

The use of databases, information centres and guidelines when planning research that may involve animals

AJ Smith*[†] and T Allen[‡]

[†] Laboratory Animal Unit, Norwegian School of Veterinary Science, Oslo, Norway

[‡] Animal Welfare Information Center, US Department of Agriculture, Beltsville, Maryland, USA

* Contact for correspondence and requests for reprints: adrian.smith@veths.no

Abstract

In many countries scientists planning research that may involve the use of animals are required by law to examine the possibilities for replacement, reduction or refinement (the Three Rs) of these experiments. In addition to the large number of literature databases, there are now many specialist databases specifically addressing the Three Rs. Information centres, with a mandate to assist scientists and lay people locate information on the Three Rs, have also been established. Email discussion lists and their archives constitute another, although less quality-controlled, source of information. Furthermore, guidelines for the care and use of animals in research have been produced both by regulatory bodies and scientific organisations. The growth of the internet has put an enormous amount of data into the public domain, and the problems of accessing relevant information are discussed. Suggestions are also given for search strategies when using these information sources.

Keywords: animal welfare, database, guidelines, information centre, laboratory animal welfare, Three Rs

Introduction

Many countries now have regulations that require researchers to document that they have conducted a search for possible alternatives when applying for permission to perform animal research. Although researchers may be experts on the scientific literature in their field, they may not have the same overview of the literature addressing the Three Rs — replacement, reduction and refinement — of Russell and Burch (1959, reprinted 1992). This situation is further complicated by the fact that much of the information on alternatives, required to reach a conclusion on the acceptability of a research protocol, is contained within specialist databases and publications. One or two simple searches in general literature databases, such as Medline, do not constitute an adequate search of the literature on the Three Rs.

Work to develop specific guidelines addressing the more complex or controversial areas of animal use in research is generally welcomed by researchers and laboratory animal personnel, but many of these guidelines have been criticised by scientists for not being science-based. Furthermore, an inherent reluctance by scientists to adopt guidelines produced by other countries, or a lack of information on existing guidelines, may hinder the implementation of the Three Rs.

This paper provides an overview of databases, journals, email lists, information centres, guidelines, regulations and policies, which provide useful information on alternative methods when planning research that may involve animals. The challenges faced by scientists searching for alternative

methods to the use of animals in research and teaching are also discussed. A brief description of the information included in this paper, and all the relevant web-links, can be found in the Appendix, pp 356-359.

Databases

Most researchers and librarians are well acquainted with literature databases such as Medline, EmBase, Agricola, CAB Abstracts and Biosis. Although they are not typically considered as databases on alternatives, they index the results of millions of peer-reviewed biomedical studies and technical papers. Consequently, these authoritative databases, and other such similar resources, should be the databases of choice when conducting an initial literature review. Furthermore, the distinction between literature databases and scientific journals is gradually disappearing with the development of free access and open access databases. For example, PubMed Central is a free archive of literature from approximately 200 biomedical and life sciences journals provided by the The National Library of Medicine, at the National Institutes of Health. The emergence of 'open access' publications (eg <http://www.earlham.edu/~peters/fos/overview.htm>, accessed 5 October 2005), where a complete version of the journal is available online with free access and with unrestricted distribution rights, ensures that research papers can be read by anyone from the day they are published. For example, BioMed Central has, at present, approximately 100 open-access journals in its collection, and even gives research

groups the option of starting their own journal (<http://www.biomedcentral.com/independent/starting>, accessed 28 September 2005).

These literature databases are particularly useful for locating information on reduction and refinement techniques. For example, because the number of animals used in a study is partially dependent upon the variability in the response of the animals, reviewing literature from similar research may assist researchers in determining the appropriate numbers of animals required. If invasive procedures are to be performed on animals, researchers should also review the appropriate veterinary literature to ensure that less invasive techniques are not available and that, for potentially painful procedures, the best analgesic and general care protocols are used. Similarly, studies that require the successive killing of animals at various time points may involve the use of large numbers of animals. The general biomedical and veterinary literature should be reviewed to determine if alternative methods, such as imaging techniques, biomarkers or other non-lethal sampling methods could be used instead. This type of information can be obtained from the large multi-disciplinary databases.

Patron access to information on the Three Rs in the large databases has been facilitated by the development of the Agricola Thesaurus for Animal Use Alternatives (<http://www.nal.usda.gov/awic/alternatives/altfact.htm>, accessed 21 September 2005) and the use of terms such as 'animal welfare', 'animal use alternatives', 'animal testing alternatives', and 'alternatives to animal testing'. When specific indexing terms are listed for individual databases, it should be understood that these terms alone are not sufficient when examining the literature for information on the Three Rs. They are listed to indicate that database compilers are aware of the legal and scientific interest in such information and have adapted their indexing strategies to accommodate these needs.

Until the mid 1990s there were few sources of information on specialised databases that addressed the Three Rs. Then, several fields, most notably toxicology and education, began developing and implementing alternative techniques into their research, testing and teaching strategies.

At the 1st World Congress on Alternatives and Animal Use in the Life Sciences held in Baltimore, Maryland, in 1993, more than 40 representatives of government, industry and animal welfare groups met to discuss the need for an international directory of organisations that would maintain databases and provided information on alternatives to animals in research. This led to the publication of the Directory of Resources on Alternatives and Animal Use in the Life Sciences in 1998 (AWIC 1998 [<http://www.nal.usda.gov/awic/newsletters/v9n1/9n1new.htm>, accessed 27 September 2005]) by the Animal Welfare Information Center (AWIC), Beltsville, Maryland, USA. The information in this Directory has since been incorporated into the AWIC website.

The first European initiative to collect such information on the Three Rs, and make it readily available to researchers, was the European Centre for the Validation of

Alternative Methods (ECVAM) workshop in Neubiberg, Munich, in September 1996 (Janusch *et al* 1997). Information experts constructed an overview of approximately 20 databases in existence at that time, categorised them according to their field, and made this information available on the internet (<http://oslovet.veths.no/databases.html>, accessed 20 September 2005). This overview has since been updated, and several other similar sites have been constructed by organisations such as Alternatives to Animal Testing on the Web (AltWeb [<http://altweb.jhsph.edu/atoz.htm>, accessed 22 September 2005]), the Fund for the Replacement of Animals in Medical Experiments (FRAME [<http://www.frame.org.uk/links/databases.htm>, accessed 28 September 2005]) and the Netherlands Centre for Alternatives to Animal Use (NCA [<http://www.nca-nl.org>, accessed 28 September 2005]).

Examples and descriptions of specialist databases

Specialist databases may be broadly grouped into four categories: 1. bibliography and literature databases; 2. clearing-houses for information on the Three Rs; 3. databases of the Three Rs within teaching and training; 4. databases of the Three Rs to research procedures.

1. Bibliography and literature databases

The two databases, 'Agricola' (produced by the United States National Agricultural Library) and 'CAB Abstracts' (produced by CABI Publishing, a division of CAB International in the UK) provide extensive coverage of the world's veterinary and laboratory animal medicine and science literature. Both databases index appropriate citations with the terms 'animal welfare' or 'animal testing alternatives' labels. Agricola also uses the terms 'animal use alternatives', 'alternative toxicity testing', 'endpoints', 'animal use reduction', 'animal use refinement', 'animal use replacement', as well as the broad category 'laboratory and experimental animals'. CAB Abstracts uses 'laboratory animal science' to allow users to focus on that specific body of literature. Searchable subjects covered by both databases include analgesics and anaesthetics, animal behaviour and animal husbandry, in addition to basic research. (CAB Abstracts is available through vendors such as Dialog, Ovid, SilverPlatter and others; users may also search CAB Abstracts, and CABI's Global Health database, by subscribing to CAB Direct at <http://www.cabdirect.org/>.)

The 'AltBib' database is a Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing and is produced by the National Library of Medicine in Bethesda, Maryland, USA. While the bibliography has not been updated since 2001, a new feature, added in September 2005, allows the use of embedded search filters to conduct live searches for alternatives in toxicology in the PubMed databases.

'Biosis' is a multi-disciplinary database that covers biomedical research, biological research, veterinary science, pharmacology and other life science topics. It contains more than 13 million records dating back to 1969. It is an extremely useful database for finding information

on alternative techniques because of its broad coverage of the life sciences. The interdisciplinary nature of the database allows scientists to find information on models and techniques that may fall outside the journals that they normally read. For example, a quick search on Biosis using the terms 'pain and models and animal' retrieved information from the journals *Alternatives to Laboratory Animals*, *Fundamentals of Applied Toxicology*, *Journal of Urology*, *The Journal of Neuroscience* and *Sciences et Techniques de l'Animal de Laboratoire*. Its useful thesaurus terms include 'animal care', 'animal husbandry', 'models and simulations', 'laboratory techniques' and 'methods and techniques'.

'EmBase' is a powerful biomedical and pharmacological database produced by the Dutch company Elsevier. With more than 11 million records, it is generally regarded as the database of choice for finding drug-related research information. Recently, it has begun indexing citations with keywords including 'human versus animal comparison', 'animal testing alternative', 'animal welfare', 'experimental animal', 'experimental models' and 'animal tissue'.

The 'Medline' database is the flagship product of the US National Library of Medicine. It is an exceptional resource containing more than 12 million records including the subjects biomedical and clinical research, veterinary medicine and science, and laboratory animal science. It indexes using terms such as 'animal welfare', 'laboratory animal science', 'animal testing alternatives', 'animal use alternatives', 'animal experimentation', 'research design' and 'animal models'.

Both the EmBase and Medline databases alone are remarkable storehouses of biomedical information, but Elsevier has combined their EmBase with the unique records from Medline to produce an enormous searchable resource of 16 million validated biomedical and pharmacological records called EMBASE.com.

'Scopus' is the world's largest abstract and indexing database, containing 27 million records covering articles from 14 000 peer-reviewed titles from more than 4000 international publishers. Users can search the internet directly from the Scopus interface using Scirus, a search engine specifically designed for scientific information (<http://www.scirus.com>, accessed 27 September 2005), giving access to over 167 million pages of relevant scientific information on the web, including author homepages, university sites and corporate information.

The AltWeb 'Pain Management' database is a collaboration between the Animal Welfare Information Center (AWIC) and Alternatives to Animal Testing on the Web (AltWeb) (see below). This database includes over 10 000 peer-reviewed citations from the more general literature databases Medline, Agricola and Agris. It covers all relevant laboratory animal species, farm animals and wildlife, and includes abstracts with information on doses of anaesthetics and analgesics, with their possible side-effects.

'TextBase' is produced by the Norwegian Reference Centre for Laboratory Animal Science and Alternatives, and provides an overview of approximately 1000 textbooks within the field

of laboratory animal science. Few of these books focus purely on the replacement of animals in research, but many of them contain science-based information that can be used to implement reduction and refinement. In addition to descriptions of the books, there are links to book reviews, publishers and internet bookstores. Many of the products are also linked to entries in the NORINA database (a Norwegian Inventory of Alternatives) (see below).

2. Clearinghouses for information on the Three Rs

Alternatives to Animal Testing on the Web (AltWeb) is an international project that was launched in 1997. Altweb has contributed greatly to the dissemination of information on existing databases via the internet. In addition to acting as a clearinghouse of information and news on the Three Rs, it has initiated the development of new resources, such as the Pain Management database (see above). AltWeb has also created a 'Humane Endpoints' database in collaboration with FRAME in Nottingham, England. This database is designed to aid investigators in implementing the earliest possible endpoint that is compatible with their research goals, to reduce pain and distress in animals. A search of the AltWeb site should be considered mandatory when seeking information on the Three Rs.

The Federation of European Laboratory Animal Science Associations (FELASA) is one of several organisations that maintain comprehensive websites serving as valuable clearinghouses. FELASA has issued recommendations on a variety of topics relevant to the Three Rs, including: the education and training of persons caring for or using research animals; health monitoring of breeding colonies and experimental units; nutrition and feeding; and the accreditation of diagnostic laboratories and training courses. In addition, FELASA currently has working groups looking at environmental enrichment, veterinary care, quality assurance systems, the ethical evaluation of animal experiments and recommendations for continuing education.

3. Databases of the Three Rs within teaching and training

The 'Alternatives in Education' database is maintained by the Association of Veterinarians for Animal Rights (AVAR). This database contains more than 7500 entries covering the subjects of anatomy, physiology, pharmacology, anaesthesia and surgery. It is easy to search and also allows browsing of all titles within a discipline. A typical record details the vendor contact information, educational level, type of medium (eg software, model), cost, and a web link. The site also links to the NORINA database (see below).

The European Resource Centre for Alternatives (EURCA) maintains a small 'alternatives' database that covers specialist alternatives for use in higher education, in particular computer programs within the subjects of physiology and pharmacology. Rather than covering all alternatives available, EURCA has chosen to offer peer-reviewed information of selected products. The EURCA database complements larger collections, such as AVAR, and the compilers cooperate closely with those of the NORINA database (see below).

The 'Norwegian Inventory of Alternatives' (NORINA) is produced by the Norwegian Reference Centre for Laboratory Animal Science and Alternatives in Oslo. NORINA 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. All products are classified by type (eg CD-ROM, video film) and area of use (eg anatomy, physiology). Items that can be loaned from animal welfare organisations are flagged with direct links to the organisation's website. Such loan schemes are offered by, among others, the Humane Society of the United States (HSUS) and InterNICHE. Products that have been reviewed, or are available free of charge, are also marked. The host website (<http://oslovet.veths.no>, accessed 20 September 2005) has recently been redesigned, facilitating combined searches in NORINA, TextBase and the rest of the web pages at the Centre.

The advantage of cooperation between database providers is obvious within the field of education. Many of the earliest animal alternatives were very simple, qualitative simulations of complex biological processes and some of these gave the impression that 'alternatives' were too simple to be of use in higher education. Nowadays, many computer simulations are extremely complex and are based on real data from experiments performed on animals. The problem of locating alternatives is made more difficult by the fact that many companies only produce one or two products, covering a very limited sector of the biomedical sciences. Therefore, without some form of database or catalogue, teachers and students seeking alternatives will have great problems in finding adequate information and gaining an overview of what has been produced worldwide.

4. Databases of the Three Rs to research procedures

'Alternatives to Skin Irritation/Corrosion Testing in Animals' is a database produced by Dr Jane Huggins containing 360 abstracts from peer-reviewed journals. The site is sponsored by the MatTek Corporation, which produces bio-engineered human tissue constructs for research and testing.

'Toxnet', produced by the National Library of Medicine in Bethesda, Maryland, USA, gives extensive coverage of alternative testing techniques. This site provides access to nine different toxicology-related databases, including 'HSBD' (Hazardous Substances Data Bank), 'IRIS' (Integrated Risk Information System), 'Gene-Tox' (Genetic Toxicology Data Bank), the comprehensive 'Toxline' (Toxicology Literature Online) and 'DART' (Developmental and Reproductive Toxicology database). The nine databases can be searched individually or together. The site also serves as a gateway to other National Library of Medicine toxicology resources.

'AnimAlt-ZEBET', a database on alternatives to animal experiments on the internet, is produced by the Centre for the Documentation and Evaluation of Alternative Methods to Animal Experiments, a unit of the German Federal Institute for Risk Assessment. It offers validated information on alternative methods in a searchable database and is available in both German and English.

Journals as knowledge databases

Much of the challenge associated with the retrieval of information on the Three Rs is caused by the fact that many scientists rarely assign keywords to their papers that reflect the Three Rs; consequently, much valuable information remains undetected. This problem is made worse by the fact that there is no specific journal for the Three Rs.

Although not strictly databases, many of the journals within the field of animal welfare in general, and laboratory animal science in particular, have extensive internet-based resources with valuable material on the Three Rs. Some of the best known journals include: *Alternatives to Laboratory Animals* (ATLA); *Animal Welfare*; *Comparative Medicine*; *Contemporary Topics*; *Institute for Laboratory Animal Research (ILAR) Journal*; *Lab Animal*; and *Laboratory Animals*.

For example, the website of *Laboratory Animals* provide online versions of working party conclusions, conference reports and key review papers, including:

- The Euthanasia Working Party Report for use with the EC Directive 86/609;
- Review papers on Refinement of the Husbandry of Birds, Mice and Rabbits;
- Guidelines on the Administration of Substances and Blood Sampling;
- Guidelines for the Reporting of Animals and Husbandry Methods (Mammals and Fish);
- Humane Endpoints in Animal Experiments;
- Recognising and Assessing Pain, Suffering and Distress in Laboratory Animals.

Several of these journals also regularly produce special issues devoted to specific themes. These issues are in themselves small knowledge databases describing the current state of the field, and should be used actively when planning animal research. Examples of these special issues include: Refining Dog Husbandry and Care — Eighth report of BVA/AFW/FRAME/RSPCA/UFPAW Joint Working Group on Refinement (Prescott 2004); Fish Models in Biomedical Research (ILAR 2001); and Immunisation Procedures and Adjuvant Products (ILAR 2005).

The demands on space in printed journals can potentially be a serious limitation to the dissemination of information on the Three Rs. Editors may demand the removal of information perceived as being less relevant to the paper's conclusions (such as animal source, health status, housing conditions, anaesthetic regimes and method of killing). However, this information may be crucial to researchers planning similar experiments or wishing to fully evaluate the ethical and scientific acceptability of the research. In the past, researchers and journals alike have 'solved' this problem by citing papers that describe the methods used in more detail. However, many of these references prove, on closer inspection, to be less than adequate because of differences in the protocol or other factors that make it impossible to compare the experiments. In recent years,

journals have started to use the internet to publish detailed protocols that are too large to be printed as part of the paper itself. These protocols, together with online reprints and searchable tables of content, have turned many journals into science-based databases that are invaluable in the search for information on the Three Rs.

Email discussion lists and archives

A large number of email discussion groups within the field of laboratory animal science behave as efficient disseminators of information on the Three Rs, even if they are not as well controlled for quality as other sources. These groups include: CompMed (Comparative Medicine), originally developed by Dr Ken Boschert and now hosted by the American Association of Laboratory Animal Science (AALAS); LAREF (Laboratory Animal Refinement and Enrichment), run by the Animal Welfare Institute (AWI) in Washington DC, USA, which provides a useful discussion forum for all aspects of animal housing; and LAWTE (Laboratory Animal Welfare Training Exchange) that allows trainers to exchange ideas on animal welfare and humane education of animal users.

Those discussion groups that have archives are the most valuable. For example, CompMed subscribers can access an electronic archive of all postings to the list since it was started in 1992. A comprehensive overview of mailing lists and other laboratory animal resources on the internet is available at the NetVet website: <http://netvet.wustl.edu> (accessed 29 September 2005).

Information centres

In recent years a number of information centres have emerged, whose mandate is to assist scientists and lay people locate information on the Three Rs. These centres include the Animal Welfare Information Center (AWIC) and the University of California Center for Animal Alternatives (UCCAA). In addition, the European Centre for the Validation of Alternative Methods (ECVAM) has produced its own collection of databases on alternatives, known as SIS (Scientific Information Service). In Europe, the European Consensus-Platform for Alternatives (ECOPA) has stimulated the establishment of a dozen national platforms so far. These platforms, with representatives from government, industry, academia and the animal welfare movement, are centres of competence within the Three Rs, which identify areas of concern in the particular country and seek to finance research into, and the implementation of, the Three Rs; for example, the 3R Research Foundation in Switzerland (<http://www.forschung3r.ch>, accessed 20 September 2005). Furthermore, many countries have centres for the Three Rs that actively cooperate with these platforms (eg Netherlands Centre for Alternatives to Animal Use (NCA [<http://www.nca-nl.org>, accessed 20 September 2005]). A full list of these centres is available at <http://ecopa.vub.ac.be/index.php?request=/content/members.php> (accessed 20 September 2005).

A range of other organisations, such as the Universities Federation for Animal Welfare (UFAW) and the Fund for

the Replacement of Animals in Medical Experiments (FRAME), act both as reference centres and animal welfare organisations with membership from the whole of society and their publications are valuable sources of information on the Three Rs.

Animal Welfare Information Center (AWIC)

The AWIC was established by the US Federal Government, and is an agency of the US Department of Agriculture, to assist scientists in finding alternatives to painful procedures performed on animals. The AWIC has produced an extensive array of bibliographies covering all laboratory, farm and exhibit animals and provides an extensive selection of links to databases, guidelines and regulatory information throughout the world. The bibliographies cover topics on husbandry, handling, alternatives and database searching, and issues of concern to animal care committees such as food and water deprivation, caging, animal numbers and humane endpoints. Detailed information on alternatives and database searching can be found at <http://www.nal.usda.gov/awic/pubs/IACUC/altdb.htm> and <http://www.nal.usda.gov/awic/alternatives/alternat.htm> (accessed 24 September 2005).

Animal Welfare Institute (AWI)

The AWI in Washington DC, has been producing quality material on the housing, husbandry, and handling of laboratory animals for many years. Their most recent databases include:

- ‘Annotated Database on Environmental Enrichment and Refinement of Husbandry for Nonhuman Primates’;
- ‘Database on Refinement of Housing and Handling Conditions and Environmental Enrichment for Laboratory Animals’;
- ‘Practical Enrichment Options for Animals kept in Research Institutions’.

Their most recent bibliographies include:

- *Environmental Enrichment for Rodents and Rabbits*;
- *Environmental Enrichment for Caged Macaques: Photographic documentation and literature review*;
- *Comfortable Quarters for Laboratory Animals*.

Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART)

ANZCCART produces an array of useful fact sheets and newsletter articles on humane techniques in restraint and handling, pain and distress, and animal species. It also has an extensive listing of links to alternatives databases throughout the world.

Center for Alternatives to Animal Testing (CAAT)

CAAT is an academic centre at the Johns Hopkins University Bloomberg School of Public Health, Baltimore, USA. Its primary mission is the implementation of the Three Rs, especially in the areas of consumer products testing and toxicology. CAAT sponsors numerous workshops on these topics and provides extensive funding for research into alternative testing techniques. The newest feature is an online course entitled Enhancing Humane

Science/Improving Animal Research (<http://caat.jhsph.edu/humanescience/login.cfm>, accessed 20 September 2005). This free online course addresses issues such as experimental design (including statistics and sample size determination), humane endpoints, environmental enrichment, post-surgical care, pain management and the impact of stress on the quality of data. Other features on the CAAT site include technical reports and proceedings from workshops.

National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)

The NC3Rs was formed in 2005 and is an initiative of the British Government that will fund Three Rs research, produce information resources and guidelines on humane techniques, and organise conferences and symposia on Three Rs initiatives. It replaces the Medical Research Council's Centre for Best Practice for Animals in Research.

The Norwegian Reference Centre for Laboratory Animal Science and Alternatives

The Laboratory Animal Unit at the Norwegian School of Veterinary Science maintains a comprehensive website with collections of links relevant to the Three Rs. The site has been extensively redesigned in 2005, integrating all the information on the site into one large database for easier and faster access. Features include lists of guidelines, the NORINA and TextBase databases (see Databases), teaching resources and information on fish as research animals, including a comprehensive list of guidelines (see Guidelines).

Guidelines

Specific science-based guidelines are invaluable when planning experiments that may involve the use of animals. However, the development of such guidelines, particularly if they are to apply internationally is often a laborious process. Obstacles include differences of opinion between member states, scientific organisations and the animal welfare movement, a lack of clear-cut scientific evidence for proposed refinements, economic constraints, and the feasibility of implementing or enforcing the guidelines.

A number of regulatory authorities have issued useful guidelines that should be considered whether they are mandatory or not in the country concerned.

Appendix A of the European Convention ETS 123

This Appendix provides detailed recommendations for the care and use of research animals in European countries that have ratified the European Convention ETS 123 (http://www.coe.int/T/E/Legal_affairs/Legal_co-operation/Biological_safety_use_of_animals/Laboratory_animals/draft%20revision%20of%20Appendix%20A.asp#TopOfPage, accessed 20 September 2005). The Appendix has recently been rewritten to take into account the needs of individual species and recent advances in our understanding of animal behaviour and related housing needs.

Policy 12 in the USA

In the United States, overseeing the use of animals in experiments is divided between several Federal agencies. However, only the Federal Animal Welfare Act (AWA) and

its regulations (9 CFR 2005), administered by the US Department of Agriculture, are enforced by law. Although the AWA regulates many aspects of animal research (rats, mice and birds bred for research are exempt), several of its provisions relate directly to the Three Rs. The law provides special requirements for two species: facilities keeping dogs must provide them with the opportunity for exercise on a routine basis, and facilities with non-human primates must have a program in place to promote their psychological well-being. The law also requires scientists to consider the Three Rs in their experiments. In general, scientists submitting protocols to an animal care committee must justify the use of the animal model, the number of animals that are being requested, provide pain relief or justify why it is being withheld, and cannot use paralytics without anaesthesia nor perform more than one major operative procedure unless justified. Any protocol that involves a painful or distressful procedure must include a written narrative that details the methods and sources used to determine that no alternative methods were available. This last requirement has been clarified by USDA's Policy 12 — Animal Care Policy Manual, Consideration of Alternatives to Painful/Distressful Procedures (United States Department of Agriculture 2000).

Policy 12 provides guidance on the definition of the Three Rs, stipulates that a database search is the most effective method of finding information on alternatives, and indicates how that information is to be presented to the animal care committee in order that they can determine whether a reasonable and good effort was made in determining the availability of alternatives. The scientist must provide the names of the databases searched, the date the searches were performed, the years covered by the searches, and the keywords or strategy used. The narrative must also discuss what type of information was found in the literature and must indicate if alternative techniques were or were not found. If an alternative method was found and is not being incorporated, that decision must be defended. The policy encourages investigators to consider the Three Rs in the planning stage of the proposed research.

Canadian guidelines

The Canadian Council on Animal Care (CCAC) has issued many guidelines on the care and use of animals in research, including the care and use of experimental animals, transgenic animals, choosing appropriate endpoints, the care and use of wildlife, laboratory animal facilities, the ethics of animal investigation, animal use protocol review and terms of reference for animal care committees. Detailed guidelines on the care and use of fish in research, teaching and testing were issued in 2005. These guidelines include appendices covering zoonoses and criteria for water quality, as well as over 120 literature references; all guidelines can be accessed via their website.

Other guidelines

Several organisations offer collections of guidelines on their websites. These include the Norwegian Reference Centre. This Centre has also produced guidelines for reporting the

results of fish experiments (Brattelid & Smith 2000), which are based on similar guidelines for mammals written by a working party of the German laboratory animal science association GV-SOLAS (Working Committee for the Biological Characterization of Laboratory Animals/GV SOLAS [Gesellschaft für Versuchstierkunde Society for Laboratory Animal Science] 1985), and guidelines for alternatives to the use of animals in teaching and training (Smith & Smith 2004). In 2005, the Centre held an international conference on Harmonisation of the Care and Use of Fish in Research in Gardermoen, Norway; a collection of guidelines and other resources was established at this conference and will be expanded in the future.

A collection of guidelines produced by scientific societies is available from AWIC at <http://www.nal.usda.gov/awic/pubs/IACUC/profguid.htm> (accessed 24 September 2005). Within the publication *Information Resources for Institutional Animal Care and Use Committees 1985–1999* (Allen 2000), many other topics of interest to a scientist developing a research protocol can be found. These include articles on food deprivation or water deprivation, pain management and humane endpoints, and statistics and animal numbers.

The Organisation for Economic Co-operation and Development has available a Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation ([http://www.oalis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono\(2000\)7](http://www.oalis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono(2000)7), accessed 27 September 2005).

The National Institutes of Health (NIH) in the US provides guidance to both NIH scientists and grant holders through the Animal Research Advisory Committee (ARAC). ARAC guidance documents can be found at <http://oacu.od.nih.gov/ARAC/index.htm> (accessed 20 September 2005) and include:

- Guidelines for Housing Multiple Species of Large Laboratory Animals;
- Guidelines for Diet Control in Behavioural Studies;
- Guidelines for Research Use of Adjuvants;
- Guidelines for Survival Rodent Surgery;
- Oocyte Harvesting in *Xenopus laevis*;
- Guidelines for Toe Clipping of Rodents;
- Guidelines for Ascites Production in Mice;
- Pain and Distress in Rodents and Rabbits: Responsibilities, Recognition and Alleviation;
- Guidelines for Endpoints in Animal Study Proposals;
- 2000 Report of the AVMA Panel on Euthanasia;
- Guidelines for Euthanasia of Rodent Feti and Neonates;
- Guidelines for Survival Bleeding of Mice and Rats;
- Guidelines for Euthanasia of Rodents Using Carbon Dioxide;
- Guidelines for the Genotyping of Mice and Rats.

The NIH Office of Laboratory Animal Welfare, which oversees grant holders, also makes available a report from the National Research Council of the National Academies

on Guidelines For The Care And Use Of Mammals In Neuroscience And Behavioural Research.

In a workshop that AWIC teaches on searching for alternatives, one of the more enlightening sections is the group discussion of the Three Rs. People assume that an alternative must be a non-animal method and the discussion of reducing animal numbers and the refinement of painful procedures allows the researchers to view their research programs in a different light. A well-designed protocol form can assist researchers in better understanding this distinction. The US Department of Defense has a protocol form with instructions that encourage military researchers to examine their scientific proposals to ensure that military research adopts the Three Rs whenever possible.

As the guidelines above indicate, the interest in the Three Rs is driven by the ability of animals to feel pain. The Animal Welfare Research Group at the Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK, has launched a new multimedia website, Guidelines for the Recognition and Assessment of Animal Pain, to help people working with animals recognise and assess pain in order to treat them appropriately. The site contains written guidelines, photographic and video evidence, and a bibliography. Topics covered include the types and causes of pain, methods used to assess pain and how the methods may be validated, and different types of treatments.

In due course, regulatory authorities are likely to publish research protocols and general decisions of principle on the internet. The Home Office in the UK has recently started this process (<http://www.homeoffice.gov.uk/comrace/animals/abstracts.html>, accessed 27 September 2005). There are a number of practical problems related to the desire for anonymity, which make it difficult to provide the detailed information that is often necessary to evaluate a protocol fully. Scientific organisations and websites designed to support decision-makers, such as the Institutional Animal Care and Use Committees (IACUC) website (<http://www.iacuc.org>, accessed 20 September 2005) in the USA should also be consulted.

Challenges when using the internet to search for alternatives

One of the problems faced by researchers when searching for information on the internet is that much of the material is not accessed by standard search engines. This hidden section of the internet, also called 'the deep web', includes documents in which the text is concealed within a format that is not always accessible to the search engines, for example, PDF (portable document format) files, information on company intranets and text contained within databases. It has been estimated that over 50% of the information in the deep web is contained within such databases (Bergman 2001). This makes it all the more important to circulate information about databases containing information on the Three Rs. Those managing databases that are available on the internet should ensure that sufficient information about the databases' content is, in some way or

another, available in a form that any internet search engine can access and catalogue.

Another problem facing internet users is the sheer volume of information that a search returns. This may appear overwhelming and important details may be missed. Some internet search engines make allowance for this by filtering out irrelevant, non-scientific information; Scirus is an example of one of these search engines (<http://www.scirus.com/srsapp>, accessed 20 September 2005). An excellent evaluation of internet search engines, constructed by Dr Krys Bottrill, is available on the website of FRAME. The FRAME site also includes valuable information on designing a literature search, covering search basics, a guide to searching the internet, internet search engines, and search terms of relevance to the Three Rs.

Advice for using bibliographic databases

When looking for information on the Three Rs in any large bibliographic database, it is useful to have a minimum amount of information first. This should include a clear understanding of the research, including the objective of the research, the proposed animal model, the names of the drugs/chemicals/organisms to be used either as pain-relieving agents or experimental compounds, a description of the animal procedures and clear endpoints. A meaningful search for the Three Rs demands knowledge of why the procedure is being performed and the expected outcome. The staff at AWIC have developed a Literature Search for Alternatives Worksheet (<http://www.nal.usda.gov/awic/alternatives/searches/altwksht.pdf>, accessed 24 September 2005) to assist researchers in this task. The Centre also organises workshops on searching the literature for information on the Three Rs.

Once this information has been gathered, it should be analysed to determine where alternative techniques might be substituted and to formulate questions that can be answered by the search. For example, if the proposed research involves killing successive numbers of animals over several weeks to track the progression of a disease process, a search for alternative techniques might focus on finding answers to the question 'Are there *biomarkers*, *non-invasive imaging* techniques or *non-lethal biopsy* techniques that will provide the same information?'

It is convenient to conduct a search using the Three Rs as a guide. The keywords used for the first part of the search will primarily come from the area of research (eg 'osteomyelitis', 'trauma') but may also include other terms. The first part of the search will examine the literature closely related to the proposed study for refinements to the proposed methods, information from similar studies that will assist with determining the proper number of animals needed and to see if the proposed work duplicates previously published experiments (this is a requirement of the US Animal Welfare Act [<http://www.aphis.usda.gov/lpa/pubs/awact.html>, accessed 24 September 2005]). Depending on the type of research, it might also be important to look for appropriate anaesthetics, analgesics, methods of restraint and other procedures.

In the second part of the search, replacement is considered. There may be some overlap with the first part of the search, in that alternative animal models may already be in hand. If not, then alternative animal and non-animal models should be considered.

There are many useful guides available that discuss this type of searching and evaluation of the results (Snow 1990; Smith 1994; Shevell & James 1995; Stokes & Jensen 1995; Kreger 1997; Allen 1999; Bottrill 1999; Kreger 1999; Langley *et al* 1999; Wood & Hart 2001; Information Managers in the Pharmaceutical Industry 2002; Allen *et al* 2004; Grune *et al* 2004).

Conclusions

Many countries have now introduced policies and regulations for animal research that encourage scientists to implement replacement, reduction and refinement in their research protocols. In response to this changing regulatory environment, many agencies, professional organisations and commercial organisations have developed guidance documents, organised Three Rs workshops and enhanced existing electronic databases in an effort to assist the research community. Consequently, there is now a large range of databases and other sources of information on alternative techniques to the use of animals in teaching, training and research. Sufficient time must be set aside to investigate the potential for implementing the Three Rs. Information specialists play an important role in this process; scientists and teachers should be encouraged to seek professional assistance when evaluating alternatives in their particular field.

These resources function together and all researchers, who are planning courses or research where animals may be used, should be broadly familiar with them. Resources such as Medline and EmBase can give scientists a general idea of where to begin their literature review as they begin gathering information on the Three Rs. Specialised databases, such as NORINA and Toxnet, allow teachers and scientists to rethink the use of animals entirely or consider a significant reduction in the numbers used. International consortiums such as AltWeb, regulatory authorities such as the UK Home Office, and national research agencies such as the US National Institutes of Health, have developed websites that provide extensive science-based guidance related to the Three Rs. For example, the guidelines on humane endpoints, developed by the Canadian Council on Animal Care, may assist a researcher in alleviating animal suffering.

The UK-based forum Focus on Alternatives (<http://www.focusonalternatives.org.uk>, accessed 20 September 2005) has produced an excellent poster illustrating the strategy that should be used when planning experiments that may involve the use of animals (<http://www.focusonalternatives.org.uk/PDFs/EarlyPlanningPoster.pdf>, accessed 20 September 2005). This flow diagram emphasises the role that authoritative databases, information centres, and guidelines should play in implementation of the Three Rs. A worked example, using this flow diagram, is available at <http://www.focusonalternatives.org.uk/PDFs/Early%20Plannig%20PosterB.pdf> (accessed 20 September

2005). The sheer amount of information available to researchers can appear overwhelming; however, by knowing where to look, how to look, and by using science-based guidelines provided by competent authorities, the incorporation of the Three Rs into a research program can simply become one part of the overall scientific process and not perceived merely as a regulatory burden.

Acknowledgements

We are grateful to Anne Cathrine Munthe and Unni Hope at the Library, Norwegian School of Veterinary Science, for information on the literature databases.

References

- 9 CFR** 2005 *Animals and Animal Products, Chapter I Animal and Plant Health Inspection Service, Department of Agriculture — Animal Welfare* parts 1–4. US Government Printing Office: Washington DC, USA
- Allen T** 1999 On-line databases: what is available? what is missing? *Animal Welfare Information Center Bulletin* 10: 11–14. <http://www.nal.usda.gov/awic/newsletters/v10n1/10n1alle.htm> (accessed 28 September 2005)
- Allen TA** (ed) 2000 *Information Resources for Institutional Animal Care and Use Committees 1985–1999*. United States Department of Agriculture, Research Service National Agricultural Library, Animal Welfare Information Center: Baltimore, Maryland, USA. <http://www.nal.usda.gov/awic/pubs/IACUC/iacuc.htm> (accessed 28 September 2005)
- Allen T, Crawford R, Jensen D, Adams K, Goodman G and Erickson H** 2004 For literature searches, is Medline enough? Response to protocol review scenario. *Lab Animal* 33: 15–16. <http://www.labanimal.com/labanimal/v33/n2/full/labanimal0204-15a.html> (accessed 28 September 2005)
- AWIC** (ed) 1998 *AWIC Resource Series number 1. Directory of Resources on Alternatives and Animal Use in the Life Sciences in 1998, 2nd Edition*. AWIC: Beltsville, Maryland, USA. <http://www.nal.usda.gov/awic/newsletters/v9n1/9n1new.htm> (accessed 27 September 2005)
- Bergman MK** 2001 The deep web: surfacing hidden value. *The Journal of Electronic Publishing* 7(1). <http://www.press.umich.edu/jep/07-01/bergman.html> (accessed 28 September 2005)
- Bottrill KA** 1999 Guide to Searching for Alternatives to the Use of Laboratory Animals, FRAME (Fund for the Replacement of Animals in Medical Experiments), UK. <http://www.frame.org.uk/Searching%20for%20Information/Search%20Guide%20Index.htm> (accessed 28 September 2005)
- Brattelid T and Smith AJ** 2000 Guidelines for reporting the results of fish experiments. *Laboratory Animals* 34: 131–135
- Grune B, Fallon M, Howard C, Hudson V, Kulpa-Eddy JA, Larson J, Leary S, Roi A, van der Valk J, Wood M, Dörendahl A, Köhler-Hahn D, Box R and Spielmann H** 2004 Report and recommendations of the international workshop 'Retrieval Approaches for Information on Alternative Methods to Animal Experiments'. *ALTEX* 21: 115–127. http://www.altex.ch/pdf/artikel/altex_3_2004_Grune.pdf (accessed 28 September 2005)
- ILAR** 2001 Fish Models in Biomedical Research. *ILAR Journal* 42(4)
- ILAR** 2005 Immunization Procedures and Adjuvant Products. *ILAR Journal* 46(3)
- Information Managers in the Pharmaceutical Industry** 2002 Searching for 3Rs information—published literature sources. http://www.impi.org.uk/i3r_v2_jul2002.pdf (accessed 28 September 2005)
- Janusch A, van der Kamp M, Bottrill K, Grune B, Anderson DC, Ekwall B, Howald M, Kolar R, Kuiper HJD, Larson J, Loprieno G, Sauer UG, Smith AJ and van der Valk JBF** 1997 Current status and future developments of databases on alternative methods. The Report and Recommendations of ECVAM Workshop 25. *ATLA* 25: 411–422
- Kreger MD** 1997 Why conduct literature searches for alternatives? *ASLAP Newsletter (American Society of Laboratory Animal Practitioners)* 30:19–23. <http://www.nal.usda.gov/awic/alternatives/aslap.htm> (accessed 28 September 2005)
- Kreger MD** 1999 The literature search for alternatives. In: Podolsky ML and Lukas VS (eds) *The Care and Feeding of an IACUC: The Organization and Management of an Institutional Animal Care and Use Committee* pp 139–152. CRC Press: Boca Raton, Florida, USA
- Langley G, Broadhead C, Bottrill K, Combes R, Ewbank R, Hawkins P, Hubrecht R, Jennings M, Newman C, Rowe S, Southtree J, Todd M and Ward L** 1999 Accessing information on the reduction, refinement and replacement of animal experiments. *ATLA* 27: 239–245
- Larson JA, Anderson DC, Ungar K and Stark P** 1995 *Directory of Resources on Alternatives and Animal Use in the Life Sciences*. Animal Welfare Information Center: Beltsville, Maryland, USA
- Prescott MJ** (ed) 2004 Refining dog husbandry and care. Eighth report of BVA/AFW/FRAME/RSPCA/UFWA Joint Working Group on Refinement. *Laboratory Animals* 38, Suppl 1
- Russell WMS and Burch RL** 1959 (reprinted 1992) *The Principles of Humane Experimental Technique*. Universities Federation for Animal Welfare: Wheathampstead, UK
- Shevell JL and James ML** 1995 Search for animal alternatives and the role of the information specialist. *Contemporary Topics in Laboratory Animal Science* 34: 65–68
- Smith C** 1994 AWIC tips for searching for alternatives to animal research and testing. *Lab Animal* 23: 46–48. <http://www.nal.usda.gov/awic/alternatives/tips.htm> (accessed 28 September 2005)
- Smith AJ and Smith K** 2004 Guidelines for humane education: alternatives to the use of animals in teaching and training. Proceedings of the Fourth World Congress on Alternatives and Animal Use in the Life Sciences, New Orleans, 2002. *ATLA* 32, Suppl 1A: 29–39. <http://www.worldcongress.net/2002/proceedings/SP4%20Smith.pdf> (accessed 28 September 2005)
- Snow B** 1990 Online searching for alternatives to animal testing *Online* pp 94–97 ISBN 0146 5422
- Stokes WS and Jensen DJB** 1995 Guidelines for institutional animal care and use committees: consideration of alternatives. *Contemporary Topics in Laboratory Animal Science* 34: 51–55, 58–60
- United States Department of Agriculture** 2000 Animal Care Policy Manual, Policy #12 — Consideration of Alternatives to Painful/Distressful Procedures. <http://www.aphis.usda.gov/ac/policy/policy12.pdf> (accessed 28 September 2005)
- Wood MW and Hart LA** 2001 Searching for the 3Rs: facilitating compliance in the bibliographic search for alternatives. *INSPEL (International Journal of Special Libraries)* 35: 191–198
- Working Committee for the Biological Characterization of Laboratory Animals/GV SOLAS (Gesellschaft für Versuchstierkunde Society for Laboratory Animal Science)** 1985 Guidelines for specification of animals and husbandry methods when reporting the results of animal experiments. *Laboratory Animals* 19: 106–108

Appendix

Category	Description	Internet address
Databases		
Agricola	Agricultural and veterinary medicine, animal welfare	http://agricola.nal.usda.gov
Agris	International information system for agricultural sciences and technology	http://www.fao.org/agris
AltBib	Alternatives in toxicology	http://toxnet.nlm.nih.gov/altbib.html
Alternatives in Education	Alternatives in teaching and training, includes software and other media	http://www.avar.org
Alternatives to Skin Irritation/Corrosion Testing	360 abstracts from peer-reviewed journals	http://www.invitroderm.com
AltWeb Pain Management	Anaesthesia and analgesia for animals	http://altweb.jhsph.edu (click on analgesia/anesthesia)
AnimAlt-ZEBET	Validated information on alternatives in animal research	http://www.bgvv.de/cd/1591
BioMed Central	Independent publishing house providing open access to papers from 130 journals	http://www.biomedcentral.com
Biosis	Biomedical research, veterinary medicine, life sciences, animal welfare	http://www.biosis.org
CAB Abstracts	Agricultural and veterinary medicine, animal welfare	http://www.cabdirect.org
EmBase	Biomedical research, pharmacology, animal welfare	http://www.embase.com
EURCA	European Resource Centre for Alternatives in Higher Education	http://www.eurca.org
Humane Endpoints	Compilation of information on humane endpoints from the literature on animal research presented as 222 abstracts with keyword search function	http://apps1.jhsph.edu/altweb/humane
Medline	Biomedical research, veterinary medicine, pharmacology, animal welfare	http://www.pubmed.gov
NORINA	Audiovisual materials and other products that may serve as alternatives or supplements to animal use in teaching and training, at all levels from junior school to University	http://oslovet.veths.no/NORINA
PubMed Central	Digital archive of life sciences journal literature at the US National Institutes of Health (NIH)	http://www.pubmedcentral.nih.gov
Refinement and Environmental Enrichment	Refinement of housing/ handling conditions and environmental enrichment for all laboratory animals	http://www.awionline.org/lab_animals/abiblio/laball.htm
Scopus	World's largest abstract and indexing database, with access to 14 000 peer-reviewed titles from more than 4000 international publishers	http://www.info.scopus.com
TextBase	Overview of more than 1000 textbooks within laboratory animals science and related disciplines	http://oslovet.veths.no/textbase
Toxnet	Provides access to nine different toxicology databases	http://toxnet.nlm.nih.gov
Overviews of databases	Collection of links to databases within the Three Rs	http://oslovet.veths.no/databases and http://www.frame.org.uk/links/databases
Journals		
<i>Alternatives to Laboratory Animals (ATLA)</i>	Development, validation, introduction and use of alternatives to laboratory animals in biomedical research and toxicity testing.	http://www.frame.org.uk
<i>Animal Welfare</i>	Welfare of farm, zoo, laboratory, companion, laboratory and wild animals	http://www.ufaw.org.uk
<i>Comparative Medicine</i>	US journal of laboratory animal science	http://www.aalas.org
<i>Contemporary Topics</i>	Journal of the American Association for Laboratory Animal Science (AALAS), including a section of refereed articles	http://www.aalas.org
<i>ILAR Journal</i>	Quarterly publication of the Institute for Laboratory Animal Research	http://dels.nas.edu/ilar_n/ilarhome/index.shtml
<i>Lab Animal</i>	Journal with emphasis on management and care of laboratory animals	http://www.labanimal.com
<i>Laboratory Animals</i>	European journal of laboratory animal science	http://www.lal.org.uk
Email lists		
CompMed	Discussion group, with archives, for over 2000 laboratory animal specialists	http://www.aalas.org/search/archives/archives.htm
LAREF	Laboratory Animal Refinement and Enrichment	http://www.awionline.org/pubs/Quarterly/winter03/0103p12.htm
LAWTE	Laboratory Animal Welfare Training Exchange	http://www.lawte.org
NetVet	Overview of email lists and other Internet resources within laboratory animal science	http://netvet.wustl.edu/compmed.htm
Information centres		
American Association of Laboratory Animal Science (AALAS)	Information resource for animal care committees	http://www.iacuc.org
Alternatives to Animal Testing on the Web (AltWeb)	International consortium that disseminates Three Rs information, produces pain management and humane endpoint databases, and provides access to full text resources	http://altweb.jhsph.edu
Animal Welfare Information Center (AWIC)	US Federal agency that produces specialised reference works on animal research and alternatives, information on the literature search for alternatives	http://www.nal.usda.gov/awic

Appendix continued

Category	Description	Internet address
Animal Welfare Institute (AWI)	Produces specialised databases, bibliographies, enrichment options, publishes <i>Comfortable Quarters for Laboratory Animals</i>	http://www.awionline.org
Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART)	Produces fact sheets and articles on humane techniques in restraint/handling, pain/distress, and animal species	http://www.adelaide.edu.au/ANZCCART
Center for Alternatives to Animal Testing (CAAT)	Sponsors numerous workshops, publishes proceedings, offers online tutorial on <i>Enhancing Humane Science</i> for scientists	http://caat/jhsph.edu
European Centre for the Validation of Alternative Methods (ECVAM)	Supports the European Commission and EU member states by promoting the scientific and regulatory acceptance of non-animal tests through research, test development and validation and the establishment of a specialised database service	http://ecvam.jrc.it
European Consensus-Platform for Alternatives (ECOPA)	Forum for the exchange between national consensus platforms, industry, science, animal welfare and regulators to enhance the development and implementation of alternatives	http://ecopa.vub.ac.be (access to all the European National Platforms)
Fund for the Replacement of Animals in Medical Experiments (FRAME)	Publishes ATLA (<i>Alternatives to Laboratory Animals</i>), funds alternatives research, produces bibliographies, provides a tutorial on searching literature for alternatives	http://www.frame.org.uk
Humane Society of the United States (HSUS)	Loan scheme for alternatives to animals in education	http://www.hsus.org
InterNICHE	Resources on alternatives to animal use in teaching	http://www.interniche.org
National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)	Promotion, development and implementation of the Three Rs in animal research	http://www.nc3rs.org.uk
Norwegian Reference Centre for Laboratory Animal Science and Alternatives	Information on laboratory animal science, the Three Rs, specialised databases, and collections of guidelines	http://oslovet.veths.no
Universities Federation for Animal Welfare (UFAW)	Publishes the journal <i>Animal Welfare</i> , <i>UFAW Handbook on the Care and Management of Laboratory Animals</i> , and the <i>UFAW Animal Welfare Series</i>	http://www.ufaw.org.uk
University of California Center for Animal Alternatives	Literature searches for alternatives, including templates for teaching and research protocols	http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm
World Congresses on Alternatives	Website, including proceedings, for the triannual World Congresses on Alternatives and Animal Use in the Life Sciences (1993–2005).	http://www.worldcongress.net
Guidelines, regulations and policies		
Antibody production		
Canadian Council on Animal Care (CCAC)	Guidelines on antibody production	http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Antibody/antibody.pdf
US National Institutes of Health (NIH)	Ascites production in mice	http://oacu.od.nih.gov/ARAC/ascites.pdf
US National Institutes of Health (NIH)	Research use of adjuvants	http://oacu.od.nih.gov/ARAC/freunds.pdf
Euthanasia		
European Commission DGXI	Euthanasia of experimental animals: Parts 1 and 2	http://www.lal.org.uk/pdffiles/LA1.pdf http://www.lal.org.uk/pdffiles/LA2.pdf
American Veterinary Medical Association	Report of the panel on euthanasia	http://www.aphis.usda.gov/ac/euthanasia.pdf
UK Home Office	Humane killing of animals	http://www.homeoffice.gov.uk/docs/hc193.html
US National Institutes of Health (NIH)	Euthanasia of rodent fetuses and neonates	http://oacu.od.nih.gov/ARAC/euthmous.pdf
US National Institutes of Health (NIH)	Euthanasia of rodents using carbon dioxide	http://oacu.od.nih.gov/ARAC/EuthCO2.pdf
Experimental design and animal numbers		
<i>Alternatives to Laboratory Animals</i> (ATLA)	Design and statistical analysis, <i>in vitro</i>	http://www.frame.org.uk/atlafn/statsguidelines.pdf
<i>Laboratory Animals</i> (UK)	Reduction of animal use	http://www.lal.org.uk/pdffiles/festing.pdf
Food or water deprivation		
UK Home Office	Water or food restriction	http://www.homeoffice.gov.uk/docs2/waterfoodguidance.html
US National Institutes of Health (NIH)	Diet control	http://oacu.od.nih.gov/ARAC/dietctrl.pdf

Appendix continued

Category	Description	Internet address
Humane endpoints		
Canadian Council on Animal Care (CCAC)	Choosing an appropriate endpoint	http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/gdlines/endpts/appopen.htm
Laboratory Animals (UK)	Humane endpoints in experiments	http://www.lal.org.uk/endpoints2.html
Organisation for Economic Co-operation and Development	Recognition, assessment, and use of clinical signs	http://www.oelis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono(2000)7
US National Institutes of Health (NIH)	Endpoints in animal study proposals	http://oacu.od.nih.gov/ARAC/Endpoints.pdf
Injections/blood samples		
European Federation of Pharmaceutical Industries Associations and European Centre for the Validation of Alternative Methods (ECVAM)	Administration of substances and removal of blood, includes routes and volumes	http://www.eslav.org/efpia.htm
University of Bergen	Blood collection using the saphenous vein: an alternative to retro-orbital collection	http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html
Laboratory Animals (UK)	Refinement of blood sampling techniques	http://www.lal.org.uk/refine.html
BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement	Refining procedures for administration of substances	http://www.lal.org.uk/pdffiles/refinement.pdf
BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement	Removal of blood from laboratory mammals and birds	http://www.lal.org.uk/pdffiles/blood.pdf
US National Institutes of Health (NIH)	Survival bleeding of mice and rats	http://oacu.od.nih.gov/ARAC/Bleeding.pdf
Literature search for alternatives		
Alternativen zu Tierexperimenten (ALTEX)	Retrieval approaches for information on alternative methods	http://www.altex.ch/pdf/artikel/altex_3_2004_Grune.pdf
Focus on Alternatives	Strategies to use when planning animal research	http://www.focusonalternatives.org.uk/PDFs/EarlyPlanningPoster.pdf http://www.focusonalternatives.org.uk/PDFs/Early%20Plannig%20PosterB.pdf
Focus on Alternatives	Accessing information on the Three Rs	http://www.focusonalternatives.org.uk/PDFs/Accessing%20Info.pdf
Fund for the Replacement of Animals in Medical Experiments (FRAME)	A guide to searching for alternatives	http://www.frame.org.uk/Searching%20for%20Information/Search%20Guide%20Index.htm
Information Managers in the Pharmaceutical Industry (IMPI)	UK resource on searching for information on the Three Rs	http://www.impi.org.uk/i3r_v2_jul2002.pdf
US Department of Agriculture	Policy 12 consideration of alternatives to painful or distressful procedures	http://www.aphis.usda.gov/ac/policy/policy12.pdf
US Department of Agriculture, Animal Welfare Information Center (AWIC)	Alternatives & searches	http://www.nal.usda.gov/awic/alternatives/alternat.htm
Pain and distress		
Federation of European Laboratory Animal Science Associations (FELASA)	Pain and distress in laboratory rodents and lagomorphs	http://www.lal.org.uk/pdffiles/FelasaPain.pdf
Laboratory Animals (UK)	Recognising and assessing pain, suffering, and distress	http://www.lal.org.uk/pain/index.html
Laboratory Animals (UK)	Refinement of animal use-assessment and alleviation of animal pain and distress	http://www.lal.org.uk/pdffiles/fleck.pdf
Royal (Dick) School of Veterinary Studies, University of Edinburgh	Recognition and assessment of pain and distress, including photos and videos	http://www.vet.ed.ac.uk/animalpain
US National Institutes of Health (NIH)	Mice, rats and rabbits: responsibilities, recognition and alleviation	http://oacu.od.nih.gov/ARAC/FinalPainDistress0704.pdf
Regulatory/professional		
American College of Veterinary Anesthesiologists	Treatment of pain in animals	http://www.acva.org/professional/Position/pain.htm
American Fisheries Society	Use of fish in research	http://www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml

Appendix continued

Category	Description	Internet address
American Psychological Association	Ethical conduct in the care and use of animals	http://www.apa.org/science/anguide.html
Australian National Health and Medical Research Council	Care and use of animals for scientific purposes	http://www.nhmrc.gov.au/publications/synopses/ea16syn.htm
Canadian Council on Animal Care (CCAC)	Guides for the care and use of experimental animals	http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/toc_v1.htm and http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/TOC_V2.HTM
Council of Europe	European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123)	http://conventions.coe.int/Treaty/en/Treaties/Html/123.htm
European Union	Legislation on the protection of animals, including the EU Directive 86/609/EEC on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes	http://europa.eu.int/eur-lex/en/lif/reg/en_register_1540.html
Federation of European Laboratory Animal Science Associations (FELASA)	Guidelines on the care and use of animals in research	http://www.felasa.org
International Association for the Study of Pain	Ethical guidelines for study of pain in conscious animals	http://www.iasp-pain.org/ethics-a.html
Society for Neuroscience	Use of animals in neuroscience research	http://apu.sfn.org/content/Publications/HandbookfortheUseofAnimalsinNeuroscienceResearch/Policy.htm
UK Home Office	UK and European legislation and guidance	http://www.homeoffice.gov.uk/comrace/animals/legislation.html and http://www.homeoffice.gov.uk/comrace/animals/reference.htm
US Department of Agriculture	Animal welfare regulations	http://www.access.gpo.gov/nara/cfr/waisidx_05/9cfrv1_05.html (see Parts 1-4)
National Research Council of the National Academies	Care and use of mammals in neuroscience and behavioural research	http://grants1.nih.gov/grants/olaw/National_Academies_Guidelines_for_Use_and_Care.pdf
US Public Health Service	Guide to the care and use of laboratory animals	http://www.nap.edu/readingroom/books/labrats
Transgenic animals		
Canadian Council on Animal Care (CCAC)	Transgenic animals	http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/TRANSGEN/TRANSGE1.HTM
European Centre for the Validation of Alternative Methods (ECVAM)	Transgenic animals and welfare issues	http://altweb.jhsph.edu/publications/ECVAM/ecvam28.htm
US National Institutes of Health (NIH)	Genotyping of mice and rats	http://oacu.od.nih.gov/ARAC/GenotypRodnt.pdf
Miscellaneous topics		
US Department of Agriculture, Animal Welfare Information Center (AWIC)	Guidelines for Institutional animal care and use committees (IACUCs)	http://www.nal.usda.gov/awic/pubs/IACUC/profguid.htm
BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement	Telemetry	http://www.lal.org.uk/pdf/Files/Web.pdf (Includes four guidance documents)
Canadian Council on Animal Care (CCAC)	Amphibians and reptiles	http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/AmphibiansReptiles.htm
Norwegian Reference Centre for Laboratory Animal Science and Alternatives	The care and use of fish in research	http://oslovet.veths.no/fish
UK Coordinating Committee on Cancer Research (UKCCCR)	Welfare of animals in experimental neoplasia	http://www.ncrn.org.uk/csg/animal_guides_text.pdf
US Department of Defense	Animal use protocol template	http://www.dtic.mil/biosys/downloads/Appendices.pdf
US National Institutes of Health (NIH)	<i>Xenopus</i> oocyte collection	http://oacu.od.nih.gov/ARAC/oocyte.pdf
US National Institutes of Health (NIH)	Survival surgery in rodents	http://oacu.od.nih.gov/ARAC/surguide.pdf
US National Institutes of Health (NIH)	Toe clipping of rodents	http://oacu.od.nih.gov/ARAC/FinalToeClip0504.pdf