[Frontiers in Bioscience S2, 5-10, January 1, 2010]

# Animal ethics in SIRS research

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## 1. ABSTRACT

It is well recognized that animals play a vital role and are indispensable to scientific and medical research. Over the years, a number of non-animal procedures have been developed. However, despite all the advances in science, as yet, no system has been evolved which can completely replace a living system to conduct basic research. There is still a need to test food, drugs, medical devices, treatment regimes etc. on some animals before they can be tested and used (if found suitable) in human beings. Even the most sophisticated technology models have failed to mimic completely the complex cellular interactions occurring in a living system. The search for a complete alternative to animal research is still on and in the mean time we can all help play our part by conducting animal research in a humane and responsible fashion. This chapter discusses the ethical issues in animal research highlighting the need to use animals conscientiously.

## 2. INTRODUCTION

The use of animals in research and teaching is a valuable privilege afforded to the scientific community by the general public that must not be abused. However, like any privilege there is a risk that it can be lost. While a vocal minority opposes it, the majority of the public support the use of animals as long as it is done as humanely as possible and it is scientifically justified. The ultimate benefits of animal research to human health and advancement of science cannot be reasonably discounted. It is conceivable that animal rights activist could succeed in their attempts to persuade the general public to withdraw their support of animal research if the scientific community fails to act responsibly in following widely recognized ethical principles, guidelines and regulations. The loss of this support would ultimately be disastrous to human health and well-being. Maintaining the welfare of animals for SIRS studies can be especially challenging due to the

potential degree of adverse affects on the animals in certain studies. An understanding and commitment to sound ethical practices and regulatory compliance is essential for all investigators and their staff who are planning animal research.

## 3. GUIDING PRINCIPLES OF ANIMAL RESEARCH

This chapter examines four aspects of the humane use of animals: (1) the '3 Rs': Replacement, Reduction and Refinement, (2) Humane Endpoints, (3) IACUC: Institutional Animal Care & Use Committee and (4) Regulatory Requirements.

### 3.1. The '3 Rs': replacement, reduction and refinement

Originally proposed by Russell and Birch[1] in 1959, these principles of 3 Rs (commonly referred to as the 'alternatives') are now internationally accepted by today's biomedical community as the basis of the care and use of animals used for research, teaching, testing or training; and everyone involved with use of animals should be personally committed to these Rs. The application of alternatives to animals used in research, testing and education has been an increasing trend in recent years and very significant work is being done in the search for alternatives. These three ethical principles should be appropriately considered while planning any research design.

The first of the three Rs, replacement, refers to efforts to use non-animal models such as *in vitro* tests, tissue culture and computer simulation whenever possible. An example of this is the use of *in vitro* cell culture techniques to investigate mechanisms of inflammation. However, the *in vivo* relevance of these findings and assessment of therapeutic efficacies of novel approaches would still require the use of animal models. This also means that researchers should try to replace the more advanced animals with animals lower on the phylogenetic tree. The biomedical community is also committed to considering the use of biological materials such as blood, serum, plasma etc. from human subjects whenever ethically and legally possible.

The second R, reduction, refers to using fewer animals for each test. For example, information in literature could be of help in deciding the minimum number of animals that are required to achieve meaningful data for a specific study. It is therefore possible in some situations to use ten animals to obtain information which had once required the use of a hundred animals. Animal studies should not be repeated or duplicated unnecessarily. Rational selection of group size, careful experimental design, maximizing use of animals, correct choice of model, minimizing loss of animals and statistical analysis if considered in the study design can help significantly reduce the number of animals[2].

The third R, refinement, means the procedures should be altered so that pain and distress to the animal subject can be minimized or eliminated. Where possible, less invasive procedures should be employed and strict attention paid to adequate anesthesia or analgesia. More sophisticated monitoring equipment, such as end tidal carbon dioxide monitors, pulse oximeters, EKG machines and blood gas machines are being used in research settings. These enable us to ensure a better physiologic state for the animals during anesthesia and surgery. With proper training of personnel, unnecessary wastage of study animals can be avoided. Refinement is the most commonly practiced of the 3 Rs. Pain-relieving drugs, non-pharmacological techniques, new diagnostic, imaging, and therapeutics techniques, environmental enrichment programs and establishment of more humane endpoints are some of refinement options that can be considered.

In addition to the conventional three Rs, there is also a fourth 'R' of research that has been proposed and is receiving increasing attention, which is 'responsibility'. The adoption of the 3 Rs in the planning process of using laboratory animals will help implement Responsibility - the fourth R. According to Dr Ronald E. Banks, it means responsibility to the research and teaching animals, responsibility to the public, responsibility to scientific and medical integrity, and responsibility to appropriate stewardship of animal resources. Responsibility to research and teaching animals does not suggest equality between human and animals; rather, it is a commitment to practicing appropriate animal care[3].

Responsibility to the public involves not only educating the public about the benefits obtained through animal facilitated investigation and the challenges and opportunities ahead, it also means listening to the public concerns, however ill-founded, and responding in a quiet and gentle manner with the truth. Responsibility to the public means that we continue with determined resolve to address issues of public health, while preparing ourselves for accusations and attacks on our facilities and ourselves. Responsibility to scientific and medical integrity involves optimal protocol design, sufficient data base searching, peer review, and professional oversight. Responsibility to appropriate stewardship of animal resources requires model selection based on the correct model system for specific issues and the use of appropriate number of animals – neither too many nor too few – to answer the question at hand.

From an ethical perspective, it is the duty of every researcher using animals to pursue all avenues to reduce the number of animals that are being used and sacrificed. We realize that the successes are always published but failures are seldom reported which leads to duplication of effort and use of larger number of animals. This hurdle can be crossed by establishment of adequate data banks reporting both successes and failures. The knowledge of proven failures in previous experimental designs could lead to decisions that greatly reduce the number of animals used in future studies.

### 3.2. Humane endpoints

Animals used in research and testing may experience pain and/or distress from a variety of sources, including, but not limited to induced diseases, procedures and toxicity. Ethical principles, guidelines and regulations

require that Institutional Animal Care and Use Committees (IACUCs) or Ethics Committees determine that discomfort to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research, and that unrelieved pain and distress will only continue for the duration necessary to accomplish the scientific objectives. Sound ethics and regulations stipulate that animals that would otherwise suffer severe or chronic pain and distress that cannot be relieved should be painlessly killed at the end of the procedure, or if appropriate, during the procedure. The concept is considered a standard of laboratory animal care that attempts to reduce animal suffering and improve the quality of research data. However, extreme caution must be exercised in order to avoid confounding the research results.

Criteria used to end experimental studies earlier in order to avoid or terminate unrelieved pain and/or distress are referred to as humane endpoints. They should ensure that study objectives will still be met even though the study is ended at an earlier point. Ideally, humane endpoints are sought that can be used to end studies before the onset of pain and distress. Endpoints are established for both scientific and humane reasons. An experimental endpoint is chosen to mark the planned end of an experimental manipulation and associated data gathering. A contingent experimental endpoint may also be used to signal euthanasia to remove an animal from the study for humane reasons. On the other hand, in experiments with unrelieved or unanticipated pain and/or distress, humane endpoints are criteria that indicate or predict pain, distress, or death and are used as signals to end a study early to avoid or terminate pain and/or distress. Ideal endpoints are those that can be used to end a study before the onset of pain and/or distress, without jeopardizing the study's objectives. However, in most cases, humane endpoints are developed and used to reduce the severity and duration of pain and/or distress[4].

The designation of accurate humane endpoints for any research model is not an easy task because there is no single set of parameters that could possibly be useful in all animal models. The specific endpoints to be used must be based on the pathophysiology of the disease process. That is why the initial designation of humane endpoints for a new model will require an intensive effort. In particular literature review reveals that there is a lack of readily available information on humane endpoints for trauma, burn, and lung injury models. As these endpoints are developed, it is vital that the information be made readily available through discussion and publication in appropriate journals.

As our understanding of animal pain and suffering grows, there is an increasing need to re-look at earlier humane endpoints and refinements of animal studies. Addressing these issues will lead to meaningful science while meeting the demands of both the scientific and lay communities. Establishing and implementing humane endpoints to minimize animal suffering in our quest to improve human health is best achieved by a collaborative effort on the part of investigators, veterinarians and animal care staff.

The intended goal of humane endpoints is to minimize the distress or suffering of research animals; however, if applied incorrectly, this well-intended concept could lead to premature decisions and inaccurate data, resulting in a waste of animal life. Humane endpoints should be assigned cautiously and preferably after preliminary studies to prevent inconclusive research. In order to accomplish this, investigators must become aware of certain concepts including: when to implement endpoints, what endpoints to consider, and how to establish the endpoints for their studies. Equipped with the basic principles of humane endpoints, investigators can make informed decisions that meet current standards of animal care while still achieving the scientific goals of their research studies.

Once humane endpoints are established, they should be defined carefully and thoroughly in the animal-use protocol that is submitted to the IACUC for review. The protocol should also establish an adequate but practical frequency of observations and describe the documentation that will be included in an animal's health record. The frequency of observations depends on the nature of the experimental manipulation or disease state and the expected rate of change in an animal's condition. There should be clear directions concerning who will be empowered to decide that a humane endpoint has been reached and the animal should receive interventional care, be removed from the study and/or euthanized. These individuals should be well trained to recognize what is normal and abnormal for the species, and they should clearly understand what is considered an acceptable or unacceptable condition as specified in the animal-use protocol. A clear designation of authority and responsibility to decide on and carry out the euthanasia is essential. Ideally, more that one person should have this authority to accommodate for absences.

Even if pain or distress is not anticipated, every protocol should contain a contingency plan for dealing with unexpected situations that may arise.

The development and use of human endpoints can reduce the severity and duration of unrelieved pain and distress. Clinical score sheets can be developed and used to establish humane endpoints for experimental studies. Score sheets are used to record and identify clinical signs and conditions associated with a particular experimental model. Single or multiple clinical signs that are predictive of the current experimental endpoint can then be used to allow for earlier and more humane endpoints.

The use of death as an endpoint is strongly discouraged and must always be well justified. Endpoints other than death must always be considered and should be used whenever the research objectives can be attained with non-lethal endpoints. Use of death as an endpoint must be justified in writing in proposals and its use must be approved by the IACUC prior to beginning a study.

### 3.3. IACUC: institutional animal care and use committee

Most of the well established scientific and educational institutions have oversight bodies governing the use of animals for research, testing and teaching. These bodies are known by various names such as Institutional Animal Ethics Committee, Institutional Animal Review Committee and in North America and Singapore as Institutional Animal Care and Use Committee (IACUC) with varying composition, functions and responsibilities depending on local legislations. The IACUCs derive their authority from the law and in accordance with the law, each IACUC is appointed directly by the Chief Executive Officer (CEO) or Institutional Official of an organization and reports only to the CEO or his delegate, an Institutional Official (IO). Therefore, IACUCs have the necessary independence to enforce regulations without undue management hindrances and play a pivotal role in maintaining high standards of research in the institution.

All protocols involving use of animals must be reviewed and approved by IACUC and experimentation may only begin after written approval from IACUC. Most IACUCs require use of a standardized protocol application form to assist the investigator in providing the information necessary to ensure compliance with both institutional policies and the regulatory requirements. In order to approve proposed research projects or significant amendments to ongoing research projects involving use of animals, the IACUC conducts a review of all the components related to the care and use of animals. It is the responsibility of IACUC to determine that research projects conform to the institutional as well as regulatory guidelines therefore, it is important for the investigator to keep in mind the following while writing their protocols:

1. Procedures with animals will avoid or minimize discomfort, distress and/or pain to the animals.

2. Procedures that may cause more than momentary or slight pain and/or distress to the animals will be performed with appropriate sedation, analgesia or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator.

3. Animals that would otherwise experience severe or chronic pain and/or distress that can not be relieved will be painlessly killed at the end of the procedure or if appropriate during the procedure.

4. The living conditions of animals will be appropriate for the species and contribute to their health and comfort with proper housing, feeding and nonmedical care of the animals.

5. Medical care for animals will be provided only by a qualified veterinarian or a suitably trained designee.

6. Personnel conducting the procedures are appropriately qualified and trained in those procedures.

Methods of euthanasia will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia[5], unless a deviation is justified for scientific reasons in writing by the investigator

For a research project application to be approved a quorum (more than 50% of committee members) should be present at the Meeting and more than 50% of the quorum should vote in favor. In Singapore, approval requires the presence of either the member representing the general community and/or the member whose primary concern is in non-scientific areas.

After review of an application, the IACUC will make one of the following decisions – approval, modifications required for approval, rejection of the application. The IACUC frequently allows researchers to reply to queries from reviewers. Research proposals falling short of Guidelines will not generally be approved and IACUC may suggest modifications before approving. Activities involving animals on approved protocols are reviewed every year for which the investigator is expected to submit a brief report. Breaches in approved protocols may result in suspension or withdrawal of approval. It is the responsibility of the investigator to inform IACUC in writing about date of completion or discontinuation of an approved project. If changes are intended in a protocol the investigator must submit an amendment for approval by the IACUC prior to implementing the changes.

### 3.4. Regulatory requirements

In addition to our personal commitments to ethical principles and guidelines while working with animals, we also have legal obligations. There are six (6) main overlapping areas of biomedical research which are influenced by the regulations. These include – animal care, human safety, industrial research, wildlife protection, environmental protection and transportation. All the countries are governed by their own regulations which might have some similarities to the regulations of other countries. It is not within the scope of this discussion to describe the specifics of all the regulations, but it is obligatory for researchers to comprehend their responsibilities under the regulations that directly impact on them. The ultimate responsibility for compliance with regulations that affect the care and use of animals lie with investigators therefore, it becomes imperative that they have a working knowledge of applicable regulations necessary to insure that proposals for funding contain all the necessary information and to assure that the conduct of all research proposals is in compliance with the requirements of both regulatory and funding agencies.

#### 3.4.1. Regulations in the United States

In the USA, there are three (3) main documents or guiding bodies, commonly referred to as ‘the 3 Big Guns’, governing animal research. These are:

1. The Animal Welfare Act (Federal Law) or AWA - Enacted in 1966 and enforced by the United States Department of Agriculture (USDA), the AWA and associated policies and regulations address standards for housing, husbandry, sanitation, veterinary care,

minimization of pain and distress, consideration of alternatives to procedures causing pain and distress, IACUC functions, record keeping, personnel training, exercise for dogs and psychological well-being for non human primates. It is the principal Federal statute governing sale, handling, transport and use of animals. AWA applies to all warm blooded vertebrate animals used for teaching, testing or research. It, at present, excludes horses not used for research, farm animals used for agricultural research, birds, rats of the genus Rattus and mice of genus Mus bred for research.

2. The Health Research Extension Act (enacted in 1985) and The Public Health Service (PHS) Policy - The PHS policies and guidelines address proper care and treatment of animals used in research and outline organization and operation of IACUC. It specifically addresses several issues associated with animal-based research including the use of tranquilizers, analgesics, anesthetics and paralytics; euthanasia; pre-surgical and post-surgical veterinary medical and nursing care of animals; and record keeping. PHS policy covers all live vertebrate animals (including birds, reptiles, amphibians and fish) used in federally funded research. The PHS requires institutions to use the Guide for the Care and Use of Laboratory Animals (Guide) as a basis for developing and implementing an institutional program for activities involving animals. Compliance with the Guide is mandated by the PHS as a prerequisite for receiving support from NIH.

3. The Public Health Service Guide for the Care and Use of Laboratory Animals[6], commonly referred to as the 'Guide', is recognized by veterinarians, IACUCs, regulators, inspectors, institutions, investigators and researchers as the standard reference on laboratory animal care and use programs. Its guidelines are based on established scientific principles, expert opinions and experience with methods and practices consistent with high-quality, humane, responsible animal care. The Guide is used as a "bible" by veterinarians overseeing animal care and use.

The Association for the Accreditation of Laboratory Animal Care International or AAALAC - While AAALAC has no regulatory authority, it is a very powerful voluntary organization that provides guidance on appropriate animal care based on existing US and internationally accepted standards of animal care and occupational health and safety. It is generally regarded that the AAALAC accreditation is the Gold Standard in animal care and use. Although, there is some cost associated with maintaining the standard and in funding the AAALAC site visit; the advantage is that AAALAC accreditation is well recognized by external organizations such as funding agencies as well as government authorities. AAALAC accredited status of a facility confirms compliance with all regulations and compliance with current internationally accepted standards of animal care and use.

### 3.4.2. Regulations in Singapore

In Singapore, animal research is governed by *Guidelines on the Care and Use of Animals for Scientific Purposes*[7]. Developed by the National Advisory Committee for Laboratory Animal Research (NACLAR) in 2004, it is a national guide which establishes the best practices in the use and care of animals for scientific purposes. NACLAR Guidelines were adapted from the best practices of Australia, Canada, New Zealand, the US and various organizations including the Council for International Organizations of Medical Sciences (CIOMS) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The NACLAR '*Guidelines*', in accordance with widely accepted scientific, ethical and legal principles, define the responsibilities of all people involved in the care and use of animals for scientific purposes. The Guideline document is organized into three sections:

1. Guiding Principles for the Care and Use of Animals for Scientific Purposes – This section addresses the overall guiding principles to promote humane and responsible care and use of animals for scientific purposes.

2. Guidelines for Institutional Animal Care and Use Committee – This section addresses the operational aspects pertaining to Institutional Animal Care and Use Committee (IACUC).

3. Training Guidelines – This section addresses the training scope and requirements for the animal users and animal facility personnel.

## 4. SUMMARY & PERSPECTIVE

The bulk of knowledge necessary for improvements of health and well-being of humans as well as animals can be gained only through *in vivo* experiments involving a wide variety of animal species. The use of animals in research and teaching brings with it a responsibility to minimize animal pain and distress. Although, there are involuntary regulations (required by law or set forth as a condition of funding) governing the use of animals in research, we must bear in mind that the best controls are established and enforced within the scientific community itself with inputs from general public concerned with the social and ethical implications of the research effort.

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**Key Words:** Ethics, animals, SIRS, 3Rs, IACUC, Review

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