APPENDIX 4: The Role of Veterinarians in the Care and Use of Animals in Research and Teaching

ANZCCART FACT SHEET

THE ROLE OF VETERINARIANS IN THE CARE AND USE OF ANIMALS IN RESEARCH AND TEACHING

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Introduction
Veterinarians play a central role in biomedical (including veterinary), wildlife and farm animal research. In Australia, on a numerical basis, they are principally involved with biomedical research. The comprehensive nature of veterinary training equips veterinary graduates to handle a diverse range of professional responsibilities relevant to the use of animals for scientific purposes and animal welfare. In the Australian context, veterinarians in scientific institutions may be divided into two main categories: those involved in the production, medicine and surgery of animals used for scientific purposes; and those involved in Institutional Animal Ethics Committees, and, of course, there are many veterinarians whose duties involve elements of both categories. The broad range of duties in the first category includes management of laboratory animal production and maintenance colonies, provision of research support, maintenance of quarantine facilities, operation of in-house pathology programs, experimental surgery, and involvement in animal house design. For those veterinarians involved with Institutional Animal Ethics Committees their role is to promote animal welfare and regulatory compliance duties include review of proposals to use animals for scientific purposes, the minimisation of pain and distress, the monitoring of animals in research and teaching, the administration of animal ethics committees, provision of advice to the institution, provision of advice to researchers, provision of consultation and advice concerning compliance with relevant legislation and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and training of research and technical personnel. Veterinarians also play a major role in the development of institutional and regulatory policy, codes of practice, regulation and monitoring.

Although principally outside the scope of this article, qualification as a veterinarian also provides a strong base for veterinary graduates to embark upon a scientific research career in such diverse fields as immunology, molecular biology, physiology, biochemistry, virology, pathology, bacteriology, parasitology, animal management, wildlife research, agricultural animal research, and exotic disease research. Indeed many are Australian veterinary graduates currently working as investigators in all these fields.

Management of Laboratory Animal Production and Maintenance Colonies
A veterinarian responsible for the management of laboratory animals uses the strong scientific knowledge provided by a veterinary degree but must acquire a number of special skills and a variety of experience in order to fulfill the duties involved in this role. Although well equipped in the general principles of preventative and clinical medicine, surgery, genetics, the scientific process, the principles of animal management, pathology, quarantine, and nutrition, the laboratory animal veterinarian needs to apply this knowledge to a range of less familiar species. The focus of undergraduate training is on the domestic and farm animal species with little attention given to mice, rats, guinea pigs, and rabbits, let alone fish, amphibia, native animals and reptiles. The laboratory animal veterinarian therefore embarks early on a steep learning curve concerning anatomy, physiology and medicine of rodents, rabbits and other unusual species. The latter includes diagnosis and treatment of diseases one has not encountered before. In some situations, more familiar species, in which he or she has received considerable training, may be encountered, but the circumstances of their housing may be vastly different in the research environment. Many additional responsibilities such as personnel management of a team of animal technicians, financial management and environmental control are part of the role of these veterinarians. It is apparent that, in addition to the experience and training of a veterinarian, it is necessary to acquire some of the skills of the production engineer and human resources manager.

The ultimate aim of the laboratory animal veterinarian is to provide to the researcher, in a timely and efficient manner, an experimental animal in a state appropriate to the intended research in terms of biological characteristics, genetic constitution and microsatellite and general health status. In recent years genetic definition, by targeting specific genes, particularly in laboratory mice, has allowed investigators to more accurately define the biological roles of genes and the genetic components of disease processes. As the effects of genetic manipulation cannot be fully known in advance, this has placed more demands on veterinarians involved with the production and monitoring of these animals.

(Continued on page 2)
Monitoring aspects will be discussed in the next section, but from a production viewpoint these animals may require special care, they may be more susceptible to disease and reproductive efficiency may be impaired. The veterinarian with experimental surgery committees performs surgery to assist investigators in their research and this may extend to development of surgical techniques for specific protocols as well as training and providing advice in surgical techniques to investigators and animal technicians in some procedures.

It may be seen that the laboratory animal veterinarian, as defined here, is something of a jack of all trades. Indeed they are masters of most of the component disciplines. It might be seen to be a section of the profession that has many challenges. Laboratory animal veterinarians will tell you that this is indeed true.

Veterinarians and Institutional Animal Ethics Committees

Institutions using animals for scientific purposes within Australia must establish one or more Animal Ethics Committees directly responsible to the institution. There are four essential categories of membership of Animal Ethics Committees defined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (this code is incorporated into the legislation of the various states in Australia). The Category A member is a person with qualifications in veterinary science and with experience relative to the activities of the institution. Veterinary membership of such committees may be in a voluntary capacity, or as a full or part-time employee of the institution, usually dependent on the size, and sometimes on the location, of the institution. In larger institutions such veterinarians may be termed the institutional Animal Welfare Officer, or the Executive Officer of the Animal Ethics Committee. The principal role of the veterinarian is to provide expert advice to the committee. Such advice may include questions regarding animal care, animal experimental manipulations, effects of experimental manipulations on the health and welfare of the animals, genetic implications, appropriate use and methods of euthanasia, and possible alternatives to animal use. The veterinarian has an important role to fill in the minimisation of pain and distress thereby significantly contributing to animal welfare and the refinement of the investigative process. In the absence of a statistician and by the nature of their training, veterinarians may contribute to statistical evaluation, which is essential to the minimisation of the number of animals used whilst obtaining a statistically valid result. In smaller institutions, the veterinarian may also bear a large part of the often quite substantial administrative load relevant to the operation of the Animal Ethics Committee.

The role of veterinarians within the Animal Ethics Committee’s sphere of activities and institutional animal welfare extends well beyond the formal ethics meetings. Training of investigators and technical staff is an important function carried out by veterinarians in many institutions using animals. This training usually includes formal instruction on compliance with the Code and relevant legislation, and extends to practical workshops in animal handling techniques, routine research procedures, anaesthetic methods, euthanasia techniques and general or specific surgical skills. This may be done in conjunction with training provided by research group leaders. The ongoing monitoring of animals and animal facilities is another essential role.

The veterinarian is the ideal person to monitor pain and distress and instigate alleviation measures when necessary. As the Animal Ethics Committee is required to provide comment on the building or modification of animal facilities the veterinarian also has a central role in playing in assisting the committee to determine the appropriateness of the housing environment.

The concept of genetic modification of animals, while enabling scientists to concentrate on specific genes essential to the disease process, has brought with it a whole new set of potential welfare issues to those entrusted with the monitoring of research using animals. Veterinarians, conversant with this discipline acting in conjunction with animal technical staff and investigators, are well-positioned to assess the welfare and genetic stability of newly created genetically modified animals. They are also best able to institute special care when necessary and to implement measures where increased susceptibility to disease is involved.

Given the sometimes sensitive nature of particular research protocols, and the unique role of veterinarians within institutions and their Animal Ethics Committees, veterinarians are often required to act as de facto "information or publicity officers" with potential for interaction with the media and the general community in public forums. The veterinarian’s role may also include that of an independent complaints officer for concerns and issues raised by staff, students and members of the general community relating to the care and use of animals for research or teaching purposes.

The veterinarian who is a member of an Institutional Animal Ethics Committee has, by nature of his or her training and experience, a unique and significant role in being able to provide beneficial advice to the Animal Ethics Committee, the investigators and/or teachers, and the institution itself whilst facilitating the accumulation of scientific knowledge in the most humane manner.

Conclusion

The question that is often asked is why veterinarians become involved with animals used for scientific purposes. There are two main reasons. First, the animal research branch of the profession is intellectually stimulating and encourages scientific curiosity. It encourages use of all of the primary disciplines of veterinary science and adds unique skills not often used in other veterinary pursuits including care of a wide variety of often unusual species, facility design for intensive animal production and experimental holding, infection control and zoonoses, scientific principles, ethics, philosophy, policy formation, animal research compliance, gene technology and OHS. Second, the desire to become a veterinarian usually stems from empathy for animals and it is that empathy that is critical for veterinarians that fulfil a role in the monitoring and care of animals used for scientific purposes. The recognition that these animals are sentient beings whose welfare is paramount is imperative and the veterinarian plays a key role in ensuring that everyone involved in the use of these animals understands this principle. The adverse effects of stress on the immune system, for example, are well documented and it is the interface between the researchers and the veterinarians that promotes the reality that good animal welfare leads to good science!


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Appendix 5: NHMRC Information for Category D members

Category D Members on Animal Ethics Committees (AECs)

As an Category D member of an AEC have you ever felt that one of the following could describe how you feel?

A fish out of water?

Out in the cold?

or even......

A shrinking violet?

Then the following brochure contains some valuable information on why you may have these thoughts, why it is natural and even necessary for you to have these thoughts - and some strategies for dealing with them.

This information is provided by the:
Animal Welfare Committee of the
National Health and Medical Research Council.
1. Why are Category D members of AECs important?

By definition Category D members bring an independent view to the AEC. They have special skills, knowledge and understanding to influence the social norms of the institution on whose AEC they serve.

2. Why is it natural that you may sometimes feel “on the outer” at AEC meetings?

According to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (The Code), the Category D person should not fit any of the other categories. By definition, your presence on a sub-committee depends on you having no connection with the institution and no research or teaching experience in areas involving animals.

Unless the committee on which you serve has more than one Category D member, you are bound to feel singular. In fact, your effectiveness depends upon it. In reality, as an important part of the decision-making process, you are by no means alone and without your presence and input on the AEC, no research can proceed.

3. What is the responsibility of the Category D members on AECs?

In strictest terms, the basic responsibilities of the Category D members are the same as those of Categories A, B and C. That is:

- To ensure compliance with the protocols of the AEC
- To protect the welfare of the animals

Less formally, your particular responsibility as a Category D member is to bring to the AEC your independent viewpoint on animal welfare issues as a reflection of the current values of the community outside the research world.

4. Am I suited to be a Category D member on an AEC?

Although it involves a lot of preparatory reading, and extra commitment of time to attend meetings, membership on an AEC can be a rewarding activity. But it is not the sort of voluntary undertaking that suits everyone.

- Most importantly, you need to be prepared to question, and possibly continue questioning, AEC chairs, scientists and anybody else who is not making complete sense to you. Rather than viewing your request for information as a personal shortcoming, see any fault as lying with the scientist who is unable to communicate in plain English. Draw confidence from the fact that it is a requirement of The Code that any protocol under discussion is understandable to you.

- Even though you may feel “outnumbered” by members from other categories, you need to have confidence to speak your mind. Remember, no other membership category brings to the AEC your particular qualities. Do not squander your opportunity to shape and influence the outcome of AEC deliberations.

- There may be disagreements or, in rare cases, conflicts at AEC meetings. They may arise amongst committee members, or with the researchers appearing before the committee, and there should be a procedure for resolving disputes. However, if the motion of maintaining a potentially unpopular position causes you concern, you may be happier declining an offer of AEC membership.

- You need to be a good listener and maintain an open mind.

- You need to assess the aspects of a new application that have importance to you, and not worry that you may not fully understand all of the technical details. These are well covered by members from other categories who will be happy to explain them to you in lay language.
That said, you are free to seek outside expert advice. The only restriction on your doing so is the need to maintain confidentiality.

- It is helpful for you to have a sense of loyalty to the institution on whose AEC you sit and to try and protect it from adverse outcomes related to the operation of the AEC. Remember, the ultimate responsibility for animals used in research and teaching lies with the institution.

- And finally, you need to be a person with people skills. Much of the success of a well-functioning committee depends on the ability of its members to interact, talk freely and respect each other's point of view.

5. What are the most important aspects of an application from the Category D perspective?

Of course, these will vary between individuals and from one AEC to the next. The following list, presented in no particular order, offers a few suggestions, none of which require specialized knowledge:

- Does the lay statement make sense to you? This is an important question. A "yes" underscores the correct functioning of the AEC. If you cannot understand the lay statement, by all means ask the researcher to present another. Be prepared for what may be a less than favorable response.

- Have the three Rs - Replacement, Reduction, Refinement - as described in The Code been considered?

- Where more than one surgery step is proposed, has consideration been given to how much recovery time is needed between operations?

- The Code dictates that death as an end point should be avoided. However, occasionally, death as an end point is an unavoidable outcome of research. You need to prepare your thoughts regarding how you would deal with such a protocol.

- Are individual animals being held for an unreasonably long time?

- Are animals being used in more than one experiment to "save" on costs or surgery?

- Is the post-operative care of the animals the best possible?

- Has the matter of pain and suffering to the animal been considered and addressed by the researcher?

- Has the researcher included the provision of appropriate pain-solvers for minimizing pain in animals recovering from surgery?

- Are you convinced that the animals will be cared for throughout the experiment, including after hours, when researchers should be both available and knowledgeable?

- Is back-up veterinary care readily available?

You may like to add your own points to make this a personal checklist.

6. Other members of the AEC have organisations and institutions to which they can turn for support. Category Ds have no such umbrella.

It's true. But despite this, category D members have access to a number of resources on which they can draw for information or advice. These include the following people:

- Animal Welfare groups
- The Chair of your AEC
- Other members of your AEC
- The researchers with protocols before the AEC
- Management of the animal facility
- The member of the institution to whom the AEC is responsible. Depending on the institution, this may be the CEO, the Director or the Vice-Chancellor.
- NEB/ARC Secretary

AND

The following publications:

- The Australasian Code of Practice for the Care and Use of Animals for Scientific Purposes (the Code)
7. Some researchers don't seem to take seriously the role of the AEC.

The attitudes of scientists in animal welfare have progressed considerably through the 80's, particularly amongst young researchers who have grown up with AECs as part of their research culture. A perceived lack of gravity from a researcher may reflect more a lack of awareness of the AEC role.

Entrenched attitudes are not easy to turn around and some investigators are very strongly attached to their views. Don't be reluctant to take a firm stand against conservatism. Try not to have unrealistic expectations of the extent and speed of change. In other words, don't give up. Remember you are more likely to affect change from within the system.

8. There appears to be no mechanism in place on my AEC for dealing with grievances related to the operation of the committee.

There should be. It is a requirement of The Code that AECs have Terms of Reference. These should include details of how decisions are reached and how disputes are settled. They should be available to all members of the AEC and also for letting by intending new members.

If you have never seen the Terms of Reference, or the operating procedures for your AEC, there is a chance that they may not exist. The Code provides useful information regarding what the terms of reference should encompass.

Does your AEC function as it should, according to The Code?

As a Category D member of an AEC, you have a responsibility, along with members from all other categories, to ensure that the committee functions properly. If you have any doubts regarding procedures on your committee, the following list may help you to focus on the problems and raise them with other members of the AEC.

- Does the AEC meet regularly to consider applications? Is the Chairman present? Is there a quorum? Remember there must be a Category D member present for a quorum.
- How does the committee handle protocols tabled on the day of the meeting?
- Do the members of the committee have access to the researcher?
- How is discussion handled by the Chair?
- Are the Categories C and D members included in discussions?
- How are emergency meetings handled? Are there requests for responses over the telephone?
- Does the Committee have an executive? Is it legal? It must include a Category C or D member. Was it agreed upon by all Committee members?
- How often does the Committee visit the animal house?
- Does the Committee follow up on projects that it has approved?
- Does the Committee produce an annual report summarizing what was approved, what happened, etc? Is this document provided to the head of the institution?
- Are there any procedures in place for handling dissent?
Members are required to acknowledge in writing their undertaking regarding maintaining confidentiality and acceptance of the terms of reference in accordance with item 2.2.8 of The Australian Code of Practice.

Confidential information means all knowledge, know-how, methods, the tangible form of the expression of ideas, all rights concerning patents (particularly patentable information), copyrights, trademarks, trade secrets, registered designs, improvements to technology.

For example:
Methods used to test a product would not generally be considered to be confidential.
Information about the product (formulation, effectiveness, new market push etc) would be considered to be confidential.

Notwithstanding the above, individual members have the freedom, whenever necessary, to discuss aspects of protocols with colleagues and co-workers. This can be done in a generic manner without discussing or disclosing confidential details that compromise potential patents or reputations.

I acknowledge that the Committee is required to operate in accordance with the “………………..AEC” Terms of Reference and will, in the course of its duties, deal with certain matters which are deemed by the Committee to be confidential or may affect intellectual property rights of other persons.

Accordingly, I undertake to treat in absolute confidence within the Committee, all information of the nature described above, transmitted to me or acquired by me arising from my membership of the Animal Ethics Committee.

Name:…………………………………………………………………………..

Signature:………………………………………………………………………

Date:…………………………………………………………………………

Witnessed:……………………………………………………………………..
Effective searching

Please note that ‘Alternatives’ may often refer to alternatives to animal use and alternative methods and thus may encompass all 3Rs: Replacement, Reduction and Refinement of the use of animals in research, testing and teaching. Two recent articles provide some very useful tips!

Article 1. ‘Effective searching of the scientific literature for alternatives : search grids for appropriate databases’\(^{(8)}\) refers to a starter website which provides lists of some main free and proprietary literature databases, separating them firstly into two main groups. 1. Animal Models and 2. Topics (with emphasis on refinement). The searcher needs to be aware of the scope and emphasis of such databases, particularly bibliographic databases. For example: PubMed emphasises articles that pertain to biomedical work rather than a comprehensive search for an animal model. AGRICOLA may be useful for accessing animal science and veterinary literature, but emphasises the USA journals, whereas CAB covers also the European literature. There are also other search engines not mentioned or proprietary, such as EMBASE.com, Scirus, Medline and BIOSIS.  This is still, however, a good place to start your search:


Article 2. ‘The use of databases, information centres and guidelines when planning research that may involve animals’\(^{(9)}\) provides well considered background and comment on the main information sources, their capacity and scope, as well as an extensive categorised list of information sources with web access. Suggestions are also given for search strategies when using these information sources. There are also links from the UK based forum Focus on Alternatives which provide an excellent poster and worked example of a simple strategy for incorporating the 3Rs when planning projects that may involve the use of animals. These can be found at:

http://www.focusonalternatives.org.uk/pdfs/earlyplanningposter.pdf
and

There are also some other main searching starter websites with guidance on how to search for alternatives at:

http://www.frame.org.uk/Searching%20for%20Information/Search%20Guide%20Index.htm,
http://altweb.jhsph.edu/searchalt.htm
and

AEC applications - investigators and teachers reporting on 3Rs searches

The US Department of Agriculture has a specific Policy #12 that refers to Consideration of Alternatives to Painful/Distressful Procedures. This policy provides further information in support of their Animal Welfare Regulations. These “require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals AND provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including refinements, reductions and replacements”.


“When a database search is the primary means of meeting this requirement, the narrative must at a minimum, include:
1. The names of the databases searched
2. The date the search was performed
3. The period covered by the search
4. The key words and/or the search strategy used”

There are requirements in the mandatory Australian Code to ‘list any potential alternatives to animal use’ under Replacement (Section 2.2.16 viii). The above-mentioned criteria could be similarly used to demonstrate this search, before explanation as to which alternatives would be used or deemed unsuitable.

Contents

The following compilation attempts to collate international 3Rs databases and websites.

**Replacement**
- General Research/Medical
- Toxicity Testing
- Education and Training

**Reduction**
- General

**Refinement**
- General Assessment and Management of Pain and Distress
- Administration of Substances
- Blood Collection
- Antibody Production
- Behavioural Experiments
- Environmental Enrichment
- Euthanasia
- Housing and Husbandry
- Humane Endpoints
- Induction of Tumours
- Surgical Care
- Animal Models in Biomedical Research
- Genetically Modified / Cloned Animals
- Wildlife and Aquatic Animals

**Other**
- Some on line journals and newsletters
- Some primary reference sources
REPLACEMENT

Replacement as one of the 3R's is defined as the substitution for conscious living higher animals of insentient material. (1) There are a number of alternative methods that can be used to replace the use of live animals in either all or part of a project. Replacement may be relative, where animals are still required to provide cells or tissue, but experiments are conducted in vitro -

- tissue culture
- perfused organs
- tissue slices
- cellular and
- subcellular fractions

These methods are well suited to studies at the tissue, cellular or subcellular level and, in these circumstances, can be cost-effective and time-saving. They also provide a level of knowledge that complements studies in whole animals. A number of organisations around the world are working towards the development and validation of replacement methods. Many of them have informative web sites:

*The European Centre for the Validation of Alternative Methods (ECVAM)*

*Interagency Coordinating Committee on the Validation of Animal Models (ICCVAM)*
http://iccvam.niehs.nih.gov/

*Interagency Coordinating Committee on the Validation of Animal Models (ICCVAM)*
http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.html

General Research/Medical

*ALTWEB - Johns Hopkins University - Center for Alternatives to Animal Testing (CAAT)*
This site provides a very substantial database and linkages to many other databases. There are also specific databases for Humane Endpoints and Pain Management plus news, information, discussion and resources from the field of alternatives to animal testing. [http://altweb.jhsph.edu/databases.htm](http://altweb.jhsph.edu/databases.htm)

*NCA-Netherlands Centre for the Alternatives*
[www.nca-nl.org/](http://www.nca-nl.org/)

*Animal Welfare Information Center (AWIC) - National Agriculture Library (USA)*

*Fund for Replacement of Animals in Medical Experiments (FRAME)*
NB: FRAME and the AWIC sites give guidance on how to search other databases, such as Medline, for information on Replacement Alternatives.

*UC Davis Center for Animal Alternatives*
Norwegian Reference Centre for Laboratory Animal Science and Alternatives
http://oslovet.veths.no/databases.html

Academie Tierschutz (4)
Akadanie.fur.Tierschutz@fbmev.de c. 150000 references –German and English

3Rs Research Foundation based in Switzerland.
www.forschung3r.ch/
3Rs link provides a comprehensive list of databases

PREX (4)
A subscription-based database host specialising in alternatives and animal welfare at Utrecht University. Databases available include Agricola, CSA Life Sciences, a veterinary subset of the CAB database, Current Citations (article details from contents pages of over 10,000 most frequently requested journals held by the British Library) and Laslite, a database covering articles which have been published in journals of laboratory animal science.
http://prex.las.vet.uu.nl/

ZEBET
Database produced by ZEBET, Berlin with over 4,000 bibliographical references to about 300 replacement, refinement and reduction alternatives in all fields of biomedicine. The record for each method includes an expert analysis and validated information of the method and the animal-based method that it replaces. This valuable information is not often available from most on-line databases. The ZEBET Database is hosted by DIMDI where it may be searched free of charge either alone or simultaneously with Medline and Towline. There is a maximum number of users supported at any one time and access is denied once this limit is reached. This site offers information in both English and German.
http://www.dimdi.de/

Toxicity Testing

National Library of Medicine (NLM) (USA)- ALTBIB and TOXNET
NLM publishes a regular annotated bibliography on alternatives to animal testing that incorporates access to nine different toxicology-related databases.

INVITTOX
This is a direct and concise site listing numerous in vitro protocols for toxicity testing.
http://embryo.ib.amwaw.edu.pl/invittox/list.html

MECI/ MEMO (4)
These databases arose out of the MEIC study (Multicentre Evaluation of In Vitro Cytotoxicity) in which the same 50 chemicals were tested by a large number of laboratories worldwide in their own in vitro systems. The MEIC database contains this in vitro data together with information on the methodology of the assays used to generate the data. Both the MEIC data base and the associated MEMO database contain published and unpublished human toxicity data for the same substances, obtained from a large variety of sources.
INVITRODERM

Database of some 200 references to in vitro alternatives to the use of animals in skin irritation testing. www.invitroderm.com/

ECOTOX

The ECOTOX (ECOTOXicology) database provides single chemical toxicity information for aquatic and terrestrial life. ECOTOX is a useful tool for examining impacts of chemicals on the environment. Peer-reviewed literature is the primary source of information encoded in the database. Pertinent information on the species, chemical, test methods, and results presented by the author(s) are abstracted and entered into the database. Another source of test results is independently compiled data files provided by various United States and International government agencies. Prior to using ECOTOX, you should visit the "About ECOTOX/Help" section of this Web Site. In addition, it is recommended that you consult the original scientific paper to ensure an understanding of the context of the data retrieved from the ECOTOX database.

Education and Training

UC Davis Center for Animal Alternatives- for Veterinary Education
This location is a good start as it links to many (but not all) of the other sites listed below.
http://www.vetmed.ucdavis.edu/Animal_Alternatives/index.htm

AVAR (Association of Veterinarians for Animal Rights) Alternatives in Education Database
AVAR maintains an extensive database on alternatives to animal use in education that can either be searched online or viewed under categories (anatomy, anaesthesia, pharmacology, physiology and surgery).
http://www.avar.org/

EURCA (European Resource Centre for Alternatives in Higher Education)
EURCA was established in 2000 to actively promote the use of alternatives to using animals in higher education and as a mechanism for effective dissemination of useful information about alternatives to the higher education community. It comprises a collection of (mostly) technology-based alternatives, in particular specialist alternatives for teaching physiology and pharmacology. Product information can be found on the EURCA website including basic descriptive information supplemented by peer-reviews and evaluations. Information about EURCA projects also can be found on the website.
http://www.eurca.org

ERIC

The world's largest bibliographic database on education research and practice. Contains over one million records from 1966 onwards.
http://ericir.syr.edu/Eric/

LAWTE (Laboratory Animal Welfare Training Exchange)
The Laboratory Animal Welfare Training Exchange (LAWTE) aims to promote an information exchange among those involved in delivering animal welfare programs to those who work with laboratory animals. Information about LAWTE activities can be found on its website as well as information about training programs, materials and services. A LAWTE listserv allows members to share ideas and to discuss specific issues.
NORINA- Norwegian Inventory of Alternatives
This site provides information on approximately 4000 audiovisual aids and other products that may be used as alternatives or supplements to animal use in teaching and training at all levels from junior school to University.

http://oslovet.veths.no/NORINA/ The site also links to various loan systems such as:

Interniche
InternICHE is the International Network for Humane Education that aims for a high quality, fully humane education in biological science, veterinary, and human medicine. InternICHE supports progressive science teaching and the replacement of animal experiments by working with teachers to introduce alternatives, and with students to support freedom of conscience. InternICHE offers a range of resources to provide information and support to teachers and students including information about alternatives. InternICHE has set up a web-based network to facilitate sharing experiences regarding the use and implementation of alternatives.

http://www.interniche.org

The Interniche publication 'from guinea pig to computer mouse: alternative methods for a progressive, humane education' is a very comprehensive resource for current information on, and availability of, an extensive range of alternative methods and models. This publication and the accompanying video, 'Alternatives in Education' that showcases examples where conventional animal use has been replaced by alternative methods in life science education can be obtained from Animals Australia

http://www.animalsaustralia.org

Humane Society US (HSUS)
This site also provides a loan system for alternatives to animal use in teaching, particularly dissection.

http://www.hsus.org/animals_in_research/animals_in_education/

REDUCTION
The goal of reduction, the second of the 3R's, is to reduce the numbers of animals used to obtain information of a given amount and precision (1). To achieve this, the Australian Code of Practice requires that:

• studies are designed to be scientifically and statistically valid
• only the minimum numbers of animals are used
• studies should not be repeated unnecessarily.

There are two important caveats:

• the principle of reduction of numbers of animals should not be applied at the expense of greater suffering to individual animals
• the number of animals used must satisfy statistical requirements - neither too few nor too many.

Remember - if reducing the numbers of animals makes it impossible to reach a valid conclusion from the experiment, this does NOT achieve the goal of Reduction. To proceed with such an experiment is inherently unethical.
On-line resources to assist in applying the principle of reduction can be found at:

FRAME Reduction Committee Training Materials on Experimental Design and Statistical Analysis.

A new website to provide modelling concepts, methodologies and data to reduce, refine or replace animals used in medical science. (3) www.bath.ac.uk/mech-eng/ark/index.html

ETHITEX
A new subscription based resource for global ethical tissue and whole animal sharing.
www.ethitex.com

REFINEMENT
The third of the 3R's – Refinement is any decrease in the incidence or severity of 'inhumane' procedures applied to those animals that still have to be used. (4).

There are two key issues:

• To assess the impact of any procedure or condition on the well-being of the animal
• Strategies to eliminate or minimise that impact.

General Assessment and Management of Pain and Distress

Assessment of pain and distress
Kuiper and Allen have compiled a comprehensive list of literature on the assessment of animal welfare and animal distress. This site offers a diverse range of topics and a variety of species are covered. Topics include: animal pain and distress, management of pain in production animals behaviour, welfare and environmental design, assessing welfare and suffering, assessment of housing systems, response patterns of fish production to stress and crib biting in horses. Species include: laboratory rodents, cattle, pigs, horses, rabbits, fish, poultry, domestic animals, farmed silver foxes and dogs.
http://www.vetinfo.demon.nl/aw/index.html

Recognition and alleviation of pain and distress in laboratory animals
A comprehensive overview about behaviour, pain, and distress in laboratory animals. The volume explores: (a) Stressors in the laboratory and the animal behaviours they cause, including in-depth discussions of the physiology of pain and distress and the animal's ecological relationship to the laboratory as an environment. (b) A review of euthanasia of lab animals-exploring the decision, the methods, and the emotional effects on technicians. Also included is a highly practical, extensive listing, by species, of dosages and side effects of anaesthetics, analgesics, and tranquillisers.
http://www.nap.edu/catalog/1542.html.

Pain management database
This database includes information about anaesthesia and analgesia for most commonly used laboratory animals, including: rats, mice, primates, dogs, cats, rabbits, pigs, guinea pigs, birds, sheep, fish, and exotic species. It provides information about available drugs and the side effects of commonly used drugs. Citations are from publications that have published laboratory animal studies or human clinical studies with relevance to animal research. This database covers the period 1990 to the present, and is updated quarterly.
http://www.altwebsearch.org/aadb/aadb_search.cfm
Pain and Distress: Recommended resources

A comprehensive bibliography of recommended resources pertaining to pain and distress in animal research. The listing includes - peer-reviewed journal articles, books, CD-ROMS, websites and videos and covers topics including definitions, biology of pain and distress, recognition and assessment, alleviation and prevention, philosophical and ethical issues, analgesia and anaesthesia, euthanasia and neonatal and foetal pain.

http://www.hsus.org/ace/18787

Strategies to achieve the goal of refinement often need to be customised to a specific set of circumstances. With increasing knowledge and experience, a number of useful guidelines have been developed to assist in minimising the impact of particular procedures and practices. This is an area where knowledge is rapidly expanding. The resources and links below, highlight the latest in these developments.

Administration of Substances

A Good Practice Guide to the Administration of Substances and Removal of Blood, including Routes and Volumes.

European Federation of Pharmaceutical Industries Association and the European Centre for the Validation of Alternative Methods. Contains information on administration volumes considered good practice for the mouse, rat, rabbit, dog, marmoset and mini-pig, animal welfare issues with different routes, repeated intravenous infusions and vehicles for administration. Includes an extensive bibliography.

http://www.eslav.org/efpia.htm

Blood Collection

Removal of blood from laboratory mammals and birds

This is the first report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement. Each workshop was intended to address a single topic, the proceedings being published in the scientific press. This report, is the first of the series. It aims to describe in detail, the most humane methods for taking blood samples from the common laboratory animal species. Methods of venepuncture are described for the following animals: cats, cattle, chickens, dogs, ferrets, gerbils, goats, guinea pigs, hamsters, horses, marmosets, mice, pigs, rhesus, rabbits, rats and sheep.


Refinement of blood sampling techniques

The following papers are from Laboratory Animals 32(4) 1998.

- Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guineapig, ferret and mink. A. Hem, A. J. Smith & P. Solberg. This method is described for blood collection from the lateral saphenous vein. It allows rapid sampling, which if necessary can be repeated from the same site without a need for new puncture wounds. The method is a humane and practical alternative to cardiac and retro-orbital puncture, in species where venepuncture has traditionally been regarded as problematic.

The papers shown above can be accessed from [http://www.lal.org.uk](http://www.lal.org.uk) Go to on-line reprints/working party reports and guidelines to download these reports.

**Blood collection using the saphenous vein**
A method is described for blood collection from the lateral saphenous vein. This enables rapid sampling, that if necessary can be repeated from the same site without a need for new puncture wounds. It is a humane and practical alternative to cardiac and retro-orbital puncture in species such as mouse, rat, hamster, gerbil, guinea pigs, ferret and mink. Description and slides of technique can be viewed at [http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html](http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html)

**Blood collection using the facial vein**

**Blood sampling in sheep**
A detailed description of collecting blood samples from the jugular vein including a series of illustrations. Can be downloaded in PDF format. [http://www.agcom.purdue.edu](http://www.agcom.purdue.edu)

The Animal Research Review Panel Guidelines on *Blood Collection* that can be found at the link to NSW Research Procedures under Policies and Guidelines, outlines specific strategies to achieve the goals of refinement when using these methods. 

**Antibody Production**

*Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies*  
An overview of adjuvants briefly explains the function of adjuvants; guidelines for use of adjuvants, particularly Freund's adjuvants; and to introduce alternative adjuvants. Topics covered include the need, function, advantages and disadvantages of adjuvants and a list of alternate adjuvants.  

**Canadian Council on Animal Care: Guidelines on antibody production**
Guidelines for production of both polyclonal (pAb) and monoclonal antibodies (mAb) to assist investigators and research support personnel to achieve an acceptable immunological result with minimal discomfort for the animals involved. Selection of the species used for polyclonal antibody production includes; rabbits, chickens, hamsters and guinea pigs. When large volumes of antisera are required, animals used include horses, sheep and goats. Rats and mice are used for monoclonal production with the overall consideration to minimise pain and/or distress to the animals.  

**NH&MRC, Guidelines on Monoclonal Antibody Production**
National Health & Medical Research Council (2001). Contains information on Replacement and Refinement of techniques and outlines issues that should be considered in AEC review.
Alternatives in Monoclonal Antibody Production (1997)
The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) has worked with scientists since 1981 to find new methods to replace the use of laboratory animals in experiments, reduce the number of animals tested, and refine necessary tests to eliminate pain and distress. The Centre promotes humane science by supporting the creation, development, validation, and use of alternatives to animals in research, product safety testing, and education.

Monoclonal Antibody Production
A report of the committee on methods of producing monoclonal antibodies, National Research Council (1999). Includes detailed discussion of the advantages and disadvantages of in vitro and in vivo methods and animal welfare issues relating to the use of the ascites method to produce monoclonal antibodies in mice. Contains an extensive bibliography.

The Antibody Resource Page
This is a source of information on antibodies for both researchers and educators and includes information on antibody suppliers, contract antibody services, educational resources, immunology/biotechnology, databases and software and antibody news. http://www.antibodyresource.com/

Further information on how to achieve the goals of Refinement in the NSW Animal Research Review Panel Guidelines on Monoclonal Antibodies can be found at the link to Research Procedures under Policies and Guidelines at the NSW Animal Ethics infolink site.

Behavioural Experiments
Methods and Welfare Considerations in Behavioural Research with Animals
This handbook is useful to the researcher who is considering different methodologies for behavioural experiments. The report contains chapters on manipulation of access to food or fluids; experimental enclosures/physical restraint; pharmacological studies; aversive stimuli; social variables; ethological approaches; and teaching with animals. An introductory chapter reviews the contributions of behavioural research to understanding a variety of medical and psychological problems, such as stress, cognitive deficits with aging, behavioural problems stemming from early deprivations, sleep disorders and pain.

Guidelines for Ethical Conduct in the Care and Use of Animals
Developed by the American Psychological Association's Committee on Animal Research and Ethics (CARE) for use by psychologists working with non-human animals.

Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research
This new book provides current best practices for animal care and use and discusses how the regulations and guidelines can be applied to neuroscience and behavioural research. The book treats the development, evaluation, and implementation of animal-use protocols as a decision-making process, not just a decision. It encourages the use of professional judgment and careful interpretation of regulations and guidelines to develop performance standards that ensure animal well-being and high-quality research.
Material covered includes ethical considerations, protocol development strategies including pilot studies, sample size, pain and distress and humane endpoints, prolonged survival studies, studies of neural injury and disease, and perinatal and behavioural studies. Copies may be purchased from National Academies Press.

**Environmental enrichment**

*General principles and information on enrichment* [2]:

*Refinement of Housing and Handling Conditions and Environmental Enrichment for Laboratory Animals (including database)*
Primates, rodents, rabbits, cats, dogs, ferrets, farm animals, horses, birds, fish, amphibians and reptiles. [http://www.awionline.org/lab_animals/biblio/laball.htm](http://www.awionline.org/lab_animals/biblio/laball.htm)

*Environmental Enrichment Information. Resources for Laboratory Animals 1965-1995*
A joint project of AWIC and UFAW; covers birds, cats, dogs, farm animals, ferrets, rabbits and rodents.

*Primate Info Net*
The University of Michigan, National Primate Research Centre, hosts this site. It contains publication search for alternatives, enrichment and other primate care, fact sheets and the option to subscribe to an on line forum. Primate Lit is a recommended bibliographic database.
http://pin.primate.wisc.edu/infoserv/forums/pef/

*The Shape of Enrichment* [2]
Particularly wildlife and exotic animals:
http://www.enrichment.org/

**Euthanasia**

This report was produced to assist personnel in assessing which method of euthanasia is the most humane and appropriate for the species of animals that are being used.

There are three main sections to this report. Section 1 deals with legislative requirements. Section 2 provides information on methods of euthanasia used for vertebrates and section 3 covers groups of species such as fish, reptiles, rabbits, rodents, birds, carnivores, large mammals, exotics and primates.
http://www.lal.org.uk/workp.html

*Humane Society- review of carbon dioxide use for euthanasia (and anaesthesia) 2002*
http://www.hsus.org/animals_in_research/pain_distress/concerns_about_carbon_dioxide_use_in_euthanasia_and_anesthesia/

*ANZCCART news December 2004 and review of carbon dioxide use for euthanasia of rodents*:

From the Journal of the Veterinary Medical Association, 218: 669-696. The recommendations in this report are intended to give veterinarians guidance in relieving pain and suffering of animals that are to be euthanased. Information is given on euthanasia of animals in research and animal care and control facilities. Expanded information on exothermic, aquatic and fur-bearing animals and added information on horses and wildlife are some of the issues covered in this report. Special considerations are given to equine euthanasia and to non-conventional species in zoos, as well as amphibians, fish, reptiles, marine animals and birds.

http://www.avma.org/resources/euthanasia.pdf

Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits

www.dpi.vic.gov.au/animalwelfare/ and go to codes of practice or scientific procedures

Housing and Husbandry

Refining rodent husbandry: the mouse
This report is the third in the workshop series. It describes ways in which existing husbandry and care of mice can be improved with the emphasis on providing environments that allow animals to express a wide range of behaviours.
http://www.lal.org.uk/pdffiles/lab1566.pdf

Refinements in rabbit husbandry
Second report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement. This report describes ways in which the current systems of housing rabbits can be improved by group housing or by enriching the environment of individual cages.
http://www.lal.org.uk/pdffiles/RABbit.PDF

Annotated Database on Refinement of Housing and Handling Conditions and Environmental Enrichment for Laboratory Animals The Animal Welfare Institute is a non-profit organisation based in Washington.
http://www.awionline.org/

Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits

www.dpi.vic.gov.au/animalwelfare/ and go to codes of practice or scientific procedures

Humane Endpoints

Pain Management and Humane Endpoints Workshop and Database
http://altweb.jhsph.edu/topics/humane-endpoints.htm

Humane Endpoints in laboratory animal experiments CD ROM
This is an excellent interactive CD ROM for education and training purposes that was presented at the 5th world congress for Alternatives in the life sciences. For further information: i.boumans@uu.nl

Pain & Distress Report
A quarterly newsletter published by the Humane Society of the United States. This is a valuable resource for researchers, members of AEC’s, and animal care personnel. It provides current information on issues relating to the assessment and relief of pain and distress including
summaries of recent developments in policies and perspectives, articles from the technical and scientific literature, publications, resources, services, upcoming conferences and web sites. Download copies of the newsletter, including back issues.

**Humane Endpoints for Animal Experiments for Biomedical Research**
Proceedings of the 1998 International conference held in the Netherlands. Edited by Coenraad Hendriksen and David Morton, contents include: human neonates and pain; criteria for humane endpoints; an applied approach to the assessment of severity; practical use of distress scoring systems; relating criteria for humane endpoints to objectives; and discussions of issues in toxicity testing, cancer research, vaccine production and re-use of animals. Download copies of the proceedings.

**Pain Management and Humane Endpoints.**
An information resource published by the Animal Welfare Information Centre (USDA) which includes a discussion paper on setting acceptable endpoints in invasive experiments, assessment and alleviation of post-operative pain, guidelines for euthanasia of mouse and rat foetuses and neonates and an extensive bibliography. Copies can be download from: [http:www.nal.usda.gov/awic/pubs/IACUC/pain.htm](http:www.nal.usda.gov/awic/pubs/IACUC/pain.htm)

**Canadian Council on Animal Care**
*Guidelines for choosing appropriate endpoints in experiments using animals for research, teaching and testing* (1998). The purpose of this document is to present guidelines for selecting an endpoint that reduces animal pain and/or distress in laboratory animals, while still satisfying the experimental design requirements for objective evaluation when animals are used in biomedical research, teaching and testing. These guidelines should be used as a guide to the ongoing process of refinement in animal experimentation. [http://www.ccac.ca/english/gui_pol/gdlines/endpts/app9to10.htm](http://www.ccac.ca/english/gui_pol/gdlines/endpts/app9to10.htm)

**Organisation for Economic Co-operation and Development (OECD).**
The purpose of this Guidance Document is to apply the principles of the Three Rs to the use of animals in regulatory toxicity tests. This document is a guide to the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. The general principles contained in this document are specifically designed to be applicable for all mammalian species used in toxicity testing and other experimental studies. [http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono(2000)7](http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono(2000)7)

**Induction of Tumours**
*UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia*
Animals with local or disseminated tumours are likely to experience pain and/or distress, thus justifying special care and attention. Associated techniques including surgical preparation, irradiation and drug administration may increase the severity of an experimental procedure. Recognising this, the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) in 1988 set up an ad hoc committee to develop guidelines for research workers using animals in experimental neoplasia. A second revision of these guidelines was published in July, 1997. [http://www.ncrn.org.uk/Csg/publications.htm](http://www.ncrn.org.uk/Csg/publications.htm)

**FDA –Center for Drug Development and Research - Oncology Tools**
Oncology Tools contains a variety of information related to cancer and approved cancer drug therapies. To make it easier for consumers and patients to obtain information about different types of cancer and treatment, this web page can be searched by specific types of cancer and by
approved drug therapies. Clinical trials and patient support groups are also available on this site.  
http://www.fda.gov/cder/cancer/ 

**Surgical Care**

**Guidelines on Rodent Survival Surgery**
National Institutes of Health Intramural Program. Outlines operative procedures as well as issues which need to be considered in pre-operative planning and post-operative management, includes helpful information about disinfectants, instrument sterilization and suture selection.  
http://oacu.od.nih.gov/ARAC/surguide.htm 

**Applying principles of Aseptic Surgery to Rodents.**
AWIC Newsletter, Vol.4, No.2, pp3-6. Terry Cunliffe-Beamer  
http://www.nal.usda.gov/awic/newsletters/v4n2/4n2.htm 

**Rodent Surgery**
Application of aseptic technique and peri-operative care. Nicole Duffee  
http://dcminfo.wustl.edu/education/rodent_surgery_ho.doc 

**Animal Models in Biomedical Research (see also GM/cloned animals)**

**Model animals for biomedical research.**
This is a web site that has been developed by the National Institutes of Health and provides information about national and international activities and major resources that are being developed to facilitate biomedical research using the animal models listed here. Species include mouse, rat and non-mammalians models in the round worm fruit fly, zebra fish and frog.  
http://www.nih.gov/science/models/ 

**European mouse Mutant archive (EMMA)**
www.emma.rm.cnr.it/ 

**Zebradish International Resource Centre**
www.zfin.org 

**Genetically Modified / Cloned Animals**

**NHMRC Guidelines for the creation, breeding, care and use of genetically modified and cloned animals for scientific purposes.**
Pending 

**Guidelines for Transgenic Research**
Canadian Council on Animal Care- guidelines for transgenic animals are provided: to assist Animal Care Committee (ACC) members and investigators in evaluating the ethical and technological aspects of the proposed creation, care and use of transgenic animals.  
http://www.ccac.ca/english/gui_pol/gdlines/transgen/transgen1.htm 

**Transgenic animal web** (*)
This site has links to useful resources. It has been developed by the University of Michigan and includes a very useful guide to searching the web for mouse models of human disease and genetically altered mouse strains.  
http://www.med.umich.edu/tamc/links.html
Refinement and reduction in production of genetically modified mice
This is the sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement and is published as a supplement to Laboratory Animals (Volume 37, Supplement 1, July 2003). This fifty-page report covers the techniques and stages involved in the production of GM mice, tissue biopsy techniques, identification methods, assessment of animal welfare, reducing the numbers of surplus mice, and strategies to refine these procedures.

For further information go to http://www.lal.org.uk, follow the links to ‘journals’ then ‘on line’ and ‘contents’.

Refinement in the collection of tail tissue for genotyping
Monash University has a practical protocol that includes promoting the collection of tissue by less invasive methods than tail tipping. Such methods include ear notching and buccal mucosal cell swabbing.

Wildlife including aquatic animals

NSW Ethics Infolink
This site provides some very practical guidelines for captive wildlife, wildlife surveys, trapping and radio tracking, collection of voucher specimens, opportunistic research on free living wildlife and use of feral animals in research.

Guidelines on the care and use of wildlife
Guidelines on the care and use of fish
Canadian Council on Animal Care - these guidelines on the care and use of fish in research, teaching and testing cover a wide range of issues including the special requirements for the management, operation and maintenance of facilities and water supply. Quality monitoring, capture and transport of fish, husbandry, health and disease and experimental procedures such as surgery, administration of substances, collection of body fluids and euthanasia are also covered. Copies can be downloaded from the following website.
http://www.ccac.ca

Fish welfare
A briefing paper prepared by the Fisheries Society of the British Isles which includes sections on how fish respond to natural stressors and assessing fish welfare.
http://www.le.ac.uk/biology/fsbi

Guidelines for the Use of Live Amphibians and Reptiles in Field Research
http://199.245.200.110/pubs/herpcoll.html

Guidelines for the Use of Fish in Field Research (2004)
A policy regarding the use of fish in field research that includes all phases of handling fish. Published by the American Institute of Fishery Research Biologists and the American Society of Ichthyologists and Herpetologists. Copies can be downloaded from: http://www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml

Guidelines for the Capture, Handling and Care of Mammals
The objective of these guidelines is to identify field methods in mammalogy that meets standards of the American Society of Mammalogists, the oldest scientific organisation devoted to the study of mammals.
http://www.mammalsociety.org

Guidelines for the Use of Wild Birds in Research
These Guidelines are formulated with consideration of animal welfare and research needs.
http://www.nmmh.si.edu/BIRDNET/GuideToUse/index.html

Live Animal Capture and Handling
Guidelines for wild mammals, birds, amphibians and reptiles
http://srmwww.gov.bc.ca/risc/pubs/tebiodiv/capt/index.htm

NSW Animal Research Review Panel Guidelines on Wildlife Research
The NSW Animal Research Review Panel Guidelines on Wildlife Research outlines specific strategies to achieve the goals of refinement when using these methods. The guidelines can be found at the Animal Ethics infolink under Policies and Guidelines.

WildPro (Wildlife Information Network), Wildlife and Ecology Studies Worldwide, Fish and Fisheries Worldwide, Aquatic Sciences and Fisheries Abstracts (AFSA) and Zoological Records may also be useful for information and literature searching.

SOME ON LINE JOURNALS AND NEWSLETTERS
A number of journals and newsletters are available on-line, either as full text articles or as abstracts or indexes. The following sites publish information that relates to the care and use of animals for scientific purposes, including the 3Rs. (1)
Furthermore, the emergence of ‘open access’ publications where a complete version of the journal is available on line with free access and unrestricted distribution rights, ensures that research papers can be read by anyone from the day they are published. See www.earlham.edu/~peters/fos/overview.htm and Biomed Central: www.biomedcentral.com/independent/starting

Animal Welfare
UFAW (Universities Federation for Animal Welfare) publish this subscription only. It has various 3Rs special editions.
www.ufaw.org.uk/

ANZCCART News
Published since 1988; complete index on-line. Full published on-line since September, 1999.
http://www.adelaide.edu.au/ANZCCART

Animal Welfare and Technology
http://www.iat.org.uk

ATLA (Alternatives to Laboratory Animals)
Published by FRAME; index to current issue available on-line.
http://www.FRAME.org.uk

AWIC Bulletin
First published in 1990; full text and all back issues available on-line.

Contemporary Topics and Comparative Medicine
Published by the American Association for Laboratory Animal Science (AALAS). Free on-line access to abstracts and index for Contemporary Topics from volume 32 (1993) and Comparative Medicine from volume 50 (2000). Full articles available by subscription.
http://aalas.org

ILAR Journal – (previously ILAR News)
PDF files of articles are available on-line from volume 38, (1997).
http://dels.nas.edu/ilar/

IVIS (International Veterinary Information Services)
The IVIS website provides free access to original, up-to-date publications organised in electronic books each edited by highly qualified editors: proceedings of veterinary meetings; short courses; continuing education (lecture notes, manuals, autotutorials and interactive websites); an international calendar of veterinary events; image collections, and much more with the help of private and corporate sponsors. International Veterinary Information Service (IVIS) is a not-for-profit organisation established to provide information to veterinarians, veterinary students and animal health professionals world wide. On-line texts of particular interest include:
• Recent Advances in Anaesthetic Management of Large Domestic Animals (E.P.Steffey)
• Recent Advances in Anaesthetic Management: Companion Animals (R.D.Gleed & J.W.Ludders)
• Zoological Restraint and Anaesthesia (D.Heard)
• Laboratory Animal Medicine and Management
  http://www.ivis.org/default.asp?CK=0

*Journal of Applied Animal Welfare Science*
Table of contents and abstracts available on-line from volume 1 (1998). The site also has a search capability for journal articles.
http://www.psyeta.org/jaaws/index.html

*Lab Animal*
Lab animal is a peer-reviewed journal emphasising proper management and care. Editorial features include animal models, breeding and research methods, animal care and nutrition, personnel and facility management, facility design, education and training and new equipment. Some articles available on-line. Search capability for full index from 1972.
http://www.labanimal.com

*Laboratory Animals*
Published by Laboratory Animals Ltd (UK). Access to articles on-line available to subscribers. Free access to abstracts and index of issues since January 2001 (vol 35) available at:
http://www.catchword.co.uk/rsm/00236772/contp1-1.htm

*Scandinavian Journal of Laboratory Animal Science*
Publication of the Scandinavian Society for Laboratory Animal Science (Scand-LAS) Online access to abstracts and index from 2001, (volume 20) can be found at:
http://biomedicum.ut.ee/sjlas/index.html

**SOME PRIMARY REFERENCE SOURCES**
3. NZ NAEAC newsletter
6. 5th World Congress for Alternatives & Animal Use in the Life Sciences. Berlin 2005
7. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2004
Animal experiments which are poorly designed and/or incorrectly analysed waste scientific resources. If the animals suffer pain or distress they are also unethical. At best such experiments may get the right answers, but at the cost of using more animals than necessary. At worst, they may give the wrong answers and many more experiments may be necessary to discover the truth. Good experimental design and statistical analysis of the resulting data provides one way of reducing the use of animals, as suggested by Russell & Burch (1959).

Unfortunately, surveys of published papers suggest that there is substantial room for improvement. For example, a meta-analysis of 44 papers on fluid resuscitation in animals found that only two said how animals had been allocated to the treatment groups, none had sufficient power to detect reliably a halving in risk of death, there was substantial scope for bias, and the results were heterogeneous due to method of inducing the bleeding, so that the odds ratios comparing survival in the control and treated group were impossible to interpret. The authors questioned whether these experiments had any relevance to human medicine (Roberts et al. 2002). In another survey of 133 papers published in a veterinary journal the authors suggested that 61% would have required statistical revision had they been submitted to a statistician before publication and 5% had such serious errors that the conclusions did not appear to be supported by the data. About 30% of the papers had detectable errors in experimental design and nearly half had errors in the statistical analysis of the resulting data. There were also errors in the presentation of the data so that in some cases it was not clear how many animals had been used (McCance 1955).

A good scientific project should have a clear strategy usually involving a number of separate experiments, and each experiment needs to be well designed. There are three main types of experiment. Pilot studies are used to test the logistics of a proposed experiment, and gain preliminary information. Exploratory studies are used to study patterns of response, without there being any clear hypothesis about the effects of a particular treatment. This type of experiment is used to generate hypotheses which can be tested in confirmatory experiments. Preferably such hypotheses will be reasonably simple, and will not require a great number of statistical tests so that the p-values resulting from statistical tests will be reasonably reliable.

Once an investigator has decided on appropriate treatment and control groups, and decided which characters will be measured, there are five requirements for a well designed experiment (Cox 1958):
1. Bias should be avoided

Bias can occur when animals in different treatment groups differ in some way which is not associated with the treatment. Obviously in an experiment with two treatment groups (say a control group and one given a drug treatment), if all the controls were females and all the treated animals were males any effect of the drug would be confused with the difference between the two sexes. It is, however, less obvious that if all the treated animals are in one cage and the controls in another, any observed differences may be due to the treatment, or they may be due to differences between the cages. For example, the animals in one cage may be fighting, whereas those in the other cage are living harmoniously.

In order to avoid bias it is essential to identify correctly the “experimental unit”, and to assign the experimental unit to the treatments using an appropriate method of randomization. The “experimental unit” is that entity which can be assigned to an experimental treatment independent of all other experimental units. Two experimental units must be capable of being assigned to separate treatments. Thus, if the treatment is given to rodents in the diet or drinking water, all animals in a cage must receive the same treatment, so the cage becomes the experimental unit. However, if animals in the same cage can receive different treatments, such as when these are given by i.p. injection, then the individual animal will be the experimental unit. If an animal can be given a treatment for a period of time, followed by a different treatment in a crossover experimental design, then the experimental unit will be the animal for a period of time.

Experimental units should be assigned to the treatments at random so as to minimize the chance that the treatment groups will differ to any great extent even before the experiment has started. Physical randomization by drawing numbered pieces of paper from a receptacle is probably the easiest method, but tables of random numbers or computer programs that produce random numbers or sort numbers in random order can also be used.

2. Experiments should be powerful

Experiments need to be sufficiently powerful to detect treatment differences likely to be of scientific interest. The first step in designing powerful experiments is to understand and control inter-individual variation among the experimental units. As far as possible, animals should be of uniform weight, age and sex. Where rodents are being used, careful thought should be given to using inbred strains rather than outbred stocks (Festing et al. 2002; Festing 2002). Such strains have many advantages. As all animals are genetically identical, the phenotypic variation is reduced compared with outbred stocks. As they stay genetically constant for long periods of time a profile of their phenotypic and genetic characteristics can be built up as a result of independent work by many individuals. The more that is known about the characteristics of a strain, the more valuable it becomes. Thus in some cases strains can be chosen which have characteristics of particular value to a given research project.

Isogenic strains are also internationally distributed so that the work can be duplicated around the world, where necessary.

Measurement error is a potent source of variation which may need to be reduced. This can often be minimized using good quality apparatus, multiple samples and repeated measurements, depending on individual circumstances. When administering drugs, each individual should be weighed and the appropriate dose should be carefully measured and administered.

Some of the variation can often be controlled by splitting the experiment up into smaller “mini-experiments” using a randomized block experimental design. Such mini-experiments are easier to handle, and experimental units which are similar can be used for each block. For example, if the animals have a greater range of body weights than would be desirable, the heaviest ones could be assigned to block one, the next heaviest to block 2 etc. Typically in a randomized block design the block size is the same as the number of treatments.

The next step is to estimate an appropriate sample size. This should be done using a power analysis or the resource equation method (Festing et al. 2002), rather than an arbitrary number such as six or eight animals per group.

The power analysis method depends on a mathematical relationship between a number of variables which are specified by the scientist. It is easiest to understand in the case of an experiment involving just two groups, the results of which are to be analysed using Student’s t-test. The scientist needs to specify the significance level to be used (often 5%), the effect size of scientific interest (i.e. the minimum difference between the two groups that the experiment should be designed to detect), the sidedness of the test (a two-sided test is used when it is not known whether the treatment will increase or decrease the character of interest compared with the control group), and the power that the experiment need to have. The power is the chance that the experiment will successfully detect the specified effect size. This is usually set at about 80-90%, but could be higher if the consequences of failing to detect an effect of the treatment are likely to be serious. For example, it is really the power of the experiment that should be of greatest interest for toxicologists because a low powered experiment will fail to detect the effects of a toxic agent. Finally, the investigator must supply an estimate of the standard deviation. As the experiment has not yet been done this has to come from a previous study. Unfortunately, this is a serious limitation of the power analysis method as the results depend critically on the standard deviation, and this can vary enormously between experiments. Thus a power analysis can only give an indication of an appropriate sample size, and some leeway in the interpretation of the results is necessary.

The resource equation (Mead 1988) provides an alternative method of sample size determination for measurement outcomes, which depends on the law of diminishing returns.
Increasing the size of a small experiment gives good returns, but once an experiment has reached a certain size adding additional experimental units provides little additional information. The method is simple and it seems to give reasonably good results, although a power analysis should be used where possible. With this method, the error degrees of freedom, E, in the analysis of variance should be somewhere between about ten and 20. For most experiments this is simply the (total number of animals) minus (the number of treatment groups). Thus if an investigator plans an experiment with four treatments (say a control and three dose levels), and plans to have eight rats per group then $E=32.4 = 28$. This suggests that the experiment is a bit too large, and the investigator could probably have as few as six rats per group. However, this method should not be used too rigorously. Sometimes experiments where $E$ is 30 or even 40 can be justified, but in such cases the investigator should be encouraged to do a power analysis where this is possible.

3. Experiments should have a wide range of applicability
Controlling variability may lead to more powerful experiments, but experiments may also need to have a wide range of applicability. An experiment done using male F344 inbred rats may not be repeatable using female DA rats. The range of applicability can often be increased using factorial experimental designs, in which more than one experimental factor is varied at a time. Such designs have many advantages, as they can provide extra information at little extra cost. They can also be used to optimize experiments which are done repeatedly, such as those involved in drug screening (Shaw et al. 2002).

It is often possible to design an experiment using both sexes or more than one strain of animals without increasing the total size of the experiment (Festing 2003). Other factors which could be varied could include diet, pre-treatments, and the effects of various drugs. Such experiments will normally need to be analysed using a multi-way analysis of variance (Festing et al. 2002).

4. Experiments should be simple
Experiments should not be so complicated that mistakes are made in their execution or statistical analysis. Written protocols should always be used. Ideally, small pilot studies should be used to study the logistics of a proposed experiment to ensure that it is feasible and to gather preliminary information.

Scientists should never start an experiment unless they have a clear understanding of how the results are to be analysed. In some cases it may be worthwhile to generate some fictitious data of the type expected from the experiment and subject this to the proposed method of statistical analysis.

5. Experiments should provide a method of estimating the level of uncertainty of the results
Experiments should normally be subjected to a statistical analysis which should provide an estimate of the reliability of the conclusions. Where means or proportions are presented these should, ideally, be accompanied by confidence intervals or some other appropriate measure of their precision. Where the data are subject to a statistical analysis, exact p-values should be presented rather than simply indicating whether the results are "significant" or not.

Other useful information
Guidelines for the design and statistical analysis of human (Altman et al. 1989), animal (Festing & Altman 2002) and in-vitro studies (Festing 2001) can be consulted to give an indication of best practice. The animal guidelines are available on the ILAR web site (www.dels.nas.edu/ilar/). A good introduction to the analysis of variance is given by Roberta & Russo (1999). Much of the material presented here is described in more detail by Festing et al. (2002). A number of excellent statistical packages are available such as MINITAB and SPSS. Spread sheets should not be used for statistical analysis. They sometimes give erroneous and non-standard output.
### APPENDIX 9: The Proposal Assessment Guide

**Acknowledgement:** Dr Carol Ginns, Melbourne University Animal Welfare Officer

<table>
<thead>
<tr>
<th><strong>Project Title</strong></th>
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<tbody>
<tr>
<td>Does it describe the work proposed?</td>
<td>Yes</td>
<td>No</td>
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<table>
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<tr>
<th><strong>Project duration</strong></th>
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<tbody>
<tr>
<td>Is the proposed duration stated?</td>
<td>Yes</td>
<td>No</td>
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<table>
<thead>
<tr>
<th><strong>Safety</strong></th>
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</thead>
<tbody>
<tr>
<td>Are there any safety issues for humans or other animals?</td>
<td>Yes</td>
<td>No</td>
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<table>
<thead>
<tr>
<th><strong>Justification for the use of animals</strong></th>
<th></th>
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<tbody>
<tr>
<td>Are the aims clearly stated?</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Is the significance of the work clear?</td>
<td></td>
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<tr>
<td>Is the outline of the project design clear?</td>
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</table>

**Do I know...**

**Broadly what is going to be done to the animals?**

<table>
<thead>
<tr>
<th><strong>Replacement</strong></th>
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<tr>
<td>Is it clear why alternatives are not being used?</td>
<td>Yes</td>
<td>No</td>
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<tr>
<th><strong>Reduction</strong></th>
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<tbody>
<tr>
<td>Are the numbers requested justified?</td>
<td>Yes</td>
<td>No</td>
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<tr>
<th><strong>Refinement</strong></th>
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<tbody>
<tr>
<td><strong>Do I know...</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Where the animals will be housed and who will care for them at all stages of the project?</td>
<td></td>
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<tr>
<td>Whether any genetically modified animals have phenotypes which require special care?</td>
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<tr>
<th><strong>Project description</strong></th>
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<tr>
<td><strong>Do I know...</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>The meaning of all the terms used?</td>
<td></td>
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<tr>
<td>The details of what will happen to each individual animal or group of animals from the beginning to the end of the project? (agents, dose rates, routes and frequency of administration, actions, anaesthesia, surgery, number of procedures per animal etc)</td>
<td></td>
<td></td>
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<tr>
<td>The potential impacts on the animals’ welfare of each procedure?</td>
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<tr>
<td>What criteria will be used to monitor the animals?</td>
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<tr>
<td>What will be done if welfare problems are identified?</td>
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<tr>
<td>How the animals will be euthanased and disposed of?</td>
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<tr>
<td>Whether early and subsequent inspections by the AWO are needed?</td>
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<tr>
<th><strong>Investigators</strong></th>
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<tbody>
<tr>
<td><strong>Do I know...</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Who will be doing the work?</td>
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<tr>
<td>What experience the named personnel have in the specific techniques described in the proposal?</td>
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<tr>
<td>What training is needed?</td>
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<tr>
<td>Who will provide the training?</td>
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<tr>
<td>How the training will be provided?</td>
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APPENDIX 10: Site Inspection List
Acknowledgement: based on a similar list by Qld DPI&F

## POINTS TO CONSIDER WHEN CONDUCTING SITE INSPECTIONS

Site inspections and monitoring are an important aspect of AEC membership that allow members to gain a first hand knowledge of the AEC approved animal housing and projects. It is also a good opportunity to meet investigators and teachers carrying out those projects plus animal facility/care staff.

- The Australian Code requires that AECs monitor all animal facilities and laboratory areas preferably on an annual basis.
- By carrying out site inspections, the AEC can be assured that the general welfare of the animals is considered and that investigators and teachers are carrying out their activities according to the requirements of the Code and any conditions specified by the AEC.
- As many members of an AEC as possible should attend site inspections and if possible include monitoring of some individual projects in the inspection.
- It is preferable for the AEC Chair to give sufficient notice of site inspections so that members can talk to the investigators about their projects. The animal facility staff should also be present, and a report of the site inspection made to the nominated person on the license prepared.
- References: Regulations- especially 16 and 23, the Australian and Laboratory Animals Codes and any relevant Victorian livestock Codes of Practice-[www.dpi.vic.gov.au/animalwelfare/](http://www.dpi.vic.gov.au/animalwelfare/) (This website will include a template for auditing in 2006, which can be used as a guide.)

Some key aspects to consider during inspections are:

- Are the animals coping with situation they are in and are pain and stressors minimised?
- Is the project being conducted according to the Code and the approved application, with any specific conditions attached?
- Are the animal facilities in good repair and of adequate standard?
- Is general hygiene, records, husbandry, pest control and euthanasia methods appropriate?
- Are the animals adequately monitored (regularly as well as associated with particular procedures) and are the relevant records available?
- Are individual animals (wherever possible) as well as cages/pens identified to indicate whether they are part of a specific project or not?
- Is individual animal housing appropriate and necessary?
- Is there opportunity for social contact and appropriate environmental complexity (enrichment)?
- Are there appropriate security and emergency backup systems (for power, water failure) in place? Is there adequate after hours/weekend monitoring and contacts?
- Are there contingency procedures for emergency treatment and euthanasia established?
- Do the paddock animals have satisfactory shade/shelter, water and body condition?
- Has any corrective action or problems been identified from previous audits/inspections?
- Is there an open and accountable attitude and is communication effective amongst teachers/investigators and animal facility/care staff?
APPENDIX 11: Example SOP format

PROTOCOL FOR COLLECTION OF FAECAL SAMPLES FROM SHEEP

Purpose
To provide fresh faecal material from a specific animal for faecal egg counts and other diagnostic investigations.

Materials and Equipment
1) Containers, 50 mL, with a screw-top lid (eg TECHNO-PLAS)
2) Vinyl examination gloves
3) Permanent ink pen
4) Stock spray marker (eg LEADER)

Method
1) Manually restrain the sheep (often best again a wall, gate or fence).
2) Put on vinyl examination gloves.
3) Gently insert the gloved index finger into the sheep's rectum and extract faeces. If there is insufficient material present, peristalsis can be stimulated by gentle massage of the upper wall of the rectum.
4) Place the collected faeces into a container.
5) Label the container with the relevant animal identification.
6) To identify sheep that have been sampled, a spray marker may be used.
7) When faecal samples are collected in the field, they should be kept cool by placing the containers in an 'Esky' with ice packs or crushed ice.

Training and Supervision
This procedure should only be undertaken by a veterinarian, or staff who have been trained in the procedure.

Welfare Risks
Gentle insertion of the finger is essential and the animal should be as relaxed as possible. This procedure is completed in a few minutes and restraint is only a minor discomfort to the animal.

Contingencies
Any damage to the rectum or anus should be reported to the animal care staff or veterinarian.
APPENDIX 12: Ethical guidelines for students in schools and laboratory classes. ANZCCART 2005

Students using animals or parts of animals in school

Introduction
You need to realize that using living, dead or even parts of animals in school is a privilege with responsibilities. Not only must you obviously avoid being cruel to animals, but you should also be genuinely concerned about the wellbeing of the animals used, and have respect for how they are adding to your education. Here are some things you should think about, talk about with your friends, and discuss with your teachers;

Principles to Consider
1. Why are animals or their parts being used?
Animals should only ever be used to teach something in schools if that is the only way you can learn about it. You should think about whether it really is the only way to learn that particular thing, or could it be done without using animals or animal parts? Students and teachers should follow the “Three Rs” - Replacement of animals with something else (eg. videos, models, computer programs etc.), Reduction of the number of animals used (eg. sharing animals with more students etc.), and Refinement (improvement) of how the animals are used (eg. can the animals be made more comfortable, can the same thing be done without having to upset, harm, or kill the animal etc.).

2. Are the animals being looked after properly?
Teachers aren't the only ones responsible for looking after the animals, you are too! This is called a "duty of care" – in other words, it is your duty to care for the animal. If you have to touch the animals it's important to pay close attention to your teacher when they show you how to properly touch or hold the animal.

3. Are you obeying the law?
There are laws about how animals can be used in schools, and part of that means that your teacher must have been given permission by an Animal Ethics Committee (AEC). To get this permission your teacher has to tell the AEC what kind of animals are going to be used, how many, what will be done with them, and why they need to use animals. You should ask your teacher about this – because the laws apply to you too!

4. What do you think about using animals or parts of animals?
You should talk about using animals in school with your friends and teachers. You should listen to their opinions and tell them yours without getting into a fight about it! Feel free to come up with ways of making your classes better. Schools must also make sure that any students who don't want to use animals don't have to, and that they are given something equally useful to do instead. You can even contact the AEC if you have other questions.

5. Are you making the most of topics that use animals?
Your teacher should give you plenty of warning and practice before you use any animals or their body parts. Make sure you pay attention to these practice sessions so that you understand exactly what you will be learning once you use the animal.

ANZCCART has the following objectives:
* to promote excellence in the care of animals used in research and teaching and to reduce any discomfort that they may experience;
* to ensure that the outcomes of the scientific uses of animals are worthwhile; and
* to foster informed and responsible discussion and debate within the scientific and wider community regarding the scientific uses of animals.

If you want more detailed information you might like to look at the "Australian code of practice for the care and use of animals for scientific purposes" (Seventh edition, 2004), which is referred to in the relevant animal welfare laws for each Australian State and is available online at http://www.health.gov.au/nhmrc/research/sa/val/code.htm
Ethical guidelines for students in laboratory classes involving the use of animals and animal tissues

Introduction
The use of animals or animal tissues in laboratory classes is a privilege that brings with it responsibilities. These responsibilities go well beyond the need to avoid cruelty to animals and involve a genuine commitment to their welfare and a respect for the contribution they make to your learning. Outlined below are principles to consider in helping you to meet these responsibilities and to derive maximum benefit from the use of animals in laboratory classes.

Principles to consider

1. Consider why animals or animal tissues are being used in the laboratory
   The justification for using animals should be to enhance educational outcomes, while recognising that at the same time there is the potential for harm to animals to achieve these outcomes. Consideration should always be given to whether the educational outcomes could be achieved without the use of animals or animal tissues. Every student and staff member should be mindful of the Three Rs (replacement, reduction, and refinement) when working with animals in a teaching environment.

2. Consider the requirements for animal welfare and animal handling
   At all times the welfare of the animal you use is your responsibility, not just your teacher’s responsibility. This can be considered as a “duty of care”. If you are required to handle animals during a laboratory class, it is important to follow the instructions of staff in the correct handling and restraint techniques for the species with which you are working.

3. Consider the regulatory environment
   The use of animals in research, testing and teaching is regulated in New Zealand by legislation under the Animal Welfare Act 1999. This Act has an underlying principle of a “duty of care”. It also requires approval from an institution’s Animal Ethics Committee (AEC) for work in the teaching environment that uses animals or animal tissues. Gaining this approval involves justification for using animals (species and number), the means by which animals will be handled and, if required, humanely killed, and the educational outcomes of the laboratory work balanced against any potential harm to the animals used. The skills of the staff involved and the supervision of the students are also evaluated. In fact, the questions raised by AECs should be those asked by each student regarding the use of animals or animal tissues in their laboratories.

4. Consider your own views in using animals or animal tissues in the laboratory
   You should discuss the use of animals and animal tissues with other students and staff. Opinions should be formed and aired, with appropriate justification, in an open and accepting environment. You should feel free to raise suggestions that might improve future laboratory classes, and, to this end, student opinion regarding the use of animals in teaching should be encouraged.

5. Consider your responsibility to make sure that good use is made of the learning opportunity
   You should know what underlying principles are being taught in the class and understand the details that illustrate those principles. This includes reading background material on lecture notes and references before coming to class, reading the laboratory manual before the class, and being generally prepared to maximise the learning experience. Use every opportunity, within the approved scope of the class, to develop manual, observational, and recording skills.

ANZCCART has the following objectives:

- to promote excellence in the care of animals used in research and teaching and thereby minimise any discomfort that they may experience;
- to ensure that the outcomes of the scientific uses of animals are worthwhile; and
- to foster informed and responsible discussion and debate within the scientific and wider community regarding the scientific uses of animals.