketamine in low doses in adult female macropods for the collection of milk and reattachment of the pouch young. This procedure not only reduced pain and distress, it also minimised the killing of pouch young. • Placement of blood flow measuring probes on the femoral artery. Because these probes are outside the abdominal cavity, the rabbits make a speedy recovery from surgery. The mask system for delivery of gas mixtures and measurement of ventilation is a major advance. It is non-invasive, non-harmful, and well tolerated by most rabbits.

Category	Comments
Refinement of techniques	• Transcutaneous immunisation rather than immunisation via injection. • We have established collaborations with other investigators in Australia who are also using the mouse model of allergic airway inflammation. This has given us the opportunity to compare our particular experimental model, and its impact on the mice, with that used in other labs. In fact, our current set up is very comparable to that used by other researchers, but we are continuing discussions to try to identify refinements. • Operative technique involved a learning curve as shown by the improvement in the mortality figures. As well as technical improvements, we have changed ventilation techniques to reduce pulmonary trauma and are now using low-volume ventilators. Additional staff have been recruited to assist in surgery and to enable better monitoring of the mice post-operatively. • To prevent death due to anaphylactic shock as a consequence of virus injection an improved regime of pre-injection steroids and anti-histamines was administered. • We have reduced the number of animals used as a result of prioritising experimental groups and restricting our present studies to one strain of mouse. • The monitoring form has been amended to include observation for skin ulceration, which was observed to be an infrequent complication of the immunisation procedure. • We upgraded our aerosol apparatus and this led to improved reproducibility. This means that we are able to obtain better-quality data using fewer mice. • Increased awareness and use of environmental enrichment. • The project developed a non-invasive technique for accessing the reproductive status, population, stress and health of great whales by using 'blow' samples. • Morphometric data and a small amount of blood were collected from Little Penguins as part of the project. The penguins are handled only once, as briefly as possible. Genetic analysis of the blood provides information on the sex of the penguin and some information about the movements and therefore population structure of the Little Penguins. Historically, banding was used to obtain movement data from penguins. It is no longer used, as it was found to increase mortality rates. Bands stay on the penguin for its lifetime and have been found to hamper swimming ability. Often the penguin has to be recaptured to read the band. • Relocation of research horses to a farm setting where horses were free to roam in a large, well-grassed paddock with other horses. • Spontaneous collection of naturally voided urine in both equines and bovines. • Indirect methods such as plot tracking to assess animal presence; giving-up densities (GUD) to assess feeding activity in the presence or absence of predators; and non-lethal uptake as a substitute measure for fox predation activity were used to minimise the impact of research on small native mammals. • Use of raked sand-plots instead of cage traps for surveying quolls. • Hair and scat analysis has been improved, and this has led to a reduction in the need to trap during wildlife surveys. The techniques adopted have been more productive and less invasive than trapping. • Wildlife study: anaesthetic regimes in the field (e.g. portable isoflurane administration). • Wildlife study: veterinarian included for anaesthetic administration and any necessary veterinary interventions. • Wildlife study: edible bait to provide sustenance for animals after capture. • Wildlife study: trapping only when weather conditions are optimal. • Wildlife study: to reduce the risk of pathogen transfer between frogs disposable latex gloves and sterilisation of instruments were used.

Category	Comments
Refinement of techniques	• Use of GPS (global positioning system) collars for collecting continuous data on large mammals reduces the need for frequent recapture. • All housed cattle have access to a bedded area. • Housed sheep in trials have access to a bedded area when protocol allows. • Housed breeder ewes and lambs have access to hay. • Sheep and cattle rations include pellets/nuts, thus reducing the amount of dust. • Earlier endpoints of animal xenograft studies have been implemented. • Additional mouse enrichment is being utilised (i.e. autoclaved cardboard tubing that is replaced at each weekly cage clean). • Traps are cleared no later than 2 hours after sunrise. • Competency-based training has been implemented for all techniques used by research staff. Certificates of competence are issued only after competency has been established against a written competency standard. With the growth of research in the last 2 years this has been a critical strategy in ensuring ongoing animal wellbeing. Key to the minimising of distress to animals used is regular review of handling and restraint techniques. • Use of microimaging for detection of abdominal tumours has been introduced, and further training by experienced collaborators is to occur this year. • Determination of analgesic drug effects that can be used to refine experimental procedures in sheep. • Convention of two continuing education workshops for staff involved in research with animals to improve Animal Ethics procedures and handling and care of experimental animals. • Development of electronic sensing program for fly strike in sheep. • Development of a computer-based experimental animal tracking system to monitor animal use and animal reuse with a view to reducing individual animal exposure to experimental procedures and to monitor animal status within the research flock at any time. • Investigation of methods for environmental enrichment for long-term housed sheep. • Application of a process of using artificial windbreaks to reduce lamb losses during inclement weather. • The AEC has paid particular attention to anaesthetic doses and analgesia doses to minimise pain, and a number of modified procedures have been adopted by researchers on the basis of the experience of other researchers with these techniques. • The AEC has distributed the publication by DB Morton (1999), Humane Endpoints in AnimalExperimentation for Biomedical Research: Ethical, Legal and Practical Aspects, which gives researchers and animal house managers criteria for decision-making in the euthanasia of unwell animals.



Animal Research Review Panel New South Wales

Annual Report

2005–06

NSW DEPARTMENT OF
PRIMARY INDUSTRIES