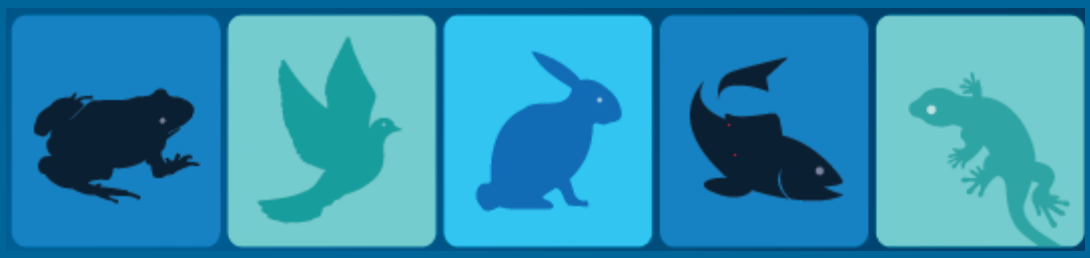
Animal Research Review Panel

Annual Report

2013 - 2014



NSW GOVERNMENT Department of Primary Industries

DISCLAIMERS

The information contained in this publication is based on knowledge and understanding at the time of writing (December 2014). However, because of advances in knowledge, users are reminded of the need to ensure that information upon which they rely is up to date and to check currency of the information with the appropriate officer of the NSW Department of Primary industries or the user's independent adviser.

The product trade names in this publication are supplied on the understanding that no preference between equivalent products is intended and that the inclusion of a product name does not imply endorsement by the NSW Department of Primary Industries over any equivalent product from another manufacturer.

ISSN 0139-9402
Prepared by Janelle Townsend and Lynette Chave

NSW GOVERNMENT | Department of Primary Industries

ANIMAL RESEARCH REVIEW PANEL

11 December 2014

The Hon Katrina Hodgkinson MP
Minister for Primary Industries
52 Martin Place
SYDNEY NSW 2000

Dear Ms Hodgkinson

In accordance with Section 11 of the Animal Research Act 1985, the Animal Research Review Panel presents its annual report covering the period 1 July 2013 to 30 June 2014.

Yours sincerely

Professor Andrew Dart
Chair, Animal Research Review Panel

ANIMAL WELFARE UNIT – BIOSECURITY NSW
DEPARTMENT OF PRIMARY INDUSTRIES – NSW TRADE & INVESTMENT
W: www.trade.nsw.gov.au / www.dpi.nsw.gov.au
Animal Welfare Inspectorial Office
Locked Bag 5123 Parramatta NSW 2124

ABN 72 189 919 072
Animal Ethics Infolink – www.animalethics.org.au
Tel: 02 9842 8090

PART ONE: ORGANISATION

1.1 The Animal Research Act 1985

The NSW Animal Research Act 1985 was the first piece of self-contained animal research legislation introduced in Australia. In introducing the legislation in 1985, the Hon. Kevin Stewart, Minister for Local Government, said that it was based on 'the twin tenets of … enforced self-regulation and public participation in the decision-making process'. It received bipartisan support in the Parliament when it was introduced in 1985 and continues to do so.

The primary aim of the legislation is to protect the welfare of animals used in research and teaching by ensuring that their use is justified, humane and considerate of their needs. The Act incorporates a system of enforced self-regulation, with community participation at the institutional and regulatory levels.

The Act establishes a system of accreditation, licensing and authorisation of organisations and individual researchers. The Act also establishes the Animal Research Review Panel (ARRP) to provide a mechanism for representatives of government, scientific and animal welfare groups to participate jointly in monitoring the effectiveness of the legislation.

The Act creates offences for conducting animal research without appropriate authorisation, with substantial custodial and financial penalties.

1.2 The Australian Code for the Care and Use of Animals for Scientific Purposes

The Australian Code for the Care and Use of Animals for Scientific Purposes (the Code) is a nationally accepted code and is included under the Animal Research Regulation. The Code is reviewed regularly by the Code Reference Group, under the auspices of the National Health and Medical Research Council (NHMRC). The Code Reference group includes representatives from NHMRC, the Commonwealth Scientific and Industrial Research Organisation, the Australian Research Council, Universities Australia, the state government ministries with responsibility for animal welfare, commonwealth government departments for the sectors of environment, education and primary industries, the RSPCA and Animals Australia. The 8th edition of the Code was released in July 2013.

1.3 The Animal Research Review Panel

The Animal Research Review Panel (ARRP) has responsibility for overseeing the effectiveness and efficiency of the legislation, investigating complaints, and evaluating compliance of individuals and institutions with the legislation. The constitution, membership and mode of operation of the ARRP are set out in the Act. The 12-member Panel has equal representation from industry, government and animal welfare groups. This allows community involvement in regulating the conduct of animal research in New South Wales. Apart from developing overall policy on animal research issues, the ARRP is closely involved in the administration of the legislation. This is achieved through evaluating applications for accreditation and licences, conducting site visits to assess compliance, and investigating complaints. The ARRP also has a role in considering amendments to the Regulation. Staff of the Animal Welfare Unit, Biosecurity NSW (the NSW Department of Primary Industries) provide executive support for the ARRP.

1.3.1 Mission statement

***** To protect and enhance the welfare of animals used in scientific research, testing and teaching in New South Wales.

* To promote an understanding within the New South Wales community of the ethical and technical issues involved in the use of animals for scientific purposes.

The strength of the ARRP lies in the diversity of expertise, opinions and ethical perspectives of its members. The development of cohesive and progressive policies has occurred as a result of this diversity. All members are employed in other fields and participate on a largely voluntary basis. Non-government members are paid fees for attending formal meetings and participating in site inspections. Members are not paid for time spent preparing for meetings and inspections, for considering applications for accreditation or licenses, or for drafting discussion papers.

1.3.2 Functions of the ARRP

Section 9 of the Animal Research Act defines the functions of the ARRP as:

- the investigation of matters relating to the conduct of animal research and the supply of animals for use in connection with animal research
- the investigation and evaluation of the efficacy of the Code of Practice in regulating the conduct of animal research and the supply of animals for use in connection with animal research
- the investigation of applications and complaints referred to it under the Act
- such other functions as the Minister may from time to time confer or impose on it.

In November 1998, the then Minister, the Hon. Richard Amery MP, conferred the following additional function on to the ARRP, pursuant to section 9 (d) of the Act:

> The consideration and comment on proposals referred to the Animal Research Review Panel which relate to the making, amendment or review of the regulations under the *Animal Research Act 1985.*

There have been no other functions formally conferred on the ARRP under section 9 (d) of the Act since it commenced.

1.3.3 Membership

The ARRP consists of 12 members appointed by the Minister on the basis of nominations received from industry, government and animal welfare groups. The nominating organisations are:

- New South Wales Vice-Chancellors' Committee: three nominees
- Medicines Australia: one nominee
- New South Wales Minister for Health: one nominee
- New South Wales Minister for Education: one nominee
- New South Wales Minister for Primary Industries: one nominee
- New South Wales Minister for the Environment (National Parks and Wildlife Service): one nominee
- Animal Societies' Federation (New South Wales): two nominees
- Royal Society for the Prevention of Cruelty to Animals (New South Wales): two nominees.

All members of the ARRP are part-time and are normally appointed for a term of 3 years.

During the 2013–14 period the membership of the ARRP was:

- A/Professor Andrew Dart (Chair) (nominated by the NSW Vice-Chancellors' Committee)
- Dr Regina Fogarty (Deputy Chair) (nominated by the Minister for Primary Industries)
- Dr Magdoline Awad (nominated by RSPCA NSW)
- Mr Peter Batten (nominated by the Minister for Education and Training)
- Ms Celeste Black (nominated by the Animal Societies' Federation). Resigned September 2013.
- Dr Mike Fleming (nominated by the Minister for the Environment)
- Professor Annemarie Hennessy (nominated by the Minister for Health). Appointed January 2014.
- Ms Emma Hurst (nominated by the Animal Societies' Federation). Appointed January 2014.
- Professor Anne Keogh AM (nominated by the Animal Societies' Federation)
- Professor Robert Mulley (nominated by the NSW Vice-Chancellors' Committee)
- Mr David O'Shannessy (nominated by RSPCA NSW)
- Professor Jacqueline Phillips (nominated by the NSW Vice-Chancellors' Committee)
- Dr Peter Rolfe (nominated by Medicines Australia)

Information on members of the Animal Research Review Panel in 2013–14 is as follows:

Professor Andrew DART(Chair) BVSc PhD Dip ACVS Dip ECVS
Dr Dart is Professor of Equine Veterinary Science and Director of the Research and Clinical Trials Unit of the Faculty of Veterinary Science, the University of Sydney. He has held positions as Director of the Veterinary Teaching Hospital and Deputy Chair and Acting Chair of the Animal Ethics Committee of the University of Sydney. Dr Dart is a Registered Specialist in Equine Surgery and has spent time in private practice and as a Clinical Academic. Professor Dart was appointed as Chair of the ARRP in December 2010.

Dr Regina FOGARTY (Deputy Chair), BVSc, PhD (University of Queensland). Dr Fogarty is the Director of the Office of Agricultural Sustainability and Food Security, a policy group within the Department of Primary Industries. Dr Fogarty has been actively involved in animal welfare issues in previous positions with the Department as Manager of NSW Agriculture's Animal Welfare Unit; as Program Leader, Intensive Livestock Products; and as Veterinary Officer (Pig Health). Dr Fogarty joined the ARRP in 2003 as the nominee of the then Minister for Agriculture.

Dr Magdoline AWAD BVSc MACVSc(Animal Welfare) GradCert Mgt(Prof Prac) CMAVA
Dr Awad is a nominee of the RSPCA (NSW). After graduating with a Veterinary Science degree from the University of Sydney, Dr Awad worked in small animal private practice before joining the RSPCA NSW in 1996 as a Veterinarian. She was Deputy Chief Veterinarian from 2004-2008 and currently holds the role of Chief Veterinarian. In 2008 she became a Member of the Animal Welfare Chapter of the Australian College of Veterinary Scientists. She has a particular interest in Shelter Medicine. She was involved in the development of the CAWS Programs (Community Animal Welfare Scheme), Indigenous Dog Health Programs as well as the Pets of Older Persons Program (POOPS) for RSPCA NSW. She became a member of the ARRP in 2008.

Mr Peter BATTEN BSc (Wool and Pastoral Sciences) (UNSW), Dip Ed (Technical) (Sydney CAE)
Mr Peter Batten was Director of the TAFE NSW – Training and Education Support – Industry Skills Unit – Orange and Granville. Peter has 30 years experience in vocational education and training with TAFE NSW including positions dealing with the welfare of animals in teaching including Program Manager Extensive Agriculture, Industry Specialist Livestock Production and Wool and Teacher of Agriculture. Peter joined the ARRP in 2008 as the nominee of the Minister for Education and Training.

Ms Celeste BLACK BA (Harvard), JD (University of Pennsylvania), LLM (Hons) (University of Sydney)
Ms Black joined the ARRP in March 2010 on nomination by the NSW Animal Societies Federation. She is a Senior Lecturer at the Faculty of Law, University of Sydney, where she developed and teaches the undergraduate law elective Animal Law. Ms Black is an executive and founding member of the Human Animal Research Network at the University of Sydney.

Dr Mike FLEMING BSc (Hons) ANU, PhD (Monash)
Dr Fleming is a nominee of the Minister for the Environment and has been with ARRP since February 2009. He is a Senior Team Leader with the Science Division of the Office of Environment and Heritage. Dr Fleming has conducted research in marsupial physiology, wildlife management and biodiversity survey. He has worked extensively in the Northern Territory and New South Wales.

Professor Annemarie HENNESSY BMdSu, PhD

Professor Hennessy was previously a member the ARRP from 2008 to 2010. She was re-appointed in January 2014. She is the director of the National Baboon Colony and an active medical teacher and researcher. She is a qualified nephrologist and specialises in general medicine, renal medicine and obstetric medicine. She is the Dean, School of Medicine, at the University of Western Sydney.

Ms Emma Hurst BA(Psy), PGDip(Psy), M(HealthPsy) .
Ms Hurst is a registered psychologist who has worked in the areas of adolescent mental health, aged care, child therapy, addictions, and health promotion research. Ms Hurst has worked in a range of settings such as mental health services, universities, and specialist early intervention services. She is particularly interested in the promotion of animal advocacy and runs a research animal rehoming service. Ms Hurst was appointed to the Panel in 2014 as a nominee of the Animal Societies Federation.

Professor Anne Keogh AM MBBS (hons), MD, FRACP, FCSANZ, FPVRI
Professor Anne Keogh is a nominee of the NSW Animal Societies Federation. She is the Senior Heart Transplant Cardiologist St Vincent's Hospital Sydney, Head of Human Clinical Research in heart failure and pulmonary hypertension, and Joint Head Clinical Research at the Victor Chang Cardiac Research Institute. She is Conjoint Professor of Medicine University of NSW, Director of two binational registries, a member of the Advisory Committee on Medical Devices, and sits on multiple global and national scientific advisory boards. She has been Trustee of Medical Advances without Animals from 2006, and has worked with a broad range of Australian and international

animal welfare groups for 20 years, Australia Day Ambassador for 7 years, past president International Society of Heart and Lung Transplantation and past president of the Pulmonary Hypertension Society of Aust and NZ which she formed in 2010. She was awarded the Order of Australia (AM) in June 2012 for services to transplantation, heart failure and animal welfare.

Emeritus Professor Robert MULLEY BA (Macquarie), MScAg (Sydney), PhD (Sydney).
Professor Mulley joined ARRP in 2008. He is a nominee of the NSW Vice Chancellors' Committee. He is Professor of Animal Science at the University of Western Sydney, and has extensive experience in husbandry and management of farmed livestock, particularly pigs and deer. More recently he has engaged in research on a range of wildlife species.

Mr David O'SHANNESSY, BSAgr.
Mr O'Shannessy is the nominee of the RSPCA (NSW). Since completing an Agricultural Science degree he has been employed as an inspector with RSPCA NSW and for a period of time was a sales representative for a veterinary pharmaceutical company. He was appointed RSPCA Chief Inspector in May 2005 and was appointed as a member of the ARRP in January 2005.

Professor Jacqueline Phillips. BVSc Hons (Uni of Syd), PhD (ANU)
Professor Phillips is a nominee of the NSW Vice-Chancellors' Committee and was appointed to the ARRP in 2010. Professor Phillips is a registered veterinarian who has worked in small animal and mixed practice. She has served on Animal Ethics Committees as a Category A member at the Australian National University (ACT) and Murdoch University (WA) . She is a Professor of Neuroscience at the Australian School of Advanced Medicine, Macquarie University. Her research is in the areas of hypertension and renal disease.

Dr Peter ROLFE BVSc, PhD
Dr Rolfe is a nominee of Medicines Australia. He is an employee of Novartis Animal Health, a registered veterinary surgeon and has had a career in research and research management and in various public and private sector roles. He currently manages research programs for the research and development of innovative pharmaceuticals for use in farm and companion animals.

1.4 Animal Ethics Committees

At the institutional level, Animal Ethics Committees (AECs) provide avenues for public participation in the regulation of animal research.

AECs are responsible for approving and monitoring research within institutions, including inspections of animals and facilities. No animal research may be carried out without AEC approval. AECs must consider and evaluate applications to conduct research on the basis of the researchers' responses to a comprehensive set of questions, including their justification for the research, its likely impact on the animals, and procedures for preventing or alleviating pain or distress. On behalf of the institution, AECs have the power to stop inappropriate research and to discipline researchers by withdrawing their research approvals. They can require that adequate care, including emergency care, is provided for animals. They also provide guidance and support to researchers on matters relevant to animal welfare, through means such as the preparation of guidelines and dissemination of relevant scientific literature. They are responsible for advising institutions on the changes to physical facilities that should be made to provide for the needs of the animals used.

The membership and duties of AECs are laid down in the NSW legislation and in the *Australian Code for the Care and Use of Animals for Scientific Purposes*, which also provides guidance on how AECs should operate.

Committee membership must include members as follows:

- Category A: a veterinarian
- Category B: an animal researcher
- Category C: a person with a demonstrated commitment to animal welfare who is not involved with the institution, animal research or the supply of animals for research
- Category D: an independent person who does not fit the requirements of the other categories, is not associated with the institution and who has never been involved in the use of animals for research.

The *Code* states that more than one person may be appointed to each category and, if a Committee has more than four members, categories C plus D should represent no less than one-third of the members.

The criteria used by the ARRP for assessment of AEC membership are documented in an ARRP policy document, *Policy 9: Criteria for the Assessment of Animal Ethics Committee Membership* (http://www.animalethics.org.au/policies-and-guidelines/operation). In examining applications from institutions for accreditation as animal research establishments, the membership of AECs are assessed to ensure they are of acceptable composition. The ARRP also assesses, and makes recommendations to the Secretary, on the suitability of all new appointments to AECs. All new AEC appointments must be approved by the Secretary. During audit inspections, the ARRP assesses the operation of the AECs.

1.5 Accreditation and licensing

The legislation requires that all applications for accreditation and animal supply licences be referred to the ARRP for consideration. The ARRP has established procedures to deal with the considerable workload this entails and has regularly reviewed and updated these procedures to take account of changes in needs and resources.

There are two components in the assessment of applicants by the ARRP:

- consideration of a written application to determine whether the applicant is complying with a limited number of fundamental requirements of the legislation
- evaluation of the applicant at a site inspection, when a much broader approach is taken.

The recommendations of the ARRP are referred to the Secretary of the Department of Trade & Investment, who has statutory authority for the issue of accreditation and licences and for imposing, altering or removing conditions of accreditation or licence.

Accreditation and licences are usually issued subject to the condition that a site inspection is satisfactory and are subject to the reporting of changes in AEC membership to the Secretary for approval. Other conditions may also be stipulated, as relevant to the operation of each institution. (See Appendix K for standard conditions on accreditation and licences).

1.5.1 Evaluation of written applications

New and renewal applications for accreditation or licences are assessed by Animal Welfare Unit staff, according to criteria developed by the ARRP. Arising from these assessments, recommendations on the applications are made to the ARRP. The ARRP considers the recommendations and then makes recommendations on the applications to the Secretary.

The ARRP may convene an Applications Subcommittee to facilitate the assessment of new applications. The subcommittee is convened on a “needs” basis. Where no need is identified by the Animal Welfare Unit for input by the Applications Subcommittee, recommendations are made by the Unit directly to the ARRP.

A small number of applications are also viewed directly and considered by the full ARRP. These include applications from individuals or organisations about which the ARRP has particular concerns, or situations where the application is sufficiently different from the norm to raise policy implications.

The criteria against which the ARRP assesses written applications are drawn from the legislation. Considerations include whether the AEC is properly constituted, whether its procedures are adequate, whether it is meeting sufficiently frequently to deal with the volume of work, and whether it is conducting inspections of the animals and facilities it supervises. The types and numbers of animals held and their accommodation are also checked, and likely problem areas are flagged for follow-up at site inspection. Similarly, numbers and qualifications of animal care staff are assessed for adequacy.

Monitoring of animal care and use by the AEC is another area of assessment. Details of AEC inspections carried out must be provided. Questions on the source and destination of animals allow the ARRP to double-check compliance with the Act’s provisions relating to animal supply.

1.5.2 Conduct of site inspections

Following the evaluation of written applications, the second phase of the process of assessing establishments is the site inspection. The aim of site inspections is to determine whether institutions and individuals are complying with the legislation. The *Australian Code for the Care and Use of Animals for Scientific Purposes* provides the criteria against which institutions are assessed. The range of items assessed includes: the membership, procedures and

activities of the AEC; animal care procedures; animal research procedures; and the physical facilities for housing and using animals. An evaluation is also made of the wellbeing of the research or breeding animals.

Audit visits are arranged in advance and usually take from 1 to 4 days per site. Large establishments with multiple sites can take up to 2 weeks to inspect. Information about inspections conducted in the 2013–14 year is provided in Appendixes C and D. The dates provided represent days on site and do not include preparation and follow-up time, which is often considerable.

Assessment begins before site inspection with an examination of written material provided by the institution or individual. This includes lists of the research applications considered by the AEC and people issued with Animal Research Authorities, AEC minutes, the AEC annual report, and records of inspections conducted, together with information about the procedures of the committee and the institutional policy on the committee's operation and decisions.

The examination is carried out by an Animal Welfare Unit Veterinary Inspector and the ARRP members who have been nominated to participate in the inspection. This pre-inspection evaluation allows likely problem areas to be identified and a general idea to be gained of how the establishment is operating.

On the day(s) of the inspection the inspection team initially looks at the animals and the facilities and talks with researchers. This examination includes assessing a broad range of items such as the physical condition of animals, animal care and management, and records related to the animals held. After examining animals and facilities, the inspection team sits in on a scheduled meeting of the AEC, which allows it to view the operation of the AEC and the interaction of its members. At the end of the meeting, time is taken to discuss with the AEC issues arising from the inspection and to solicit feedback from AEC members. Additional important considerations are how the committee liaises with researchers and whether it has developed its own policies or guidelines for procedures of particular concern, such as blood collection techniques, methodology for monoclonal antibody production, and standards for wildlife transportation and the recognition and relief of pain.

A meeting is usually held with the head of the institution at the beginning or end of the inspection. Any serious concerns are immediately referred to the institution at the appropriate level.

As soon as possible after the inspection, a detailed report is prepared. The report covers an evaluation of the AEC and an assessment of the animals' wellbeing, housing and holding, and their care and monitoring. Once the ARRP has considered the report, recommendations may arise to impose additional conditions on the accreditation or licence. For example, a condition may be that appropriate post-operative procedures must be implemented.

In addition to conditions for accreditation or licence (which are mandatory and must be implemented), the ARRP report usually contains a number of recommendations—for example, for more effective operation of the AEC, for improvement of the management of research within the institution, or for improvement of the animal facilities. Implementation of recommendations is not mandatory, but the institution is required to advise on how it has responded to the recommendations. If the recommendations have not been implemented, then the reasons for this must be explained.

Inspection reports also provide an opportunity for the ARRP to commend the institution, individual researchers or animal attendants for initiatives that raise the standards of the overall operation of the research facility or for techniques or facilities that enhance the welfare of research animals.

The ARRP also conducts revisits to institutions (and individuals) that have been inspected previously and where particular concerns were raised during the inspection. The primary purpose of these revisits is to evaluate the responses to the recommendations and conditions imposed.

The ARRP aims to carry out full audit visits for all institutions approximately every 4 years, as well as unannounced visits by inspectors to follow up problems. Reinspections concentrate more on procedures rather than facilities, unless new facilities have been built. Announced and unannounced spot checks and visits to look at specific aspects of operation may be carried out between full visits.

1.6 The Animal Research Act in schools and TAFE

The Animal Research Act allows the use of animals for educational purposes when there is a demonstrated educational benefit, when there is no suitable alternative, and when the least number of animals is used, with the least impact on their wellbeing. Although animals are used for educational purposes in many situations, their use in schools and TAFE colleges presents special issues, such as mechanisms for approval and monitoring of animal use across the State. Their use also presents opportunities to promote in students an understanding of the ethical and technical issues involved with the use of animals.

1.7 Administration

The Animal Welfare Unit of Biosecurity NSW is a section within the NSW Department of Primary Industries. The functions of the Animal Welfare Unit cover:

- animal research issues under the *Animal Research Act*, including providing executive services to the ARRP
- general animal care and cruelty issues under the *Prevention of Cruelty to Animals Act,* including the operation of the Animal Welfare Advisory Council under the Minister for Primary Industries
- animal display issues under the *Exhibited Animals Protection Act*, including the operation of the Exhibited Animals Advisory Committee
- Departmental animal welfare activities.

The Animal Welfare Unit can be contacted at:

Animal Welfare Unit – Biosecurity NSW
NSW Department of Primary Industries
Locked Bag 5123
PARRAMATTA NSW 2124
Phone: (02) 9842 8090

or at the NSW Department of Primary Industries Head Office:

Animal Welfare Unit - Biosecurity NSW
NSW Department of Primary Industries
161 Kite Street
Locked Bag 21
ORANGE NSW 2800
Phone (02) 6391 3149
Fax (02) 6391 3740
E-mail: animal.welfare@dpi.nsw.gov.au

In the 2013–14 financial year the following staff were assigned, at various times, to provide inspectorial and/or executive support to the ARRP (amongst their other duties).

Orange:

Suzanne Robinson, BRurSc, EMPA, GradCertEmergencyMgt, Senior Manager, Animal Welfare
Amanda Paul, BVSc, MACVSc (Animal Welfare), Veterinary Officer (part-time)
Tammy Kirby, Acting Licensing Clerk
Jen Anderson, Acting Branch Support Officer (July – September 2013)
Justeene Cleary, Acting Branch Support Officer (September 2013 – June 2014)
Jo Collins, Branch Support Officer (part-time) (March – June 2014)

Sydney:

Lynette Chave, BVSc, Leader, Animal Research
Peter Johnson, BVSc, PhD, Veterinary Officer
Janelle Townsend, Branch Support Officer (part-time)

PART 2: REPORT ON WORK AND ACTIVITIES

2.1 Administration and planning

Administrative functions have varied from activities such as assessments of licensing and accreditation to formulating the ARRP's operational plan for 2013–14. The appendixes to this annual report contain details of many of the operational and strategic functions of the ARRP. These include the dates of, and attendance at, ARRP meetings (Appendixes A and B); dates and attendance of ARRP members at inspections of accredited research establishments and animal supply licence holders (Appendixes C and D); the ARRP Strategic Plan 2011–14 (Appendix E) and Operational Plan for 2013–14 (Appendix F); and ARRP operating expenses (Appendix I).

2.1.1 Strategic Plan 2011–14

During 2011-12 the ARRP revised its 3-year strategic plan. The plan identifies the primary goals of the ARRP and strategies for achieving these goals.

Details of the Plan are given in Appendix E.

2.1.2 Operational Plan for 2013–14

The ARRP Operational Plan for 2013–14, including performance status for each activity, is provided in Appendix F.

2.1.3 Liaison with organisations and individuals

The ARRP liaises with organisations and individuals to offer advice and to facilitate the implementation of legislative requirements and adherence to replacement, reduction and refinement principles.

During the 2013-14 year the main method of liaison was via discussions during, and feedback after, site inspections. Additionally recommendations were made in the process of assessing Accreditation and Licence applications.

2.2 Assessment of applications

In 2013–14 there were 133 accredited animal research establishments and 41 holders of animal suppliers' licences.

During 2013–14 the ARRP considered and made recommendations to the Secretary on:

- 7 new applications for accreditation
- 43 renewal applications for accreditation
- 7 renewal applications for animal suppliers' licences.
- 5 extensions to existing accreditations and/or animal suppliers' licences.

In 2013-14 the ARRP revised its criteria for the assessment of applications to take into account the revised 8th edition of the *Australian Code for the care and use of animals for scientific purposes*.

2.2.1 LD50 testing

LD50 is a toxicity test used to determine the dose or concentration of a test substance—that is, the lethal dose—that is expected to kill 50% of the animals to which it is administered. For the purposes of the NSW *Animal Research Act, 1985* the definition of LD50 has been broadened. Included are all tests in which a potentially lethal dose of a substance will be administered and is expected to kill a proportion of the individuals in any group of animals to which it is given. In NSW such tests may be undertaken only under the approval of a properly constituted Animal Ethics Committee, with the concurrence of the Minister for Primary Industries. Applications for permission to conduct LD50 tests are evaluated by an ARRP subcommittee. Members of the subcommittee in 2013–14 were Mr Batten and Professor Dart. The subcommittee makes recommendations to the ARRP, which in turn advises the Minister.

In 2013–14 the subcommittee considered one application (six tests) from an Accredited Animal Research Establishment.

The testing is used in quality control during the manufacturing of vaccines and in the development of new vaccine formulations. The majority of the tests are related to the manufacture of clostridial vaccines, used to protect livestock and companion animals against tetanus, enterotoxaemia, black leg and black disease that are rapidly fatal if contracted by unvaccinated animals. One of the tests is required for quality control of batches of equine salmonella vaccine, used to protect horses against salmonellosis. The ARRP recommended to the Minister that she approve the application on the following conditions:

1) Data is provided in graphical form by 31 January 2015 with figures comparing 2012, 2013 and 2014 calendar years on the following:

   a) The number of animals used for each quality control test in relation to a relevant measure to be determined by the establishment. The measure should provide information on the trends in numbers of animals used over time.

   b) The number of animals used for development and research over time, with an explanation of the purpose eg replacement of a test, refinement of a procedure.

   c) The total number of animals produced in relation to numbers of animals actually used in tests.

   d) The number of animals that die in tests and the number euthanased as an early end-point in tests.

2) Any application for Ministerial concurrence to conduct LD50 tests between April 2015 and April 2016 must be presented by the establishment to the Animal Welfare Unit by 31 January 2015.

3) The company continues, in consultation with the AEC, to identify and implement refinements to lessen the impact of existing approved tests on animals and methods of reducing the numbers of animals used in existing approved tests or replacing animal tests with alternatives and reports upon these to the Animal Welfare Unit by 31 January 2015.

2.3 Assessment of changes to AEC membership

All establishments are required to advise the Animal Welfare Unit of changes to AEC membership. The ARRP assesses and makes recommendations to the Secretary on the suitability of the qualifications of the new members for the categories of membership to which they are nominated.

The qualifications of AEC members are assessed in accordance with the requirements set out in the *Australian Code for the Care and Use of Animals for Scientific Purposes* and ARRP Policy 9: *Criteria for Assessment of Animal Ethics Committee Membership (http://www.animalethics.org.au/policies-and-guidelines/operation/criteria-for-assessment)*.

In the 2013–14 year the ARRP assessed and made recommendations to the Secretary on the appointment of 53 members of Animal Ethics Committees.

2.4 Assessment of accreditation and licensing responses

The ARRP assesses and makes recommendations to the Secretary on responses from accredited animal research establishments and licensed animal suppliers to conditions and recommendations arising from site inspection and / or placed at the time of accreditation and licence application.

In the 2013–14 year the ARRP considered 41 responses from accredited animal research establishments and licensed animal suppliers.

2.5 Subcommittees

The ARRP appoints subcommittees to deal with particular issues. They explore issues in depth and have discussions with relevant members of the scientific and broader communities. Subcommittees provide reports and recommendations to the full ARRP for consideration. Membership of subcommittees is largely drawn from the ARRP. External members of subcommittees are occasionally co-opted on a voluntary basis. Activities of subcommittees in the 2013–14 year included:

- Evaluation of applications for LD50 testing (Professor Dart and Mr Batten)

- Preparation for the 2013 Animal Ethics Seminar (Professor Dart, Dr Fogarty and Mr Batten)
- Preparation for the 2015 Animal Ethics Seminar (Dr Fogarty (Chair), Professor Dart, Professor Keogh, Ms Hurst, Professor Hennessy)

2.6 Statistics on animal use

The Animal Research Regulation requires accredited research establishments (other than schools) and animal research authority holders to record and submit information on the number of animals used in research each year.

The requirements for reporting on animal use provide data on the numbers of animals used in all research projects in NSW, reported against the purpose of the research and the types of procedures in which they were involved. The aim of collecting these statistics is to give some indication of the level of 'invasiveness' of the procedures on the animals and to provide data on the use of animals in research. Aspects of the system include:

1. The recording of an animal in all projects in which the animal is used.
2. The recording of animals for each year in which they are held in long-term projects.
3. The recording of the types of procedures used (giving an indication of the impact of procedures), combined with the recording of the purpose of the research.

The categories used are based on those planned to be used in a future national database. Figures are collected on a calendar year basis rather than by financial year.

Appendix G of this report summarises animal usage in 2013.

In addition to information on numbers of animals used, information is collected on initiatives in the areas of reduction, replacement and refinement of animal use. A summary of this information is provided in Appendix H.

As an additional means of monitoring accredited animal research establishments, the annual reports of AECs are required to be submitted with the submission of annual statistics. The *Australian Code for the Care and Use of Animals for Scientific Purposes* requires that each AEC must submit a written report on its activities at least annually to the governing body of the institution for which it acts. In the 2013-14 year, the ARRP carried out an assessment of these reports, and provided feedback to the AECs and institutions.

2.6.1 Lethality testing

Accredited research establishments must keep figures on lethality testing and submit these to the ARRP. Lethality testing is defined as *'any animal research procedure in which any material or substance is administered to animals for the purpose of determining whether any animals will die or how many animals will die'*. Lethality tests include, but are not limited to, LD50 tests (see item 2.2.1). Figures on lethality testing are included in Appendix G of this report.

2.7 Support for Animal Ethics Committees

The ARRP and the Animal Welfare Unit continue to use various means to support AECs in performing their duties. These means include the conducting of site inspections; the writing of policies, guidelines and fact sheets where a need is identified; the holding of seminars for AEC members and researchers; the maintenance of a website dedicated to animal research issues (Animal Ethics Infolink) and the supply of advice over the telephone or by correspondence.

The ARRP is used as a reference source by the State's AECs, for example as a source of information on successful policies developed at other institutions.

2.7.1 Register of candidates for AEC membership

Finding interested and suitable members has been a problem experienced by a number of AECs. Categories C (Animal Welfare) and D (Independent) have presented the most difficulty. To help AECs to maintain the required membership, the ARRP suggested the establishment of a register of AEC members interested in joining other AECs. The Animal Welfare Unit has established a list of names, contact details and the categories that individuals believe they can represent. This list is available to all NSW AECs, but has remained short for a number of years.

2.7.2 Animal Ethics Seminar

An Animal Ethics Seminar was held in October 2013. In previous years the seminars were primarily attended by AEC members and AEC executive officers. It was decided by the ARRP to broaden the 2013 seminar to include animal researchers. This approach was successful as reflected in the increased number of attendees at the seminar compared to previous years.

In an effort to ensure that the programme for the meeting would meet the needs of AECs, comment was sought from all NSW AECs on topics they wished to discuss and the format for conducting the meeting. Valuable feedback was used, in conjunction with comments gathered from evaluation forms completed at previous meetings, to structure a programme accordingly. The members of the ARRP subcommittee working on this project were Professor Dart, Dr Fogarty and Mr Batten. Other members of the ARRP, including Professor Keogh AM and Professor Phillips assisted with ideas for the programme and contacting potential presenters.

The programme for the day was an interesting one with presentations over a diverse range of subjects. Associate Professor Anthony Hannan gave an informative presentation on the effects of enrichment on mouse models of brain disorders. Representatives from a variety of establishments provided insight into specific animal welfare practices employed for an assortment of species from pigs to zebra fish.

A central section of the day looked at the value of translational research versus basic science, with Professor S Bruce Dowton and Professor Mark Connor, both of Macquarie University, presenting the “opposing views” on this topic.

Other presentations included the role of Animal Welfare Officers, and the way in which wildlife survey data is collected and collated.

The Australian Catholic University again generously hosted the meeting at its MacKillop Campus, North Sydney.

Information on the 2013 seminar and on previous seminars can be found at the Animal Ethics Infolink website at: http://www.animalethics.org.au/animal-ethics-committees

2.7.3 Schools Animal Ethics Committee

A mutually beneficial discussion occurred when the Animal Welfare in Schools Adviser attended a meeting of the Panel. The purpose of the discussion was to give the Panel members some background on the operation of the Schools AEC and to explain the development of a revised list of approved procedures linked to educational outcomes. The legislation requires that this list of procedures be approved by the Panel.

After some amendments the revised procedures were approved at a subsequent meeting of the Panel. The Panel commended the operation of the Schools AEC and the contributions of the Animal Welfare in Schools Adviser.

2.8 Website: Animal Ethics Infolink

Development and maintenance of a website by the ARRP - ‘Animal Ethics Infolink’- is aimed at assisting researchers, teachers and members of Animal Ethics Committees to access information about the operation of the animal research legislation in NSW. In addition to specific information about this legislation, including ARRP policies and guidelines, this site provides general information about legislation in other states and countries and links to many sites from which useful information promoting the humane care and use of animals for scientific purposes can be sourced. The website also gives the broader community access to information about animal use for research and teaching in NSW.

The website has been developed and is maintained in conjunction with the Animal Welfare Unit. The Animal Ethics Infolink site is accessible at www.animalethics.org.au .

2.9 Site inspections

A list of dates of site inspections undertaken in 2013–14 is provided in Appendix C, and a list of ARRP members attending is given in Appendix D. There were 27 establishments inspected over a period of 28 working days. The length of these inspections ranged from one day to two days.

The ARRP aims to carry out a routine inspection of each accredited animal research establishment approximately every 4 years to maintain personal contact with institutions, AECs and researchers, and to carry out a complete audit of institutional operation under the *Animal Research Act 1985*.

The ARRP places a major focus on reviewing the operation of AECs, to ensure that AECs, investigators and institutions understand their responsibilities under the Animal Research Act and the Code. The conduct of research procedures and the conditions in which animals are held also receive close scrutiny during site visits.

2.10 Policies and guidelines

The ARRP and Animal Welfare Unit produce policies and guidelines to aid researchers, AECs, research establishments, animal suppliers and members of the broader community to understand and comply with the requirements of the animal research legislation. These documents can be found by following the links from the ARRP's website, Animal Ethics Infolink, www.animalethics.org.au (see Appendix J for a list of guidelines and policies).

New policies and guidelines are produced to fill needs identified by the ARRP.

When first published, guidelines and policies are sent out to AECs and other groups as appropriate (such as user groups and animal welfare organisations) for comment. The documents are then reviewed in the light of the comments received.

The following policies were revised during 2013-14:

ARRP Policy 5: Annual reporting by Animal Ethics Committees to accredited animal research establishments

ARRP Policy 5A: Accredited animal research establishment support for Animal Ethics Committees

ARRP Policy 6: Differentiation between animal research and veterinary treatment

ARRP Policy 14: The use of restricted drugs and the conduct of restricted acts of veterinary science in animals

The revision of ARRP Policy 14 on the use of restricted drugs contained information on the use of pentobarbitone in the field for euthanasia of animals. Such use is governed by the Poisons and Therapeutic Goods legislation. Liaison with the NSW Ministry for Health and the Office of Environment and Heritage resulted in amendments to procedures to allow wildlife researchers in the field to use pentobarbitone for the humane euthanasia of animals under specified circumstances. Pentobarbitone is a common drug of use for euthanasia of animals in a veterinary setting. The ability of researchers to use this drug in the field has been seen as a positive step for animal welfare, as previously researchers had limited options for the humane euthanasia of animals – for example where animals were found injured.

During the 2012-13 year an issue was raised with the ARRP about publication of the results of a study using baboons for shoulder surgery. As the research had occurred 20 years previously, it was decided there was no value in investigating the particular study. However, the ARRP agreed that it raised the broader issue of how AECs make judgements about the value of research, especially for projects with high impacts on the animals involved. It was decided that the development of a guideline document on this issue could be of assistance to AECs. In developing this guideline it was intended that steps would include assessing existing literature and carrying out a survey of AECs for feedback. In the 2013-14 year a review of literature on how AECs make judgements about the value of research was carried out. The ARRP considered the literature review and developed a series of questions from this that were distributed to Animal Ethics Committees. The results of the survey are to be collated to assist in the development of the guideline document.

2.11 Review of the Australian Code for the Care and Use of Animals for Scientific Purposes

A review of the 7th edition of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* was progressed by the NHMRC in the 2012-13 year. The 8th Edition of the Code was published in July 2013 with a name change to the *Australian Code for the Care and Use of Animals for Scientific Purposes.* The revised Code and information on changes between the 7th and 8th editions are available on the NHMRC website at: http://www.nhmrc.gov.au/guidelines/publications/ea28

2.12 Initiatives in replacement, reduction and refinement

Information collected from the 'Annual Return on Animal Use' submitted by each research establishment and independent researcher includes information on techniques developed or used by the establishment to replace, reduce and refine animal use in research and teaching. The adoption of such techniques is actively encouraged by the ARRP. A list of some of the initiatives can be found in Appendix H.

2.13 Complaints

A formal process for making specific complaints about animal research is set out in sections 22, 28 and 42 of the *Animal Research Act 1985*. The process allows any person to make such a formal complaint. The complaint must be made in writing to the Secretary, who refers the complaint to the ARRP for investigation. The ARRP is bound to investigate formal complaints and to make recommendations to the Secretary for disciplinary action (if it is considered warranted) or dismissal of the complaint. Both the complainant and the individual or institution being investigated have a right of appeal to the Administrative Decisions Tribunal.

The ARRP also has a policy of responding to informal complaints. These may involve varying degrees of investigation, from formal interviews to requests for documents or unannounced visits to animal holding facilities. Complaints may arrive from a variety of sources: the RSPCA may refer matters that fall outside its jurisdiction; ARRP members may raise matters brought to their attention by members of the community; public concern may be expressed in the media; and complaints may be raised in direct correspondence to the Minister for Primary Industries, the ARRP, or the Animal Welfare Unit.

A summary of the complaints considered and finalised in the 2013–14 reporting period is as follows:

Research carried out using dogs

Correspondence was received expressing concern at procedures carried out on dogs as reported in a research publication. In particular, concern was expressed about procedures carried out on conscious dogs.

The AEC that had approved the work was contacted for information. The information obtained showed that the research had been approved and carried out between 1989 and 1996. The procedures conducted on the conscious dogs were similar to those done for diagnostic and therapeutic reasons on conscious human patients.

In considering the matter it was noted that the approval and conduct of the research had occurred many years ago. However, the Panel expressed, as a general principle, the need to promote the ongoing development of the ethical consideration of research by all AECs to ensure that justification is manifest in the deliberations of the committees. This issue was to be addressed in the guideline document being developed by the Panel on how AECs make judgements about the value of research.

APPENDIXES

Appendix A: Dates of ARRP meetings 2013–14

Meeting number	Date of meeting
203	17 July 2013
204	25 September 2013
205	4 December 2013
206	6 March 2014
207	8 May 2014

Appendix B: Attendance of members at ARRP meetings 2013–14

	Meeting number				
Member	203	204	205	206	207
Professor Andrew Dart (Chair)	*	A	*	*	*
Dr Regina Fogarty (Deputy Chair)	*	*	A	*	A
Dr Magdoline Awad	*	*	*	*	*
Mr Peter Batten	*	*	*	*	*
Ms Celeste Black	*	A	N/A	N/A	N/A
Dr Mike Fleming	*	*	*	*	*
Professor Annemarie Hennessy	N/A	N/A	N/A	*	*
Ms Emma Hurst	N/A	N/A	N/A	*	*
Prof Anne Keogh	A	A	A	*	*
Professor Robert Mulley	*	*	*	*	*
Mr David O'Shannessy	*	A	*	*	*
Professor Jacqueline Phillips	A	*	*	*	*
Dr Peter Rolfe	*	A	*	*	*

* = Present

A = Absent

Appendix C: Dates of Inspections July 2013 – June 2014

Date
5, 8 July 2013
2-3 July 2013
7-8 August 2013
14-15 August 2013
4 September 2013
5 September 2013
16 September 2013
26 September 2013
5 December 2013
11-12 December 2013
8 April 2014
16 April 2014
29 April 2014
7 May 2014
21 May 2014
28 May 2014
2 June 2014
4 June 2014
16 June 2014
17 June 2014
18-19 June 2014
24 June 2014

Appendix D: Attendance of ARRP members at site inspections 2013–14

Member	Number of days spent on site inspection
Professor Andrew Dart (Chair)	-
Dr Regina Fogarty (Deputy Chair)	2
Dr Magdoline Awad	4
Mr Peter Batten	-
Ms Celeste Black	2
Dr Mike Fleming	3
Professor Annemarie Hennessy	-
Ms Emma Hurst	5
Professor Anne Keogh AM	3
Professor Robert Mulley	3
Mr David O'Shannessy	4
Professor Jacqueline Phillips	2
Dr Peter Rolfe	4

Appendix E: Animal Research Review Panel Strategic Plan July 2011 – June 2014

* Numbers on the right refer to items from 2013/2014 Animal Research Review Panel Operational Plan that address the strategies.

Goals and Strategies	
Goal 1: **Effective and efficient implementation of the statutory requirements of the Animal Research Act 1985, the Animal Research Regulation 2010 and the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* .**	
1.1 Maintain a system to accredit and licence all establishments and individuals in NSW conducting research and teaching using animals.	1.1
1.2 Maintain a programme of site visits to effectively monitor compliance with the legislation.	2
1.3 Review the methods of conducting site visits and documentation of these methods on a regular basis to help ensure high standards of efficiency, effectiveness and consistency.	2.5
1.4 Identify and implement adjuncts to inspections to better ensure compliance with the legislation.	2.5 3
1.5 Monitor compliance with the Act, Regulation and Code of Practice with respect to the conduct of animal research and teaching and the supply of animals for research and teaching.	1 2
1.6 Active participation in national reviews of the Code of Practice to ensure that it is effective in regulating the conduct of animal research and teaching and the supply of animals for research and teaching.	5.1
1.7 Prepare an annual report to Parliament on the operations and achievements of the Animal Research Review Panel.	1.4
1.8 Maintain and review the system for collection and analysis of statistics on animal use for research and teaching, to ensure that it provides useful information which accurately reflects the use of animals, without imposing an undue administrative burden on institutions or Government.	1.5
1.9 Maintain a system for receiving and investigating complaints relating to the requirements of the legislation.	1.2
1.10 Provide opportunities to the research, teaching, veterinary, animal welfare and lay communities to provide feedback on the activities of the Animal Research Review Panel and respond appropriately.	2 3
1.11 Maintain a system to consider and make recommendations on applications for permission to carry out LD50 tests.	1.3
Goal 2: **The principles, processes and responsibilities in the *Australian Code of Practice for the Care and***	

Use of Animals for Scientific Purposes **are actively embraced by all involved wherever animals are used.**	
2.1 Promote an understanding of the roles and responsibilities of institutions in supporting the effective operation of their AECs.	2 3 4
2.2 Promote an understanding of the roles and responsibilities of institutions in actively pursing programmes for researchers and teachers that underpin their responsibilities under the Code of Practice.	2 3 4
2.3 Ensure there is effective participation by researchers and teachers, veterinarians, animal welfare representatives and independent representatives in a formal review of the justification and merit for all proposals for the use of animals for scientific purposes.	2 3
2.4 Promote and foster interaction between AECs and researchers/teachers.	2 3
2.5 Promote an appreciation of the ethos underpinning the Code of Practice through visits and all communications from the Animal Research Review Panel to institutions, AECs, researchers/teachers and animal care staff.	2 3 4
2.6 Promote an understanding of the roles and responsibilities of AECs through encouraging participation in AEC training programmes.	2 3 4
2.7 By identifying problems and suggesting remedies, provide assistance to institutions, AECs and researchers/teachers to ensure that the principles, processes and responsibilities in the Code of Practice are actively embraced.	2 3
2.8 Promote discussion and understanding of key technical and ethical issues and foster interaction between AECs by maintaining a programme of meetings of members and Executive Officers of AECs and participating in AEC meetings during site inspections.	2 3.4
2.9 Review the membership and operation of individual AECs to ensure they are operating effectively.	1.1 2
2.10 Develop and promulgate evidence-based guidelines to assist AECs, researchers and teachers to effectively implement the 3Rs.	4
2.11 Promote a critical review of the operation of AECs by the institution with a view to maximising their effectiveness.	2 4
Goal 3: **Researchers and teachers considering using animals are aware of and actively apply the principals set out in the Act, Regulation and the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*.**	
3.1 Promote an understanding of the roles and responsibilities of researchers/teachers through participation in education programmes, to foster an awareness of ethical and scientific issues and the implementation of the 3Rs.	3 4

3.2 Maintain the “Animal Ethics Infolink” website as a resource for AECs, researchers and teachers and members of the community.	3.1
Goal 4: **Methods that complement or replace animal use are used wherever possible.**	
4.1 Encourage AECs critically to assess the adequacy of researchers’/teachers’ attempts to identify alternatives to animal use.	2 3
4.2 Encourage greater awareness of the use of alternatives to animals in research and teaching.	2 3
4.3 Collate and disseminate information on alternatives to animal use.	3.1
4.4 Promote consideration of funding for development and validation of alternatives.	
Goal 5: **Procedures involving animals are regularly reviewed and refined to minimise the number of animals required and to reduce the impact on individual animals.**	
5.1 Encourage a critical review of the design of projects before applications are submitted to AECs.	2 3 4
5.2 Ensure close scrutiny by AECs of breeding programmes to minimise overproduction of animals.	2 3 4
5.3 Ensure close scrutiny by AECs of the competence of researchers to carry out specific procedures.	2 3 4
5.4 Promote the critical evaluation of the monitoring of animals being used in procedures.	2 3 4
5.5 Promote the critical evaluation by AECs and researchers of the impact of the type of housing / holding on experimental animals and awareness of its implications for experimental results.	2 3 4
Goal 6: **When animals are used in research and teaching, their well-being is promoted and there is the anticipation, prompt recognition and alleviation of pain and distress.**	
6.1 Promote the implementation of strategies which will foster the well-being of animals and which will foster the development of appropriate risk management assessments related to pain and distress in animals.	2 3 4

6.2 Ensure that AECs and researchers/teachers focus on the possible impact of procedures at the planning stage and implement appropriate strategies for monitoring and alleviation.	2 3 4
6.3 Promote awareness by researchers / teachers and animal care staff of signs of well-being, pain and distress in animals.	2 3 4
6.4 Promote the use of appropriate analgesia and anaesthesia by facilitating access by researchers/teachers to information resources.	2 3 4
6.5 Promote awareness of the effects of handling and other interactions with humans on levels of pain and distress and the use of strategies to minimise adverse impacts.	2 3 4
6.6 Monitor and identify deficiencies in anticipation, recognition and relief of pain and distress during site visits and ensure deficiencies are rectified, including by provision of pre-operative analgesia where appropriate.	2
Goal 7: **High standards of housing and routine care are established for animals used in research and teaching.**	
7.1 Evaluate housing and routine care through the ongoing site visit programme.	2
7.2 Develop and disseminate evidence based guidelines for housing and routine care.	4
7.3 Actively participate in the development and review of appropriate national and international standards for housing and routine care.	5.1
Goal 8: **Animals used are supplied in accord with the legislation**	
8.1 Identify areas of non-compliance through scrutiny of records during site visits and investigation of complaints.	1.2 2
8.2 Develop and disseminate appropriate educational material.	3 4
Goal 9: **The community (research, teaching, veterinary, animal welfare and lay) has access to information about animal use for research and teaching in NSW.**	
9.1 Provide information in the annual report on ARRP activities and achievements, areas of concern to the Animal Research Review Panel and statistics on animal use.	1.4 1.5
9.2 Identify options for disseminating information about specific issues of interest and concern both broadly and to specific groups (researchers, teachers, veterinarians, animal welfare, lay).	3 4

9.3 Review and maintain a web site for the dissemination of information.	3.1
9.4 Provide opportunities for and encourage the community (researchers, teachers, veterinarians, animal welfare, lay) to have an input into legislative review, development of standards for housing and care and policy development.	3 4
9.5 Ensure that information about animal use provided by the Animal Research Review Panel is in lay terms where appropriate.	
9.6 Encourage institutions to provide information about their animal use direct to the general community.	
Goal 10: **The approach to administration of animal research and teaching is harmonised between State and Territory regulatory and funding bodies.**	
10.1 Promote interaction between State and Territory regulatory and funding bodies.	

Appendix F: ARRP Operational Plan July 2013 – June 2014

Activity	Measure of Performance	Time Frame	Status
1. Mandatory			
1.1 Review incoming applications for accreditation and licence	Recommendation to Director-General	3 months (new) 2 months (renewal)	Applications processed and recommendations made to the Secretary
1.2 Investigate formal and informal complaints	Recommendation to Director-General	Interim or final recommendations within 3 months	2 complaints received. 1 complaint finalised.
1.3 Review incoming applications to conduct LD50 tests	Recommendations to Minister	3 months	All applications reviewed and recommendations sent to the Minister.
1.4 Prepare annual report for 2012-2013	Report submitted to Minister	December 2013	Report prepared.
1.5 Prepare statistics on animal use for 2012	Statistics collated	December 2013	Statistics collated.
2. Inspections			
2.1 Conduct site visits of accredited animal research establishments on a 3 – 4 yearly basis (for those establishments in-State, active and with own AEC)	Number of establishments inspected Number of days for inspections	Ongoing	27 28
2.2 Inspect new establishments applying for accreditation prior to or within 2 months of accreditation (for those establishments in-State, active and with own AEC)	Number of new establishments inspected	Ongoing	N/A
2.3 Review and send inspection reports	Reports sent	Within 3 months of inspection	Reports sent.
2.4 Follow up “problems” identified at inspection or on review of applications for accreditation or licence	Problems rectified	Within 12 months	Problems followed up as per “Site Inspection / Accreditation responses” section of ARRP agendas.
2.5 Assessment of 2012 AEC annual reports	Assessment carried out	September 2013	2012 reports assessed and feedback provided to establishments
3. Education			
3.1 Maintain ARRP website	Site maintained	Ongoing	Website maintained.
3.2 Develop training material for researchers/teachers via reference group	Reference group meetings held	To revisit once revised Code of Practice published.	To consider July 2014
3.3 Consider content of AEC learning package in light of researcher training material developed.	Content considered	After development of researcher training material.	To consider July 14
3.4 Hold Animal Ethics Seminar	Seminar held	October 2013	Seminar held 2 October 2013.
4. Policies and guidelines			
4.1 Develop policies/ guidelines where strong need identified (maximum of 2)	Developed as need identified. Develop guideline to assist AECs in assessing research value / justification.		None identified. Literature review carried out and AEC survey commenced.

4.3 Revise current policies and guidelines	Continue programme of revision. Finalise Policy 14 (restricted drugs) revision	Ongoing	Revised Policies 5, 5A , 6 (and 14). Finalised February 14
5. Additional			
5.1 Publication of the 8th edition of the Australian code for the care and use of animals for scientific purposes	Assess amendments required to legislation / policies/ guidelines.	December 13	Amendments to legislation, policies and guidelines commenced.
5.2 Continue liaison with NHMRC	Contact with NHMRC maintained	Ongoing	NHMRC presentation at Animal Ethics Seminar October 2013

Appendix G: Animal use statistics 2013

Note: Statistics on animal use are collected on a calendar-year basis.

The following graphs, one for each **purpose** (see table below) show the numbers of animals used against the category of **procedure** (1–9; see below). The categorisation of procedures aims to give some indication of the 'invasiveness' or 'impact' of the work on the animals involved. **Species** are grouped as indicated below.

Some animals (e.g. those used to teach animal-handling techniques) are used in a number of projects. Animals that are re-used are counted in each project for which they are used. In welfare terms, this gives a more meaningful indication of animal use.

The system includes the collection of statistics on the observation of free-living animals. This causes a large number of animals to be recorded in procedure category 1 ('observation involving minor interference'). For example, an aerial survey of birds can include many thousands of individual animals.

After the graphs, statistics are given on the lethality testing performed in 2013.

Animal species categories used for collection of data

Laboratory mammals	Mice
	Rats
	Guinea Pigs
	Rabbits
	Hamsters
	Ferrets
	Other laboratory mammals (not primates)
Domestic mammals	Sheep
	Cattle
	Pigs
	Horses
	Goats
	Deer
	Cats
	Dogs
	Other domestic mammals
Birds	Poultry
	Exotic Captive
	Exotic Wild
	Native Captive
	Native Wild
	Other birds
Aquatic animals	Fish
	Cephalopods (reporting not mandatory)
	Crustaceans (reporting not mandatory)
Amphibians	Amphibians
Reptiles	Lizards
	Snakes
	Turtles and Tortoises
	Other reptiles

Primates	Marmosets
	Macaques
	Baboons
	Other primates
Native mammals	Macropods
	Possums and gliders
	Native rats and mice
	Dasyurids
	Wombats
	Koalas
	Monotremes
	Bandicoots
	Bats
	Other native mammals
	Seals
	Whales and dolphins
Exotic feral mammals	Camels
	Cats
	Cattle
	Goats
	Hares
	Horses
	Mice
	Pigs
	Rabbits
	Rats
	Dingo/Wild Dogs
	Foxes
	Other exotic feral mammals
Exotic zoo animals	Exotic zoo animals

PURPOSE
1. Stock breeding Breeding protocols to produce new teaching or research stock. Include the animals used to produce progeny and any breeders or progeny culled in the process, NOT the final progeny themselves (as these will be counted under the protocol in which they go on to be used).
2. Stock maintenance Holding protocols for animals maintained for use in other protocols. These animals may be maintained under an ethics authority because they require special management. If they are not held under an authority (e.g. normal stock animals kept mainly for commercial production, but occasionally used in research), then they are counted in the protocol only where they are used for teaching/research. *Examples:* *Fistulated ruminants that are maintained under a holding protocol for use in other short-term feeding trial protocols* *A non-breeding colony of diabetic rats held for research in other protocols*
3. Education Protocols carried out for the achievement of educational objectives. The purpose of the protocol is not to acquire new knowledge but to pass on established knowledge to others. This would include interactive or demonstration classes in methods of animal husbandry, management, examination and treatment. *Examples* *Animals used by veterinary schools to teach examination procedures such as pregnancy diagnosis*
4. Research: human or animal biology Research protocols that aim to increase the basic understanding of the structure, function and behaviour of animals, including humans, and processes involved in physiology, biochemistry and pathology.
5. Research: human or animal health and welfare Research protocols that aim to produce improvements in the health and welfare of animals, including humans.
6. Research: animal management or production Research protocols that aim to produce improvements in domestic or captive animal management or production.
7. Research: environmental study Research protocols that aim to increase the understanding of the animals' environment or its role in it, or aim to manage wild or feral populations. These will include studies to determine population levels and diversity and may involve techniques such as observation, radio-tracking, or capture and release. *Examples* *Pre-logging or pre-development fauna surveys*
8. Production of biological products Using animals to produce products other than e.g. milk, meat, eggs, leather or fur. *Examples* *Use of a sheep flock to donate blood to produce microbiological media* *Production of commercial antiserum* *Production of products, such as hormones or drugs, in milk or eggs from genetically modified animals* *Quality Assurance testing of drugs*
9. Diagnostic procedures Using animals directly as part of a diagnostic process. *Examples* *Inoculation of day-old chicks with Newcastle Disease virus to determine virulence* *Blue-green algae toxicity testing* *Water supply testing using fish*
10. Regulatory product testing Protocols for the testing of products required by regulatory authorities, such as the APVMA. **If the product testing is not a regulatory requirement (e.g. if it is part of a Quality Assurance system only), those animals should be included in the appropriate Purpose category selected from above.** (This would normally be Purpose Category 8 in the case of QA testing.) *Examples* *Pre-registration efficacy or toxicity testing of drugs and vaccines*

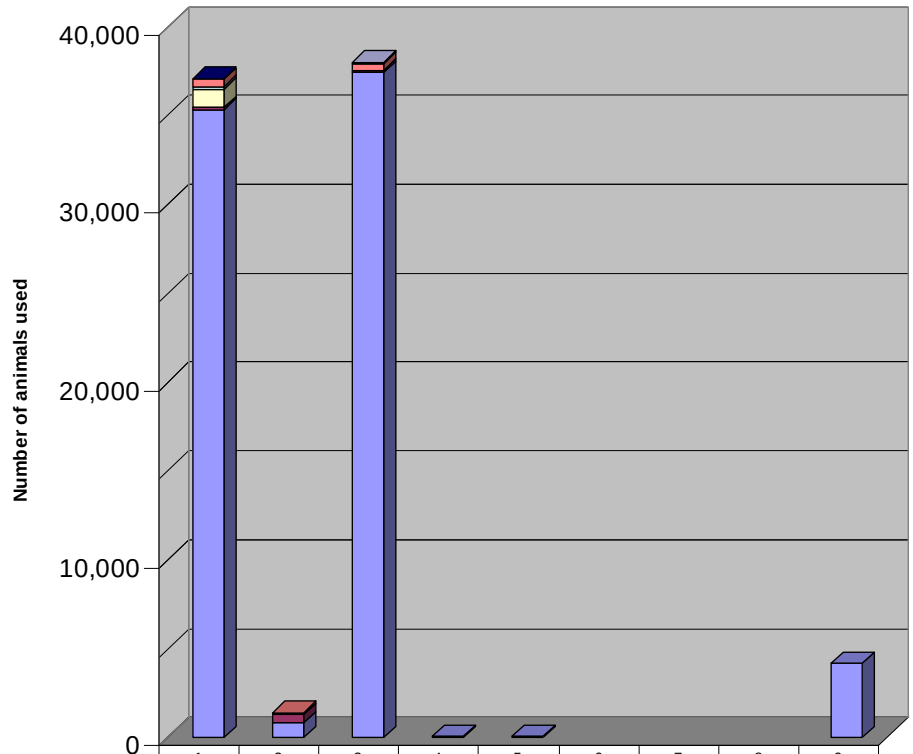
Data collection: procedure categories and guidelines used for classification

1: Observation involving minor interference	**6: Minor physiological challenge**
Animals are not interacted with, or, where there is interaction, it would not be expected to compromise the animal's welfare any more than normal handling, feeding, etc. There is no pain or suffering involved.	Animal remains conscious for some, or all, of the procedure. There is interference with the animal's physiological or psychological processes. The challenge may cause only a small degree of pain/distress, or any pain/distress is quickly and effectively alleviated.
2: Animal unconscious without recovery	**7: Major physiological challenge**
Animal is rendered unconscious under controlled circumstances (i.e. not in a field situation) with as little pain or distress as possible. Capture methods are not required. Any pain is minor and brief and does not require analgesia. Procedures are carried out on the unconscious animal, which is then killed without regaining consciousness.	Animal remains conscious for some, or all, of the procedure. There is interference with the animal's physiological or psychological processes. The challenge causes a moderate or large degree of pain/distress that is not quickly or effectively alleviated.
3: Minor conscious intervention	**8: Death as an endpoint**
Animal is subjected to minor procedures that would normally not require anaesthesia or analgesia. Any pain is minor and analgesia usually unnecessary, although some distress may occur as a result of trapping or handling.	This category applies only in those rare cases where the death of the animal is a planned part of the procedures. Where predictive signs of death have been determined and euthanasia is carried out before significant suffering occurs, the procedure may be placed in category 6 or 7.
4: Minor surgery with recovery	**9: Production of genetically modified (GM) animals**
Animal is rendered unconscious with as little pain or distress as possible. A minor procedure such as cannulation or skin biopsy is carried out and the animal allowed to recover. Depending on the procedure, pain may be minor or moderate and postoperative analgesia may be appropriate. Field capture by using chemical restraint methods is also included here.	This category is intended to allow for the variety of procedures that occur during the production of genetically modified animals. As animals in this category may be subjected to both minor and major physiological challenges and surgical procedures, this category reflects the varied nature of the procedures carried out. It effectively includes **all** animals used in GM production, other than the final progeny, which are used in a different category of procedure.
5: Major surgery with recovery	
Animal is rendered unconscious with as little pain or distress as possible. A major procedure such as abdominal or orthopaedic surgery is carried out and the animal allowed to recover. Postoperative pain is usually considerable and at a level requiring analgesia.	

The following graphs (one for each purpose) show the numbers of animals used against the category of procedure (Categories 1 to 9).

Purpose: Stock Breeding

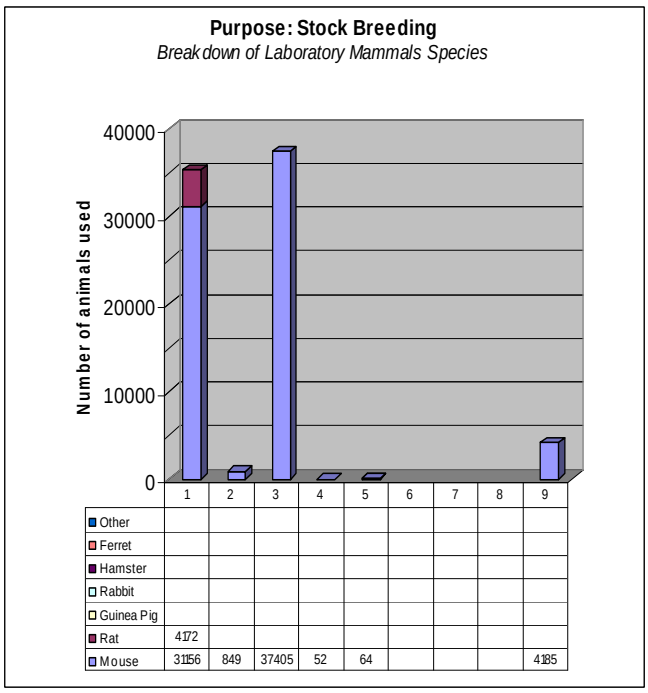
Breeding protocols to produce new teaching or research stock.
Only includes the animals used to produce progeny, NOT the final progeny.

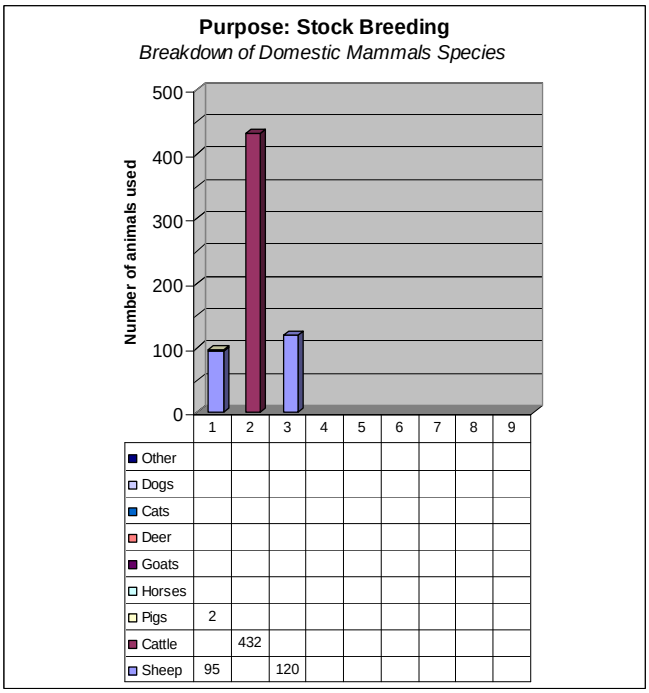


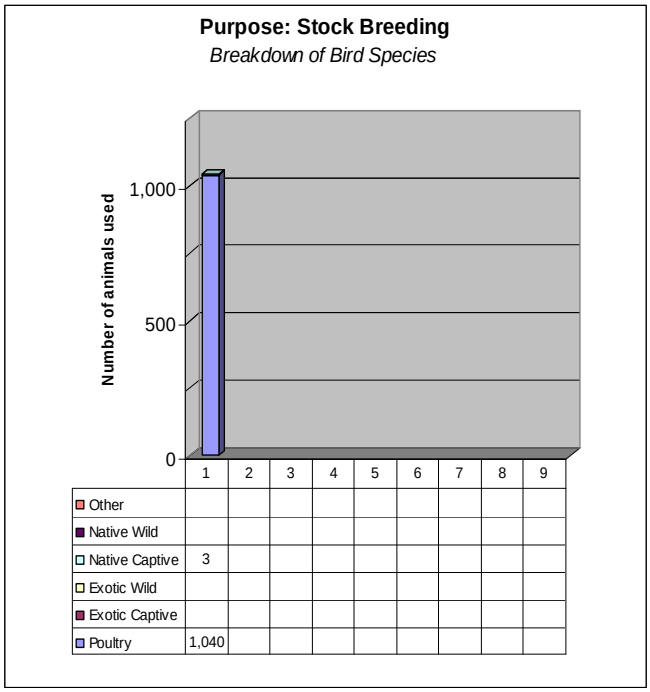
	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral	1								
Nat Mam	57		57						
Primates									
Reptiles	390	125	399						
Amphibians									
Aquatic	170								
Birds	1043								
Domestic	97	432	120						
Lab Mam	35328	849	37405	52	64				4185

Procedure

Refer to following page for a further breakdown of species.

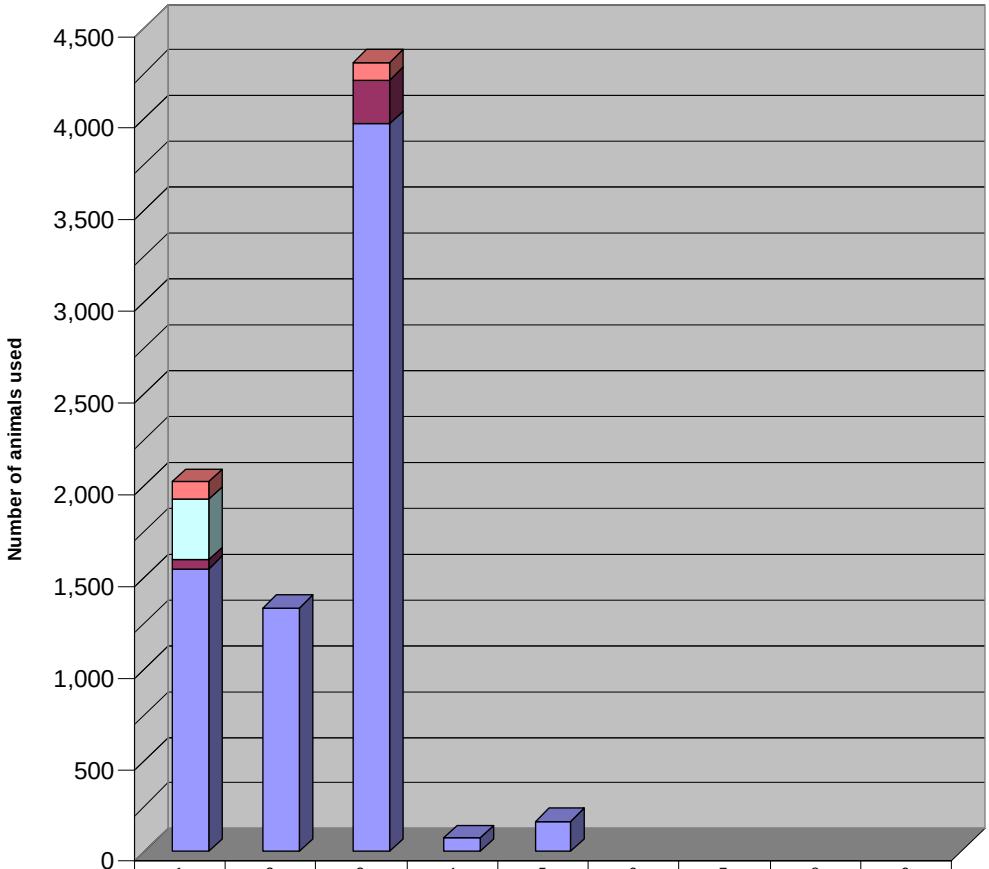

Purpose: Stock Breeding
Breakdown of Laboratory Mammals Species
Number of animals used
40000
30000
20000
10000
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit
Guinea Pig
Rat 4172
Mouse 31156 849 37405 52 64 4185


Purpose: Stock Breeding
Breakdown of Domestic Mammals Species
Number of animals used
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9
Other
Dogs
Cats
Deer
Goats
Horses
Pigs 2
Cattle 432
Sheep 95 120


Purpose: Stock Breeding
Breakdown of Bird Species
Number of animals used
1,000
500
0
1 2 3 4 5 6 7 8 9
Other
Native Wild
Native Captive 3
Exotic Wild
Exotic Captive
Poultry 1,040

Purpose: Stock Maintenance

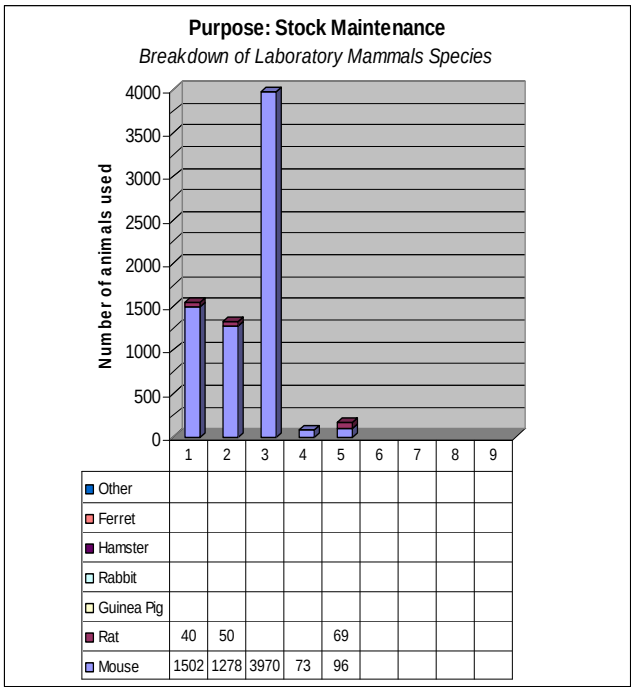
Holding Protocols for animals maintained for use in other protocols .

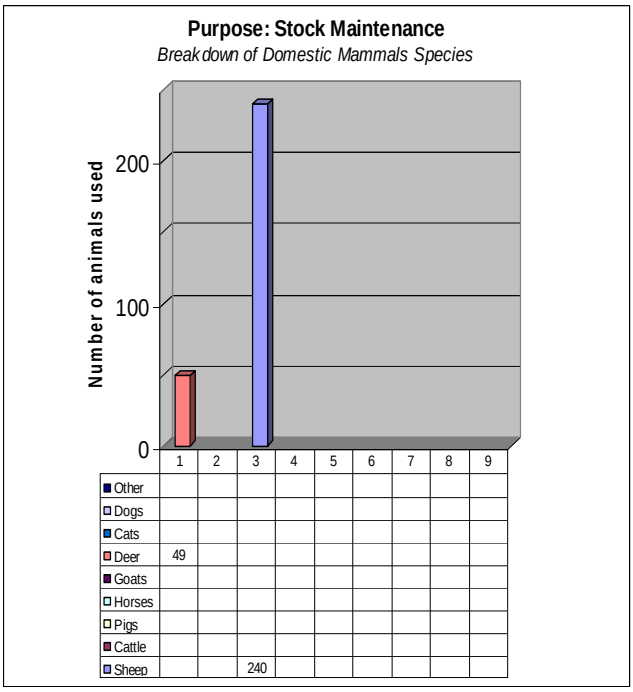


	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral									
Nat Mam									
Primates									
Reptiles	96		96						
Amphibians									
Aquatic	331								
Birds									
Domestic	49		240						
Lab Mam	1542	1328	3970	73	165				

Procedure

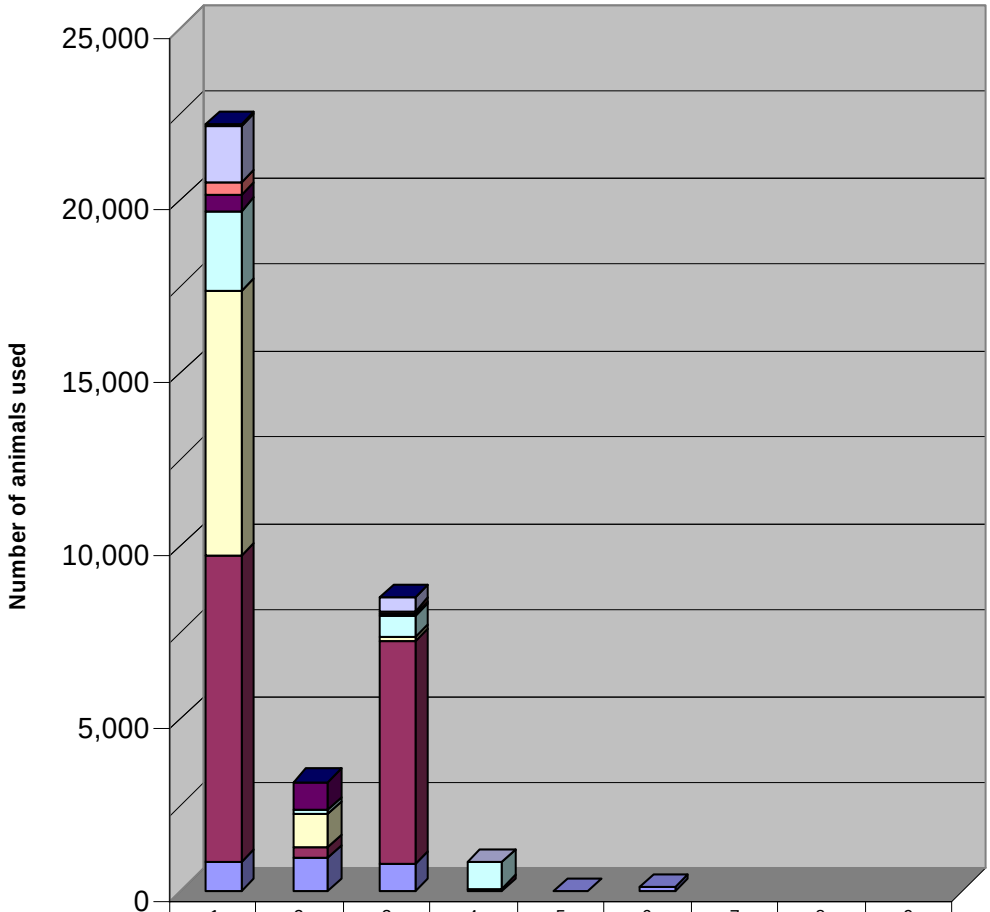
Refer to following page for a further breakdown of species.


Purpose: Stock Maintenance
Breakdown of Laboratory Mammals Species
Number of animals used
4000
3500
3000
2500
2000
1500
1000
500
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit
Guinea Pig
Rat 40 50 69
Mouse 1502 1278 3970 73 96


Purpose: Stock Maintenance
Breakdown of Domestic Mammals Species
Number of animals used
200
100
0
1 2 3 4 5 6 7 8 9
Other
Dogs
Cats
Deer 49
Goats
Horses
Pigs
Cattle
Sheep 240

Purpose: Education

Protocols carried out for the achievement of educational objectives, including interactive or demonstration classes in methods of animal husbandry, management, examination and treatment.

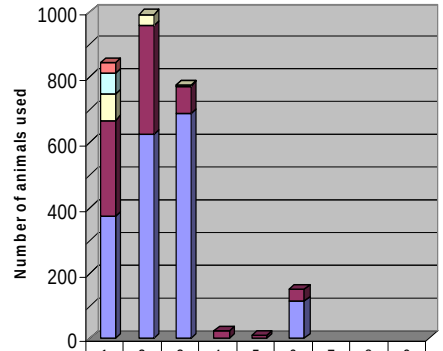


	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral	77	2	5						
Nat Mam	1,622	10	392	7					
Primates									
Reptiles	344		91						
Amphibians	470	800	38	10					
Aquatic	2,338	111	640	747					
Birds	7,630	998	120						
Domestic	8,895	272	6,455	85					
Lab Mam	846	993	778	25	9	150			

Refer to following page for a further breakdown of species.

Purpose: Education

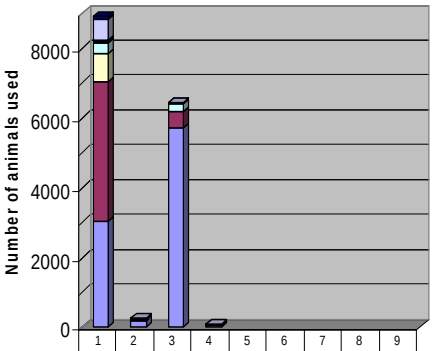
Breakdown of Laboratory Mammals Species



	1	2	3	4	5	6	7	8	9
Other									
Ferret	34								
Hamster									
Rabbit	65								
Guinea Pig	78	35	4						
Rat	293	332	85	25	9	36			
Mouse	376	626	689			114			

Purpose: Education

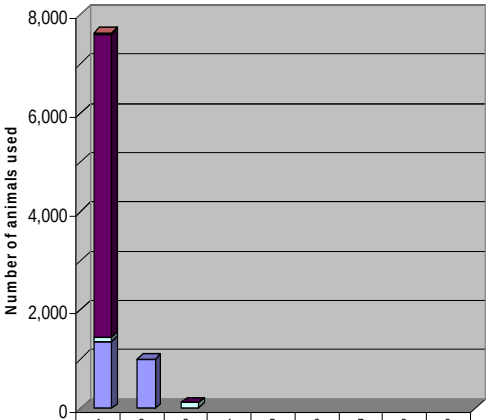
Breakdown of Domestic Mammals Species



	1	2	3	4	5	6	7	8	9
Other	54								
Dogs	613	46	35	19					
Cats	47		23						
Deer									
Goats	26	4							
Horses	299	11	202						
Pigs	832	18	5						
Cattle	3991	1	484	12					
Sheep	3033	192	5706	54					

Purpose: Education

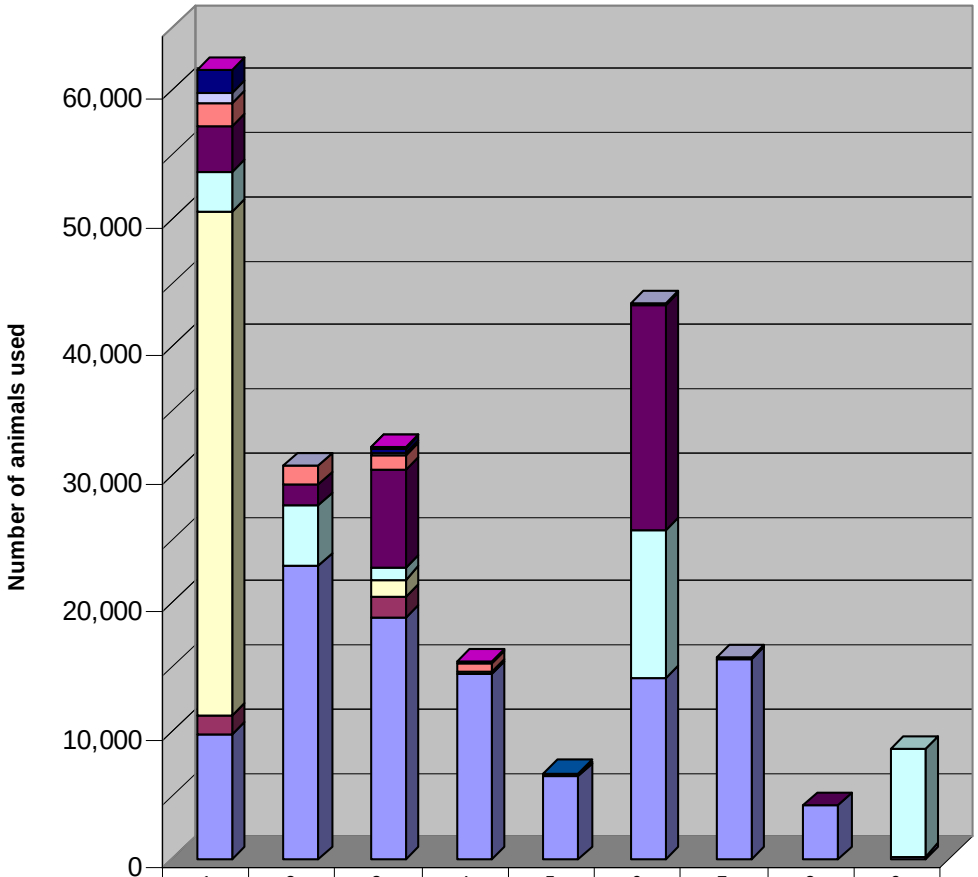
Breakdown of Bird Species



	1	2	3	4	5	6	7	8	9
Other	33								
Native Wild	6,136		5						
Native Captive	99		113						
Exotic Wild									
Exotic Captive	2								
Poultry	1360	998	2						

Purpose: Research - Human or Animal Biology

Research protocols which aim to increase the basic understanding of the structure, function and behaviour of animals, including humans, and processes involoved in physiology, biochemistry and pathology.



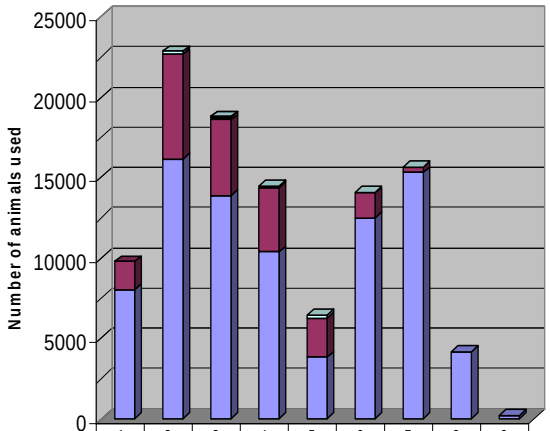
	1	2	3	4	5	6	7	8	9
Exotic Zoo	11		9	13					
Exotic Feral	1772		336	7					
Nat Mam	806	77	175	61		32	68		
Primates				11	3				
Reptiles	1875	1391	1177	747		214			
Amphibians	3582	1730	7659		34	17531	3	6	
Aquatic	3006	4690	901	39		11560			8311
Birds	39353	23	1399	14	20				
Domestic	1444	27	1673	34	223				
Lab Mam	9779	22840	18780	14484	6449	14087	15647	4195	233

Procedure

Refer to following page for a further breakdown of species.

Purpose: Research - Human or Animal Biology

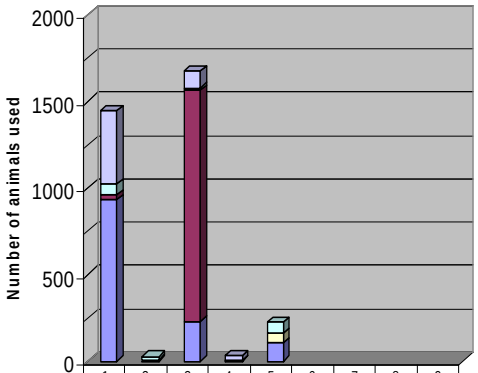
Breakdown of Laboratory Mammals Species



	1	2	3	4	5	6	7	8	9
Other									
Ferret									
Hamster									
Rabbit		127	30	88	183	40	6		
Guinea Pig		83	161	32					
Rat	1739	6460	4709	3,984	2,430	1517	321		
Mouse	8040	16170	13880	10380	3836	12530	15320	4195	233

Purpose: Research - Human or Animal Biology

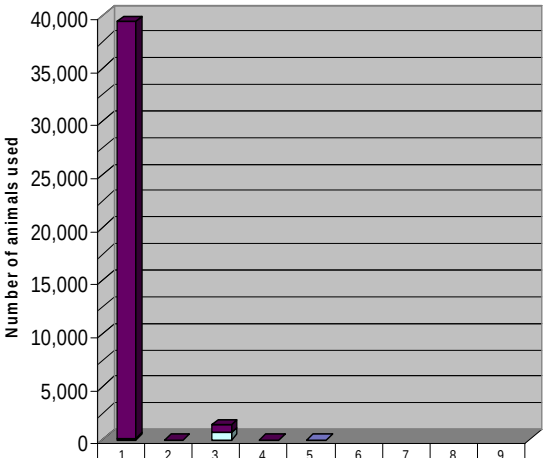
Breakdown of Domestic Mammals Species



	1	2	3	4	5	6	7	8	9
Other									
Dogs	422		94	32					
Cats									
Deer									
Goats									
Horses	66	18	11		66				
Pigs				2	51				
Cattle	23		1340						
Sheep	933	9	228		106				

Purpose: Research - Human or Animal Biology

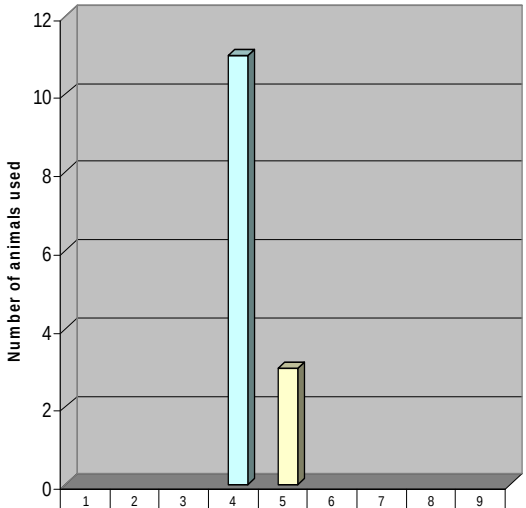
Breakdown of Bird Species



	1	2	3	4	5	6	7	8	9
Other									
Native Wild	39,243	23	772	9					
Native Captive	38		618	4					
Exotic Wild	64		9	1					
Exotic Captive									
Poultry	8				20				

Purpose: Research - Human or Animal Biology

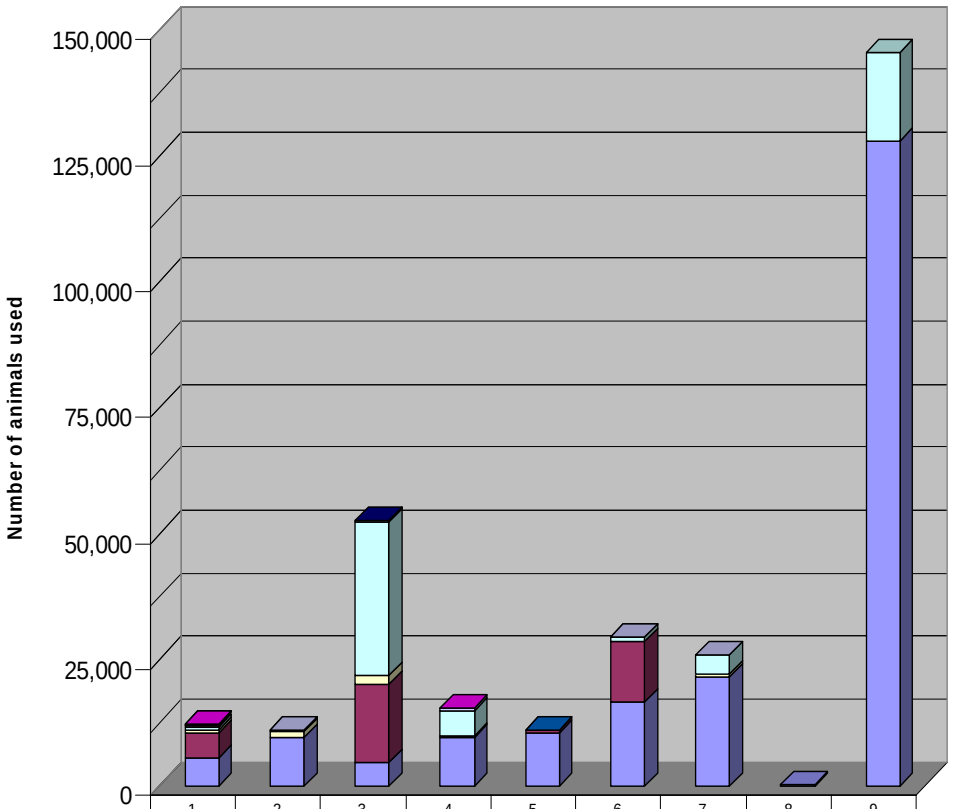
Breakdown of Primate Species



	1	2	3	4	5	6	7	8	9
Other				11					
Baboons					3				
Macaques									
Marmosets									

Purpose: Research - Human or Animal Health & Welfare

Research protocols which aim to produce improvements in the health and welfare of animals, including humans .



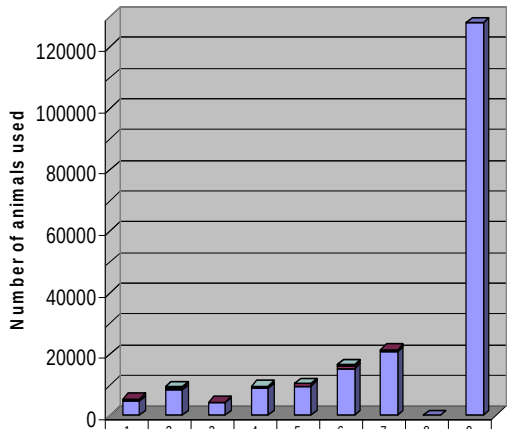
	1	2	3	4	5	6	7	8	9
Exotic Zoo	27			12					
Exotic Feral	19		31						
Nat Mam	295	1	65	633		2	72		
Primates			4	1	3				
Reptiles	39		1						
Amphibians		10			49				
Aquatic	620	410	30593	4978		796	3838		17606
Birds	611	1091	1781	4			540		
Domestic	5100	89	15489	336	431	11970			
Lab Mam	5383	9459	4537	9526	10468	16598	21542	147	127843

Procedure

Refer to following page for a further breakdown of species.

Purpose: Research - Human or Animal Health & Welfare

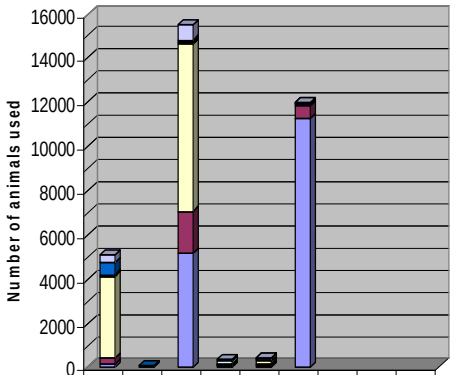
Breakdown of Laboratory Mammals Species



	1	2	3	4	5	6	7	8	9
Other									
Ferret									
Hamster									
Rabbit		11		16	26	10			
Guinea Pig		270		202	66	112			
Rat	640	781	245	368	690	1076	768		
Mouse	4743	8397	4292	8940	9,686	15400	20774	147	127843

Purpose: Research - Human or Animal Health & Welfare

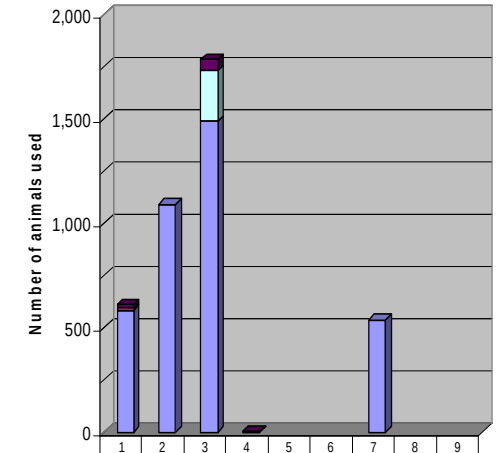
Breakdown of Domestic Mammals Species



	1	2	3	4	5	6	7	8	9
Other									
Dogs	363		670	77	34	27			
Cats	542	6	112						
Deer									
Goats									
Horses	66		90	136	95	54			
Pigs	3716	28	7547	12	130	56			
Cattle	258		1924	12	96	540			
Sheep	155	55	5146	99	76	11293			

Purpose: Research - Human or Animal Health & Welfare

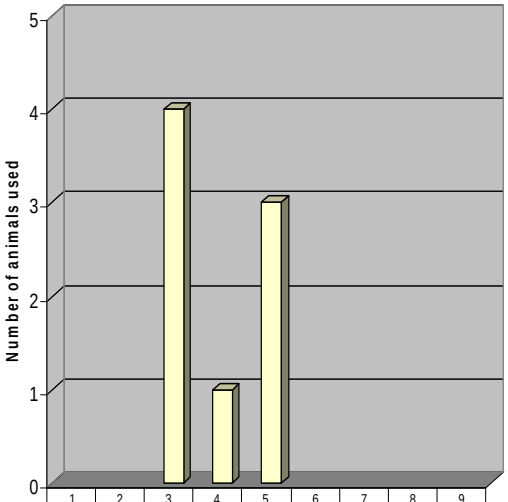
Breakdown of Bird Species



	1	2	3	4	5	6	7	8	9
Other									
Native Wild	15		49	2					
Native Captive			242	2					
Exotic Wild									
Exotic Captive	11								
Poultry	585	1091	1490				540		

Purpose: Research - Human or Animal Health & Welfare

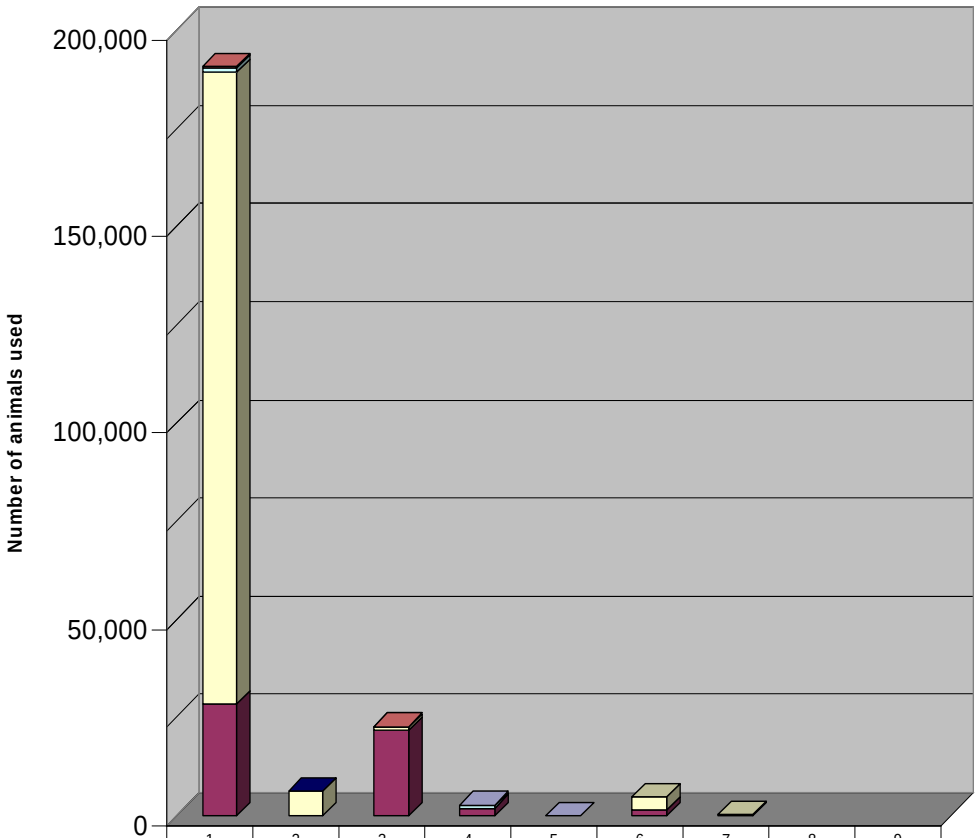
Breakdown of Primate Species



	1	2	3	4	5	6	7	8	9
Other									
Baboons			4	1	3				
Macaques									
Marmosets									

Purpose: Research - Animal Management or Production

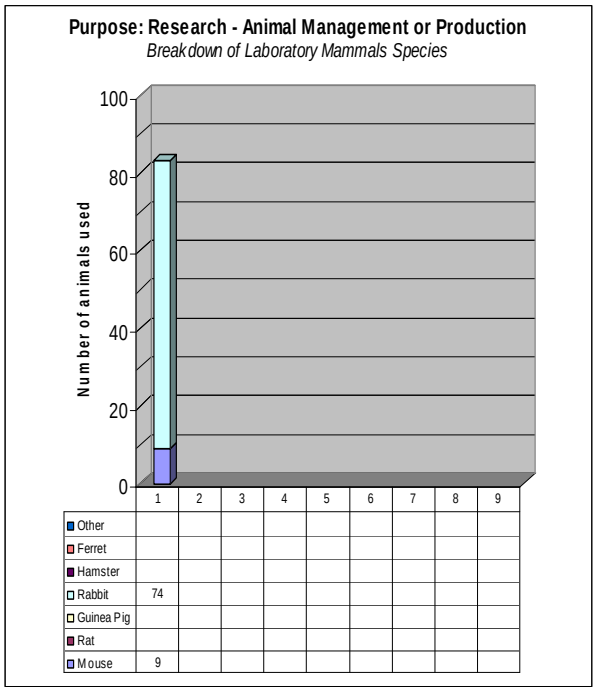
Research protocols which aim to produce improvements in domestic or captive animal management or production .

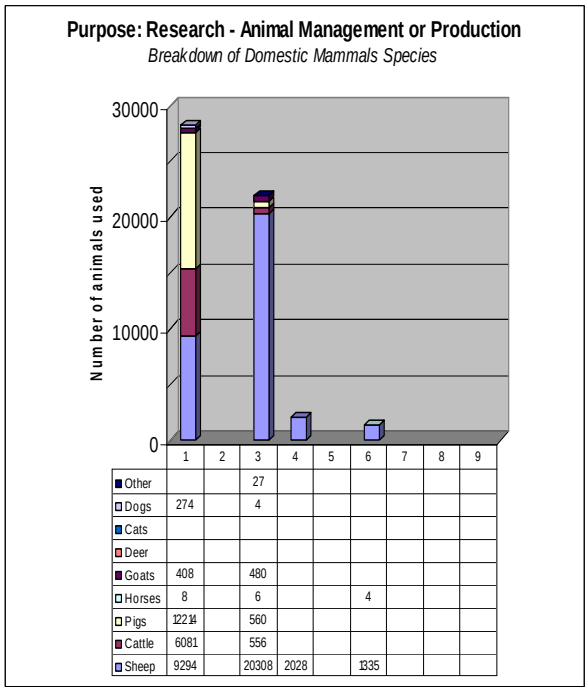


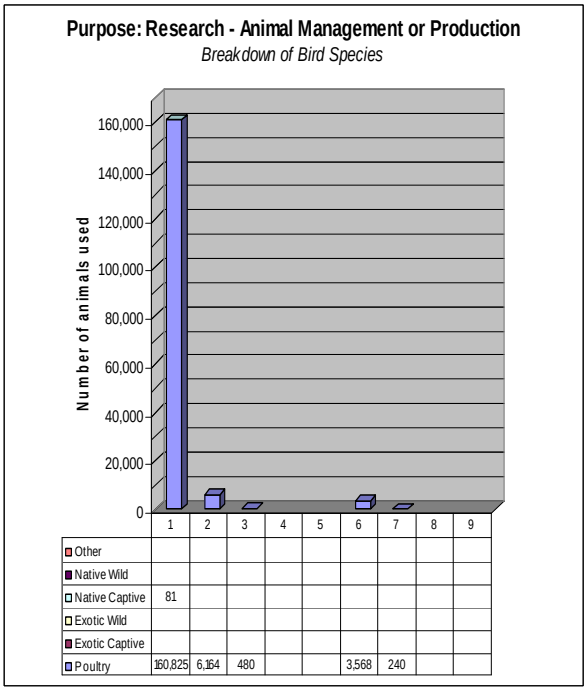
	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral		97							
Nat Mam				22	10				
Primates									
Reptiles	540		1						
Amphibians									
Aquatic	1036		314	721					
Birds	160906	6164	480			3568	240		
Domestic	28279		21941	2028		1339			
Lab Mam	83								

Procedure

Refer to following page for a further breakdown of species.

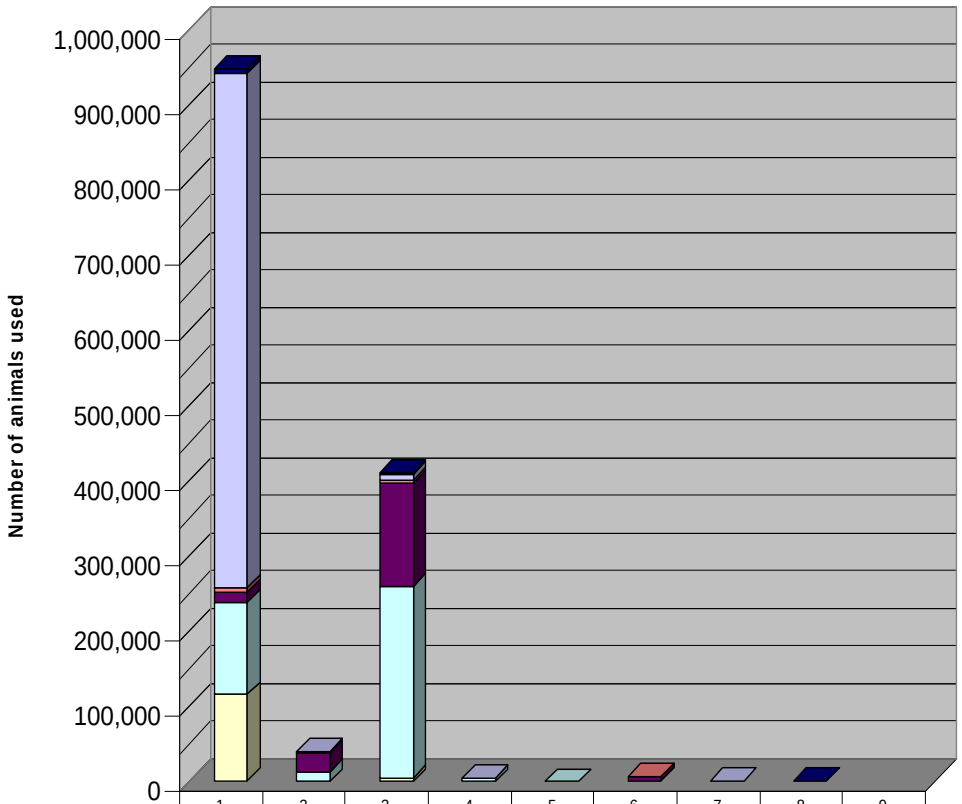

Purpose: Research - Animal Management or Production
Breakdown of Laboratory Mammals Species
Number of animals used
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit 74
Guinea Pig
Rat
Mouse 9


Purpose: Research - Animal Management or Production
Breakdown of Domestic Mammals Species
Number of animals used
30000
20000
10000
0
1 2 3 4 5 6 7 8 9
Other 27
Dogs 274 4
Cats
Deer
Goats 408 480
Horses 8 6 4
Pigs 12214 560
Cattle 6081 556
Sheep 9294 20308 2028 1335


Purpose: Research - Animal Management or Production
Breakdown of Bird Species
Number of animals used
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
1 2 3 4 5 6 7 8 9
Other
Native Wild
Native Captive 81
Exotic Wild
Exotic Captive
Poultry 160,825 6,164 480 3,568 240

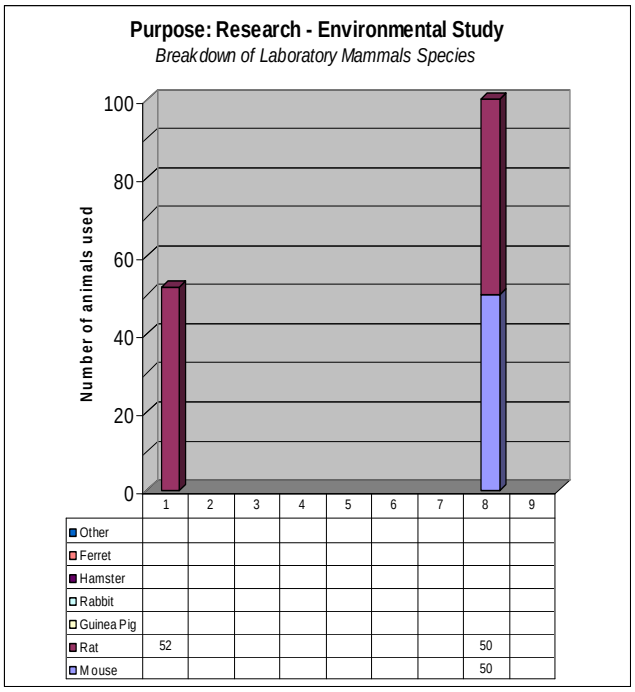
Purpose: Research - Environmental Study

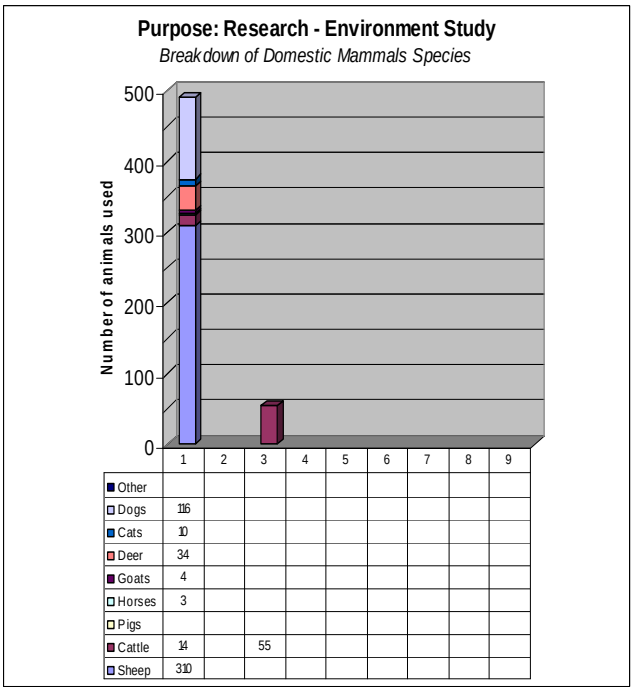
Research protocols which aim to increase the understanding of the animals' environment or its role in it, or that aim to manage wild or feral populations .

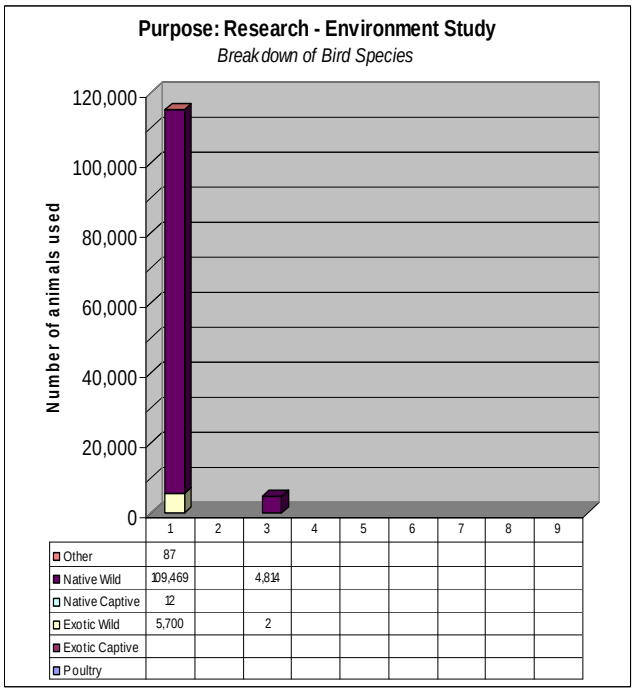


	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral	5627		1333					104	
Nat Mam	685399	453	7276	132			40		
Primates									
Reptiles	5295	488	4213			55	97		
Amphibians	14501	25573	137220			5400			
Aquatic	121761	13304	255371	5,270	53	1068		231	
Birds	115268		4816						
Domestic	491		55						
Lab Mam	52							100	

Refer to following page for a further breakdown of species.

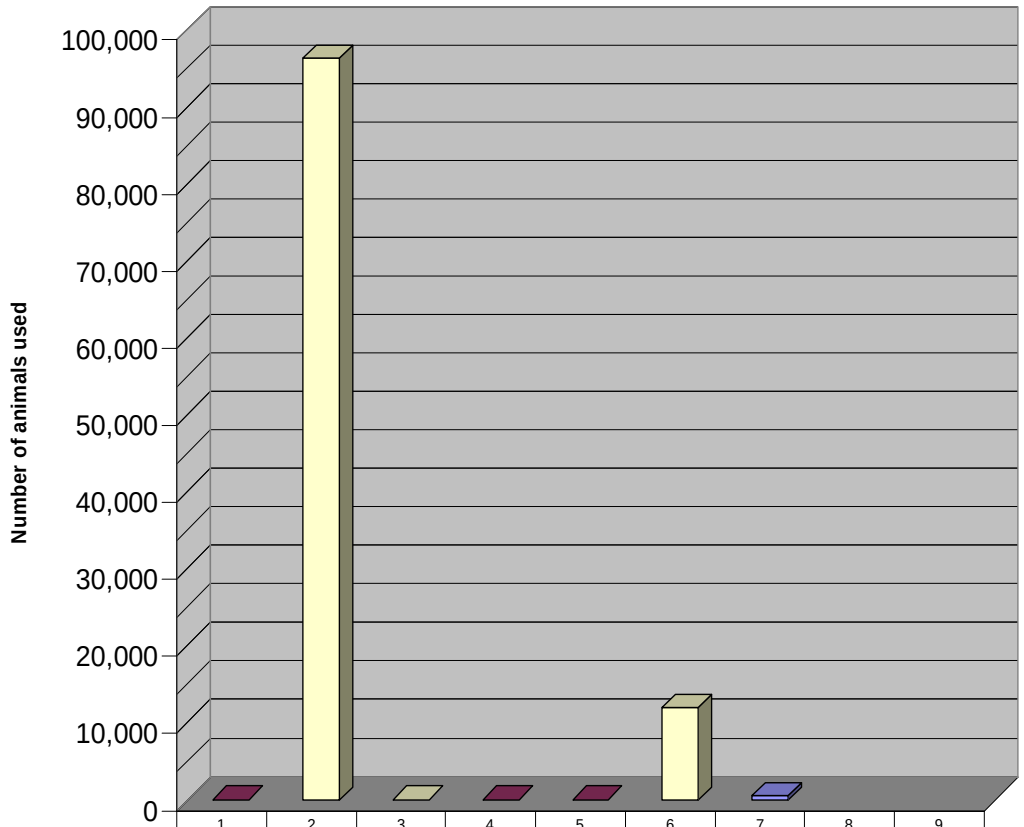

Purpose: Research - Environmental Study
Breakdown of Laboratory Mammals Species
Number of animals used
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit
Guinea Pig
Rat 52 50
Mouse 50


Purpose: Research - Environment Study
Breakdown of Domestic Mammals Species
Number of animals used
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9
Other
Dogs 116
Cats 10
Deer 34
Goats 4
Horses 3
Pigs
Cattle 14 55
Sheep 310


Purpose: Research - Environment Study
Breakdown of Bird Species
Number of animals used
120,000
100,000
80,000
60,000
40,000
20,000
0
1 2 3 4 5 6 7 8 9
Other 87
Native Wild 109,469 4,814
Native Captive 12
Exotic Wild 5,700 2
Exotic Captive
Poultry

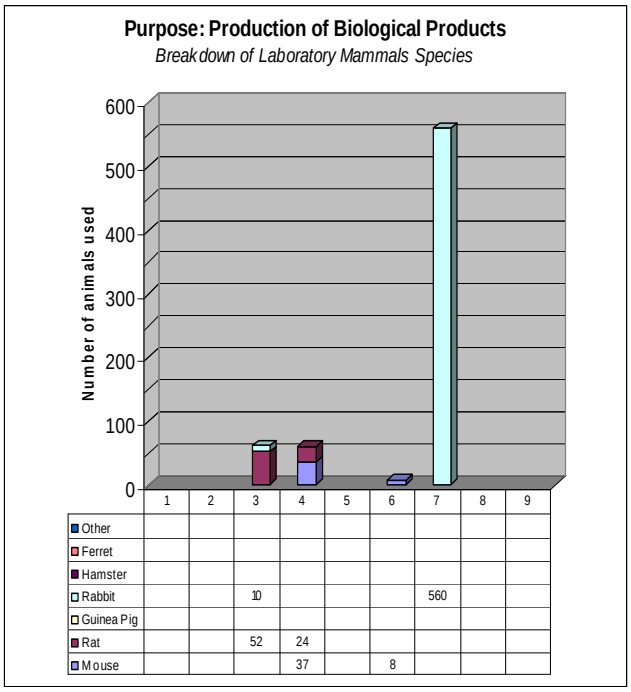
Purpose: Production of Biological Products

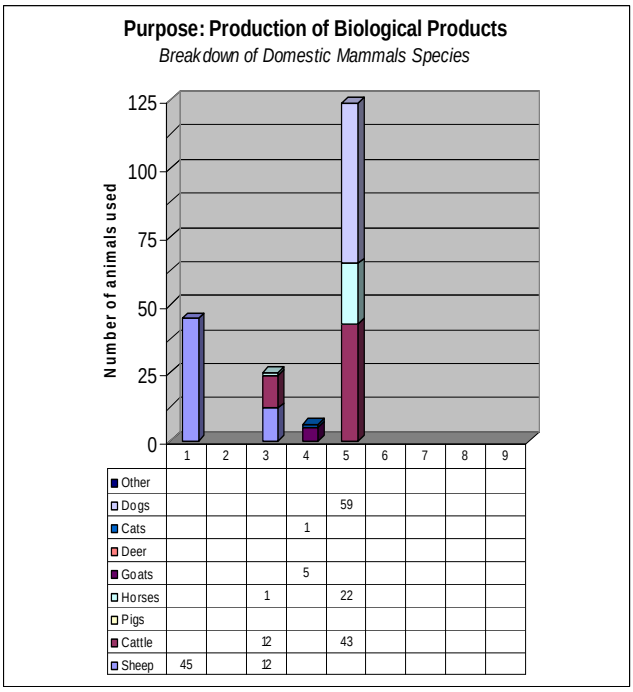
Use of animals to produce products (other than normal milk/meat/egg, etc).

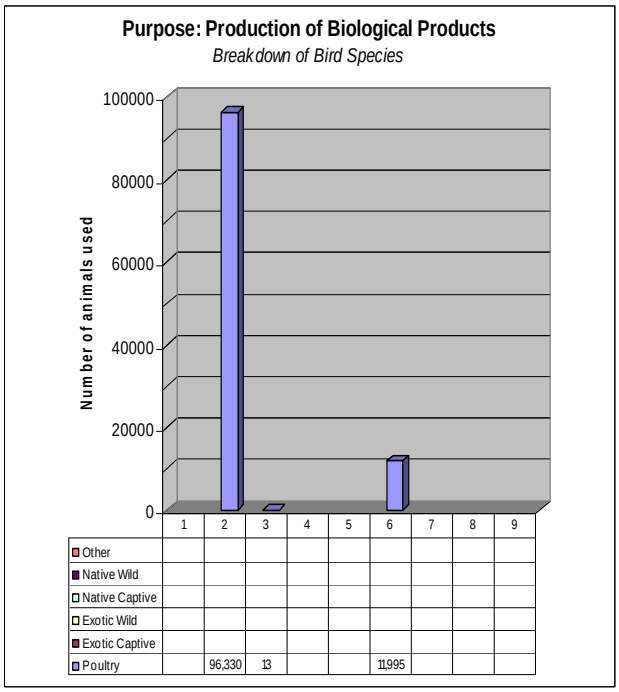


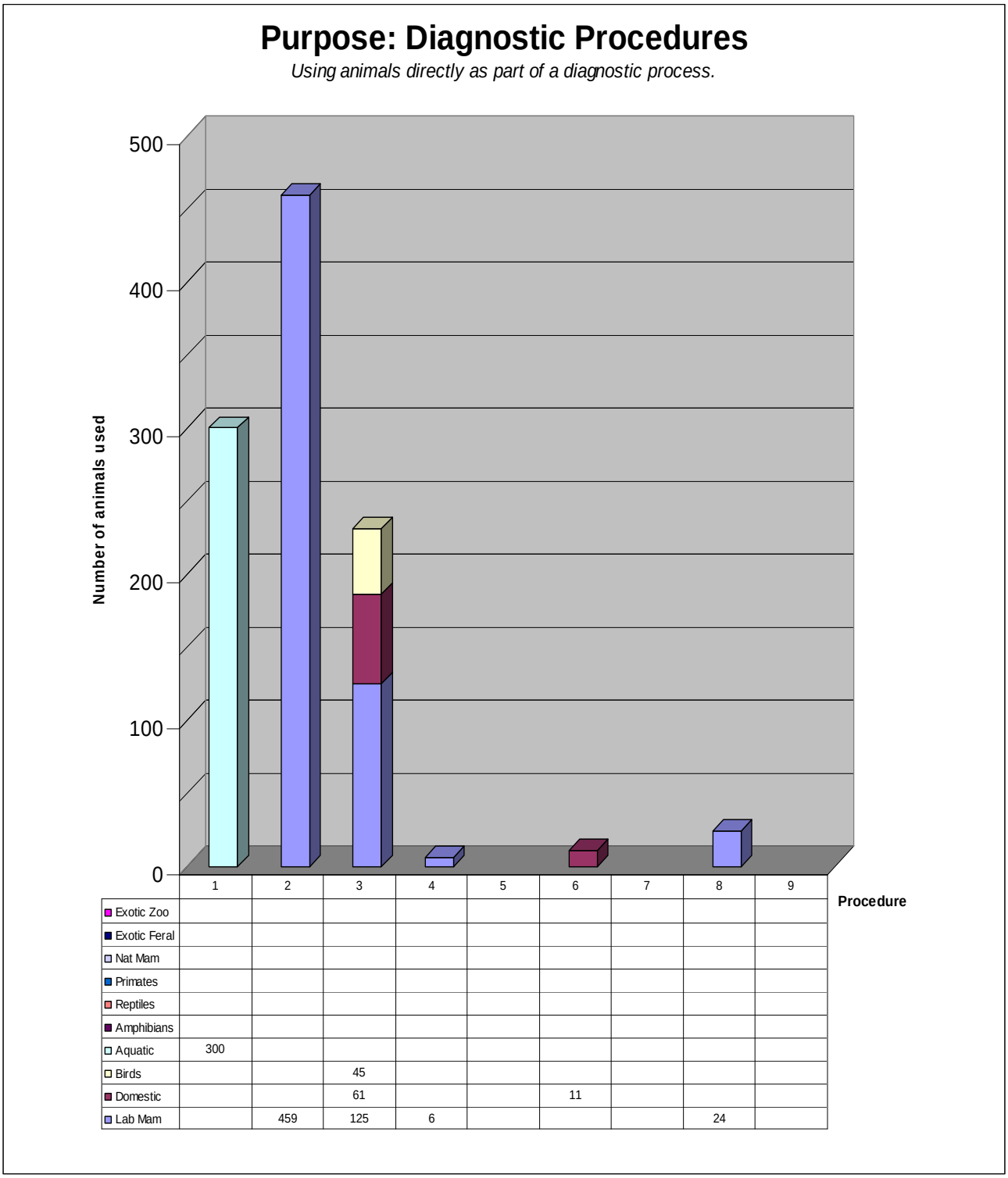
	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral									
Nat Mam									
Primates									
Reptiles									
Amphibians									
Aquatic									
Birds		96,330	13			11995			
Domestic	45		25	6	124				
Lab Mam			62	61		8	560		

Refer to following page for a further breakdown of species.

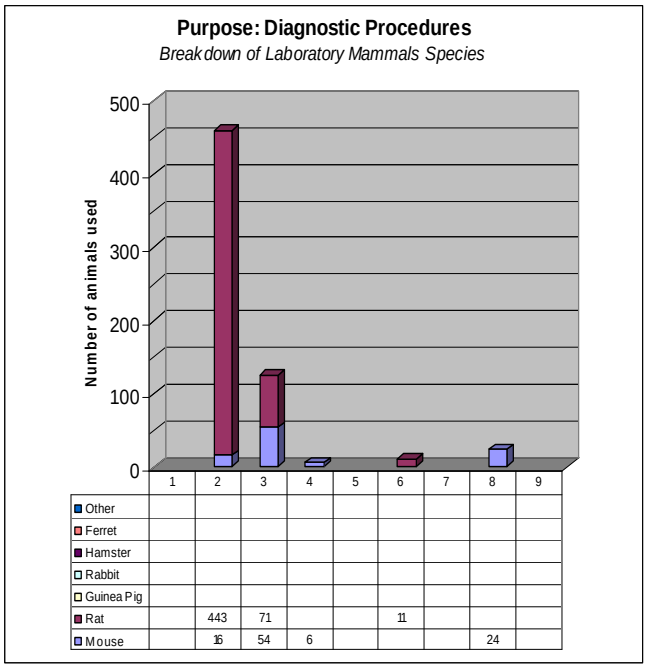

Purpose: Production of Biological Products
Breakdown of Laboratory Mammals Species
Number of animals used
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit 10 560
Guinea Pig
Rat 52 24
Mouse 37 8

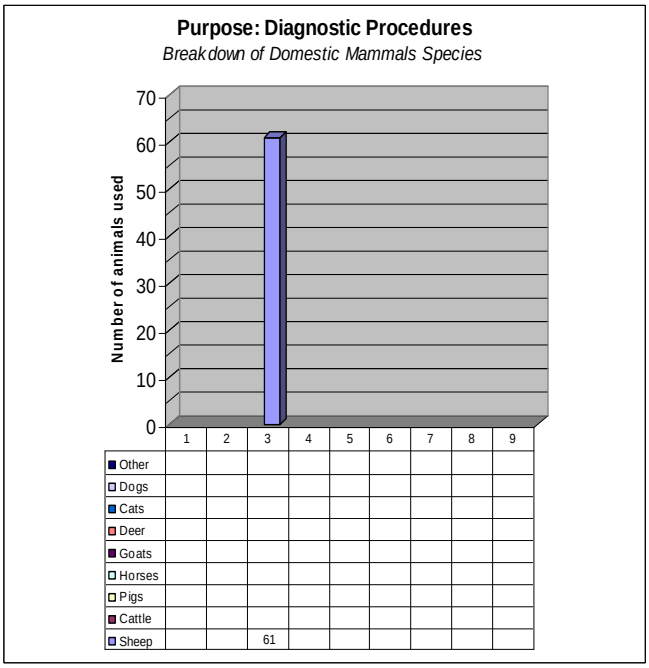

Purpose: Production of Biological Products
Breakdown of Domestic Mammals Species
Number of animals used
125
100
75
50
25
0
1 2 3 4 5 6 7 8 9
Other
Dogs 59
Cats 1
Deer
Goats 5
Horses 1 22
Pigs
Cattle 12 43
Sheep 45 12

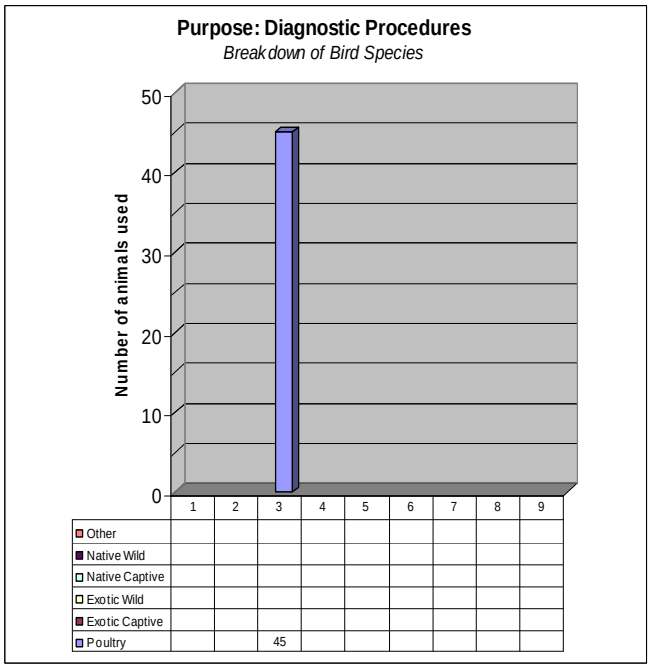

Purpose: Production of Biological Products
Breakdown of Bird Species
Number of animals used
100000
80000
60000
40000
20000
0
1 2 3 4 5 6 7 8 9
Other
Native Wild
Native Captive
Exotic Wild
Exotic Captive
Poultry 96,330 13 11,995



Refer to following page for a further breakdown of species.

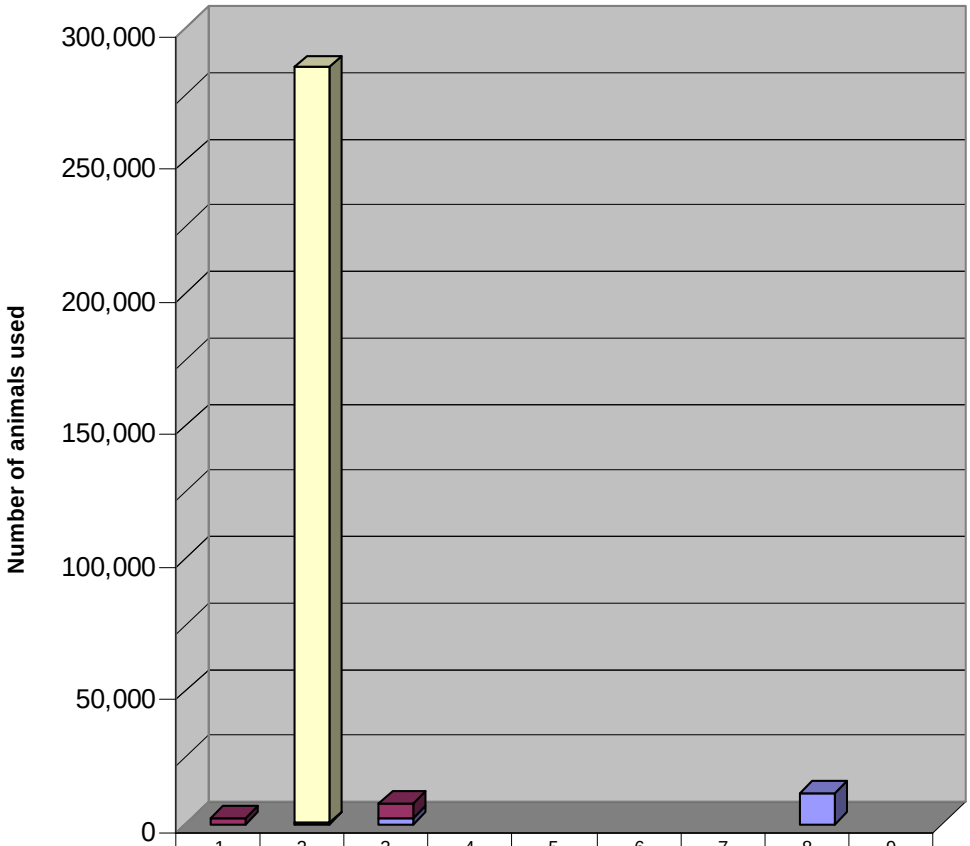

Purpose: Diagnostic Procedures
Breakdown of Laboratory Mammals Species
Number of animals used
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit
Guinea Pig
Rat 443 71 11
Mouse 16 54 6 24


Purpose: Diagnostic Procedures
Breakdown of Domestic Mammals Species
Number of animals used
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9
Other
Dogs
Cats
Deer
Goats
Horses
Pigs
Cattle
Sheep 61


Purpose: Diagnostic Procedures
Breakdown of Bird Species
Number of animals used
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9
Other
Native Wild
Native Captive
Exotic Wild
Exotic Captive
Poultry 45

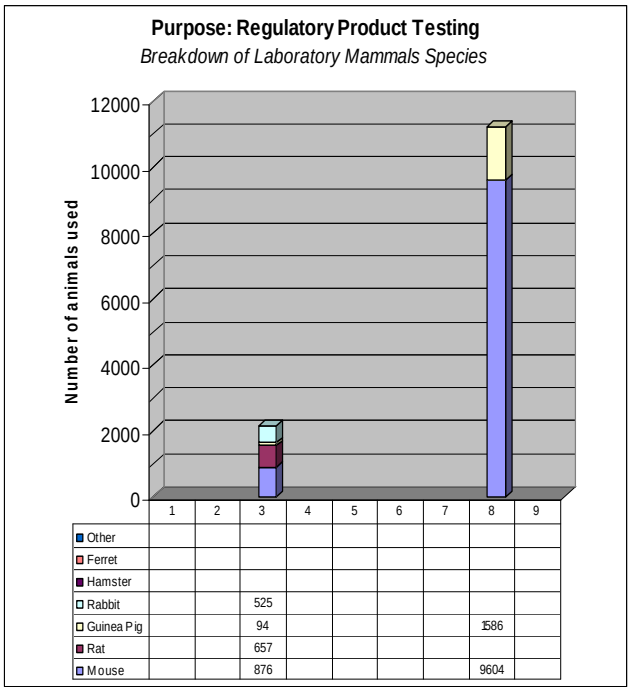
Purpose: Regulatory Product Testing

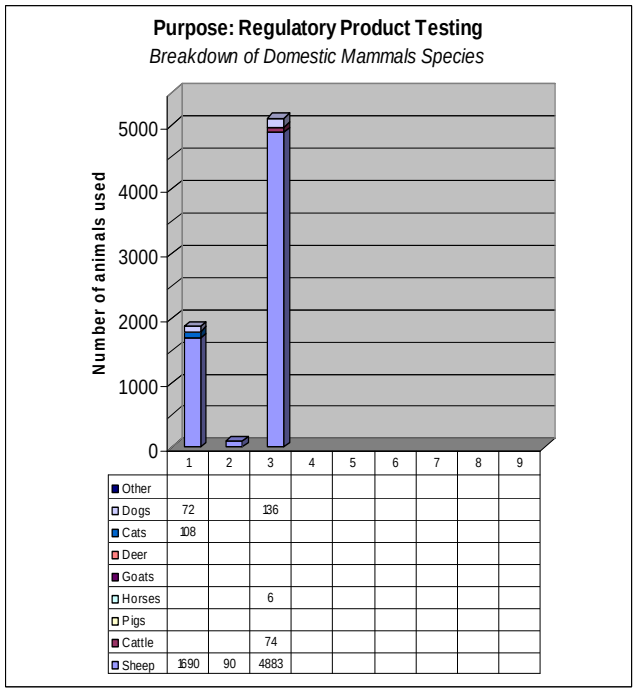
Protocols for the testing of products required by regulatory authorities.

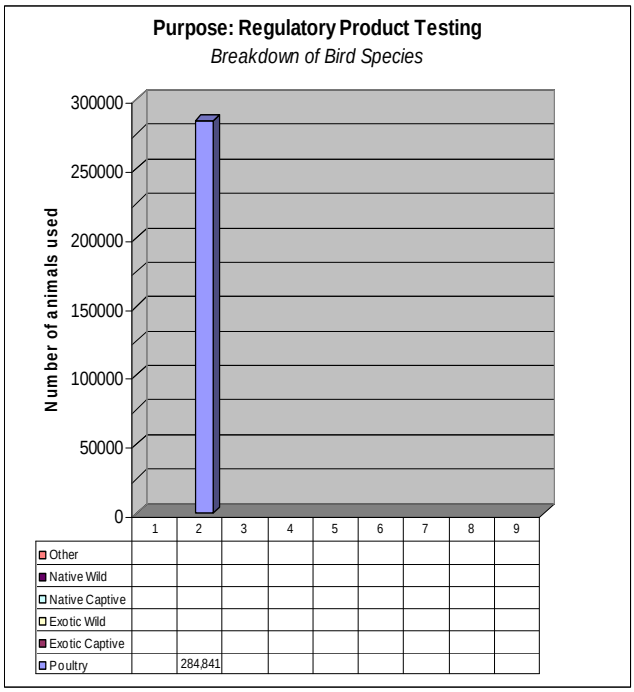


	1	2	3	4	5	6	7	8	9
Exotic Zoo									
Exotic Feral									
Nat Mam									
Primates									
Reptiles									
Amphibians									
Aquatic									
Birds		284841							
Domestic	1870	90	5099						
Lab Mam			2152					11190	

Refer to following page for a further breakdown of species.


Purpose: Regulatory Product Testing
Breakdown of Laboratory Mammals Species
Number of animals used
12000
10000
8000
6000
4000
2000
0
1 2 3 4 5 6 7 8 9
Other
Ferret
Hamster
Rabbit 525
Guinea Pig 94 1586
Rat 657
Mouse 876 9604


Purpose: Regulatory Product Testing
Breakdown of Domestic Mammals Species
Number of animals used
5000
4000
3000
2000
1000
0
1 2 3 4 5 6 7 8 9
Other
Dogs 72 136
Cats 108
Deer
Goats
Horses 6
Pigs
Cattle 74
Sheep 1690 90 4883


Purpose: Regulatory Product Testing
Breakdown of Bird Species
Number of animals used
300000
250000
200000
150000
100000
50000
0
1 2 3 4 5 6 7 8 9
Other
Native Wild
Native Captive
Exotic Wild
Exotic Captive
Poultry 284,841

LETHALITY TESTING – 2013

The *Animal Research Act 1985* defines a 'lethality test' as *'an animal research procedure in which any material or substance is administered to animals for the purpose of determining whether any animals will die or how many animals will die'*. Lethality tests include, but are not limited to, LD50 tests.

The following are the figures reported on animal use for lethality testing in 2013.

Species	No. used	No. died/ euthanased	Procedure	Justification	Alternatives
Mice	4,242	1,221	Serum neutralisation test in mice: Susceptible animals are challenged with test toxin/antibody dilutions to determine antibody titre.	Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. Testing of stability batches and new products for formulations.	This test is based upon regulatory guidelines. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop *in vitro* assays which may be used to replace existing *in vivo* assays subject to regulatory approval of these replacement assays.
Guinea Pigs	1,585	416	Vaccinated animals are challenged with test organism in order to demonstrate protection and hence vaccine efficacy.	Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. Assessment of in-process or development material to determine suitability for further manufacture.	This test is based upon regulatory guidelines. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop *in vitro* assays which may be used to replace existing *in vivo* assays subject to regulatory approval of these replacement assays.
Mice	3,084	1,623	Total Combining Power test in mice: Susceptible animals are challenged with test antigen/toxin/antibody dilutions to determine potency of antigen preparations.	In-process testing of vaccine constituents to allow evaluation of suitability for further manufacture.	This test is based upon regulatory guidelines. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop *in vitro* assays which may be used to replace existing *in vivo*

					assays subject to regulatory approval of these replacement assays.
Mice	2,124	1,162	L+ titration in mice: Susceptible animals are challenged with test toxin in order to determine potency of antigen preparation	In-process testing of production and development antigen growths to allow stop/go decision during manufacturing process.	This test is based upon regulatory guidelines. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop *in vitro* assays which may be used to replace existing *in vivo* assays subject to regulatory approval of these replacement assays.
Mice	180	60	Challenge of vaccinated mice with target organisms to demonstrate efficacy of vaccine.	Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release.	No alternatives available at this time.
Cane Toads	9	9	Testing humane euthanasia of cane toads by freezing with and without pre-refrigeration using surgical implanted EEG loggers to monitor brain activity and internal probes to measure body temperature. To evaluate the potential of refrigeration alone as a euthanasia method.	In depth studies on the possibility of simple refrigeration compared with refrigeration and freezing have not been conducted. We used animals from a different protocol to maximise their use and to determine whether refrigeration alone could be used as a euthanasia method which would be much simpler in practice for large numbers of toads and would potentially obviate the risk of compromising welfare that may be associated with freezing.	None available
Mice	111	44	In order to assess the contribution of specific bacterial and host factors to disease, it is a standard microbial procedure to “knock out” the virulence gene under study, and then compare the virulence of the knock out strain and the parental wild type strain in an animal	The contribution of specific virulence determinants to the pathogenesis of microbial pathogens can only be assessed in a live model of virulence. As mucosal and tissue barriers as well as a functioning immune system are required, these studies can only be conducted in live mammals (i.e. mice)	No alternatives exist, which effectively mimic the mucosal and tissue barriers as well as a functioning immune system observed in live mammals.

			model. Transgenic or wild-type mice were subcutaneously infected groups of 10 mice with knock out and wild type strains and monitored mice deaths over a course of 10 days to assess the contribution of the knocked out gene product to the virulence of *S. pyogenes*. Moribund mice were euthanized. At the end of te 10 day experiment, all remaining mice were euthanized.		
Mice	36	1	Animals are fed a high fat diet for 6 weeks to induce obesity. Mice are then administered one of three doses of azoxymethane, 2.5, 5 or 7.5 mg/kg a week for 6 weeks. Mice are maintained on a high fat diet for another 14 weeks and then sacrificed to determine the extent of colon polyp development.	This is the only method to determine the optimum dose of chemical to induce colon polyps in the majority of mice, with an acceptable mortality rate. Effective doses are dependent on many factors, including mouse strain (C57BL/6) and diet (high fat diet). This study will determine the optimum dose to generate polyps in an acceptable number of mice, with the lowest possible mortality. This will reduce the number of animals required for future studies.	There are no other alternatives.
European Rabbit	107	51	Testing a new method of killing rabbits by igniting a mixture of LPG and oxygen that has been pumped into a warren. The effectiveness and humaneness of this method has not been tested.	Rabbits are a major pest and they have significant impacts upon primary production and the environment. Although biological control is still effective across most of Australia there are still localised areas where other methods need to be used. The most common methods of management, poison baiting and warren ripping, are not suitable for all areas and alternative methods are still sought. In this study a new device, that claims to be effective and humane, is being tested. The manufacturers of the device (the 'Rodenator') claim that ignition of the	Initial testing of the device did not involve the use of animals. An artificial warren was constructed to test if the device could produce explosive pressures that were likely to result in rapid loss of consciousness quickly followed by death. Lethality trials proceeded only after it was clear that the device was capable of providing a humane death.

				gases causes a high pressure shock wave that humanely kills any rabbits within a warren. However, the effectiveness and humaneness of this method has not been tested. The purpose of the study is to examine the veracity of this claim.	
Mice	1,704	788	The mice were infected with the rodent malaria parasite at the dose $1x10^3$ infected red blood cells by intraperitoneal injection. Signs for disease develop between 7 to 10 days post inoculation. Mice which developed signs of severe anaemia (increase breathing) or neurological signs (fitting, coma) or which were unable to right themselves at physical checks three times daily were euthanized by cervical dislocation. Some animals die between checks. Surviving animals are retained for breeding and genotyping.	This project aims to uncover why children in endemic areas die from malaria infection while other survive. Using a murine model of malarial infection, we are aiming to uncover the host genetics contribution to malaria resistance. Ten cohorts of mice were used to investigate the role of the red blood cell and platelets on resistance to malaria infection including cerebral malaria, We have found that the platelets play a crucial role to combat the infection and we in the process of determining the mechanisms of resistance to be able to translate our findings into a clinical practice. We have also found novels drug therapeutic targets throughout our experiments. 8 survivor mice were retained for breeding.	Unfortunately, there is no other existing model to replace the need to carry out the malaria infections in mice, although our group is utilising cutting-edge sequencing technologies and combining multiple experiments to reduce the number of mice to infect with the malaria parasite.
Mice	10,612	6,335	Approximately 200 ENU affected breeding males produced 6723 weaned progeny. These were infected with the rodent malaria parasite at the dose $1x10_3$ infected red blood cells by intraperitoneal injection. Signs for disease develop between 7 to 10 days post inoculation. Mice which developed signs of severe anaemia	Malaria is a disease that kills more than 1 million children annually. In endemic areas, some people die from malaria while others survive the infection. Unfortunately, we still know little about the mechanisms underlying the host resistance to malaria infection. In order to better understand this complex phenomenon, we have performed a large-scale ENU (N-Ethyl-N-Nitrosourea) dominant mutagenesis screen for genes that when mutated, render normally susceptible mice resistant to malaria. From this screen we	Unfortunately, there is no other existing model to replace the need to carry out the malaria infections in mice, although our group is utilising cutting-edge sequencing technologies and combination with other experiments to reduce the number of mice to infect with the malaria parasite.

			(increase breathing) or neurological signs (fitting, coma) or which were unable to right themselves at physical checks three times daily were euthanized by cervical dislocation. Some animals die between checks. 306 Surviving animals are retained for breeding and genotyping.	have discovered genes controlling haematological and immunological pathways that are novel determinants in the host response to malaria infection. The major goals of this project are to 1) determine the biological basis of the resistance-causing mutations, and 2) validate the genes as potential antimalarial targets.	
Black Rat	50	50	Investigation of the susceptibility of exotic rodents on an island to brodifacoum by assessing the minimal amount that individuals need to ingest to cause death, and measuring the time interval between ingestion and death. Commensal rodents were captured and subjected to various degrees of brodifacoum in a series of no-choice feeding trials.	The eradication of ships rats and house mice on the island is required to protect the biodiversity of the local ecosystem and remove identified threats to a number of threatened species on the island. The success of an eradication programme depends upon 100% of target individuals consuming toxic baits and dying as a result. A non-toxic form of the bait has been shown to be palatable to the island rodents. As a result of extensive domestic use of brodifacoum the possibility exists that a proportion of the rodent population are resistant. Feeding trials with the toxic bait were identified as the only method by which resistance and hence suitability of the selected poison could be determined. In approving this protocol the AEC took into consideration the high importance of a successful rodent eradication programme to the future conservation of native biodiversity on the island. Every effort was made by the AEC to ensure the potential for suffering of the experimental animals was minimised e.g. extensive consultation with the applicants prior to approval, exploration of alternatives to death such as in vitro testing of clotting times, use of housing that complied with the ARRP rodent housing	No. The aim was to determine the operational efficacy of the rodenticide on a specific population targeted for eradication. The alternatives – blood clotting response tests and genetic testing – do not supply this information.

				guidelines, high frequency of animal monitoring, and determination of humane endpoints. The results of this research have provided critical knowledge about brodifacoum resistance in the island rodent population which will underpin the design, evaluation and success of the eradication programme.	

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 for the 2013 reporting year. They deal with '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).

Replacement, Reduction and Refinement
For the protocol being carried out in NSW, it is not possible for the researchers to use an alternative to live animals as observing animals is fundamental to species distribution modelling and existing data is sparse for fish assemblages in the study area. The sampling of fish assemblages in this research is non-destructive. An example of refinement includes: The operation of the backpack electrofisher only by highly trained and experienced researchers. The researcher involved has over 3 years of professional experience using this method. Also, in order to avoid any risk to non target vertebrates, if any of these are seen in the sampling reach, electrofishing will cease until the area is clear of non-target animals. In terms of reduction, the number of fish identified, temporarily held, and returned to the stream is dependent upon both the abundance of fish at the survey sites, and the degree of fishing effort that is required to determine (with a certain level of statistical confidence) the species present and their relative abundance. To minimise the impact of the research on fish, no more than the necessary degree of fishing effort to achieve robust results will be used.
The establishment has maintained an ongoing program aimed at rationalising testing which has focused on eliminating Quality Control testing which is not essential to meet product release requirements. This program covers both lethal and non-lethal testing. In addition, in all cases where clear test outcomes are not obtained upon initial testing, a critical assessment is made to confirm the necessity to perform repeat testing before re-testing occurs. It should however be noted that whilst progress has been made in this regard, there remains a minimum amount of testing necessary to meet regulatory requirements for the assessment of in-process and final product prior to release. Increasing emphasis is being placed by the establishment on the development of in-vitro testing to reduce the use of animals for our vaccine testing. The establishment has relocated a PhD scientist to run our in-vitro program and this person commenced work in January 2013. Our in-vitro strategy was presented to the APVMA last year and it was met with full support. We believe the planned program of work will take 5 years. Applications will be made to the APVMA when new assays have been developed and validated. Updated specifications for all finished product vaccines showing the removal of safety testing references were submitted to the APVMA following a request from them in November 2012. We are pleased to advise that the APVMA has granted the elimination of target safety testing for our finished product vaccine for animal species in Australia and New Zealand.
Accommodation of research horses in a large paddock on a professional horse spelling/pre-training farm. Re-homing of retired research horses to suitable new owners. Spontaneous collection of naturally voided urine for the purpose of drug analyses. In-vitro simulation of the equine metabolism of designer anabolic steroids using horse liver
The tamoxifen doses injected to the female are non-toxic for them. Although tamoxifen injection in adult mice show no complications, we found that injections to pregnant females have an adverse impact on foetuses from E15.5 onward at the dose required to induce recombination. In order to decrease the lethal effects of tamoxifen in these foetuses, we will co-inject tamoxifen and progesterone. Progesterone has been effectively used to offset tamoxifen toxicity (Chong et al., Cell Stem Cell. 2011). Doses are adjusted to favour maximum cre activity and maximum embryo viability. In any case, embryos are harvested before toxicity. These injections appear to cause spontaneous abortion of embryos, which are usually resorbed. In order to decrease this impact we have found that co-injection of Progesterone at a dose of 0.5mg per 1mg of Tamoxifen will decrease the rates of abortions. We also learnt that delivery of foetuses by C-section at (E18.5-E20.5) with Co-foster (using a separate recently

delivered mother) has been used to obtain usable mice. We will use this method to minimize numbers of animals needed to obtain experimental result."

"Care will be taken when giving IP injections to pregnant mice; injection sites will be rotated to avoid inflammation or pain in one area. A very fine gauge needle will also be used to minimise discomfort. Investigators are experienced in this procedure."

"We seek to meet the principles of Replacement, Reduction and Refinement. We will ensure that all investigators are thoroughly trained in the procedures to be performed. We will also endeavour to minimize the numbers of animals used through good experimental design, and through sharing tissues between researchers."

"All paradigms to analyse the animals' motor functions are based on natural behaviours. In the beam walking test soft bedding material is put under the beams so that any animal that falls off is not injured."

Whenever possible, in vivo imaging will be used to take serial measurements of mammary development and/or tumour progression/regression. In vivo imaging assists us in minimising the number of mice used by allowing the collection of large amounts of data from individual mice. Furthermore, the quantitative nature

of the data reaches statistical significance faster than with non-quantitative measurements."

"The use of transplant techniques for many of our experiments greatly reduces the number of mice used for studies; transplants are generally more reproducible than transgenics and thus require fewer mice to reach statistically significant findings. Breeding of transgenics and knockouts generates large numbers of mice with the incorrect genotype that are simply euthanized; to obtain females carrying 3 trangenes for example, only 6.25% of offspring carry all 3 genes. In comparison, transplantation allows the generation of the exact number and type of transgenic for a given study."

"The use of in vivo imaging further reduces the number of mice used, because longitudinal studies can be run on a single cohort of mice."

"Transplantation also only generates a single tumour focus, whereas tumour-susceptible GM mice can often generate multiple tumours, including in critical (and cryptic) organs such as the blood system."

"Because the data is of higher quality, In vivo imaging also reduces animal distress by allowing us to carefully monitor tumour load in mice, and terminate experiments before mice become moribund, with associated toxicities."

"Since the start of 2009 our group has included a dedicated animal technician who has wide experience of animal handling, procedures, welfare and training. This has ensured that our experimental colony is monitored to a high standard and any animal welfare issues are identified immediately."

"Breeding two healthy genetically modified animals can lead to the generation of animals carrying a novel genotype – either homozygous for a mutation, doubly heterozygous for inactivating mutations or overexpressing constructs. In these cases, phenotypes will be carefully monitored to avoid distress and pain, and to establish an optimal ethical endpoint for experiments. Sick or stressed animals will be sacrificed and autopsied to establish the cause of any genetic phenotype."

"Mice studs have to remain singly housed for a long time. Companion females should be provided to them when available. Our mice are used regularly and are rarely on their own. If we anticipate a long period of inactivity, ovarectomised companion females will be provided to them when available. Some male studs may be culled and replaced, as they cannot be housed together with other males without fighting."

"Anaesthesia is potentially a significant physiological insult, which can affect cardiac haemodynamics, respiratory mechanics and fluid balance. All the procedures involving anaesthesia either gaseous or injectable are performed following the AEC guidelines. We aim to maintain the animal physiology as close to normal physiology parameters, and to ensure there is close monitoring of the anaesthesia at all times. Anaesthesia will be monitored by checking blinking reflex, relaxation of the body musculature, reaction times to tail and toe-pinching. All doses of anaesthetics should abolish the blinking reflex and induce relaxation of the body musculature. Mice under a medium anaesthesia should not react to tail-pinching. Recovery will be carried out on a heating pad (23-25 degrees C.) with the animals lying on an insulating material (hand towel or plastic back bench liner) placed in the cage over the bedding. Animals will be carefully monitored until the righting reflex is regained."

"We have proposed to use fewer animals per group (6) compared to previously (10) for 07/02."

"The experiments are designed to achieve overlapping aims. We have designed the experiments to measure multiple endpoints at the same time (eg analysis by FACS and microscopy) to maximise the number of readouts from the minimum number of mice. Experiments were designed to involve the minimum amount of disruptions to the mice and minimum number of manipulations. For example, we have previously optimised the protocol for intravital microscopy by practising on cadavers."

"From our previous experiments with the Colon 26 cachectic mice we have extensively defined the timepoints at which features of cachexia and molecular changes occur. Our main interest is in the early stages of cachexia preceding the extreme weight loss and impact on animal welfare once cachexia is fully developed. Therefore, we have optimised the endpoints of these experiments so that the majority of mice are culled at a point when only mild weight loss has occurred and before they get very sick due to general loss of condition."

"Mice are administered a dose of LPS to stimulate systemic innate immune response, which increases the permeability of the blood-testis barrier. Previously reported techniques to transplant cells into the male germ line required testicular surgery (Harma et al., 2002; Ikawa et al., 2002; Kojima et al., 2008; Nagano et al., 2001; Ogawa et al., 1997; Russell, Saxena, and Weber. 1987), whereas we hope to circumvent this procedure and minimises the surgical pain and stress to animals by direct injection into the testis through the dermis (attached publication Amaral et al., 2011). A small volume will be injected into each testicle to limit the pressure on the testes and reduce leakage of lentiviral solution post-injection."

"We use analgesics after surgery to assist with recovery. All animals receive enriched cage environments, which reduces stress."

"The experimental designs used in this project have been calculated to minimise the number of animals used whilst maintaining adequate statistical power. The procedures used in these experiments involve either non-invasive monitoring of tumour progression and/or minor surgery on terminally anesthetized non-recovery animals. The use of whole body in vivo imaging will be used at all time, when appropriate for experimental question/outcome, this significantly reduces the number of mice used because longitudinal studies can be run on a single cohort of mice."

"Conditional systems also reduce the number of animals that will be used. We will only use animals where there is no alternative, but where necessary, animal experiments will be guided by replacement experiments, for example, in complex 2 and 3D tissue culture systems (as outlined in the proposal for 3D-organotypic models which are a vital component of this project). We currently obtain rat tails from A/Prof XXX and utilise these to create 3D-organotypic assay to mimic in vivo condition whenever possible/appropriate for investigation. Utility of these intermediate 3D assays helps to inform in vivo investigation thereby minimising animal use at early stage of study."

"When mice are euthanased tissues are halved and used for both flow cytometric and histological analysis, and blood is collected after death for serum antibody analysis. Other tissues such as lymph nodes may be shared between researchers. These procedures minimize the number of animals used."

"The use of retroviral transduction of bone marrow cells greatly reduces the numbers of mice required to investigate gene function by circumventing the need to generate independent transgenic lines."

"In the case of sub-cutaneous tumours, if a small amount of skin necrosis appears, which is normally very rare (necrotic spot less than 2 mm diameter), we will apply iodine to prevent infection and dry the wound rather than repeat with additional animals. In the case of more extensive necrosis the animal will be sacrificed."

"During the procedure animals will be handled for as little time as possible to reduce any unnecessary stress. We will use analgesia and aseptic techniques for surgery and during post-operative recovery animals significantly deviating from the norm will be euthanased to minimise suffering. We will collect several tissues (e.g. fat, liver, muscle) from the same animals to allow a reduction in the total number of animals needed for these studies"

"Although the spinal cord surgery described in this proposal may provoke pain and stress, we carefully optimized the injury protocol to minimize the distress. Our simple surgical method allows us to complete the procedures quickly, thereby the time taken under anaesthetic is reduced. The forceps and iridectomy scissors we use in this project are very fine, minimizing collateral tissue damage during the procedure. A sponge holding the fish during the procedure is moist and prevents the fish from dehydration that would otherwise occur during the procedure. As soon as the surgery has been

finished, fish will be carefully revived by squirting water over the gills with a pipette. The survival rate in our spinal cord surgery model is very high (>80%), which helps to reduce the number of animals used in the project."

"The vast majority of other experiments in this project simply require the generation of embryos by natural breeding methods, which generate little or no stress to animals. Microinjections will be performed with one-cell stage embryos, which unlikely sense any pain or stress during the procedure. We will generate transgenic constructs of which expression is monitored by fluorescence protein expression. This technique will enable us to enrich embryos that possibly carry integrated transgenes, contributing to reduce the number of fish that will be raised as founder candidates."

"The use of zebrafish as a vertebrate model system can be defined as an act of replacement. Since zebrafish are externally fertilized and embryos can be easily collected outside the mother, the proposed studies do not require interventions that would be otherwise necessary if mammalians were used to perform similar studies."

"In general, we intentionally perform all surgical procedures on level 1 so as to minimize any stress from transport. The recovery room and the holding room are located within the behavioural phenotying facility, with limited access. We generally are as quiet as we can be in the behavioural phenotyping facility so that mice are not startled by our movements. Wherever possible we let mice move around freely during infusions – we watch the carefully to ensure that the tubing does not get entangled and cause discomfort. If multiple drugs are proposed for use, we always infuse them as co-injections to reduce stress on animals."

"All animals that undergo surgery (cannulation) will receive Bupenorphine injection prior to anaesthetic."

"Analgesia and optimal surgical technique will be used to minimise the pain and stress caused by surgical procedures. We will try a scrotal approach to vasectomy to assess if this is a better technique than the abdominal approach. Criteria that will be used to assess the approach will include weight gain post surgery, behaviour (activity, rearing), presence of facial grimace and any incidence of wound breakdown. Close monitoring of post surgical mice and newly generated GM mouse lines will be used to detect and treat or euthanize mice that are suffering at an early stage."

"The CRISPR and TALEN techniques allow for a targeted insertion of GM material into the mouse genome. Due to the targeting any pups carrying the genetic modification will be identical. This is in contrast to traditional transgenic lines generated by microinjection where the GM gene inserts randomly and with varying numbers of repeats (copies). This older method of making transgenic mice meant that each pup carrying the genetic modification was unique and would start a new mouse line. Multiple lines are generated in this way and need to be bred and tested to see which line is the most useful. The CRISPR and TALEN targeting will reduce the breeding required after microinjection. In addition the CRISPR technology allows for several genes to be altered at a time reducing the breeding required to generate mouse lines with multiple genetic alternations."

"Sperm and embryo freezing both eliminate the need to continuing to breed unwanted mice just to keep the line available. Sperm freezing is superior to embryo freezing in that only 4-6 male mice are required for freezing. Most lines will be frozen using sperm. Where embryos or sperm can be imported instead of live mice the need to bred up a colony prior to rederivation is avoided."

"Animals used for training are not specifically bred for this purpose. All animals used for training are surplus stock that would otherwise be euthanized. This includes mice that are the wrong sex, wrong genotype or retired breeding stock. We will also utilise surplus experimental animals such as time-mated females where only pups are required."

"Shipping embryos or sperm instead of live mice will remove the animal welfare problems that can occur during the transport of live mice (especially on long haul international flights). Companion females improve the welfare of single housed male mice. All researchers undergo specific training, which will assist them with recognition of pain and distress in laboratory animals. Internet resources, such as http://www.ahwla.org.uk and http://www.procedureswithcare.org.uk are utilised to assist with training. Alternatives such as suturing pads, in house training videos are used in training to optimise the preparation of students before they handle live mice. To minimise the impact on the animals during training, many procedures are performed on deeply anaesthetised animals."

"We have discussed all aspects of the application so that our work has as minimal impact on the animals as possible, and have been guided by the latest NHMRC Code For The Care and Use of Animals for Scientific Purposes 8th Edition (2013) and the NHMRC 'Guidelines to promote the

wellbeing of animals used for scientific purposes: The assessment and alleviation of pain and distress in research animals."

"Where possible, we have reduced animal numbers required for the project. For example, we will use animals from Aim 1 in subsequent aims as they will have only been subjected to observation for litter size, viability and general health. By performing general phenotyping at the first opportunity, we will be able to carefully assess whether the new mouse lines have any adverse health effects (whether related to our hypothesis or otherwise) and therefore refine our experiments into the physiological role of GPR37L1."

"Investigators involved in the study will be trained and supervised by competent staff until deemed competent themselves. All animals undergoing surgical procedures will be treated with the appropriate anaesthetic and analgesic to minimise pain and distress. Animals will be closely monitored for signs of distress by investigators and staff. This will ensure compliance with ethical endpoint requirements."

"In vitro techniques will be used wherever possible. For example, basic proliferation studies have already been performed in vitro to ensure that a basic effect will be seen in vivo. However artificial cell culture cannot provide all the complex interactions observed in the tumour microenvironment (as these are not fully understood) and as such animal models are necessary here. The number of animals used in each treatment group is the minimal amount required the meet statistical power requirements, whilst allowing for an approximate graft rejection rate of 20%. This will reduce the number of animals required whilst ensuring that the project does not need to be repeated unnecessarily."

"To reduce the total number of animals required for this project we chose the zebrafish model. A similar study could be conducted using mice although due to their lower tolerance for ENU many more mice would be required to obtain F2 families with a similar genetic composition (e.g. 50,000 to 100,000 mice). Furthermore, it may not be possible to accurately model complex disease in mouse embryos so the screen generation (F3) may need to be raised to adulthood, further increasing the animals required at least 10-fold (e.g. 500,000 to 1,000,000 mice)."

"ENU mutagenesis protocols have been optimised by labs around the world to minimise the stress response in the treated fish. Current protocols recommend wrapping the fish container in foam during ENU treatment and during the recovery period to prevent light and noise from reaching the fish. Additionally, steps will be taken to minimize the handling of fish post-ENU treatment (e.g. slow removal and addition of water for the washes)."

"We have designed our experiments to minimise pain and stress to the animals. We are using a mouse model (A^{vy}) in which the obesity/diabetes phenotype and the epigenotype of the responsible allele are tightly linked to the mouse coat-colour phenotype and thus can be assessed non-invasively, and in which obesity occurs spontaneously and does not require any dietary, pharmacological or genetic manipulation. In assessing the transgenerational effects of parental obesity on offspring, we have chosen procedures that are as non-invasive as possible, and predominantly involve post-mortem analysis of blood/serum and tissues."

"We also run our experiments to utilize existing mice as much as possible and hence minimize the number of mice required. We use the stock A^{vy} colony as the control population for Aim 1, and as the feeder population for Aim 2 (i.e. to produce yellow A^{vy}/a males and females and corresponding control a/a mice). We will also use excess mice generated by Aim 2(a) as the breeding mice for Aim 2(c). We are cognizant of the fact that our investigations into the epigenetic behaviour of A^{vy} (Aim 1) necessarily require very large numbers of mice (as most mice display a different epigenetic state of A^{vy} than that we are interested in, so are excess to requirements), so we constantly monitor the progress of the experiment and adjust mouse production as necessary. We also make efforts to use excess mice, by for example collecting oocytes/early embryos from them."

Techniques developed or adapted to:

It is a prerequisite for all applicants to address the 3Rs as part of their AEC application and for the AEC to specifically review these parts of the application. This process resulted in the identification of a number of improvements for animal welfare and usage through replacement, reduction and refinement.

Summary of examples of implementation of the 3Rs reported by researchers in 2013:

Reduction:

- Investigators often generate a tissue bank so that tissues not required for immediate use, can be

preserved for future.

- Where animals are used in a teaching, a teacher will try to ensure that a group of students rather than a single individual is assigned to each animal
- When designing a project investigators will check on previous studies to ensure that work is not being unnecessarily duplicated.
- Investigators seek the advice of a biostatistician where appropriate.
- Pooling of samples is undertaken whenever possible
- Animal colonies bred and housed on campus are currently being relocated and managed through a central breeding system at LARTF.
- Experiments are sometimes run in parallel using just one control group
- A pilot study is often employed by an investigator before proceeding further

Refinement:

- The capture of native animals and aquatic life is always undertaken with minimal interference to animals i.e. they are released as soon as data has been collected, at the point of capture, and at a time when environmental conditions are best suited
- All procedures are carried out by competent, qualified technicians or experienced investigators/ staff following AEC approved SOPs, or industry best practice.
- Inexperienced personnel work under the direct supervision of experienced personnel until competent.
- The use of timed release mechanisms on GPS collars removes the need to recapture animals in wildlife studies
- Pitfall traps are provided with shelter materials for animals (e.g. sand, leaf litter, cloth and PVC piping) while waiting to be released. Traps are checked in the early each morning and each evening to avoid animals spending more time than necessary in captivity.
- Insect surface sprays are used around pitfall traps to reduce irritation from ants e.g. in Mallee ecosystems.
- A number of experiments have been designed to measure physiological parameters employing minimal handling of animals, while others are purely observational.
- Where appropriate animal behaviour is captured using motion detecting cameras placed at a distance so as not to disturb animals
- Where certain procedures are being undertaken they are conducted away from other animals.
- Exposure times for testing behavioural responses in experiments is limited to as short a time as possible
- Proper attention is paid to good housing, care, feeding, handling, transport, and monitoring of each species at all times
- Where possible social isolation of animals undergoing experimentation is avoided and attention is always paid to proper monitoring for procedures
- Studies are not prolonged unnecessarily.
- The AEC always tries to ensure that projects are designed to minimise the need for repeat procedures, and stress on animals.

Replacement

- Occasionally a study can be limited to one sex where there is a marked difference between the daily activities of each
- Studies are co-ordinated where possible to enable the sharing of tissues

- Sharing with other investigators use of surplus animals.
- Storage of tissue and carcasses for ready use by other investigators or as a teaching resource, in some instances decreases the overall number of animals required.
- Conducting a pilot study has proved to be helpful before moving to larger scale experiments.
- Undertaking observational studies rather than capturing animals has proved useful in a number of instances
- Similarly implanted tracking devices have assisted with wildlife studies.
- Where applicable in vitro studies are used
- Results from previous studies are used to reduce the number of animals required for some experiments.
- Given the unique nature of research, and the large number of studies which involve wildlife and aquatic species, there are generally no alternatives to the use of live animals.

For other work it is a regulatory requirement for research to be conducted on live animals before human trials can commence.

Comparison of methods for measuring methane production from cattle in grazing systems.
Refinement
Variation request to change the way the canister that releases nitric oxide are attached to a halter on the cattle. This request will make it safer for the animals.

Refine: Continuing experience in handling poultry which are particularly prone to stress.

Staff enter bird houses and communicate in quite manner for the welfare of chickens.

Monitoring system (DAS) installed in 2013 to monitor bird temperatures off site.

Use of foil blankets in transport tubs (transferring of eggs from incubator to OPU) have led to better hatching and less fluctuations in temperature,

Reduce: Control groups are shared whenever logistically possible and negative control groups are reused to reduce the number of chickens used.

The establishment continues to use surplus SPF males from the breeding farm for *Eimeria* oocyst production. The increased yields from these older surplus birds have reduced chick requirements by 2 to 10 times.

Where possible, staff will replace the use of live animals with video or synthetic / cadaver models, or by creating computer models.

Power analyses are frequently submitted as part of the application (sometimes at the request of the Animal Care and Ethics Committee) which demonstrate how researchers and teachers calculate the most suitable number of animals required to give valid data.

Applications include justification by researchers and teachers for appropriate handling techniques where necessary, avoidance of pain and distress to the animals and how pain or distress will be alleviated if the animal shows signs of displaying either state.

The establishment initiated a programme, approved by the AEC, to research tick feeding behaviour and collect *Ixodes holocyclus* saliva for analysis. This had initially been done using ticks engorged on dogs, however an *in vitro* method of feeding the ticks was devised which replaced the need for canine hosts.

- With the increases availability of computer simulations, the university is moving away from the traditional model of having students individually dissect an animal. The current model is for undergraduate students to access a computer/video demonstration, with a hands-on aspect of learning how to correctly handle an animal
- The university also actively encourages researchers working together to develop projects that can be run in parallel, which uses different tissues of the same animals in order to reduce the overall number of animals.

Researchers are also asked to provide power analysis to demonstrate an understanding of how to ensure that the minimal number of animal replicates is used.

- An investigator is analysing aspects of data from a large controlled trial funded by the Co-Operative Research Grant in 1997 that involved the collection of several measurements from 84 animals over a 13 week period. The analysis of this data replaces the use of live animals for the collection of this data.
- An investigator is using rumen fluid collected during another study to examine rumen bacteria, and blood also collected from these animals for animal genotyping. This is replacing the need for use of additional live animals for collection of these samples. The investigator is also using blood samples from another project for animal genotyping which is reducing the use of live animals.
- An investigator combined data from animals from two projects conducted in 2005 for one chapter of her PhD to re-analyse this data and reduce the need for a live animal study.
- As part of an investigator's PhD she collaborated with another establishment and used rumen samples collected during one of their Flexible Feeding Systems trials for analysis of rumen bacteria. This replaced the need for her to collect samples from other live animals.

The number of animals required in a number of the above listed studies was reduced by the use of partial factorial experimental designs. The use of partial factorial designs reduces the number of animals required by increasing the study power.

When considering each application the AEC scrutinises each application to ensure the number of animals to be used is minimal and that the impacts are reduced as much as possible.

Specimens and tissue samples collected are often advised/required to be shared with other institutions if possible to aid Reduction in the number of animals collected.

The AEC also attempts to reduce the amount of by-catching during field work involving trapping and the PI has sometimes been asked to consider alternative trapping methods of modifying experiments in order to reduce the amount of by-catch.

The AEC usually requires monitoring sheets to be used for laboratory animals with clear intervention parameters and key triggers for intervention to Refine experiments to reduce any adverse effects on animals.

New and improved technology has continued to be developed during the year, in an attempt to improve the efficiency of stored serum processing to hopefully reduce the numbers of animals used in the production process.

Replacement

- Preliminary laboratory screening of potential formulations was conducted using a 'model sheep' prior to this study being undertaken.

Refinement

- Dogs were handled by experienced personnel who the dogs were familiar with. The normal husbandry routine was maintained as far as possible, including access to group exercise outdoors in a grassy paddock several times per week.
- All procedures were undertaken by personnel with previous experience in sheep husbandry. Immediately prior to euthanasia, sheep were isolated and humanely euthanased in a fixed location which was visually and audibly obscured from other sheep.
- Dogs were kept in their regular housing and were handled by familiar trained personnel.
- Sheep were kept in groups of five and could see other sheep during the conduct of procedures. All procedures were standard farm husbandry procedures carried out by experienced personnel.
- The results of study period 1 (application of test formulation containing a vehicle and fluorescein only) were assessed prior to undertaking study periods 2 and 3 (applying test formulations containing the active ingredient).

- This was a field trial which followed normal management practices of sheep producers. Care was taken when handling animals to avoid distress. Animals were weighed and volume checks performed on drenching guns to prevent under/ over dosing of animals.

- The plasma collected during each bleed was stored and utilised in several studies, thus reducing the number of times an animal is bled.

Reduction

- Six animals in a cross-over design was considered the least number of dogs able to achieve statistically relevant data.

- This study utilised the minimum number of sheep required by regulatory agencies. Once the two untreated control sheep were euthanased and relevant tissues had been collected for the purposes of this study, additional tissues were harvested and stored for future method development studies. This will negate the need to euthanase further sheep for future studies.

- This was a pilot study conducted to determine the most suitable formulation for use in a field trial. Determining the most appropriate formulation at this point reduces the number of formulations tested in field studies, therefore reducing the number of sheep required.

- By designing this study to proceed in three study periods, it is ensured that animals are not treated unnecessarily. Results from each study period are assessed treating further animals.

The animal's owner/ manager was provided with the FEC and LD results from this study. This allowed the owner/ manager to assess the necessity of drenching the remainder of the mob and what product to use without having to perform additional faecal sampling.

Only sites that had an average representative FEC of over 300 epg were included in the study in order to reduce the inclusion of sites that may be statistically irrelevant.

We make available samples that are collected opportunistically from collection animals and wildlife under our care or that have died. Access to this important material reduces the need for additional interference with animals and has benefited many collaborative researchers through the years.

- Researchers are encouraged to share tissues wherever possible. This is facilitated by staff.
- Breeding programs are designed and maintained to produce stock for orders only to reduce numbers and overproduction
- Genetically modified animals are bred as the desired genotype as far as possible to reduce numbers.

Animals used for courses are shared between multiple participants to achieve the best learning outcome whilst reducing overall numbers of animals used.

Researchers are required to apply the 3Rs (replacement, reduction and refinement) at all stages of their research. Some researchers report on field work that involves mainly observation and low impact to animals within the study. Where research involves housing animals and performing procedures, researchers report the use of extensive in vitro experiments prior to the use of animal models, conducting power analyses to identify the least amount of animals required for experimentation to achieve statistical power, the use of highly skilled personnel and housing of animals in a safe, stress free environment where they can socialise. Animals are provided with clean food and water as well as environmental enrichment. Any procedures performed on the animals are conducted away from the other animals to minimise any distress, and strategies addressing the minimisation of pain are incorporated into protocols reviewed by the AEC.

Following is a list of specific examples of strategies to address the 3R's reported by researchers this year:

Reduction

- As the litter sizes always vary, animals from smaller litters are pooled and animals from larger litters are shared between researchers to maximise usage of available tissues.
- The number of animals used has been reduced by increasing the number of students in each teaching group.
- We have been using the inner ear from the contralateral side of these animals to perfect our post-mortem imaging technique and to provide baseline data, rather than using more animals.
- Tissues from some animals have been used for several different protocols to reduce animal numbers.
- We have recently introduced multichannel flow cytometry which would make the experiments more efficient and the animal numbers will further decrease.
- We have reduced the number of animals used by maximising our recording success. We have purchased a new infra-red camera that allows us to visualize cells more clearly during recording and fluorescently image them to confirm their location prior to recording. This means that we are able to essentially target our cells more efficiently – giving us more time to collect recordings from each animal. We have also refined our electrophysiological setup by purchasing a new perfusion pump which allows us to improve the health of our tissue during recordings by allowing a higher flow rate of oxygen containing solution. This means that we have longer to record from each animal – increasing our yield.

Replacement

- Where appropriate, protein samples taken from one set of tissues are shared amongst researchers to maximise usage of samples and hence avoid use of additional animals.
- We have used tissue cultured cells as a substitute for animal tissue until this time. We used no animal tissue in this year.
- Use of embryonic stem cells instead of embryos for optimisation of new protocols.
- Multiple organs are sampled from all euthanized animals, including controls. In this way we have built up a “Tissue bank” that can be used for some histopathological, gene expression and biochemical investigations, thereby reducing the number of mice used.
- We have replaced traditional techniques for measurement of blood pressure in the anaesthetized rat with a telemetry system that allows us to measure blood pressure in the conscious freely moving animals. This allows higher quality data to be obtained, and multiple minor protocols can now be performed in the same animal, allowing for a reduction in the total number of animals.

Refinement

- This project involves the use of non-invasive faecal sampling to determine the endocrine status of study animals, thereby negating the need to capture and bleed individual animals to achieve the same end-point.
- We use remote detection cameras to record the natural foraging behaviour of wild animals within urban environments. This negates the need for capture or interference with animals.

The focus of our studies is on rodent behaviour. We have pioneered in Australia the use of a totally automated system that
(i) removes the need for animal handling during the experiment,
(ii) allows mice to be continuously housed in social groupings,
(iii) provides reproducible data, thereby reducing the number of mice used in the experiments.

Leucocyte migration and immune regulation in murine intestine

Use of colonoscopy allows us to follow individual animals longitudinally, when assessing colitis
An in vitro model of appendiceal regulatory T cells is being developed which may allow some murine experiments to be abandoned (but will still require a smaller number of mice to be

sacrificed for derivation of cells)

(Replacement, Reduction, Refinement)

Glucocorticoid-regulated genes in paediatric acute lymphoblastic leukaemia

Most of our experiments are conducted using in vitro model systems. In vivo models are necessary to generate the initial data that can then be followed up and confirmed using in vitro models, thereby refining and reducing the number of animals required. We can frequently replace the use of animals by conducting a cell culture experiment, but ultimately hypotheses need to be confirmed in vivo. (Reduction)

In-vivo immune challenge of mice

Prior to the in vivo study a large amount of work was carried out using immortalised cell lines to obtain proof of principle. We try to obtain as much data from each mouse as possible, as well as using each mouse for the in vivo challenge we collect the spleens in order to carry out ex vivo analysis. For some preliminary experiments with murine primary cells we use spleens or lymph nodes donated by other researchers when they are sacrificing mice. (Reduction)

Studies of rodent neural stem cells in the developing brain

For some experiments it was possible to culture and expand T cell in vitro rather than using more mice. T cells that could not be used in experiments were frozen and stored in nitrogen vapour phase tanks for further use. In some cases these cells were used for preliminary experiments. (Reduction)

Pharmacology Animal Practical classes, (This application includes 5 Teaching Practical Classes)

Over the past decade animal use has declined, through the introduction of computer practicals, however there is a need for a small number of animals to demonstrate
important principles. Numbers are minimised by using a demonstration (audiovisual) aid, and by scheduling classes such that one animal can provide tissue to several
classes.
The Department regularly reviews the educational value of all classes.
Moreover tissue is also offered to researchers to make the most efficient use of the preparation.
Animals are housed for the minimum time required. (Replacement, Reduction)

Treatment of Lung Cancer using DNA-directed RNA interference (ddRNA) Technology

Establishing the clinical orthotopic model with the lung cancer cell lines used within our research group we were able to determine the minimum number per group necessary for statistical analysis which allows us to both refine and reduce the number of animals necessary for our experimental designs. (Reduction, Refinement)

The role of the microtubule regulating protein stathmin in mediating tumour migration/invasion in neuroblastoma

To reduce and/ or replace animal numbers we first investigated the effects of silencing stathmin expression on the migration and invasion of neuroblastoma cells using a collection of different neuroblastoma cell lines in vitro. These experiments were performed at least n=5 to give statistical power. Only after confirming the importance of stathmin in regulating neuroblastoma migration and invasion in vitro, did we decide to assess its role in vivo. This ensured that only the absolute minimum mouse numbers were used for this study. (Replacement)

Pharmacochemical inhibition of GSK-3beta to treat Graft Versus Host Disease (GVHD)

1. The intensive in vitro testing of the effect of small molecule inhibitor of GSK3b on allo-reactivity of human T cells was conducted using a number of different immunological assays. This allowed us to optimise the design of in vivo experiments and obtain statistically significant results using small groups of mice(n=5).
2. To minimise the number of animals to be used in vivo, human T cells from several donors were first tested in vitro for their allo-reactivity and response to GSK3b inhibitor. T cells from the most responsive donor were used further on in our in vivo experiments. (Replacement)

Effect of age on spinal fusion in a rabbit model

We acquired aged animals that were surplus to other researchers projects (Reduction)

Electrical stimulation and extracellular recording from the rabbit retina

The experimental techniques were continuously refined and great care was taken to ensure consistent and extended survival of the cells in vitro. Each experiment was planned and executed to address as many research questions as possible, to minimise the number of animals used. The experimental results obtained in this study will be used for computational modelling work carried out by our research group. These models will ultimately be used to predict the retinal behaviour during electrical stimulation and may reduce or even replace the animal use. (Replacement, Reduction, Refinement)

Molecular regulation of telomere length, cell immortalisation and malignant transformation

During the course of our studies, we also made some refinements to our protocols that allowed us to minimize animal usage:
A) We developed a new vector system for inducible gene suppression in vivo. When applied in our study of neuroblastoma, we showed this system was tightly controlled by addition of doxycycline to tissue culture media. By feeding mice doxycycline laced food, we were also able to control the vector and timing of gene suppression in vivo. This provided opportunity for attaining more rigorous data on the affects of gene suppression on tumour growth, greater statistical power and ultimately the need for fewer mice.

B) Another refinement made to our neuroblastoma study was an adjustment to the criteria for measuring tumours. According to our original protocol, tumour size was monitored by measurement of the largest diameter. However, we found that the tumours generated by the neuroblastoma cells grew in an unusual flat elliptical shape, with very little depth. This meant that mice were being culled while tumour volume was relatively small, and there was not sufficient time from the start of treatment to the end point to gauge the affect of the treatment. In consultation with the Director of Animal Care, we were able to define a more appropriate measure of tumor size that enabled us to time to gather the data needed without any adverse consequence on the health of the animals. C) In relation to our study of gene expression changes implicated in immortalization of leukaemia cells, we applied for an amendment to use NOD-SCID TCRgamma Chain knockout (NSG) mice instead of NOD-SCID mice. NSG have a gene deletion that impacts on immune function to enable more efficient engraftment of human cells than occurs in NOD-SCID mice without this genetic modification. The application of NSG mice in our study resulted in engraftment of genetically modified myeloid cells in 100% of the primary recipient mice and most of the secondary recipients. This high rate of engraftment provided statistical rigor in the results, without the need for a repeat experiment and use of additional mice. (Reduction, Refinement)

Paediatric Preclinical Testing Program - Leukaemia

Where possible we run “double experiments” where we test 2 single agent compounds simultaneously. This allows us to use a single group of mice as vehicle controls for both compounds, thereby reducing by half the number of control mice required. Additionally we harvest the leukaemia cells from the spleens of vehicle control mice from any experiments we run for use in in vitro experiments conducted by the Leukaemia Biology program. This allows us to maintain a bank of cells within the lab and minimises the number of experiments we need to run solely to expand the in vitro cell stocks. (Reduction)

The neural substrates of extinction

At the level of experimental design, where possible, we strive to use within-subject designs that increase statistical power for detecting a null hypothesis, and therefore, reduce the number of subjects required to address questions of interest. At the level of data collection, we use procedures and parameters that have been refined through years of experience, and a very detailed understanding of the literature. This experience is passed on to new students in the laboratory through rigorous teaching and supervision. (Reduction)

Freshwater fish

Over the life of the project we refined electrofishing procedures and improved equipment, and thereby the potential for fish injury. Also through additional training of staff at workshops and seminars presented by internationally recognised experts in this field we were able to both improve sampling and reduce distress to fish.

We also changed the tag types and included the use of the smaller, less-invasive VI Alpha tags

VI Alpha tags are 3 mm x 3 mm x 1 mm thick fluorescent plastic tags that are injected using a fine syringe needle. They are less invasive than external dart tags and are particularly useful when dealing with sensitive fish species and those species unlikely to be caught by anglers. External dart tags provide a phone number for anglers to call if they capture a tagged fish and therefore need to be larger and more visible to anglers. We have had reports of fish that were tagged with external dart tags being captured up to 85 km upstream from their tagging location more than 18 months after they were tagged, so this method is useful. However, to proactively enhance animal care, we have switched to VI Alpha tags where possible. (Refinement)

Developmental origins and lineage tracing of mouse mesenchymal stem cells and Hematopoietic stem cells

Indirectly we are paying close attention to experimental design. Because of the dynamic of embryology and physiology, it is not possible to conduct research of true value to human health without use of animals. Nevertheless, we use in-vitro experiments extensively to supplement our understanding of the stem cells. In particular, in this project, we use cultured cells and a colony forming assay to interrogate the nature of stem cells in vivo. Furthermore, we are often able to conduct multiple experiments on tissue from a single animal. Due to our refined experiment techniques we have used less number of animals than we requested this year. (Replacement,

Reduction)

A metabolomic approach to brain receptor and transporter function III

We obtained and installed a new NMR spectrometer in 2013 equipped with a cryoprobe and refrigerated sample changer. This instrument gives considerably better signal to noise for our samples and we have been able to reduce the number of animals used per experiment by about 20%. (Reduction)

Determination of the acute effect of phenytoin or dofetolide on the developing rat fetal heart in vivo using ultrasound

The animals were used as planned. At the end of the protocol, four rats (2 males and 2 females) which were retired breeders surplus to the requirements of the experiments were transferred to the training ethics project. The males had performed their function in the study and the females had failed to fall pregnant after mating. (Reduction)

Metabolic profiling of the Kynurenine pathway in experimental autoimmune encephalomyelitis mice

We have used styptic powder to prevent excessive bleeding during tail bleed. We find that this method stops the bleeding much quicker than the conventional sterile cotton mesh pad. This minimized the handling time and allowed the mice to have a quicker recovery. (Refinement)

Establishing xenograft models of high-risk acute leukaemia for preclinical research

In an attempt to minimise the number of mice used we harvest the leukaemia cells from the spleens of vehicle control mice from any efficacy experiments we run for use in in vitro experiments conducted by the Program. This allows us to maintain a bank of cells within the lab and minimises the number of experiments we need to run solely to expand the in vitro cell stocks. (Reduction)

The role of the Basolateral and Central Amygdala in the representation of motivationally significant events

We make use of within-subject designs wherever possible (based on the scientific merit of between and within subject designs), use the appropriate numbers for studies based on statistical power, and make use, again where possible of reversible inactivation of brain regions, rather than induction of permanent lesions. (Reduction, Refinement)

Combining propranolol and vincristine for the treatment of neuroblastoma

Although we initially anticipated the use of 200 mice for this project, our results reached statistical significance and the study was interrupted after using only 137 animals in total. (Reduction)

Ecology of pack ice seals on the Antarctic Peninsula

Biopsying remotely using a darting system reduced stress in the seals (Refinement)

Little Penguin (Eudyptula minor) Conservation Genetics and Ecology in NSW

During the course of this project, we aimed at minimising the impact our surveys have on the penguins studied by first trialling the least invasive method to obtain reliable genetic samples. Unfortunately, shed feathers proved an unreliable source of DNA and lucked feathers yielded only limited amounts of DNA, whereas a very small blood sample (less than 100uL) gave reliably high yields of good quality DNA. Additionally, we are testing a method to estimate survival rated of penguins based on burrow occupancy rather than mark-recapture. We are currently analysing data from our study and a similar study conducted in Western Australia. We are also collaborating with other penguin researchers to make the most of data that is already available and minimise numbers of animals that have to be used in this project. This was also done by running a power analysis to find the minimum sample size that would allow us to reliably detect existing effects. As this project is focusing on wild populations of a native species, the use of animals will never be replaced completely, but we are refining methods and reducing our impact throughout the project. (Reduction, Refinement)

Training program on the proper care and use of animals in scientific research

Videos and on-line tutorials of trainings on acceptable laboratory animal handling and procedures are continually used not only to minimise the number of animals required for training, but also to refine these techniques. Mice used for training on injections at the ACE courses are fully anaesthetised, and were subsequently humanely killed while under anaesthesia to minimise pain and distress. A number of euthanased animals were re-used by other researchers either for other training purposes or for tissue collections. Excess animals from other approved projects (reason: wrong genotype, excess breeders, wrong gender, etc) were transferred to this ethics protocol to reduce the overall number of animals used in research at the establishment. (Replacement, Reduction, Refinement)

Regulation of cell structure and proliferation by the actin cytoskeleton

Where possible, we share genetically modified mice between projects. This takes place during tissue collections and isolation of cells for tissue culture. Furthermore, where possible we isolate primary cells from the genetically modified mice and perform experiments in vitro in order to minimise the use of mice. (Replacement, Reduction)

Muscle contraction and in vivo gene delivery

To reduce the number of mice in the project, we use both left and right muscles from each mouse: one muscle is contracted and the other muscle is the uncontracted control. (Reduction)

Regulation of glucose metabolism by the actin cytoskeleton

To reduce the number of mice in the project, each mouse is used for multiple non-invasive experiments and multiple measurements are performed on each animal (e.g., behavioural testing, glucose and insulin tolerance testing, blood collection, tissue collection for protein expression and localisation and tissue pathology). (Reduction)

Contribution of metabolic dysfunction to cancer cachexia and tumour progression

We have requested numerous modifications to use non-invasive imaging techniques to track tumour growth over time. This includes MR imaging, micro-ultrasound, and luciferase imaging. Utilising these techniques has drastically reduced the numbers of animals required, as we do not need to cull animals at repeated timepoints to gain data regarding the kinetics of tumour growth over time, and having paired measurements provides superior data. (Reduction)

Multifunctional Nanocarriers for Improved Treatment of Neuroblastoma

We have developed and characterized 3D tumour spheroids models and have shown them to be a superior system for screening nanoparticle efficacy in vitro. As such, a number of nanodelivery systems which may have orginally progressed to in vivo studies due to promising results obtained from traditional 2D tissue cutlure, have been excluded and as such, we have only progressed 2 systems to the in vivo testing phase. (Replacement)

Physiology Teaching Animal Practical Classes, (This application includes 6 practical classes involving animals)

We have always strived to reduce the use of animals. A number of classes, especially those practical classes involving a large group of students, have been converted to a computer simulated experiment with the advent of better audio-visual equipments and more computers in student teaching labs. For example, the "Skeletal Muscle" practical is done as a demonstration practical with 2 cane toads, thus reducing the number of animals. Even though, the student enrolments have risen, the requested number of animals is the minimum to enable the groups of students to acquire practical skills in, and understanding of the physiological systems being taught. (Replacement)

Development of RNAi Therapeutics for the Treatment of Non-Small Cell Lung Cancer

To reduce and/ or replace animal numbers we first investigate the toxicity and gene silencing efficacy of all our nanoparticles in a range of different NSCLC cell lines in vitro. These experiments are performed at least n=5 to give statistical power. Only after confirming which nanoparticles demonstrate the highest cell uptake and gene silencing efficacy in vitro do we consider testing their efficacy in vivo. This ensures that only the absolute minimum mouse numbers are used. (Replacement, Reduction)

Gene therapy to enhance cochlear implant performance

We have substituted animal use for a high-throughput cell culture model to optimise electroporation parameters and configurations. (Replacement)

Physiological significance of the transient receptor potential ion channels (TRP) in mouse cochlea

Personnel experienced with cochlear tissue were employed to ensure minimal animals were required for tissue extraction (reduce and refine protocol and animal number requirement). Tissue from aged wildtype C129/SvEV animals not suitable for this project was share used. We used cell culture to practise and refine the electrophysiology protocols prior to animal experiments (refine and replace). (Replacement, Reduction, Refinement)

Role of Gtf2ird1 and gene family members in Williams-Beuren syndrome

through refinement of our Golgi staining methodology, we have switched from using a cross involving Gtf2ird1 knockout mice with GAD67-GFP mice in order to visualize Purkinje neurons to a system that uses silver staining. This refinement has considerably reduced the amount of breeding and mouse production. As part of the same modification we decided to restrict our future studies to the mouse lines that have provided the maximum scientific value and cease work on those that were less informative. This has focused our activities and reduced our mouse usage to its minimum while still retaining maximum output. (Reduction, Refinement)

Activating a tumour suppressor for leukaemia therapy

With the lack of in vivo efficacy, we have started using an in vitro co-culture model where AML cells are grown on bone marrow cells to examine the reasons behind the lack of efficacy and to identify other agents that might act synergistically with PP2A activators.

This reduced the usage of animals. (Reduction)

Passive and Active Properties of the Retina

All of the data acquired as part of this study has contributed towards improved computational models of retinal activation. Ultimately this may lead to a reduction in animal usage. Further, sharing of tissue with other projects has led to a greater than 50% reduction in total numbers of animals used (exclusively) for this project. (Replacement, Reduction)

A novel method of studying spinal cord cysts: MR imaging in posttraumatic syringomyelia

We used the minimum number of animals required to evaluate the relationship between MRI and histologically measured syrinx size, and the longitudinal design minimised the total numbers required as animals were re-used at each time point. This new method for studying posttraumatic syringomyelia enables us to observe syrinx development and enlargement with a non-invasive technique in animals at multiple time-points. It will substantially reduce animal numbers required in future experiments. (Reduction, Refinement)

Alcohol, endotoxin and the pancreas

Reduction: We have optimised the method for stellate cell isolation so that an adequate yield of cells is obtained from one rat pancreas. This is in contrast to earlier work, where pancreas from two rats had to be pooled for sufficient cell yield. Thus, we have effectively halved the number of animals required per experiment.

Refinement: Cell culture methodologies have been refined in order to maximise the number of different experimental parameters assessed per cell preparation. This has resulted in a significant decrease in the number of cell preparations required, thereby reducing the number of animals.

(Reduction)

Novel Neural Interfaces and Instrumentation for Stimulation and Monitoring of Retinal Activation in a Vision Prosthesis

We utilised eight (sheep) cadavers from other studies and obtained cadavers from abattoirs for preliminary work we had originally anticipated to have required animals specific to this study. Further, data from the study has influenced computational models that will ultimately reduce numbers in the future. (Replacement, Reduction)

Predictive fear learning in mice

We have done this two ways. First, as always, our experimental designs are such that we use within-subject designs to reduce the number of animals used. Second, we are employing a measure of learned fear which is assessed in a completely automated fashion. This increases significantly the reliability of observations and will reduce the groups sizes required in future experiments to detect experimental effects. (Reduction)

Immune regulatory mechanisms in murine intestine

Use of colonoscopy allows us to follow individual animals longitudinally, when assessing colitis

An in vitro model of appendiceal regulatory T cells is being developed which may allow some murine experiments to be abandoned (but will still require a smaller number of mice to be sacrificed for derivation of cells). (Replacement)

Electrically excitable neuronal cultures for assessing biomaterial performance

A stable astrocyte culture was developed which reduces the number of male post natal mice required for the project. (Reduction)

MRP1, MRP3 and CXCR4 in neuroblastoma metastasis

Following ACEC approval, we have changed from administering doxycycline in the drinking water to using a doxycycline-containing diet. This is published to be more palatable to the mice, therefore avoiding any risk of dehydration. (Refinement)

The effect of acute injury on renal biomarker expression in adenine-induced chronic kidney injury

The use of animals is critical to this study. However, measures were still taken to reduce the number of animals used in this project. This was achieved by conducting pilot studies with smaller animal numbers until doses and time-course of chronic kidney disease were established. This was also achieved by consulting the literature carefully to avoid any repeat experiments. So far the pilot studies have been successful and the results will be used to increase sample size. Also careful monitoring of animals to ensure less impact and stress was undertaken, which meant no animals died or were excluded from the study. These refinements to the way animal research was conducted was pivotal in ensure less animal numbers were being used. (Reduction, Refinement)

Pharmacology Teaching practical classes

Over the past decade animal use has declined, through the introduction of computer practicals, however there is a need for a small number of animals to demonstrate important principles. Numbers are minimised by using a demonstration (audiovisual) aid, and by scheduling classes such that one animal can provide tissue to several classes.
The Department regularly reviews the educational value of all classes.
Moreover tissue is also offered to researchers to make the most efficient use of the preparation.
Animals are housed for the minimum time required. (Replacement, Reduction, Refinement)

The Effects of Chronic Nicotine Administration and Withdrawal on Measures of Impulsivity

Though validating the use of this new equipment prior to conducting a full experiment we are refining our approach and therefore the number of animals required in future experiments.

(Reduction)

DREADDS and the control of learning and motivation

At the moment it is not possible to replace animals in this project - the kinds of experiments that we are conducting using novel technologies can only be done in non-human animals. However, by using this technology we are refining our approach to manipulating the rat brain. In the past, for example, we would implant chronic indwelling stainless steel cannulae to pharmacologically manipulate a brain region. Our new DREADD approach refines this by removing the need for any cannula at all. In addition, we use within subjects designs to reduce the number of animals required. (Reduction, Refinement)

Gene therapy via close-field electroporation

We have substituted animal use for a high-throughput cell culture model to optimise electroporation parameters and configurations. (Replacement)

Staff training for experimental techniques and refinement of techniques used.

Where possible all mice that have reached their max hold time and are due to be culled are recommended for use for dissection or terminal procedures such as cardiac bleed to maximize the use of animals available without the need of using more. (Reduction)

The effect of high energy diets on learning and memory

In the laboratory, issues of refinement, reduction and replacement are addressed in two ways.

At the level of experimental design, where possible, we strive to use within-subject designs that increase statistical power for detecting a null hypothesis, and therefore, reduce the number of subjects required to address questions of interest.

At the level of data collection, we use procedures and parameters that have been refined through years of experience, and a very detailed understanding of the literature. This experience is passed on to new students in the laboratory through rigorous teaching and supervision. (Reduction)

Application of novel sutureless technology for eye surgery

Given the positive outcomes of the study so far, we have significantly reduced the number of animals. These reductions have been a consequence of a quicker healing time due to use of the technology and tighter mean samples reducing the number of animals exhibiting 'out-lying data' during the statistical analysis.

The project continues, investigating the gradual healing process within the now establish 0-24 hour time period. (Reduction)

Temperature, climate and the response of lizards with temperature-dependent sex determination

We house experimental animals in breeding groups, which reduces the number of males necessary for reproduction. (Reduction)

Replacement

- Video and images for demonstration of fish disease management.
- Generation of anatomical 3D models for the zebrafish to replace use of live animals

Refinement of animal use

- Use of observational only applications
- In 2013, there was an incident report received by the AEC regarding animals that had died as a result of an experiment involving a critical thermal minimum test procedure. The animals would have been experiencing high thermal stress prior to capture as there had been no rain in the Sydney region for approximately 2 months and the local bushfires had been underway for a few days. The changes to the procedure include a longer period of acclimatisation prior to performing these trials and providing the animals with the opportunity to rehydrate and increase body conditioning prior to experiments being conducted.
- Non-invasive monitoring techniques such as use of cameras for identifying habitat use by animals in preference to traditional trapping methods this has minimised the need for animal handling.
- Increasing use of target remote infra-red cameras to replace/supplement trapping for wildlife surveys and monitoring.
- Utilisation of advanced technology which reduces size and weight of tracking devices.
- Upgrades to animal housing facilities: Upgrades to Animal House Facility – use of a BAS system (building automation system). The BAS system will be used for monitoring temperature set-points and other variables such as humidity within the rooms. Alarm notifications will be improved. Installation of Ro water system to overcome issues with water quality and in particular issues with copper in the tap water.
- Improvements to housing and segregation of animals captured from the wild and housed, reducing the risk of introduction of potential pathogens to natural populations when animals are released back into the wild (respective government authorities permitting).
- Donation/Rehoming of animals unable to be returned to the wild to suitable organizations such as Zoos, Wildlife and Conservation Parks.

Reduction

- Continuous review of data obtained during experiments to refine our estimates of group variability and repeat power analysis to determine if sample size may be reduced in subsequent experiments.
- A number of researcher are utilising pilot studies to optimise animal numbers – often statistically significant results can be obtained with smaller numbers of animals.
- Data from previous studies are utilised to reduce the number of animals required.
- The establishment continues to encourage researchers to harvest and share tissues. In instances where animals have been humanely killed specimens are donated to the museum or other researchers.
- Sharing of tissues or storage of samples for re-use in future protocols where possible.
- Re-use of animals for research that would have been already in the laboratory for other research in order to reduce the number captured from the wild.
- Collection of samples such as hair, mouth/sternal gland/pouch swabs and blood from animals captured for other routine health checks minimising handling and use of wild animals.

  Blood samples collected from animals brought to the Animal Hospital by members of the public. In instances where a blood sample would normally be taken as part of standard prognosis and treatment procedures for wildlife.

Holding of Aquaculture Broodstock

No refinement was possible during this project; however a review of the project and its aims and

goals e.g. maintenance of a healthy brood stock has led to the following recommendation:

Future projects in which broodstock are being held for lengthy periods of time must incorporate factors which enrich the experience for the fish. Our fish were held in large tanks without any habitat features, structures etc. The inclusion of these habitat features must be incorporated in future projects.

Aquaculture Futures Initiative

Refinement: Application of improved husbandry techniques and holding systems for managing captive broodstock, including self-cleaning systems and water quality monitoring and alarm systems and use of nutritionally balanced diets. Water treatment systems to eliminate pathogens from incoming water.

Effects of repeat exposure of fish therapeutics on fish health

The results of this project that identified best disease management and treatment protocols for common diseases in Murray cod aquaculture will be used in all commercial aquaculture facilities to improve cod health. These practices will also be adopted for research stocks of Murray cod.

Techniques to REDUCE number of animals used:

Approved training protocol used animals that have completed their purpose in other protocols to REDUCE the total number of animals used.

Approval was given by the ACEC to transfer mice from one protocol to several other protocols to allow the use of animals in more than one protocol and thereby REDUCE the total number of animals used.

Techniques to REFINE procedures:

The research for Protocol X was approved in stages. An initial pilot study to ensure the research was viable and to REFINE techniques prior to continuing onto the main body of research.

The research for Protocol X was amended at the ACEC approval stage to change to whole body irradiation instead of just brain irradiation. This will enable researchers to gather more data using less animals.

Biostatistician Advice to the ACEC and Researchers

A biostatistician continues to support the ACEC and researchers throughout 2013 and into 2014 as our Biostatistician and advisor. He has provided invaluable advice regarding issues relating to the number of animals required for ensuring that valid statistical data are obtained from animal experimentation.

Wherever possible in pen studies control groups were shared between studies to reduce the number of animals required.

Replacement:

- Use of audio-visual material such as videos, slides, interactive computer programs;
- Use of abattoir specimens and cadavers;
- Use of plant tissue as a replacement for animal tissue for certain enzymatic assays;
- Routine husbandry procedures to be performed on animals coordinated with teaching

activities;

- Use of animals killed in road accidents.
- The use of differentiated stem cells rather than cells derived from primary cultures derived from animal tissue.

Refinement

- Improvements to animal housing and management;
- Training of researchers;
- Use of monitoring checklists to identify, action and report adverse events and the development of an adverse event form.
- The use of less invasive procedures e.g. sand pads rather than trapping.
- Use of an Observational Only - Field Research Form (No Trapping, Handling or Spotlighting).

Reduction

- Sharing of tissue among researchers;
- Obtaining more data from the use of fewer animals by combining objectives.

Close scrutiny of the numbers of animals requested in applications and progress reports to the Committee

---

The AEC constantly promotes the 3R's and is mindful of these principles when considering all applications:

Replacement: The Committee appreciates the need to minimise the use of animals whenever possible and aims to only sanction the use of animals where in vitro methods are unable to meet the requirements of a particular research investigation.

The Committee encourages preparatory in vitro assessments (including the use of animals/tissues from any animals euthanized for other reasons) and literature reviews to determine whether what they are considering has not been done before.

Reduction: When planning animal studies, bio-statistician input to ensure minimum animals are used to achieve the anticipated outcome.

Careful planning by researchers to breed only sufficient animals for experimental use and to maintain the line.

Sourcing animals externally rather than breeding in house.

Use of pilot studies to validate a process or procedure and if successful, be able to utilise the results as part of the overall study.

Refinement: Appropriate environmental enrichment and appropriate housing for the species eg. Paddocks versus pens for sheep. Effective monitoring to detect pain and distress and provide appropriate treatment to minimise suffering following surgery.

- Replacement

  1. Development of *'in-vitro'* assays in place of animal testing.
     'In-vitro' assays have been developed as a replacement to animal testing.
     Example 1: ELISA based *in vitro* assays have been developed for *Clostridium septicum, Clostridium botulinum* which traditionally required animal models for testing.

- Reduction:

1. Reducing number of animals in a study should be considered in line with valuable scientific outcomes and animal welfare impacts. The establishment has recently developed a single pig challenge model for erysipelas in pigs in which multi strains were tested on animals (pigs) using published literature. This sort of approach will minimise number of required for vaccine efficacy trials in the future.
2. A Biometrician is always consulted to adequately power each and every study in order to avoid repetition of poorly designed studies that will results in extra animal use.

3. Minimise animal numbers while maximise their use. Studies are designed in such a way that negative controls are built within each treatment group, so that extra group of negative controls can be avoided. For example, when two vaccines containing different antigens are assessed, one of the groups will be a negative control to the other group and vice versa.

4. The project team consists of scientist, biometrician, veterinarians and regulatory colleagues who will perform rigorous assessment of each and every animal study to ensure the study designs are scientifically sound.

On-going techniques to minimise the impact on animals in teaching include:

Replacement

- Use of cadavers – leg parts of horses sourced from local abattoir for use in shoeing and/or hoof health in equine studies.
- Mannequins, audio-visual materials, taxidermed and preserved specimens were used as substitutes for live animals
- Ear-tagging of sheep is practised on cardboard and leather.
- Injection pads used to practise medication injection for a range of species.

Refinement

- Prior to field work activities, students are familiarised with both the animals and the research techniques to be used. This includes visits to zoos, aquaria and museums, demonstrations of the use of equipment and DVDs showing the use of the research methods. Actual field work is kept to a minimum.
- For native animals, handling is by the licensed person only, with students observing the techniques.

Reduction

- Keeping a minimum number of animals on campus required to simulate a mini colony.
- The number of occasions that an animal is handled is minimised e.g. lambs are tagged and drenched at the same time to avoid having to re-capture.

Additional strategies identified during 2013:

Replacement

- Pegs used to simulate fingerlings when teaching counting and bagging techniques.
- Use of heads of sheep, cattle, goats and pigs, sourced from abattoir, for use in assessment of the effectiveness of humane destruction of animals.
- Use of pig fat to practise injection techniques.

Refinement

- During shearing training the learner shearer begins by shearing only part of the sheep, with the professional shearer completing the clip, to reduce handling time, injury and stress.
- Horses are monitored for behavioural changes and replaced regularly. Horse usage is rotated to prevent overuse.

- Instruction to students on their obligations and responsibilities with regard to animal welfare during enrolment/induction period. Students are required to provide written acknowledgement of their understanding.

Reduction

- Simulated penning of sheep by demonstration.

Use of ultrasound equipment to capture images (cattle) for replay to students.

Animals are re-used where possible. In particular many animals euthanized after reaching a pre-determined study end point have had tissues taken for histological studies different to the primary study in which the animal was used. Cadavers are kept frozen/formalin preserved for 1-2 months following euthanasia for the opportunity to re-use the animals for histological studies.

Refinements

- Performing pilot studies to determine potential problems in an experiment.
- Minimising variables such as disease, stress, diet, genetics, etc., that may affect experimental results.
- Early identification of pain and distress by preventative approaches e.g. careful planning, and taking action for relief, or early treatment.
- Receiving adequate training prior to performing a procedure
- Using appropriate anaesthetics and analgesics for potentially painful procedures
- For rodent oral gavage procedure, use of flexible plastic feeding tubes over rigid metal tubs.
- Scheduling timing of surgeries for proper post-operative monitoring (not too late during the day and no surgery on Fridays)
- Use on antibiotics in human cell lines culture to avoid contamination and resultant ill health in injected animals
- Score sheets for all monitoring during approved surgical procedures have been introduced to refine the process and appropriately identify and manage pain and distress in the animals.

Reduction

- Meetings (and/or open communication) with research groups to discuss colony management to ensure breeding is optimised for experimental or maintenance production only thereby minimising the generation of unrequired animals through breeding strategies.
- Performing appropriate literature searches and consulting with colleagues to ensure that experiments are not duplicated
- Designing studies to utilise animals as their own controls and consulting with a statistician to use only the numbers of animals required to achieve significance
- Tissue sharing encouraged and promoted throughout the facilities
- Data and resource sharing – archiving of genetically modified mice thru cryopreservation also facilitates transfer of frozen mouse sperm and embryos to other facilities or collaborators; less breeding for seldom-used strains; animal tissue sharing between research groups whenever possible
- Use of in vitro fertilization (IVF) techniques to obtain two-cell embryos for freezing rather than breeding up the colony to achieve enough number of female mice for superovulation and later embryo collection
- Use of audio-visual aids, instead of live animals, for education purposes

Replacement

Consideration to be given to performing experiments in cell/tissue culture models

- Refinement: Further utilisation of Go Pro Cameras, baited camera traps or infra-red cameras has occurred in both our terrestrial wildlife research and fisheries research to reduce the amount of handling and stress to animals.
- Reduction: In some field research applicants have been encouraged and successfully used the minimum number of replicates required to ensure scientific validity. More use of automated recording devices to detect rather than capture frogs has also helped reduce the use of animals. For first Year biology classes, the Principle Investigator has refined the teaching activity by placing students in groups which has also helped reduce the number of animals observed.

Furthermore, six sea mullet were transferred from research ARA On-going Holding of Sea Mullet Broodstock, which is now completed to the AEC approved teaching activity, ARA Sampling Fish for Use as Classroom Specimens. This transfer was approved by the ACEC.

Replacement

An in vivo model system which closely resemble human physiology was utilised after preliminary investigations examined signalling in 400 human cancer tissue samples and 15 human cancer cell lines.

Subsequent to gaining approval from the Animal Care and Ethics Committee a search of the literature found that alternative strategies could be used to answer the research question. This made the study possible in humans negating the need to use animals to answer the specific research question upon which the approved study was based. As a result the animal based research did not proceed as it was considered unethical given that there was a viable alternative.

The justification for using an in vitro assay to measure the potency of batches of an oral vaccine was strengthened by a project.

Refinement

Ongoing refinement of face mask anaesthetic administration to eliminate dead space and maintain physiological blood gasses.

A new stroke model was used to provide smaller strokes with shorter surgical times and better post anaesthetic recovery.

The identification of an improved stroke model during a project with a reduction in the time under anaesthetic, less impact on the animals and a reduction in number of animals used.

A reduction in the number of animals through the use of computer simulations, videos and the provision of data in preparation for a teaching course.

The initial use of cell lines and fresh melanoma isolates to test a hypothesis and only then applying the knowledge gathered from in vitro studies in animal models.

Animal numbers were reduced by 50% by utilising within animal controls. Endpoints for the protocol were set so that mice were euthanased before the tumour burden became high enough to compromise the well-being of the animal.

A reduction in the number of murine melanoma cells injected subcutaneously per mouse to minimise the impact of tumour burden on the animals.

The modification of a procedure for cannula implantation considerably improved the durability of implanted cannula and reduced adverse impacts on animals.

The potential impact on animal welfare was reduced by well trained and highly experienced researchers conducting all of the procedures and animals being constantly monitored according to the specific monitoring checklist for each experiment.

Advancements in surgical techniques led to shorter anaesthesia times and reduced surgical trauma.

The use of a new endoscope to carry out colonoscopies on mice assisted in the refinement of animal experiments by allowing for the measurement of the severity of inflammation and damage to the animals colon at various time points throughout the experiment. Previously, the severity of

disease in animals could only be determined by using indirect observations (weight loss and appearance/behaviour) and could not actually quantify the amount of damage to the GI tract until the experimental end point.

Animals were unable to be provided with analgesics during the progression of a disease as a project was testing an analgesic formulation, however providing environmental changes reduced the impact of the disease on the animals.

Training was provided for all staff on a protocol to refine procedures and reduce adverse impacts on the animals. All animals were monitored closely at the beginning of new experiments for any adverse impact.

The formalin test was optimized by using a very low formalin dosage not previously used in the literature.

The use of light and short isoflurane anaesthesia reduced struggling and the amount of physical restraint needed for the injection of dunnarts.

Anaesthesia can carry its own risks to an animal as it is a longer procedure and involves more manipulation and potential stress to the animal. The use of anaesthesia was eliminated from a protocol in order to enhance the safety and well-being of animals during the pre-sensitisation of mice to TNBS colitis. Mice could be safely shaved using the Wahl Bella Cordless trimmer while being held firmly in a scruff.

A mechanism was used to achieve a more accurate assessment of endoscopic scores while minimising the duration of anaesthesia exposure. This improved the efficiency of technique, while protecting animal safety and welfare.

Changing to the oral administration of finasteride and ganaxolone from an injection reduced discomfort and stress on the animals.

Photographs of dorsal colour patterns were used to identify individuals instead of using PIT tags due to the low number of bell frogs detected during surveys last season. This meant that animals did not need to be captured and micro-chipped for identification.

The refinement of surgical techniques (e.g. modification of analgesics, use of antibiotics, improved monitoring of sterilization efficacy, refined surgical procedures) and post-surgical care routines (e.g. targeted saline administration, electronic heat pads for mouse temperature maintenance, refined bladder expression techniques, improved post-surgical monitoring) successfully improved the survival and recovery of animals as well as significantly reducing the number of adverse events.

Reduction

There were significant reductions in the number of animals being used by attaining more parameters per animal.

The identification of an improved stroke model during a project with a reduction in the time under anaesthetic, less impact on the animals and a reduction in number of animals used.

A reduction in the number of animals through the use of computer simulations, videos and the provision of data in preparation for a teaching course.

The initial use of cell lines and fresh melanoma isolates to test a hypothesis and only then applying the knowledge gathered from in vitro studies in animal models.

Animal numbers were reduced by 50% by utilising within animal controls. Endpoints for the protocol were set so that mice were euthanased before the tumour burden became high enough to compromise the well-being of the animal.

A high proportion of students in the course studied invertebrates which minimised the number of higher order animals used.

Mouse lung primary fibroblasts were used for FBLN-1C inhibition in order to optimize FBLN-1C AO concentration.

Running experiments at the same time and sharing controls across experiments cut down on mouse numbers.

The use of fewer animals through the assessment of neural, immune and reproductive measures

in one animal.

While working up/optimising anatomical techniques, tissue was collected from another approved protocol post-mortem. This tissue was not optimal for the group's experiments, which is why it was not always used, but it allowed for the development of techniques without the unnecessary use of animals.

The number of animals used was reduced through the use of the AIA model, which is the standard model used for arthritis research, as this model is reliable and predictable.

Control animals were shared between experiments to reduce the overall number of animals required.

A method was developed which allowed one euthanised mouse to be the source for at least two experiments.

The use of the same animals to answer different research questions by taking the heart blood and brain of the same rat for cytokine assessment and cFos Immunohistochemistry.

Multiple experiments were performed on sperm or eggs derived from the same animal. Cryopreservation protocols were tested and separate experiments were conducted on androgenesis, IVF or nuclear transfer procedures were undertaken on the same batch of sperm/eggs.

The number of mice that were going to be used was reduced by replacing adult females with juvenile females. More oocytes could be obtained from juvenile mice and protein extraction techniques could be modified so as to require less oocytes and therefore less mice.

Tissue was shared with another research group and was stored for future use.

All of the procedures conducted as part of a protocol were conducted by personnel experienced in all of the techniques. This maximised the data generated from each animal, thereby reducing the overall number of animals required for the study.

The adoption of new protocols for collecting RNA and purchasing/using a new more sensitive qPCR machine optimized micro-array and gene expression characterization which reduced the number of animals required.

An additional complementary experimental system to investigate the relative importance of the pT286 and pT253 pathways in stroke damage has been developed. This involves using cultured neuroblastoma cell lines (derived from a nerve cell cancer) and allows some types of experiments to be carried out without using animals.

The development of a microslice model to study early events initiated by ischaemia greatly reduced the number of animals required.

REPLACEMENT

<u>Tissue Sharing:</u>

- Animals that were scheduled for approved euthanasia were made available for tissue harvest, new technique training or post mortem technique training.
- At the conclusion of appropriate research protocols, some animals were retained for animal handling training.

<u>In vitro technology:</u>

- Animal use was reduced to zero for the final 12 month reporting period of an approved protocol. In vitro stem cell technology was employed to investigate insulin secreting pancreatic cells despite having authority to conduct the research using live animals.
- Harvesting nerves at the conclusion of approved, non-recovery experiments to facilitate nerve transmission studies in tissue baths.

REFINEMENT

<u>Monitoring:</u>

- On multiple occasion, monitoring record sheets were requested to be tailored to individual

protocol requirements

- The AEC now requests a minimum number of 2 personnel be listed for animal monitoring on all protocols

Enrichment:

- Wood blocks
- Seeds
- Straws
- Substrate maintenance
- Companionship for vasectomised mice

Training:

- Rodent handling
- Injection techniques
- Anaesthesia
- Monitoring – anaesthetic and post-operative

Procedures:

- Multiple experimental procedures were applied during non-recovery experiments to increase data yield per animal recruited.
- Common procedures were standardised and endorsed as AEC SOP's
- Following a request for justification to recruit multiple models of glaucoma into a proposed research protocol, approval was given for a single model of glaucoma.
- Drug doses on a proposed application for research were refined from a 10 fold range to a single, precise dose.
- Proposed cull criteria that included the loss of 20% in body mass in growing animals was refined to 10% weight loss in aged matched controls.
- A proposal to recruit multiple models of inflammation was refined to approve a pilot study involving a single model of inflammation. Approval for additional models would be assessed following provision of pilot study results. Similarly, justification for 3 models of optic nerve injury was sought prior to approval.
- Pilot studies were recruited for multiple new surgical procedures to assess impact before exposing larger numbers of animals to potential unknowns.
- Shark tagging procedures were refined to utilise a smaller tag whose process of application had significant welfare benefits when compared to the superseded procedure. Similarly, the Committee regularly recommended the use of NanoTransponders over the traditional, larger microchips
- Justification for a proposal to collect 150microL of finch blood was requested and ultimately refined to 100microL.
- Justification for a proposal to automatically repeat an invasive ocular procedure after a set period of time was requested. The AEC refined the procedure to be repeated only after a measured drop in ocular pressure was detected.
- On multiple occasions, the AEC sought clarification on the feasibility of reducing the number of repeat anaesthetics by incorporating more than one procedure per anaesthetic.
- The AEC refined historical disinfection protocols using only alcohol to now incorporate more effective disinfection (chlorhexidine in alcohol)

REDUCTION

Numbers:

- Genotyping and cryopreservation of experimentally significant ENU mutations has reduced

the number of animals required to maintain breeding colonies of these strains.

- The proposed number of animals to be used was questioned when it seemed the logistics and practicality of performing the experiment was the limiting factor to how many animals would be required.

Replacement

- Program appointed a staff member to review the use of animals in teaching. The outcome of this review has resulted in the following:

- The toad gastrocnemius practical being replaced with a muscle stimulation and fatigue activity developed by ADInstruments that the students perform on themselves with their consent.
- The rabbit smooth muscle practical being replaced with an ADInstruments laboratory using gut smooth muiscle of earthworms to demonstrate principles of pharmacology. The school has spent $60K purchasing new equipment to run this practical. In addition, there has been considerable time invested by staff in getting this prac up and running for TI. Earthworms are readily available and an appropriate anaesthetic protocol is used.

Reduction

- AEC has requested that animal facility reports include the proportional wastage of animals (i.e. those produced by breeding colonies that were humanely killed as not required for research purposes). This has formed part of the AECs attention to its role of overseeing the potential wastage associated with in-house breeding colonies.
- In 2013, the AEC focused its attention on rodent breeding projects and after reviewing the number of animals used for experimental purposes presented as a percentage of the total number of animals produced for a breeding colony, discontinued the approval to breed hopping mice at the University. This continued focus on the wastage associated with in-house breeding programs has significantly reduced the number of animals being used by the University.
- The University continues to encourage researchers to harvest and share tissues in instances where animals have been humanely killed.

Refinement:

- The AEC worked with researchers to develop a refined general anaesthetic protocol for skeletal muscle function testing – a non-recovery procedure. The outcome of this project was that the anaesthetic protocol was considered so successful that it was also adopted for recovery procedures.
- A new practice of castrating male mice that were excess to needs (i.e. would otherwise have been humanely killed) was developed. The castrated mice are used as companions for entire males that were traditionally housed in social isolation due to concerns of aggression between entire males.

The AEC has continues to request conditions of approval to include the presence of either the Animal Welfare Officer or Animal Facility Manager (a Veterinarian) to oversee high impact or new scientific procedures at the University.

- The use of microtracers (RF-Tracers, Micro-Tracers Inc, Tall Bennett Group Pty Limited, Mona Vale) has enhanced assuredness of feed identity at the pen, providing a rapid check on correct feed allocation to treatment groups. This approach limits the need to repeat trials that have ambiguous results.
- Enrichment of pens with perches may improve bird leg strength and is encouraged for use in trials requesting use of layers and considered for broilers.
- Careful selection of birds from donor flocks that possess/lack maternal antibodies arising from vaccination or wild challenge for certain diseases.
- Use of a litter moisture measuring device to provide more objective readings and is convenient

to use, thereby allowing faster implementation of corrective actions to improve bird health and welfare.

An individual sample weighing for large birds has also be used to decrease stress on birds when handled at larger weights.

Research involving the use of cats and dogs in NSW was undertaken in association with active Veterinary cases and made use of excess from routine samples as far as possible. In this way the use and impact on animals was reduced.

Example of replacement

- Use of computer simulation in subject in place of rats and cats.
- Use of road kill in first year practicals
- Use of fish from fish market and existing fur pelts for heat transference models

Examples of techniques adopted to refine procedures

- Use of remote underwater video instead of trapping and releasing fish as a less intrusive research method.
- Use of pilot studies to refine techniques before large numbers of animals are used.

Examples of techniques adopted to reduce the number of animals used

- The practice of sharing tissue from deceased rats and mice with other researchers eg blood, skin, brains, lenses, livers and hearts.
- Transfer of unused animals between protocols instead of ordering additional animals.

Training protocol makes use of excess rats and mice that have not been used for experiments and that would otherwise be euthanized to train researchers in various techniques, thus minimising number of animals required in their research applications and providing Certificates of Competency.

When projects are assessed by the Committee they pay particular attention to justification for animals use.

In the majority of applications that this committee assesses (primary production) the opportunities for replacing the use of live animals is limited. As a consequence, the Committee points out to each researcher their responsibilities under the Code and the Animal Research Act.

When assessing projects the Committee takes the view that if there are procedures that can minimise adverse impacts then they must be used unless it can be justified that the procedure would invalidate the experimental data. For example, the Committee requires SOPs to be adhered to for all procedures that are not specific to the project (such as blood sampling or condition assessment), and in situations where a procedure is unique to a project that the investigators write an SOP if the procedure is repeatedly used.

The Committee requires that all projects include biometrical support from a qualified biometrician to reduce the likelihood that planned experiments will lead to ambiguous results. The requirement of obtaining biometrical support has resulted in principal investigators having to clearly justify the number of animals needed and provide evidence to support the experimental design and sample size.

- Close monitoring of animals and development of monitoring checklists to identify adverse reactions in animals. The AEC will place conditions on projects at the approval stage to ensure that any pain or distress to animals is alleviated quickly in projects where it is impossible to eliminate this completely.
- Use of experienced veterinarians and other staff.
- Restraint time and dose rates kept to a minimum.
- Adoption of less stressful methodologies.
- Suitable housing provided and maintained including controlled environment facility.
- Use of adjuvants known not to produce adverse reactions.

- Procedures used routinely so that animals become accustomed.
- Procedures performed under anaesthesia or sedation when appropriate.
- Close scrutiny of the number of animals requested and Biometrician's comments reviewed to ensure numbers are adequate to obtain the desired statistical outcomes, to minimise the number of animals involved in trials and to ensure that trials do not have to be repeated unnecessarily.
- Reduction in number of animals used – researchers in a protocol have moved to PCR to reduce the number of animals used.
- Re-use of animals – researchers in protocols have transferred rabbits to other research institutes for possible future use.
- Close scrutiny of the volume of blood collected.
- Use of the saphenous vein method as the standard technique for blood collection in rodents.
- A number of studies conducted on animals at the owner's property to minimise any possible stress.
- Similar studies have shared the same control animals.

Environment enrichment has been introduced for pigs and rabbits.

- A current asbestosis study is using the transgenic mouse strain, MexTAg299. The MexTAg299 mice develop mesothelioma more rapidly than wild-type mice after asbestos exposure, with 100% incidence (as compared to 30% incidence in wild-type mice). Thus, use of this transgenic mouse line will give this asbestos study a greater chance of success, using a smaller number of mice, over a shorter study period, than if wild-type mice were used.
- For a mouse breeding protocol, the dam was re-mated if the previous pregnancy was not successful, instead of purchasing additional breeders.
- A current detector-dog study involves the use of several dog and handler teams at each trial, which reduces the amount of work and time required from each animal. The trials take place at the training unit, which is a familiar location for the dogs. The dogs are monitored constantly for safety concerns and stress. The handler determines whether the dog is capable of the tasks required of them on the day, and if they are deemed incapable, the dog is not used for the trials scheduled that day. Regular communication with the dog handlers ensures the most reliable methods of investigation are used for the trials.
- During the conduct of another detector-dog study, additional students supervised by the same Investigator were encouraged to attend and observe the training sessions and use the observational data in future experiments, thus reducing the requirement for future additional studies**.**
- In a mammalian wildlife study, existing relevant literature was reviewed for possible replacement opportunities prior to sampling any mammals. Appropriate sampling techniques were also identified in order to use as few mammals as possible and to minimise stress to any mammals that were used. Following mammal sampling, a post-hoc power analysis was performed to determine if the sampling design was adequate to correctly detect any differences between habitat types. In doing so, the research team could adjust the design as a compromise between maintaining statistical power and reducing the number of sites used and animals captured. At all times, the welfare of any captured mammal was supported: this included the provision of bedding material in each trap and protection from adverse weather with the use of heavy duty plastic bags; trapping was not conducted in extreme weather conditions such as in heavy rain; all traps were checked within two hours of first light and closed during the day. Because of this time allowance, only four sites were sampled at a time so that all traps could be checked within the given time frame; handling times were kept to a minimum and conducted by a competent handler. Fur cutting was chosen as a temporary marking technique as this method does not cause injury to the animal. No mammals were removed from the site of capture or held for prolonged times.
- In one study assessing the benefits of a test dietary supplement, the researchers noted that as much data as possible was generated using cultured endothelial cells prior to "proof of concept" in-vivo assessment in laboratory rodents. The researchers consulted the consultant biostatistician for assistance in determining the appropriate sample size for the different groups

in the study. This ensured the study used the minimum number of animals required to provide statistical significance.

- Researchers conducting a wildlife study stated that there were no alternatives to animals for their project, however it was noted that only those species that were biologically suitable for the study were used. The minimum number of animals was used to ensure statistical and scientific validity for the study. Handling time was brief to minimise distress to animals, and all animals were released at their site of capture.
- In a study investigating spinal injuries, the Chief Investigator advised that she has developed tissue culture models which can partly replace animal experiments; furthermore, the procedures had been refined through a reduction in the severity of the induced spinal injury. Tissue is made available to other researchers, and unaffected mother rats may be transferred to animal-facility protocols for training purposes after the pups are used.
- In a diabetes study, the researchers advised that, using methodologies refined over several years, the team now achieves a consistent incidence of type 1 diabetes of 80%. This allows the team to accurately predict the numbers of mice required for each treatment group, thus reducing the number of animals required overall to attain significant data.
- In a study investigating the physiology, ecology and behaviour of the seadragon, *Phyllopteryx taeniolatus* the research team maximises *in situ* observations and molecular analysis of these fishes to generate their data. The molecular analysis of seadragons has been refined so as to require sampling of as few animals as possible while still providing the project with sufficient data to analyse the phylogeography of the species and therefore expand on the ecological understanding of these fish. Great care and consideration has been placed on the best techniques to obtain genetic samples from seadragons. As members of family Syngnathidae are protected, the team utilise the most efficient manner of sampling, which is to take an appendage clip; this minimally invasive process requires no animals be sacrificed for muscle tissue, and is quicker and faster than taking a fin clip (Planas et al 2008).
- Researchers conducting a fish ecology study advised that they had been able to reduce the number of treatments in the relevant controlled spearing experiment; this reduced the number of individuals that required spearing (32 versus 42), while still yielding publishable results. In the controlled spearing experiment, we selected juveniles of a commonly-occurring species which exhibits high natural mortality; the study's induced mortality (32 individuals) was therefore insignificant to wild population size. The aquarium experiment involving further spearing events was abandoned once it became apparent that the study would not yield substantially different information from the field experiment. This reduced the number of individuals used to a lethal endpoint by 180. Great care was taken in surveying the organisms while using the seine net. The organisms, especially on a hot day, are placed in buckets fitted with aerators to minimize any undue mortality. Species that are protected such as the *Stigmatopora argus* are usually processed and returned to the water first as well as those known to be more fragile.
- Another fish researcher advised that the team had increasingly been using underwater cameras to reduce collections/negative behavioural effects of divers on fishes, and that survey techniques were continually refined to reduce any negative effect on fish behaviour. In other studies, the researcher had been able to refine the project to focus on two of the most common fish species of interest, thus avoiding the need to involve all the species originally proposed; the team had utilised a limited number of specific sites as replicate areas for surveys and collections – which had reduced the overall impact of the project on fish communities in Sydney.
- In a study involving wallabies, the Chief Investigator reported that the team had been able to reduce the time animals spent in cage traps and handling time, and they had also improved the design of datacollection collars to reduce the impact on the wallabies, and the time required to attach them.

In a study investigating liver disease, the researchers advised that excess female mice generated from the breeding phase were allocated to the training protocol in the animal house. Furthermore, through refinement of the experimental protocol, less liver tissue was now required for the experiments, which had halved the number of animals used under this protocol in recent years.

Along with the establishment's continued review of our Standard Operating Procedures, the main focus

of the AEC in 2013 was the review of the Fauna Survey Standard Operating Procedures (SOPs) Manual and the assessment of 'high' risk projects (i.e. those not included in the SOPs)

The AEC reviewed the changes to the 8th edition of the Australian Code for the Care and use of Animals for Scientific Purposes for incorporation in operating practices.

Due to an increase in anoxic conditions for aquatic fauna assessments reported during the year, the SOPs for bait trapping were amended to advise in waters with low oxygen levels, where other trapping methods have been exhausted, the use of bait traps is permitted.

The SOPs were amended to highlight the importance of only using gill netting as a method should all other options be exhausted. In addition, the AEC conducted an audit of a high risk project.

Replacement: The Committee continues to maintain a Biological Non-Human Tissue Database through which researchers are able to share excess tissue, thus replacing the use of live animals with the use of stored tissue. In addition, to make these tissues more widely available, the Committee has joined the Ethitex tissue sharing database which facilitates tissue sharing throughout Australia.

Refinement:

- The Committee continues to encourage researchers to undertake a pilot study if the impact of the proposed study interventions on animal health and well-being is unknown.
- Animal House veterinary managers review protocols with researchers in order to optimise anaesthesia protocols (including monitoring) and analgesia.
- Scoring systems for monitoring of experimental animals have been developed and refined, with the aim of minimising potential pain and distress that animals may experience as part of certain research related procedures.

Reduction: The Committee has minimised animal usage by the following techniques:

- Careful scrutiny of animals requested to ensure that sufficient numbers are used to provide a statistically valid result, thus preventing the need for repeat experiments and use of additional animals,
- Approval of new techniques for embryo freezing rather than continuous breeding to maintain lines,
- Re-use of animals, where appropriate, after extended recovery interval,
- Making surplus tissue available through a Biological Non-Human Tissue Database and seeking prior agreement from investigators to make surplus tissue available,
- Consolidating breeding protocols to ensure no over-breeding which in turn reduces the need for culling.
- Rederivation: Animal facilities optimise fostering process and thereby minimise the numbers of female mice used for fostering purposes.
- Training: Animal facilities use mice for training purposes that were identified with an undesired genotype (hence would have been euthanized regardless).

Sharing: Where possible, mouse lines are shared between different research groups to avoid unnecessary breeding.

Replacement

In February 2013 an adviser on Alternatives to Animal Research joined the AEC. The AEC has requested a number of researchers meet with this adviser when possible replacements are identified at the time of new protocol submission. This has allowed some elements of animal experimentation to be replaced; or has instigated a side by side study with the intention to move to a non-animal model once results can be obtained accurately through a non-animal model.

Reduction

The Australia Phenomics network is utilised for the cryopreservation of mouse strains that are no longer required or will not be required for an extended period of time. This securely maintains

strains for future use and avoids the unnecessary breeding of rodents to solely maintain a strain.

Prof X developed a new fluorescent target array assay. This assay greatly decreases the number of mice required to test a hypothesis. A publication on this work is currently under submission with the 3Rs Journal Altex.

The establishment and ACT government has purchased new small animal imaging equipment. The optical imager and micro CT will reduce the number of animals used by allowing imaging of tumors at different time points, previously mice would have to be allocated to every time point and sacrificed at that time. Each imager has its own isoflurane vaporiser to safely maintain anaesthesia during imaging. The equipment is available for use by all researchers in the ACT.

Refinement

New imaging equipment as mentioned above.

Mandatory training for all staff and students working with animals continues to remain in place.

Buccal swabbing is being used for the collection of DNA from amphibians and reptiles in the place of toe or tail clipping.

There is a continued requirement for the use of clinical monitoring score sheets and analgesics.

The availability of isoflurane anaesthetic set ups has increased; with the majority of anaesthesia now undertaken with isoflurane.

Examples of refinement

1. Projects with novel animal models, published/established animal models being used for the first time, and/or by a research group without any experience in a model, are required to undertake pilot studies to demonstrate that the animal model functions as expected prior to commencing treatment experiments.
2. Requirement for in vitro toxicity and/or efficacy data prior to giving permission to place biomaterials into animals or administer experimental treatments.

Examples of reduction

Retired male and female breeders and stud males used as source of pancreatic islet cells in another project

1. Sourcing of external professionals to assist and/or teach in new and/or modified techniques
2. Regular review of procedures via various industry communities such as ANZLAA and ANZCCART
3. Provision of animals with unwanted genotype to research groups for training purposes
4. Development of In-Vitro techniques to replace the use of animals
5. Improved peri-and-post operative analgesia to reduce pain from surgery
6. Compulsory awareness training for use of environmental enrichment

Communal tissue banking platforms for access to tissue outside of the establishment.

The methodologies for projects are generally constrained by the regulatory requirements for pre-clinical testing of human therapeutics. Nevertheless, the Company always looks to implement these improvements in animal use. Examples include:

- The Company has replaced the standard guinea pig skin sensitising maximisation test with the local nymph node assay), which represents a significant improvement in terms of animal welfare. The advantages of LLNA over the guinea pig skin sensitising method are:
  - It greatly reduces animal trauma and discomfort, being considerably less invasive then the maximisation test which involves repetitive shaving, dermal application and intradermal injection of Freund's adjuvant.
  - It has a shorter test duration i.e. of 12 days, which includes 5 days acclimatisation period and 6 days of actual treatment.
  - It potentially uses fewer animals.

Sighting studies using small numbers of animals are routinely carried out to determine the optimal

dose levels of test items before a main study involving greater numbers of animals is initiated.
Method development to assess multi-stage blowfly larval implants on sheep skins and on sheep to develop a new product to provide rapid speed-of-kill in conjunction with long term protection against sheep blowfly. Setting up a new model with multiple stage larvae there were a few unknown factors which included how the larvae would behave with multistage larvae together, how big the strike would get, how aggressive would the strike be, how long it could be run before having detrimental effects on the sheet etc. Skins were used instead of sheep and the strikes as single stages individually and in combination (multiple stages) could be studied and run for a longer time period without causing harm. Being controlled and on skins it was possible to set up more strikes than if they were put on the sheep. This has resulted in a partial replacement, as well as reduction in the use of animals and the ability to screen more compounds in a shorter time frame which constitutes refinement.
• Continues use of remote controlled infrared digital cameras and acoustic recording devices instead of, or in addition to, trapping to detect species presence of absence. • Researcher training in microchip insertion continued. • Phasing out of toe clipping in small animals. • Trial of unmanned aerial drones for conducting waterbird surveys rather than manned aircraft (noise) or foot-based surveys (disturbance to habitat). Use of secure outdoor enclosures furnished with native habitat for rearing endangered frogs in the location of their natural range instead of the artificial environment of indoor tanks at breeding facility.

Appendix I: ARRP expenses

Note: The following figures do not include the time and costs incurred by individual ARRP members—and met at their own expense—for work such as maintenance of the Animal Ethics Infolink website, planning for the AEC members meeting, and input into the development of guidelines. In addition, support provided to members by their employing establishments (e.g. salaries paid by government departments for their employees' time spent on ARRP business) is not included in the figures.

Fees and retainers	**2,306.30**
Travel and subsistence	**1,504.48**
Stores (including catering) and printing	**1,523.53**
Freight and postage	**1,248.46**
TOTAL	**6,582.77**

Appendix J: ARRP policies and guidelines

(Available from http://www.animalethics.org.au)

Policies

2. Payment of External Members of Animal Ethics Committees (revised 15/5/2009)
3. Procedures Prohibited under the NSW Prevention of Cruelty to Animals Act (revised 24/4/2009)
4. Non-Research Animals at Accredited Animal Research Establishments (revised 4/8/2010)
5. Annual Reporting by Animal Ethics Committees to Accredited Animal Research Establishments (revised 24/1/2014)

5A. Accredited Animal Research Establishment Support for Animal Ethics Committees (revised 8/5/2014)

6. Differentiation between animal research and veterinary treatment (revised 8/5/2014)
8. Establishment of Protocols for Grievance Procedures
9. Criteria for Assessment of Animal Ethics Committee Membership
10. Emergency Procedures
11. Formal Agreements between Accredited Research Establishments sharing Animal Ethics Committees
12. Frequency of Animal Ethics Committee Meetings
13. Inspections by Animal Ethics Committees
14. The use of restricted drugs and the conduct of restricted acts of veterinary science in animal research (revised 27/2/2014)
15. Orientation of New Members of Animal Ethics Committees
16. Conflict of Interest with Membership of Animal Ethics Committees

Guidelines

1. Opportunistic Research on Free-Living Wildlife
2. Captive Wildlife
3. Individuals and Institutions Engaged in Collaborative Research
4. Use of Animals in Post-graduate Surgical Training
5. Collection of Voucher Specimens
6. Use of Pitfall Traps
7. The Use of Feral Animals in Research
8. Teaching Artificial Insemination and Pregnancy Testing in Cattle
9. Radio Tracking in Wildlife Research
10. Wildlife Surveys
11. Guidelines for Tick Serum Producers
12. Animal Research Model Application Form
13. Guidelines for the Production of Monoclonal Antibodies
14. Guidelines for the Care and Housing of Dogs in Scientific Institutions
15. Blood Collection
16. Supervision of Animal Supply by Animal Ethics Committees
17. Training Personnel
18. Guidelines for the Housing of Rabbits in Scientific Institutions

19. Teaching Cervical or Vaginal Artificial Insemination of Sheep
20. Guidelines for the Housing of Rats in Scientific Institutions
21. Guidelines for the Housing of Guinea Pigs in Scientific Institutions
22. Guidelines for the Housing of Mice in Scientific Institutions (April 2012)
23. Guidelines for the Housing of Sheep in Scientific Institutions

Appendix K: Standard conditions for accreditation and animal supply licence

The following are standard conditions that are placed on establishments seeking accreditation as animal research establishments and licences as animal suppliers. Additional conditions are added on a case-by-case basis.

Accreditation

1. That any site inspection is satisfactory.
2. Details of changes to Animal Ethics Committee membership (including the qualifications of new members and the categories to which they are appointed) must be provided to the Animal Welfare Unit of the NSW Department of Primary Industries within 30 days of membership changes. The revised composition of the AEC must meet the approval of the Secretary, Trade & Investment.
3. Rabbits should be housed in groups in pens. Rabbits may only be housed in cages with the express permission of the AEC on the basis of compelling evidence for the need to use such housing. Lack of space or facilities for pens should not be considered sufficient justification for the use of cages. Where rabbits are held in cages, these cages should be enriched by methods such as pair housing in double cages. (*Australian Code for the Care and Use of Animals for Scientific Purposes Clauses 3.1.5, 3.1.6, 3.2.13*) (See ARRP Guideline 18: Guidelines for the Housing of Rabbits in Scientific Institutions (http://www.animalethics.org.au/policies-and-guidelines/animal-care))
   (For establishments housing rabbits)
4. Unless precluded by the requirements of specific projects, chickens should be provided with housing that meets their behavioural needs including straw or other suitable bedding to cover the floors of cages, perches and dust bathing substrate.
   (For establishments housing chickens)
5. Dogs should be housed in accordance with ARRP Guideline 14: Guidelines for the Care and Housing of Dogs in Scientific Institutions (http://www.animalethics.org.au/policies-and-guidelines/animal-care).
   (For establishments housing dogs)
6. Unless otherwise approved by the Animal Ethics Committee, animals should be housed in accordance with the ARRP guidelines on animal housing for specific species found at: http://www.animalethics.org.au/policies-and-guidelines/animal-care.
7. Unless otherwise approved by the Animal Ethics Committee, wildlife studies should be carried out in accordance with the ARRP guidelines on wildlife research found at: http://www.animalethics.org.au/policies-and-guidelines/wildlife-research .
8. Animals (other than exempt animals) may only be obtained from a licensed animal supplier (see http://www.animalethics.org.au/policies-and-guidelines/animal-supply).
9. It is essential that the AEC members are provided with a copy of the inspection report of {date} and that the AEC is involved in the assessment of, and provision of responses to, the conditions, recommendations and observations contained in this report.
   (Added after inspection)
10 A response to conditions {xx} of the inspection report of {date) must be provided to the Animal Welfare Unit of the NSW Department of Primary Industries by {date—within 3 months of inspection report being sent}.
   (Added after inspection)

Animal Supply Licence

1. That any site inspection is satisfactory.
2. The documented procedures and methods of record keeping, as required under clauses 2.5.11, 2.5.12, 2.5.15 (vii) and 3.2.2 of the Australian Code for the Care and Use of Animals for Scientific Purposes, must be submitted by the supply unit to the AEC for approval.

3. To assist in monitoring the management of breeding colonies, the supply unit must provide regular reports to the AEC, for review, on the fertility, fecundity, morbidity and mortality of all breeding colonies. The frequency of such reports should be at least 6 monthly and more often if determined necessary by the AEC.
4. To help ensure that overproduction is avoided, the supply unit must provide regular reports to the AEC, for review, on the number of animals culled and the reasons for these numbers. The frequency of such reports should be at least 6 monthly and more often if determined necessary by the AEC.
5. Any breeding which involves animals which have been the subject of genetic modification (involving the introduction of foreign DNA into cells or whole animals) must comply with clauses 2.4.26, 2.4.27 and 3.3.24 of the Australian *Code for the Care and Use of Animals for Scientific Purposes*.