The Three Rs: The Way Forward

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Laws that mandate replacement alternatives, reduction alternatives, and refinement alternatives (the Three Rs) in scientific research have been passed in the United Kingdom, Germany, the Netherlands, the United States, and the European Union over the past decade. Full implementation of this newly developed legislation depends upon scientists' ability to understand animal welfare issues and to accept the legitimacy of the public's interest in the conduct of science. The European Centre for the Validation of Alternative Methods (ECVAM), established by the European Commission in 1991 to promote the scientific and regulatory acceptance of alternative methods, recently sponsored a workshop to discuss the current status of the Three Rs and to make recommendations aimed at achieving greater acceptance of the concept of humane experimental technique.

Twenty-one scientists professionally committed to the Three Rs were invited to attend the conference, which was chaired by Michael Balls, head of ECVAM, and Alan M. Goldberg, director of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT). The conference was held in Sheringham, Norfolk, UK on May 30, 1995–June 3, 1995. A report based on the conference was published by ECVAM (*1*) in December 1995 and is excerpted as follows.

Origins of the Three Rs

The Three Rs originated in a proposal made in 1954 by Charles Hume, founder of the Universities Federation for Animal Welfare (UFAW), that the UFAW should undertake a scientific study of humane technique in laboratory animal experiments. The project was managed by a committee under the chairmanship of Sir Peter Medawar, the Nobel prize winning immunologist, with William Lane-Petter, Secretary of the Research Defence Society of Great Britain, among its members. Christine Stevens, founder of the Animal Welfare Institute (AWI) in the U.S., provided financial support for the project. W.M.S. Russell, a zoologist, and R. L. Burch, a microbiologist, were appointed to carry out the work, which led to the publication of the book *The Principles of Humane Experimental Technique* (*2*) in 1959.

At the time of the book's publication, Charles Hume commented that

> This deserves to become a classic for all time, and we have great hopes that it will inaugurate a new field of systematic study. We hope that others will follow up the lead it has given, and that a generalised study of humane technique, as a systematic component of the methodology of research, will come to be considered essential to the training of a biologist.

Hume's predictions regarding the book's impact have been realized as the concepts of replacement alternatives, reduction alternatives, and refinement alternatives have become established in law. However, at the present time, a thorough working knowledge and acceptance of the principles of humane experimental technique among scientists in general remains at best elusive and at worst ignored.

Scientific and Ethical Justification

Current legislation in Europe and the United States decrees that all proposed use of laboratory animals should be subject to review to determine whether such use appears to be scientifically and ethically justifiable. Individually and collectively, such laws not only recognize Russell and Burch's concept (*2*) but place legal and moral obligations on all concerned to replace, reduce, and refine laboratory animal experimentation wherever possible.

The degree to which proposed animal use is reviewed varies from country to country. For example, in the United Kingdom, a working party of the Institute of Medical Ethics concluded that a project using animal subjects should only be done when the review committee ascertains that the aim of the project is worthwhile; that the experimental design of the project is such that there is a good likelihood of achieving the stated aims; that the aim could not be achieved using methods or subjects that were morally more acceptable and that produced no less scientifically acceptable results; and that the likely benefits of the project are worth the costs to the animals in terms of pain and suffering (*3*).

In the United States, the Animal Welfare Act requires that all procedures involving animals be reviewed by an institutional animal care and use committee (IACUC). The IACUCs are required to ensure that the protocols are worthwhile, that they use the minimum number of animals necessary, and that the investigators document that they have adequately considered alternatives to any procedure that causes more than momentary pain or distress (either with or without the use of anesthetics). Guidelines for searching for alternative procedures have been prepared to assist investigators and IACUC members in these considerations (*4*).

The Three Rs

Reduction Alternatives

The term reduction alternatives describes methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures or for obtaining more information from a given number of animals so that, in the long run, fewer animals are needed to complete a given research project or test. The greater the number of animals used, the greater will be the overall costs in terms of animal suffering. Therefore, the number of animals used should be the minimum that is consistent with the aims of the experiment.

There is evidence that poor experimental design and inappropriate statistical analysis of experimental results leads to inefficient use of animals and scientific resources in toxicological research (*5,6*). Previous studies of statistical methods used in other areas of biomedical research reveal similar findings (*7*). In some cases, the level of statistical expertise appears to be so low that investigators are either unaware of the potential value of obtaining statistical advice, or they are unable to obtain appropriate statistical advice because there are so few biometricians with experience in their field of interest.

A basic understanding of experimental design and statistics is necessary for all scientists. For investigators with no previous training in statistics, this level of expertise can probably be obtained from an introductory course. There are many texts on statistical methods, which can be used for both learning purposes and as reference books. Biomedical research workers should have more detailed training in biometrics and sta-

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tistics so that they can act as consultants to other investigators in their own institutes.

Refinement Alternatives

Refinement alternatives encompass those methods that alleviate or minimize potential pain and distress and enhance animal well-being. Distress is an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and, therefore, shows maladaptive behavior. The stressors may induce physiological, psychological, or environmental stress. Pain results from potential or actual tissue damage, such as that caused by injury, surgery, or disease, and can lead to distress (*8–10*).

Much potential pain and distress can be avoided or alleviated with the proper use of anesthetics, analgesics, and tranquilizers. This critical component of any comprehensive program of veterinary care provides for frequent observation of the animals by trained veterinary staff to detect and relieve pain and distress. However, a substantial number of animals used in research and testing experience unrelieved pain and distress.

At present, we do not have a convenient and standardized way of objectively assessing animal pain and distress. Rather, the assessment is generally based on subjective clinical signs of abnormal behavior and appearance. Although the implementation of refinement alternatives depends largely on the ability of scientists to observe and understand the behavior and needs of laboratory animals, many experimenters are as lacking in ethological knowledge as they are in statistical training. The best approach to pain and distress is to assume that a procedure that inflicts pain and distress in human beings will inflict at least as much pain and distress in animals, unless there is evidence to the contrary.

Very little research funding is available to support efforts to investigate and refine experimental techniques and scientific procedures. Furthermore, there is no readily available up-to-date knowledge base on refinement. Techniques that are developed to refine a procedure are frequently not reported in the scientific literature or are established simply as standard operating procedures (SOPs) within an institution. To establish best practice and to advance the implementation of refinement alternatives, it is important to share such experience, data, and SOPs. Sharing of data and theories is normally accomplished via the scientific literature, but there has been a marked lack of opportunity to discuss and provide information on refinement alternatives in the main biological journals. Consequently, scientists are not sufficiently aware of the concept of refinement alternatives and, in general, they do not recognize the importance of refinement in their research. The concept of recognizing, minimizing, and eliminating pain and distress in laboratory animals should be included in training programs for all persons involved in the care and use of laboratory animals. Details of refinement and animal welfare considerations should routinely be included in scientific papers and publications.

Replacement Alternatives

Replacement alternatives encompass those methods that permit a given purpose to be achieved without conducting experiments or other scientific procedures on animals. Russell and Burch (*2*) distinguished between relative replacement, e.g., the humane killing of a vertebrate animal to provide cells, tissues, or organs for *in vitro* studies and absolute replacement in which animals would not need to be used at all, e.g., the culture of human and invertebrate cells and tissues.

The range of replacement alternative methods and approaches includes the improved storage, exchange, and use of information about previous animal experiments to avoid unnecessary repetition of animal procedures; use of physical and chemical techniques and predictions based upon the physical and chemical properties of molecules; use of mathematical and computer models; use of organisms with limited sentience such as invertebrates, plants and microorganisms; use of *in vitro* methods including subcellular fractions, tissue slices, cell suspensions, and perfused organs; and human studies including use of human volunteers, postmarketing surveillance, and epidemiology.

In many areas of the biomedical sciences, *in vitro* methods are increasingly used as the methods of choice in place of animal studies, not because they provide precisely the same information, but because they offer the best scientific approach. Russell and Burch (*2*) discussed the relative merits of fidelity and discrimination models, noting that high-fidelity models, as exemplified by the use of rodents and other laboratory mammals in toxicity testing, are used because, in their general physiological and pharmacological properties, they are similar to humans. High discrimination models, on the other hand, "reproduce one particular property of the original, in which we happen to be interested" (*2*).

Russell and Burch (*2*) warned of the high-fidelity fallacy and of the danger of expecting discrimination in particular circumstances from models that show high fidelity in other, more general terms—a prediction illustrated by recent analyses of the differing molecular responses to certain chemicals by the rat, the mouse, and the human. Russell and Burch (*2*) pointed out that the fidelity of mammals as models for man is greatly overestimated; however, replacement alternative methods must be based on good science, and extravagant claims that cannot be substantiated must be avoided. The development and acceptance of replacement alternatives for both research and testing must be based on a sufficient understanding of the molecular and cellular mechanistic basis of what is being studied or measured, i.e., on sound science.

Education and Training

The successful implementation of the Three Rs depends upon the education and training of those involved in research and testing. Education is defined as the didactic presentation of the information and theories of animal use that will contribute to the development of proper attitudes toward the use of animals in scientific procedures. Training is defined as the acquisition of practical knowledge and skill directly associated with animal handling and procedures.

The objective of the education and training is to provide sufficient information to allow scientists to conduct animal procedures to high standards of both science and animal welfare, following proper evaluation of the scientific and ethical considerations that should govern the use of laboratory animals. Coursework should contribute to a scientist's ability to design experiments properly and to plan research strategies, to become competent in animal handling and the performance of scientific procedures, to make decisions with regard to the ethics of using animals in experiments, and to determine whether alternatives are available.

A description of the course on animal experimentation and alternatives currently offered at Utrecht University in The Netherlands (*11*) and the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA) (*12*) and the U.S. National Research Council (*13*) could serve as prototypes for the development of courses in other countries.

The Way Forward

The use of the term alternatives to encompass all of the Three Rs is now widely accepted in many countries, enshrined in legislation, and incorporated into the names of various centers throughout the world. However, some scientists see its use as being driven by political and social forces rather than by scientific issues. This is partly due to a lack of appreciation of the basis of the Three Rs concept as proposed by Russell and Burch (i.e., that scientific excellence and the greatest humanity in the use of laboratory animals are inextricably linked) (*2*). It also stems

from a defensive attitude among some scientists, perhaps resulting from the campaigns of some antivivisection organizations and from insufficient dialogue among the scientific and animal protection communities.

In the mid-1990s, the question we face is whether there will be a revolution in thinking and practice, which is what is needed if the principles of humane experimental technique are to be brought fully and effectively into operation. Much has been achieved, but there is still considerable room for progress and improvement.

The Sheringham workshop participants propose several general recommendations:

- Existing laboratory animal protection laws should be fully implemented.
- All countries should have a legal framework that actively incorporates the Three Rs into all animal-based research, testing, and education.
- There should be formal and informal mechanisms for the education and training of academic, industrial, and government scientists and officials in the Three Rs to ensure compliance with the spirit and letter of laboratory animal protection legislation and regulations.
- There should be international discussion and agreement on what levels of animal suffering should not be permitted in any circumstances, regardless of any likely or potential benefits.
- It is unacceptable to export scientific work involving laboratory animals to avoid scientifically realistic, but more stringent, animal welfare codes.

The participants of the Sheringham workshop unanimously reaffirmed the principles put forth by Russell and Burch (*2*) that humane science is good science and that this is best achieved by vigorous application of the Three Rs. The only acceptable animal experiment is one that uses the smallest number of animals and causes the least possible pain or distress, is consistent with the achievement of a justifiable scientific purpose, and is necessary because there is no other way of achieving that purpose. Any proposed experiment on animals should be subjected to prior and effective expert review by an ethics committee. Scientists should be better informed about the Three Rs concept and should be encouraged to see it as an opportunity for reaping benefits of every kind—scientific, economic, and humanitarian. Only in this way can the aspirations of all those who have worked for the good of both human and animal welfare be achieved at last.

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