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The Three Rs: The Way Forward

The Report and Recommendations of ECVAM Workshop 11[1,2]

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Preface

This is the report of the eleventh of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM), which was established in 1991 by the European Commission. ECVAM's main goal, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures which would enable it to become well-informed about the state-of-the-art of non-animal test development and validation, and the potential for the possible incorporation of replacement alternative tests into regulatory procedures. It was decided that this would be best achieved by the organisa-

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[1]ECVAM — The European Centre for the Validation of Alternative Methods. [2]This document represents the agreed report of the participants as individual scientists.

tion of ECVAM workshops on specific topics, at which small groups of invited experts would review the current status of various types of *in vitro* tests and their potential uses, and make recommendations about the best ways forward (1).

The workshop on The Three Rs — The Way Forward, held in Sheringham, Norfolk, UK, on 30 May to 3 June 1995, under the co-chairmanship of Michael Balls (ECVAM) and Alan M. Goldberg (Johns Hopkins Center for Alternatives to Animal Testing [CAAT], Baltimore, MD, USA), had a wider aim. The principal objectives of this workshop were 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 and, in the interests of both scientific excellence and the highest standards of animal welfare, the more active implementation of reduction alternatives, refinement alternatives and replacement alternatives.

The invited participants were individuals actively and professionally committed to the Three Rs, and we were privileged to have William Russell and Rex Burch, who developed the Three Rs approach in the 1950s, as participants in the workshop.

The opening ceremony was held in Sheringham Town Hall, where Rex Burch has practised as a microbiologist since the early 1970s. Since this was the first time that Russell and Burch had attended a scientific conference together for nearly forty years, the proceedings were recorded on videotape (the VHS tape can be borrowed from ECVAM and a JVC version is available from CAAT). The rest of the workshop was held at the Links Country Park Hotel, West Runton.

Introduction

The origins of the Three Rs concept

What are now known as the Three Rs of Russell and Burch, *replacement, reduction* and *refinement*, have their origins in a proposal made in 1954 by Charles Hume, founder of the Universities Federation for Animal Welfare (UFAW), that 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, with William Lane-Petter, Secretary of the Research Defence Society, among its members. It was international from its outset, since Christine Stevens, of the Animal Welfare Institute (AWI) in the USA, provided financial support and made frequent visits to UFAW while the study was being conducted.

W.M.S. Russell and R.L. Burch were appointed to carry out the work. This led to their book, *The Principles of Humane Experimental Technique* (2), which provided a wealth of information and many remarkable ideas and insights, most of them as relevant today as they were more than 35 years ago. The book has recently been reprinted (3), and copies can be obtained from UFAW.[1] It was in this book that Russell and Burch presented the concept of the Three Rs. They defined *replacement* as "any scientific method employing non-sentient material which may in the history of animal experimentation replace methods which use conscious living vertebrates", *reduction* as a means of lowering "the number of animals used to obtain information of a given amount and precision", and *refinement* as any development leading to a "decrease in the incidence or severity of inhumane procedures applied to those animals which have to be used".

Nobody can recall precisely when the Three Rs concept arose (4), but it was sometime between 1955 and 1957. UFAW held a symposium on *Humane Technique in the Laboratory* (5) in 1957, and it was then that the concept of the Three Rs was first discussed in public. More about the origins of the Three Rs concept can be found in a talk given by Charles Hume in Washington in October 1959 (6), when he said of *The Principles of Humane Experimental Technique:*

"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 train-

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ing of a biologist."

What is perhaps the central message (the "humanity criterion") of *The Principles of Humane Experimental Techique* (2) is spelled out on page 157 of the book:

"If we are to use a criterion for choosing experiments to perform, the criterion of humanity is the best we could possibly invent."

"The greatest scientific experiments have always been the most humane and the most aesthetically attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful."

The evolution of the Three Rs concept

Despite its originality and scholarship, and the involvement of many distinguished scientists in the discussions leading up to its publication, Russell and Burch's book had little obvious impact on thinking or practice in the early years after its publication. In fact, its authors did not meet each other again for about 30 years, when they were "rediscovered" by a new generation of reformers.

However, in 1969, one particularly significant development did take place — the foundation by Dorothy Hegarty of the Fund for the Replacement of Animals in Medical Experiments (FRAME), specifically to advance Russell and Burch's vision that humanitarian and scientific benefits would result from the systematic and rational application of the Three Rs approach. FRAME saw reduction and refinement as achievable in the short term, but decided to focus its own activities primarily on replacement as the ultimate, long-term goal (7). FRAME was to succeed in establishing itself in the middle ground between the antivivisectionists and the defenders of animal-based research, with a positive message, based not on confrontation, but on support of the Three Rs concept.

In the 1970s, there were a number of other significant events. For example, there was a substantial increase in laboratory animal use in the early part of the decade, which led to great public concern in Great Britain, and an Animal Welfare Year campaign, involving many animal welfare organisations, was organised to mark the centenary of the *Cruelty to Animals Act 1876* (8), the law under which experiments on animals were allowed and regulated. This led in turn to the formation of the Committee for the Reform of Animal Experimentation (CRAE), which had as its principal goal the reform of the 1876 Act (9). The concept of alternatives was also taking hold in the USA, as a result of the efforts of the AWI (who distributed Russell and Burch's book), United Action for Animals, and the Humane Society of the United States.

Meanwhile, David Smyth, a distinguished physiologist, was conducting a survey on the Three Rs for the Research Defence Society, which led to another important landmark, the publication of his book on *Alternatives to Animal Experiments* (10). Smyth provided a Three Rs definition of *alternatives*, which has since been widely accepted:

"All procedures which can completely replace the need for animal experiments, reduce the numbers of animals required, or diminish the amount of pain or distress suffered by animals in meeting the essential needs of man and other animals."

A number of particularly important changes began to take place at the beginning of the 1980s. In the USA, animal activist Henry Spira launched a campaign to abolish the Draize eye irritancy test, with the worldwide support of a coalition of 400 animal organisations. In Europe, discussions began which were later to lead to the *Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (11) and *Council Directive 86/609/EEC of 24 November 1986 on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes* (12).

Meanwhile, the British Home Secretary, Merlyn Rees, said that he would consider listening to proposals for the reform of the 1876 Act, but only if animal welfare organisations would agree on a policy among themselves. An alliance was therefore formed between CRAE, FRAME and the British Veterinary Association (BVA; 9). A set of BVA/CRAE/FRAME proposals were submitted in 1983 (13), which greatly influenced British Government thinking, as revealed in two White Papers (14, 15). In what was a

very dramatic development at the time, the 1985 White Paper contained a commitment to the Three Rs concept, in these words:

"Animal experiments that are unnecessary, use unnecessarily large numbers of animals, or are unnecessarily painful, are indefensible."

Members of the BVA/CRAE/FRAME alliance were invited to act as advisers to the British Government during the preparation and passage through Parliament of the *Animals (Scientific Procedures) Act 1986* (16), which was supported by both the Conservative and Labour parties and replaced the 1876 Act. The 1986 Act set up a project and personal licensing system, as well as an independent Animal Procedures Committee (APC), which can give advice to the Government, whether or not it is wanted. The Act contains two particularly important clauses (16):

5(4). In determining whether and on what terms to grant a project licence the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme of work to be specified in the licence.

5(5). The Secretary of State shall not grant a project licence unless he is satisfied that the applicant has given adequate consideration to the feasibility of achieving the purpose of the programme to be specified in the licence by means not involving the use of protected animals.

Directive 86/609/EEC (12) spelled out its Three Rs basis in Article 7, as follows:

7.2. An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.

7.3. When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected.

7.4. All experiments shall be designed to avoid distress and unnecessary pain and suffering to the experimental animals.

Similar words are used in the Council of Europe Convention (11).

In Germany, when the national legislation on animal protection was changed in 1987 to meet the requirements of *Directive 86/609/EEC*, a clause was inserted which requires the Federal Government to present a report (the *Tierschutzbericht*) to the Bundestag every two years, to document the progress made with respect to the implementation of animal protection measures. This puts pressure on the relevant government institutions to take the necessary steps to implement current legislative requirements for animal protection. According to the German animal protection act (the *Tierschutzgesetz*), nobody is allowed to cause pain, suffering or harm to an animal without good reason. The legislation closely resembles *Directive 86/609/EEC*, with an additional provision which prohibits animal experimentation for developing tobacco products, washing detergents and decorative cosmetics.

In The Netherlands, the *Act on Animal Experimentation* was adopted in 1977. Items included in this Act which are of importance with regard to the implementation of the Three Rs are:

a) Mandatory registration of animal use.
b) Prohibition of the use of an animal for a purpose that could be achieved equally by using *in vitro* methods or other non-animal procedures.
c) The requirement that persons involved in animal experimentation are shown to be competent. Education and training in the field of laboratory animal science, including ethics and alternatives, is mandatory for scientists and animal technicians.
d) The requirement that institutions where animal experiments are conducted must be licensed.
e) The requirement that a certificated animal welfare officer be appointed in association with the licence for the institute.
f) Mandatory use of anaesthetics and analgesics when appreciable pain is anticipated. Their use may only be omitted when this would jeopardise the purpose of the experiment.

At present, the 1977 Act is being revised; the amended Act will include provisions relating to Animal Experimentation Committees (AECs; see section on *Scientific and Ethical Justification).*

Following the adoption of the *Act on Animal Experimentation*, several initiatives were undertaken. A Department of Laboratory Animal Science was established at Utrecht University in 1983; in this Department, research and education programmes have been developed which are specifically directed toward further implementation of the Three Rs, and courses on laboratory animal science are routinely held for scientists. In 1987, the Dutch Alternatives to Animal Experiments Platform was established, through which government, industry and animal welfare organisations cooperate in order to stimulate the development and use of alternative methods. The main task of the Platform is to advise the Government on the funding of research projects concerned with the development of alternatives. The Netherlands Centre Alternatives to Animal Use (NCA) was established in Utrecht in 1994, as a national information centre on alternatives. The main objective of the NCA is to stimulate the development, validation, acceptance and use of alternative methods; that is, the NCA supports the Platform in seeking to realise its goal.

In the USA, the Johns Hopkins Center for Alternatives to Animal Testing (CAAT) was founded in 1981, with the support of a grant from the Cosmetic, Toiletry and Fragrance Association, to address the major issues facing the development of alternatives (17). CAAT's initial focus was on establishing a small grants programme, through which it has funded the development of new *in vitro* systems, and of assays that could ultimately be used for product safety testing, by fundamental research scientists. Over the years, CAAT has become a visible advocate of the Three Rs, and it fulfils a unique role in the USA in liaising with scientists from academia, industry and governmental organisations. In this capacity, CAAT has organised regular scientific symposia and has played a major role in bringing together diverse groups to formulate a framework for the validation of alternative methods for product safety testing (18).

During the early 1980s, the campaigns against the Draize and LD50 tests, and simultaneous attempts to pass legislation in the USA to promote the use of alternatives, focused industrial and congressional attention on alternative methods. Public pressure led to the revision and strengthening of the *US Animal Welfare Act* and the *Public Health Service Policy on the Humane Care and Use of Laboratory Animals* (19), both of which incorporated the requirement that consideration be given to the Three Rs before any research involving the use of animals was started. The concept of alternatives was also promoted via legislation relating to the role and activities of the National Institutes of Health (NIH).

In 1986, a report by the US Congress Office of Technology Assessment on *Alternatives to Animal Use in Research, Testing and Education* (20) provided evidence of the broad scope and potential of the Three Rs concept of alternatives and, in the same year, the *Health Research Extension Act* gave legislative force to the revised Public Health Service Policy on animal research. In Europe, the European Research Group for Alternatives in Toxicity Testing (ERGATT) was also established in 1986.

A set of *International Guiding Principles for Biomedical Research Involving Animals* were published in 1985 (21); the basic principles are outlined in Table I. Thus, by the end of the 1980s, new laws were in place in various parts of the world, which not only recognised Russell and Burch's concept, but placed legal and moral obligations on all concerned to seek to replace, reduce and/or refine laboratory animal experimentation wherever possible. Full implementation of these laws and the development of replacement alternatives became the next challenges.

In 1993, the US *NIH Revitalization Act* included statements drafted and supported by animal protection organisations and by several large corporations which promote the concept of alternatives. In particular, the Act authorised the establishment of an Applied Toxicology Program within the National Institute of Environmental Health Sciences (NIEHS), which eventually developed into the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM). At the same time, a group of scientists

Table I: International Guiding Principles for Biomedical Research Involving Animals

I.	The advancement of biological knowledge and the development of improved means for the protection of the health and well-being both of man and of animals require recourse to experimentation on intact live animals of a wide variety of species.
II.	Methods such as mathematical models, computer simulation and *in vitro* biological systems should be used wherever appropriate.
III.	Animal experiments should be undertaken only after due consideration of their relevance for human or animal health and the advancement of biological knowledge.
IV.	The animals selected for an experiment should be of an appropriate species and quality, and the minimum number required, to obtain scientifically valid results.
V.	Investigators and other personnel should never fail to treat animals as sentient, and should regard their proper care and use and the avoidance or minimisation of discomfort, distress, or pain as ethical imperatives.
VI.	Investigators should assume that procedures that would cause pain in human beings cause pain in other vertebrate species although more needs to be known about the perception of pain in animals.
VII.	Procedures with animals that may cause more than momentary or minimal pain or distress should be performed with appropriate sedation, analgesia or anaesthesia in accordance with accepted veterinary practice. Surgical or other painful procedures should not be performed on unanaesthetised animals paralysed by chemical agents.
VIII.	Where waivers are required in relation to the provisions of article VII, the decisions should not rest solely with the investigators directly concerned but should be made, with due regard to the provisions of articles IV, V and VI, by a suitably constituted review body. Such waivers should not be made solely for the purpose of teaching or demonstration.
IX.	At the end of, or when appropriate during, an experiment, animals that would otherwise suffer severe or chronic pain, distress, discomfort, or disablement that cannot be relieved should be painlessly killed.
X.	The best possible living conditions should be maintained for animals kept for biomedical purposes. Normally the care of animals should be under the supervision of veterinarians having experience in laboratory animal science. In any case, veterinary care should be available as required.
XI.	It is the responsibility of the director of an institute or department using animals to ensure that investigators and personnel have appropriate qualifications or experience for conducting procedures on animals. Adequate opportunities shall be provided for in-service training, including the proper and humane concern for the animals under their care.

Taken from Howard-Jones, (21).

from key regulatory agencies in the USA established the Interagency Regulatory Alternatives Group (IRAG; an *ad hoc* committee), to discuss the implementation of alternatives in the regulatory sector.

In 1989, Alan Goldberg and Bert van Zutphen decided to initiate a series of world congresses devoted to alternatives and animal use in the life sciences (covering the Three Rs in research, testing and education), which were to be held every three years. The first World Congress was held in Baltimore, USA, in November 1993, and was attended by 725 people (representing academia, industry, and government and animal protection organisations) from 24 countries (22). The second World Congress is to be held in October 1996 in Utrecht, in The Netherlands, while a third World Congress, to be held in Italy in 1999, is already being planned.

It is clear that significant changes have occurred in the planning and conduct of biomedical research projects. In Great Britain, where reasonably accurate statistics on animal use are available, the data indicate that the use of animals increased by an average of 6% per year between 1937 and 1971, to a total of over 5.5 million (23). From 1972 to 1978, the number of animals used remained relatively stable, and then animal use began to decrease by an average of 5% per year from 1979 onwards. The increase in animal use was driven largely by the search for new drugs and the expansion of the pharmaceutical industry. Since the mid-1970s, the use of animals in commercial, government and university/hospital laboratories in Britain has dropped by 65%, 56% and 26%, respectively. In The Netherlands, total animal use has declined by 50% since 1978 (24). While the data for the USA are less reliable, it has been argued that animal use has also decreased significantly in American laboratories, despite funding for biomedical research having increased during this period (25).

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 expectations of Hume, Russell, Burch, Lane-Petter, Medawar, Stevens, and all the others involved in the original UFAW project, are to be met, and 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.

Scientific and Ethical Justification

All proposed use of laboratory animals should be subject to review, to determine whether such use appears to be scientifically and ethically justifiable. In some circumstances, the "alternative" may simply be not to undertake the animal procedure at all. Where the necessity of conducting certain animal procedures cannot be justified sufficiently on scientific or ethical grounds, the project proposal should be rejected. Guidelines have been prepared to assist review committees in assessing whether alternatives have been adequately considered (26).

In their consideration of the ethics of using animals in biomedical research, a Working Party of the Institute of Medical Ethics (UK) concluded that "a research project involving animal subjects should take place only when it can be shown:

a) that the aim of the project is worthwhile;
b) that the design of the project is such that there is the strong possibility that it will achieve the aim;
c) that the aim could not be achieved using morally more-acceptable and scientifically no less-acceptable alternative subjects and procedures; and
d) that the likely benefits of the project are substantial enough in relation to the suffering likely to be caused to the animals used (that is, the likely benefits of the research should be 'weighed' against the 'costs' to the animals involved)"(27).

It is these four main points, which include the need to consider the potential for using alternative methods, which should be addressed to the satisfaction of the reviewers (who generally include biomedical scientists, veterinarians, ethicists, and community representatives with an interest in animal protection).

The UK system involves licensing specific persons both with respect to the projects to be undertaken and for their personal use of laboratory animals (28). It provides a comprehensive and vigorous system of controls when taken together with the formal certification (designation) of heads of establish-

ments where experimental animals are used and the common species are bred. The *Animals (Scientific Procedures) Act 1986* also provides for the appointment of inspectors in the Home Office (the government department equivalent to the ministry of internal affairs in other countries). Home Office Inspectors check all designated establishments to ensure compliance with, or to report on non-compliance with, the 1986 Act, or with the terms and conditions of licences or certificates issued under the Act.

Uniquely in Britain, individual Home Office Inspectors statutorily review projects and protocols, and advise the Minister (in practice, his officials) on the costs *versus* benefits, with the aim of ensuring that only properly justified work is licensed; these are functions performed by ethics review committees in other countries. Where appropriate, views on proposed research projects are sought from other Inspectors and, occasionally, from external assessors or the APC. Some special categories of work are mandatorily referred to the APC, for example, work with primates and on cosmetics, and applications to use animals for microsurgical training. Home Office Inspectors only make recommendations to government officials but, in practice, their advice is usually accepted. Inspectors have almost no formal enforcement or executive powers with regard to licensees, but can order the immediate humane killing of animals they consider to be suffering excessively. Experimenters rarely disregard the views of Home Office Inspectors on the extent to which practical outcomes match the detailed protocols authorised by project licences.

In Germany, the Department of Agriculture, which is responsible for animal protection, adopts the general philosophy that, even if it is more expensive to use a non-animal method than to conduct an animal procedure, a lower cost is not sufficient justification for using animals. However, in 1994, the highest constitutional court in Germany ruled that an animal experiment which is scientifically justifiable cannot be prohibited for ethical reasons, interpreting this to be in compliance with both the national legislation and *Directive 86/609/EEC.*

In The Netherlands, the performance of animal experiments is not permitted unless the protocol has been reviewed and approved by an AEC. According to the proposed revision of the 1977 *Act on Animal Experimentation*, the chairperson and at least two other members of such a committee must be independent (that is, they must not have a working relationship with the institutions for which the protocol is reviewed). Furthermore, the AEC must also include experts on ethics and on alternative methods, and the composition of the committee must be approved by the National Committee on Animal Experimentation. The main tasks of an AEC are to evaluate whether the expected benefit of the proposed experiment outweighs the likely suffering of the animals concerned, and to ascertain that the feasibility for implementing the Three Rs has been adequately taken into account when preparing the protocol. AECs are also required to evaluate the competence of the persons involved in the design and performance of the experiments. Rejection of a proposed research protocol by the AEC can only be overruled by the National Committee on Animal Experimentation.

In the USA, under both the *Animal Welfare Act* amendments of 1985 (regulations approved in 1989) and the Public Health Service revised policy, Institutional Animal Care and Use Committees (IACUCs) are required to review and approve all animal research proposals before the research is allowed to proceed. The IACUCs are expected to ensure that approved animal research protocols are worthwhile, that they use the minimum number of animals necessary, that animal pain and distress are minimised, and that, in any procedures likely to cause animal pain and distress (whether or not anaesthetics or analgesics are used), principal investigators document that they have established "that alternatives were adequately considered"(29).

While the manner in which IACUCs pursue their duties varies, the inspectors enforcing the Act have paid particular attention to the requirement that investigators document the lack of alternatives, and to the section of the regulations which reads:

"Research facilities will be held responsible if it is subsequently determined that an alternative procedure was available to accomplish the objectives of the proposed experiment . . . or if it is subsequently determined that an experiment is unnecessarily duplicative and that a good-faith review of available sources would have indicated as much."

As a result, the consideration of alternatives in the USA, while not necessarily embraced enthusiastically, is becoming routine during the preparation of research proposals.

Selection of Appropriate Animals

In whatever country the research is to be conducted, in designing a project the investigator should first consider whether the aims of the project could be realised by using *in vitro* techniques or less sentient animal species, such as insects or nematodes. The replacement of one animal species with another, particularly if the species which is then used is non-vertebrate, could also be considered to be an alternative method (30). Therefore, the model selected should be the lowest phylogenetic species, and also the least sentient species, which will allow the scientific objectives to be realised. If the use of living vertebrates is considered to be essential, the aim should be to use the minimum possible number of animals (see the section on *Reduction Alternatives)*, and to use strategies which will ensure that the animals which must be used are subject to the minimum discomfort (see the section on *Refinement Alternatives*).

A variety of strains of certain species are available. For example, over 400 inbred strains of mice and over 200 inbred strains of rats have been developed. These provide a wide range of phenotypes which are of potential value in many areas of research. The choice of strain for a particular project should largely be governed by a knowledge of its characteristics, and by the need to control phenotypic variability. In most cases, isogenic (inbred or F1 hybrid) animals are more suitable than outbred stocks, because of their high phenotypic uniformity, long-term stability, identifiability, and detailed background information on their characteristics (31).

However, in biological assays against standards (where phenotypic uniformity is especially important), although F1 hybrids are usually more uniform than inbreds, there are no *a priori* grounds for choosing the most suitable strain or cross for a particular assay, and further studies of the type described by Hendriksen *et al.* (32) should be undertaken, preferably as part of ongoing studies to minimise the number of animals which are used for each assay and the degree of discomfort caused by current assay procedures.

Where uniformity is important, it can also be promoted by controlling the environment in which the animals are reared and used. As Chance discovered many years ago (reviewed in Russell & Burch [2]), this does not mean keeping the environment uniform and constant in all respects, but rather keeping it uniform, constant and appropriate in certain key respects. Reduction here generally coincides with the concept of refinement. For example, as described by Fox (33), "handling weanling female rats for three days prior to experiments using the Steelman-Pohley method of follicle-stimulating hormone assay reduced the variability of their response", so that "about twice as many non-handled rats would be required in an assay to obtain the same degree of precision as with handled rats".

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 in an experiment, the greater will be the overall costs, in terms of animal suffering (27). Thus, the number of animals used should be the minimum which is consistent with the aims of the experiment. However, past experience in the area of regulatory toxicity testing, for example, suggests that laboratory animal welfare considerations and common sense do not always prevail (for example, in LD50 testing; 34). Saving of time or personal convenience, or other non-scientific reasons, are not sufficient justification for using more animals than the minimum necessary to obtain meaningful results. Proper statistical design, prior to undertaking the study, and appropriate analysis of the resulting data, may make it possible to obtain results of comparable precision by using fewer animals.

The precision of an experiment depends mainly on the sample size and the "error" variance and not on the body weight of the

test animal. The use of more animals on the grounds that they are smaller and less expensive is not scientifically justifiable. If a test has to be conducted in a rodent and in a non-rodent species and the test guideline specifies the use of four dogs, then the use of more than four rats cannot be scientifically justifiable. It is recognised that problems with a protocol may be encountered once the experiment is under way. To prevent the continuation of unsuccessful animal experiments without review, acceptable limits for failures in protocols, and the actions to be taken if these occur, should be specified.

Careful attention should also be given to the type of endpoint to be used. Qualitative endpoints (for example, dead/alive) often involve severe animal pain and distress, and generally provide less information than do quantitative measurements.

Research strategy

Relatively little consideration has been given to research strategy since Russell and Burch (2) discussed the random screening of potential new pharmaceutical agents under this heading. Such screening is now largely done by using *in vitro* systems, which is one reason for the decrease in the numbers of animals used in the last decade. However, research strategy is also important in other contexts. In particular, it may be necessary to carry out small pilot studies which can be reviewed before committing animals and resources to major experiments. The statistical guidelines developed by Muller *et al.* (35), which include a detailed discussion of possible research strategies, should be brought to the attention of biomedical investigators.

Experimental design and statistics

Optimum experimental design and statistical considerations may suggest that a particular protocol employs an insufficient number of animals, and that more are needed to provide a satisfactory answer to the question being posed. However, this should still lead to an overall reduction in animal use, since experiments which use too few animals will generally not achieve their desired objectives, and will frequently need to be repeated with a larger number of animals.

Regulatory tests

International harmonisation of protocols in regulatory testing should lead to an overall reduction in the use of animals, provided that there is a reasonable compromise on acceptable sample sizes. Such harmonisation should provide an opportunity to review the design and sample sizes required in regulatory experiments, since, in some cases, sample sizes appear to have been decided in an arbitrary manner, without taking statistical considerations into account. Where possible, the requirements should be formulated in terms of acceptable confidence intervals, rather than by specifying the numbers of animals needed, so that where greater control of phenotypic variation is possible, the number of animals can be reduced.

Efforts at international harmonisation should target not only the protocols for particular tests, but also the specific requirements for those tests. This would reduce the numbers of animals used by minimising the array of tests required.

Non-regulatory experiments

There is evidence that poor experimental design and inappropriate statistical analysis of experimental results is leading to inefficient use of animals and of scientific resources in toxicological research (36–38). This is in agreement with previous studies of statistical methods used in other areas of biomedical research (37–41). However, more investigation is needed to determine whether appropriate experimental design and statistical analysis are employed in other areas of research, such as experimental surgery, pharmacology, biochemistry, experimental immunology, and microbiology.

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.

The "named statistician"

In some countries, a "named veterinarian" must be appointed to supervise some aspects of laboratory animal welfare. In view of the importance of good experimental design and appropriate statistical analysis in underpinning high quality research, and the potential savings in terms of the numbers of animals which are used, consideration should be given to the need for a full-time or part-time "named statistician" to be associated with

research facilities. This statistician would undertake to be available to all investigators who needed advice on experimental design and statistical analysis of experimental data, and possibly would have some statutory obligation to meet regularly with investigators to discuss current research projects.

Education in statistics

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 a course involving the equivalent of about 30 hours of lectures and associated practical work. However, an equivalent level of expertise could also be obtained by reading and by using computer-assisted learning techniques. Books by Cox (42) and Cochran & Cox (43) provide a good introduction to experimental design; Cohen (44) deals specifically and in detail with the problem of determining the appropriate size for an experiment. There are many texts on statistical methods, which can be used both for learning purposes and as reference books (45–48). There is also a need for some biomedical research workers to have a more detailed training in biometrics/statistics, so that they can act as consultants to other investigators in their own institutes.

Refinement Alternatives

Refinement alternatives encompass those methods which alleviate or minimise potential pain and distress, and which 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 behaviour (49). 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. These terms and concepts have been defined and discussed previously (49–51).

Pain and distress can result from both experimental and non-experimental causes. Potential sources of experimental pain and distress include: improper or prolonged restraint, experimental infections, chemical-induced toxic effects, surgical and experimental procedures, post-operative pain, and improper euthanasia techniques. Non-experimental sources include: naturally occurring infectious and non-infectious diseases, suboptimal environmental conditions, improper handling, stressful housing situations (for example, social isolation, barren cages or pens), injuries sustained during fighting, and injuries associated with the housing or caging.

Much potential pain and distress can be avoided or at least alleviated with the proper use of anaesthetics, analgesics and tranquilisers, which is a critical component of any comprehensive programme of adequate veterinary care. Such a programme provides for frequent observation of the animals by trained veterinary staff, to detect and appropriately relieve pain and distress. However, a substantial number of animals used in research and testing experience unrelieved pain or distress. All experimental protocols should be sufficiently detailed with regard to the type and severity of likely adverse effects, the times of peak occurrence, humane endpoints, and the remedial actions to be taken.

In The Netherlands, a serious attempt has been made to categorise animal experiments on the basis of the severity of pain and distress experienced. In 1993, 51.4% of the animals were reported to have experienced either no or minor discomfort, 26.1% experienced moderate discomfort, and 22.5% experienced severe discomfort (with a fifth of these receiving drugs to prevent or relieve the pain or distress; 24).

The percentage of animals experiencing unrelieved pain and distress from non-experimental causes is not known. Recent advances in science and technology, and in laboratory animal medicine, offer significant opportunities to develop alternative methods which may further reduce or eliminate unrelieved pain and distress. It is proposed that research, testing and education facilities are encouraged to improve and optimise the well-being of laboratory animals, for example, by:

a) The procurement and maintenance of animals free of pathogenic organisms; this requires effective vendor surveillance, quarantine, health monitoring, disease investigation and preventive medicine programmes.

b) The provision of optimal caging and husbandry procedures (appropriate to the physiological and behavioural needs of the species), such as avoiding caging animals singly whenever feasible and practical.
c) The provision of optimal environmental conditions, with minimal variations in temperature, humidity, etc.
d) The provision of enrichment/exercise programmes where they are appropriate and will be beneficial.

Need for assessment measures of animal pain and distress

At present, we do not have a convenient and standardised way of objectively assessing animal pain and distress. Rather, the assessment is generally based on subjective clinical signs of abnormal behaviour and appearance. The approach to animal pain and distress is to assume that a procedure which 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 (52, 53).

There are wide variations among different countries with respect to assessments of the extent of pain and distress caused by particular husbandry and experimental approaches. There are no internationally defined standards on animal pain and distress or agreed cut-off points, although some working guidelines have been produced and disseminated widely (for example, 54). There are a number of specific national guidelines on procedures which cause particular concern, such as those on cancer research produced by the UK Coordinating Committee on Cancer Research (55), on antibody production (56), and on lethal endpoint screening tests for antimicrobial agents (57). The number of techniques or scientific procedures that are commonly used and are of significance probably amount to no more than one or two hundred.

Before adverse effects on animals can be assessed, one must be able to recognise such effects via either behavioural or physiological measures. Several such measures have been suggested (for example, 58–60), but have not been widely adopted. In order to prioritise which husbandry and experimental procedures need to be refined and modified, appropriate objective measures of adverse effects on animals need to be developed, validated and aggressively disseminated (52).

International harmonisation

Attitudes differ as to which scientific procedures lead to animal pain and distress, and the severity of the pain and distress they cause. Efforts to identify these differences, to determine the underlying reasons for them, and to harmonise international standards as much as possible should be undertaken.

Research support for refinement alternatives

Very little research funding is available to support efforts to investigate and refine experimental techniques and scientific procedures. Some *ad hoc* funding has supported workshops on antibody production, adjuvant use and infectious disease models. The BVA Animal Welfare Foundation, FRAME, the RSPCA and UFAW formed a Joint Working Group on Refinement in 1989, with the intention of setting up a series of workshops to discuss ways in which common laboratory procedures could be refined. The first and second reports were on the removal of blood from laboratory mammals and birds (61) and on refinements in rabbit husbandry (62), respectively. Nevertheless, no sustained source of funding is available to support the relatively modest research projects which could provide the impetus to develop an appropriate measure of adverse effects on animals, and which could provide essential data on the actual impact of particular scientific procedures and experimental techniques. Some research funding is available to support animal husbandry modifications and environmental enrichment (especially for primates, dogs and cats), but there is little funding available to explore environmental enrichment in rodent housing, despite the fact that rodents constitute 85% of all laboratory animals used in experiments.

Dissemination of information on refinement alternatives

Scientists are not sufficiently aware of the concept of refinement alternatives and, in general, they do not recognise the importance of refinement in their research. The concept of recognising, minimising and eliminating pain and distress in laboratory animals should be included in training programmes for all persons involved in the care and use of laboratory animals. Details of

research facilities. This statistician would undertake to be available to all investigators who needed advice on experimental design and statistical analysis of experimental data, and possibly would have some statutory obligation to meet regularly with investigators to discuss current research projects.

Education in statistics

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a) The procurement and maintenance of animals free of pathogenic organisms; this requires effective vendor surveillance, quarantine, health monitoring, disease investigation and preventive medicine programmes.

refinement and animal welfare considerations should routinely be included in scientific papers and publications (63). Refinement alternatives have multiple benefits, and the promotion of their implementation should include the publication of appropriate review articles which document the scientific, economic and humane benefits.

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.

Since the pioneering work of Chance (64), there have been several publications on the assessment of distress and refinement procedures (2, 33), which should be made as widely known as possible (65–67). In particular, experimenters should be familiar with the important general survey by Claassen (68). As pointed out by Gardner & Gardner (69), acceptance of the very real need for more research in distress assessment and refinement should not discourage us from making urgently needed immediate improvements in cases where there are obvious physiological or behavioural indications of stress, and therefore of distress, due to factors such as overcrowding, social isolation or restraint.

Auditing system

Auditing of the implementation of refinement alternatives at the institutional level is rarely carried out, although it can easily be done by requiring investigators to provide basic information for review by ethics committees/IACUCs, or by independent review by animal welfare officers, laboratory animal veterinarians, or government inspectors. Such data can significantly enhance the development of further refinements, and raise awareness of the concept of refinement and its importance.

Replacement Alternatives

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

It was discussed whether animal organotypic and primary cell culture should be classed as reduction, since an animal would still have to be used, whereas serial cell culture, for example, the use of permanent cell lines, would be classed as replacement. It was thought to be preferable to continue to use Russell and Burch's approach, partly because the use of different terms for non-human vertebrate primary cultures on the one hand, and human and invertebrate primary cultures and non-human vertebrate serial cell cultures on the other, would lead to confusion, and partly because the humane killing of an animal does not represent an experiment or a regulated scientific procedure in most countries.

Nevertheless, statistics on animal use should include the numbers of animals killed specifically for the purpose of providing cells and tissues for *in vitro* studies, and for the production of subcellular fractions, such as liver S9 preparations for use in metabolism and mutagenicity studies. Account should also be taken of the culling of animals surplus to requirements, which can be very high in the breeding and supply of rodents.

Replacement alternative methods and approaches

The range of replacement alternative methods and approaches includes the following (27, 70, 71):

a) The improved storage, exchange and use of information about animal experiments already carried out, so that unnecessary repetition of animal procedures can be avoided.
b) The use of physical and chemical techniques, and of predictions based on the physical and chemical properties of molecules.

c) The use of mathematical and computer models, including: i) modelling of quantitative structure-activity relationships, i.e. taking advantage of correlations between molecular structure and biological activity in the prediction of the potential desired and undesired effects of series of related chemicals; ii) molecular modelling and the use of computer graphics, for example in actively designing drugs and other chemicals for specific purposes; and iii) modelling of biochemical, physiological, pharmacological, toxicological and behavioural systems and processes.
d) The use of "lower" organisms with limited sentience and/or not protected by legislation controlling animal experiments, including invertebrates, plants and microorganisms; for example, the use of bacteria in genotoxicity testing.
e) The use of the early developmental stages of vertebrates before they reach the point at which their use in experiments and other scientific procedures is regulated.
f) The use of *in vitro* methods, including subcellular fractions, short-term maintenance of tissue slices, cell suspensions and perfused organs, and tissue culture proper (cell and organotypic culture), including human tissue culture.
g) Human studies, including the use of human volunteers, post-marketing surveillance and epidemiology; for example, skin patch testing in humans before marketing, and monitoring consumer response after marketing, as alternatives to the animal testing of cosmetic products.

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 to tackling the questions being asked. An example of this would be the use of tissue, cell and subcellular preparations *in vitro* for screening candidate compounds for pharmacological activity. The information provided by the *in vitro* methods may ultimately have an outcome similar to that provided by the animal studies used in the past (for example, the identification of lead compounds).

Replacement alternatives in research and testing

A distinction also needs to be made between the replacement of animal use in fundamental biomedical research and in regulatory testing (72). In the former case, scientific methodology evolves mainly through an informal publication and peer-review acceptance and/or improvement process. In the latter case, however, formal validation of the replacement alternative method in terms of its relevance and reliability for its stated purpose is likely to be necessary (18, 73), since national or international laws, guidelines or regulations will need to be modified, if the non-animal test is to gain wide acceptance as a replacement for the animal test. In addition, as practised at present, regulatory testing often requires the induction of adverse effects, and even of considerable animal suffering, which are integral to the test procedure and are therefore unavoidable.

It follows from this that those of us who are concerned that the Three Rs concept should be implemented as fully as possible, should welcome the trend toward the use of non-animal methods in fundamental research. However, in the case of regulatory efficacy and toxicity testing, research specifically aimed at providing validated replacements for the currently accepted animal test procedures and testing strategies should be conducted, and the value of such research should be recognised by the scientific community at large. This would be fully consistent with one of the hopes expressed by Russell and Burch in the conclusion to their book, i.e. that their efforts "would stimulate some experimentalists to devote special attention to the subject"(2).

A great deal of effort is being put into the development and evaluation of replacement alternative methods for use in testing, by industry and academia, often with the financial support of animal welfare organisations and/or government funds specifically earmarked for this purpose. Until now, the rate of progress has been slow, partly because a series of barriers must be overcome, in addition to the legislative/regulatory barrier referred to earlier (74). The most important of these are the validation barrier and the scientific barrier.

The validation barrier

The validation of new tests and testing strategies in terms of assessing their relevance and reliability is difficult, and the hurdles placed in the path of replacement alternatives must necessarily be high, if mistakes are to be avoided which could have dis-

astrous human health and environmental consequences and thus delay the achievement of our objectives (75). However, these hurdles must be fair, especially as the animal tests we are seeking to replace have not themselves been subjected to formal, independent and objective evaluation in terms of their relevance, reliability and applicability. Validation should be seen as a continuous process, and the principles and criteria involved and the correct practices to be followed are still being debated (18, 73, 76).

One of the greatest problems in planning validation studies in the area of toxicity testing is finding *in vivo* data of sufficiently high quality for use in evaluating the predictive value of the results obtained in *in vitro* tests. This has led to recommendations that an International Reference Chemical Data Bank be established (76, 77), to provide open-access listings of chemicals, backed by first-class toxicological data reviews, safety advice and a source of chemicals of known purity. The European Centre for the Ecotoxicology and Toxicology of Chemicals (ECETOC) has established task forces for providing chemicals for use in validation studies on alternatives to the Draize eye and skin irritation/corrosivity tests (for example, 78). The US National Toxicology Program (NTP) publishes the results of all of its studies, both electronically on the Internet and in book form.

Of course, the most appropriate way to assess a new *in vitro* method is not necessarily to use *in vivo* data from laboratory animals as the reference standard, but to compare results from both methods to those obtained in human (clinical) studies. Unfortunately, finding human data of sufficient quality is a major problem, and those data which do exist are often proprietary. Moreover, generating new human data is fraught with ethical and logistical considerations.

The scientific barrier

Replacement alternative methods must be based on good science, and extravagant claims which cannot be substantiated must not be made about them. One of the most fascinating sections of Russell and Burch's book is their discussion on the relative merits of fidelity and discrimination models (2). 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 "more consistently like us than are other organisms". High discrimination models, on the other hand, "reproduce one particular property of the original, in which we happen to be interested". The *Limulus* amoebocyte lysate (LAL) test is one such model. The use of discrimination models in toxicity testing, for example, is represented by the currently available *in vitro* systems and other replacement alternatives, which are more suitable for answering a specific question about the mechanism of a toxic effect or toxic response in a particular cell type than for answering the more general question: "Is this chemical likely to be toxic in ways which we cannot envisage?"

Russell and Burch warned of the high fidelity fallacy and of the danger of expecting discrimination in particular circumstances from models which show high fidelity in other, more general, terms (2). They pointed out that the fidelity of mammals as models for man is greatly overestimated, and concluded that the assumption that "mammals are always the best models" for man "is maintained with special stubborness in some special fields (such as that of toxicity testing)". They went on to say that the most important consequence of the high fidelity myth is that it "ignores all the advantages of *correlation*", whereby "the responses of two utterly different systems may be correlated with perfect regularity", despite other differences between them. The argument about fidelity, discrimination and correlation test systems is still going on today.

Ultimately, there is only one way forward — 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. Hence, the current trend toward a more mechanistic approach should be welcomed, encouraged and financially supported.

Several specific recommendations relating to the development and use of replacement alternatives in production and testing are given at the end of this report (see *Conclusions and Recommendations).*

Education and Training

The successful implementation of the Three Rs heavily depends upon the education and training of those persons involved in research and testing. A distinction must initially be made between "education" and "training". Education is defined here 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 skills directly associated with animal handling and procedures. Both education and training are necessary for the implementation of all of the Three Rs.

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 which should govern the use of all laboratory animals. All people involved in performing animal experiments should have appropriate practical training, to help ensure that they are technically competent to carry out the procedures. Most countries require that those conducting animal research be competent and trained in the techniques that they are using or plan to use. Training for those in professions that require expertise in animal handling should be accomplished through apprenticeships (that is, "on-the-job" training) and, in such cases, animals should only be used in order to perfect specific skills which cannot be achieved in any other way.

The education and training 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. The concept of refinement alternatives, and the obligation to make them publicly available, should also be emphasised in education and training programmes.

The workshop participants identified five separate groups for which education and training are necessary:

a) animal technicians;
b) scientists, including laboratory animal veterinarians;
c) directors of animal facilities and animal welfare officers;
d) national and regional inspectors; and
e) members of ethics committees/IACUCs (scientists and lay representatives).

Course content and format

The type of education and training referred to below is intended primarily for students preparing for an advanced degree in biomedical research. Such courses should emphasise that the aim of the training is to improve scientific quality as well as to educate scientists with respect to the legal requirement for using alternatives whenever possible. A description of the course on animal experimentation and alternatives currently offered at Utrecht University in The Netherlands has been published recently (79). In addition, the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Education in Europe, and the National Research Council in the USA, have published guidelines for the education and training of persons working with laboratory animals (80, 81). All of these publications serve as excellent prototypes for the development of courses in other countries.

There are several ways in which a course on animal experimentation and alternatives can be packaged. The first is the standard written syllabus with accompanying reference materials (for example, 82). Demonstration videos can also be used. Other possible formats include interactive CD-ROM and the Internet, the use of both of which is becoming more widespread. These latter two formats have the potential to reach a greater number of scientists.

Education in ethology

It is clear that the progress of implementing reduction and refinement alternatives depends largely on the ability of scientists to observe and understand the behaviour and needs of their laboratory animals. At present, it would seem that many experimenters are as lacking in ethological equipment as they are in statistical knowledge. For example, many experimenters have an indiscriminate reaction to work with mazes, some regarding it as harmless and some as stressful. In fact, if a rat is put in a maze when not particularly hungry, he is given an interesting opportunity to exercise the special talent

of his species, that of making a cerebral map of his spatial surroundings. Such a rat, exploring the maze thoroughly, will learn it, so that later, if the maze is altered, he can soon spot new blind alleys or short cuts. If a rat is put in a maze when severely hungry, he will run it with anxiety and distress, becoming conditioned to one route and sticking to it thereafter, even when conditions are changed and it no longer leads to reward. These two cases have been used as classical examples of relaxed and stressful behaviour, respectively, by Russell (83).

One very useful guideline for mammalian behaviour supplied by ethology is this: anything that leads to learning is harmless or positively enjoyable; anything that leads to conditioning involves some stress and, therefore, distress. A very effective short course in ethological observation was designed some years ago for medical students by Chance & Mackintosh (84). It originally required the use of two rats, but could easily be adapted to a video.

Replacement alternatives in education

Students should not be forced to use animals for any purpose. Replacement alternatives for use in education have been described by Fosse (85). These include models, films and videotapes of procedures, interactive computer programs, software simulations, courseware on compact discs and interactive laser discs, and virtual reality programs. The NORINA database contains information on those alternatives which are available (86). Resources are required to produce these materials and to make them more widely available.

Informing Scientists about the Three Rs Concept

The use of the term *alternatives* to encompass all of the Three Rs is now widely accepted in many countries, and is incorporated into the names of various centres, for example, CAAT, ECVAM and the NCA, and journals, such as *AATEX (Alternatives to Animal Testing and Experimentation* — Japan), *ALTEX (Alternativen zu Tierexperimenten* — Switzerland), *TAR (The Alternatives Report* — USA), and *ATLA (Alternatives To Laboratory Animals* — UK). However, some scientists see its use as being driven by political and social forces exclusively, rather than being relevant to scientific issues (71). 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, and of the great potential value of alternative methods (87). It also stems from a defensive attitude among some scientists, perhaps resulting from the campaigns of some antivivisection organisations and from insufficient dialogue among the scientific and animal protection communities (88).

Scientists should be better informed about the Three Rs concept, and should be encouraged to see it as an opportunity, rather than as a threat. At the conclusion of their book, Russell and Burch expressed the hope that experimenters would be stimulated "to devote special attention to the subject . . . to work in full awareness of its existence and possibilities"(2). For some, including the participants in this workshop, the Three Rs has become a subdiscipline within the biological sciences, but this is not generally recognised. Such recognition should be encouraged and promoted through scientific societies, academic journals and funding agencies. Various governments have already given a lead, by creating such bodies and centres as ECVAM, ICCVAM and ZEBET, the German Federal Government's Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments. The journals specifically devoted to the Three Rs, such as *AATEX, ALTEX, TAR* and *ATLA*, should be made more readily available to scientists by their institutional libraries.

Informing the General Public

Although many members of the general public are concerned about the use of animals in research, testing and education, it is clear that they are not typically well-informed about the Three Rs approach to the ethical issues raised by animal experimentation. There are several reasons for this: the Three Rs approach has not been embraced by many of the protagonists in the vivisection controversy (neither scientists nor animal protectionists); some individuals and organisations who have embraced the Three Rs have not

made a point of informing the public of this approach; and those who have made the effort have often found the media unreceptive, preferring instead to portray confrontations between those holding diametrically opposed views. Indeed, it is easier for all concerned to convey a message in the form of simple slogans, such as *Stop Animal Experiments* (89) or *Most People See a Rat — We See a Cure for Cancer* (90), than it is to explain the principles of reduction, refinement and replacement as a blueprint for reconciling the interests of science and animal welfare.

Why should supporters of the Three Rs consider informing the public about this approach? Their efforts can reach members of the public who are sympathetic, and who can then offer moral, financial and political support to the cause. We know that members of the general public are hearing about more confrontational approaches to the animal research controversy; unless they also hear about the Three Rs, our approach will be perceived as being irrelevant to this issue.

Many organisations concerned with promoting the Three Rs have sought to inform the public about their activities, and about the alternatives approach in general (for example, 91). These efforts have entailed producing leaflets, brochures and other written material (92); taking advantage of media opportunities to appear on radio or television programmes, or in print; speaking to students and community groups; and placing advertisements. The Internet is providing organisations with a new opportunity to make their message widely and freely available. An unexplored possibility is for one or more organisations to independently produce a high quality video programme, which can be shown on educational television stations and be distributed to interested individuals and organisations.

Past efforts to inform the general public about the issues relating to alternatives have had mixed outcomes. Accordingly, pro-alternatives organisations should devise and implement such efforts carefully, with due consideration to past successes and failures.

Perhaps the most important specific target audiences within the general public are teachers and students in schools and colleges. In the UK, there has been a surge of interest since animal experimentation became a subject in secondary (high) school social studies and biology syllabuses. This has led many organisations to produce special publications, and FRAME was invited by an educational publisher to produce two booklets to convey an objective, middle-ground position on animal experimentation and on alternatives (93, 94). With the support of industrial companies, a copy of each booklet was given free of charge to every secondary school in Britain. It is particularly important that material for schools and colleges should be designed to help young people make up their own minds on this issue (the approach adopted, for example, in the newsletter *CAATALYST*, which is produced by CAAT for 11–14 year olds), rather than to seek to persuade them. In addition, while more-detailed material might usefully be provided for teachers, care should be taken not to be seen to want to instruct them in how to teach their subjects.

One very successful way for organisations to encourage individual students to become actively involved in the Three Rs is the provision of support for temporary employment in laboratories or in the offices of the organisations themselves. Replacement alternative methods can be very suitable for undergraduate, as well as graduate, research projects.

There are occasions when concerted action by groups of organisations with similar philosophies and policies could be the best way of getting a message across to a large audience. For example, advertisements could be placed in newspapers or magazines in the light of specific events. Organisations which were willing to join in such concerted actions might also agree on a common logo, which would become more widely recognised the more it was used.

Special Considerations

Vaccines and other immunobiologicals

The development, production and quality control of vaccines and other immunobiologicals was recognised as being an important area for the implementation of the Three Rs, since large numbers of animals are used which usually suffer significant pain and distress as a result of the experimental procedures employed. Most of the animal tests undertaken are documented in either a com-

pendium, such as the European Pharmacopoeia, or in guidelines produced by national control authorities and international regulatory bodies (for example, by the World Health Organisation), as well as in national product licences.

For a variety of reasons, interest in both the Three Rs and in vaccine quality control strategies has grown in the past decade. This has led to a reduction in the use of animals for vaccine quality control purposes. Reviews of recent developments, and recommendations for further initiatives, have been published (95, 96).

Steps should be taken to facilitate further implementation of the Three Rs concept with respect to the development, production and quality control of immunobiologicals, especially in relation to testing requirements. Potency tests based on a challenge procedure are of particular concern. Lethal endpoint potency tests for tetanus and diphtheria vaccines are still documented in some pharmacopoeias, and are therefore still performed in some countries, although alternative non-lethal endpoint tests are also permitted and are widely used by more advanced countries. Lethality endpoints should be replaced with other assessment measures. Validated *in vitro* test systems, such as enzyme-linked immunosorbent assays (ELISAs) or serological systems based on *in vitro* models, should be employed, to reduce and refine the use of animals. When challenge of experimental animals is unavoidable, clear clinical symptoms should be taken as being equivalent to the lethality endpoint, and animals showing such symptoms should then be killed humanely (95).

A proposed alternative approach for estimating potency (97) should be evaluated. In this approach, a distinction is made between: a) estimation of the immunogenic potency of the first few batches obtained from a seed lot; and b) monitoring the consistency of subsequent batches. The use of animals is limited to the first few batches, while monitoring the consistency of the quality of subsequent batches is undertaken by using *in vitro* methods (97).

Although there is no *in vitro* model available for replacing the Abnormal Toxicity Test in vaccine safety testing, the value of this test is questionable, since its lack of specificity hampers reliable interpretation of the results obtained (95). Efforts should also be made to minimise the numbers of mice used for pertussis vaccine testing by standardising the procedures employed.

It was emphasised that the development of reduction, refinement and replacement alternative methods is the responsibility of all those involved in the production and quality control of vaccines and other immunobiologicals (that is, both industry and government).

Transgenic animals

The production, breeding and use of animals genetically modified via the application of transgenic techniques will undoubtedly increase significantly in the future. This new technology offers great potential scientific benefit, in terms of the understanding of fundamental biological processes, the nature, diagnosis and treatment of various diseases, the production of useful biological products, and the husbandry of disease-resistant animals. Animal transgenesis may also contribute to the implementation of the Three Rs, by reducing animal numbers, by permitting the replacement of more-sentient species with less-sentient species, by reducing animal suffering in other ways, and by increasing the relevance of laboratory animal use. For example, in the USA, several transgenic mouse models are currently being investigated by the NTP with respect to their usefulness in evaluating the potential carcinogenicity and mutagenicity of chemicals (98). Carcinogenicity studies employing these models are completed in six months or less, compared with the standard two-year bioassay, and involve fewer animals per dose group. In addition, the animals are not subject to age-related morbidity and mortality.

Despite these kinds of developments, some observers are of the opinion that the use of transgenic animals will lead to an overall increase in animal use, rather than to a decrease. There is also legitimate concern that the control of the production, breeding and use of transgenic animals is not adequately provided for in current legislation for the protection of laboratory animals. For example, there is as yet no broad agreement on whether only the creation of transgenic animals, and/or their breeding, and/or their use in specific experiments should be treated as regulated procedures, or whether transgenic animals should be protected throughout their lives, whether or not they are ever

used in specific experiments. The system adopted in the UK, whereby strict controls are applied to the breeding of transgenics until it can be shown that no special health and husbandry problems are involved, is to be recommended.

Molecular geneticists involved in producing transgenic animals must always be aware of the animal welfare implications of their experiments, and of their legal and moral responsibilities (99, 100). Concern that this is not always the case has been highlighted by the occurrence of unexpected, sometimes very severe, adverse effects in transgenic animals. There are also ethical concerns about transferring genes between species, particularly when the genes involved are human genes (101). Moore & Mepham (102) have suggested recently that the actual and potential implications of the increased use of transgenic animals may result in the need for a fundamental reappraisal of the mechanisms whereby permission to perform scientific procedures on animals is considered and granted.

Special protection for selected animals

A fundamental tenet of the refinement principle is that the least sentient species suitable for the proposed experimental work should always be selected. However, there is no general agreement on how sentience can be measured or how degrees of sentience can be compared. For example, is a marmoset more sentient than a rat? In some legislation, special consideration is given to non-human primates, cats and dogs. In Britain, this special consideration is also extended to *Equidae*. It can be argued that there are two different justifications for this approach.

Firstly, in the case of the higher primates (Old World monkeys and Hominids), whatever is meant by "sentience" or, in the words of *Directive 86/609/EEC*, "degree of neurophysiological sensitivity"(12), it is commonly accepted that these animals are placed at the top end of any spectrum or scale. They therefore deserve special consideration, both in their own right, and also because, if we cannot ensure the proper application of laboratory animal protection laws and regulations to them, we are unlikely to be successful in providing adequate care for rodents, birds and fish.

Secondly, cats, dogs and horses have been bred over many centuries to be companion animals for human beings, and it can be argued that, since they have been bred to be dependent on us, we have a specific moral obligation to them. That is certainly how the vast majority of members of the general public would be likely to view the situation, and both animal experimentation and the Three Rs must be viewed in a social, as well as in a scientific, context. Nevertheless, there is a strong counter-argument that all laboratory animals should be given equal protection, and that all forms of speciesism should be avoided.

Benefit and suffering[1]

It is implicit, if not explicit, in all the regulations governing laboratory animal use that the purpose of the work must be worthwhile, and that it must be viewed in relation to the suffering caused to the animals used.

The British 1986 Act requires that the *likelihood* of a beneficial outcome of a proposed programme of work is assessed in advance, and that, where it would involve vertebrate animals which might be caused to suffer pain, distress and/or lasting harm, a weighing of likely benefit *versus* likely suffering must be made, before permission is given for the experimental work to proceed (16, 27, 103).

Cost–benefit analysis is part of our everyday lives, and it involves judgement on the basis of evidence, rather than automatic decisions based on precise measurements. Applying this kind of approach should not be new to scientists, since it is an essential part of the peer-review system for granting research funding.

Who should be involved in assessing and weighing benefit and suffering will depend on the particular system for regulating animal experimentation. The ultimate moral responsibility should lie with the scientists who propose to perform the studies. However, institutions, funding agencies, governments and the community in general should all have some input (27). In the UK, the Home Office Inspector would discuss this question with a project licence applicant, and would advise the Home Secretary, with whom the ultimate legal responsibility lies

[1]*The word "suffering" has been used as a generic term for "undergoing, experiencing or being subjected to pain, distress and lasting harm".*

(104). In other countries, IACUCs or animal ethics committees contribute to the discussion or even make decisions.

This is a topic which deserves further consideration, especially with respect to the ways in which benefit and suffering should be assessed and weighed. Four main schemes have been proposed or are in use: that suggested in *Lives in the Balance*, the report of the Institute of Medical Ethics (UK) Working Party (27); the Dutch model developed by the Department of Animal Problems, Leiden University (105); a model proposed by Porter (106); and the system already used by the British Home Office Inspectorate for the assessment and weighing of benefit and suffering, which was described in the 1993 report of the APC (107).

It is widely agreed that there are levels of animal suffering which could never be justified on scientific grounds, but there is no agreement as to what those levels are. It is certainly possible to perform procedures in one country which would not be permitted in another. This is a matter which deserves urgent international discussion and agreement.

The setting of targets

One way of making progress is to agree that specific targets should be met, especially if a date for meeting a target is also agreed. Whether or not a target is met, the fact that it exists can influence policy and stimulate action, as has been seen in response to the Sixth Amendment (*Directive 93/35/EEC* [108]) to the European Union (EU) Cosmetics Directive, *Directive 76/768/EEC* (109, 110).

In 1993, the Member States of the EU agreed that everything possible should be done to achieve a reduction of 50% in the use of vertebrate animals for experimental and other scientific purposes by the year 2000 (111). It has also recently been suggested that agreement should be sought in the EU on phasing out the use of non-human primates as laboratory animals by the year 2005 (112). Further targets which could be suggested would be an end to potency and toxicity tests involving lethal endpoints, to the use of the Draize eye irritancy test, and to all procedures which result in substantially severe effects.

Concluding Remarks

The workshop participants unanimously reaffirmed the principles put forward by Russell & Burch (2), that humane science is good science and that this is best achieved by vigorous application of the Three Rs: reduction alternatives, refinement alternatives and replacement alternatives. Thus, the only acceptable animal experiment is one which uses the smallest possible number of animals and causes the least possible pain or distress which is consistent with the achievement of a justifiable scientific purpose, and which is necessary because there is no other way of achieving that purpose. Any proposed experiments on animals should be subjected to prior and effective expert review by an ethics committee or an equivalent body. The Three Rs should be seen as a challenge and as an opportunity for reaping benefits of every kind — scientific, economic and humanitarian — not as a threat.

It was proposed that, if funds could be obtained, an animal welfare information unit for Europe should be established at Sheringham, under the direction of Rex Burch; this would liaise with the corresponding centre in the USA. Such an initiative could help meet some of the specific recommendations made in this report, as well as playing an important role in the education of the general public.

It was agreed that contact would be maintained by the workshop participants, and that a further meeting of the Sheringham Group would take place on the occasion of the Second World Congress on Alternatives and Animal Use in the Life Sciences, to be held in Utrecht in October 1996. One of the principal aims of this meeting will be to review the steps taken by the participants in the workshop, individually and collectively, to see that its report and recommendations have been publicised and implemented. In addition, it was agreed that an informal meeting of societies and organisations committed to the implementation of the Three Rs should be arranged at the Second World Congress, with a view to the possible formation of an international federation.

Conclusions and Recommendations

General

1. Existing laboratory animal protection laws should be fully implemented.
2. All countries should have a legal framework which actively incorporates the Three Rs into all animal-based research, testing and education.
3. 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.
4. Before proposing any programme of work involving laboratory animals, scientists should ask themselves whether the project is worth doing in the first place, and, if so, whether the problem could be approached in a different way, for example by using *in vitro* methods or animals of lower sentience.
5. Any proposed experiments on animals should be subjected to prior and effective expert review, both for scientific merit and animal welfare considerations. All scientists and institutions concerned should take steps to ensure that proposed programmes of work involving animals which do not have to pass through an external peer-review process are nevertheless subjected to effective evaluation for scientific merit and necessity.
6. Institutions should be required to appoint one or more persons responsible for ensuring that the Three Rs are fully taken into consideration when programmes of work on animals are proposed and during the experimental work itself.
7. It should be recognised that the assessment and weighing of the likely benefit and likely animal suffering involved in a proposed programme of work is an essential part of the process whereby permission for the work to proceed is/is not granted. Further studies should be undertaken on how this assessment and weighing could be conducted, as a basis for international agreement and harmonisation.
8. There should also 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.
9. It is the responsibility of the investigator to choose and justify, on scientific and animal welfare grounds, the animal species and strain which is most suitable for the proposed investigation.
10. It is unacceptable to export scientific work involving laboratory animals to avoid scientifically realistic, but more stringent, animal welfare codes.
11. Discussions should be encouraged at the national and international level with a view to setting targets and time limits for the achievement of specific goals in the reduction, refinement and replacement of the use of vertebrate animals in experiments and other scientific procedures.
12. There is a need for the involvement in animal welfare issues of more people with initial training in the life sciences and postgraduate training in biometry/statistics.

Reduction alternatives

13. In cases where a choice between species is possible, there is generally no scientific justification for using more of the smaller species than of the larger one.
14. Research strategy should be considered carefully, with a view to reducing the numbers of animals used. The example of Hendriksen *et al.* (32), in choosing strains of laboratory mice in order to minimise the numbers needed in specific biological assays, should be followed for those assays which use large numbers of animals and which are unlikely to be replaced with *in vitro* alternatives in the near future.
15. The design of regulatory testing procedures, including the sample sizes required, should be reviewed regularly, possibly as part of international harmonisation.
16. Substantial reduction in animal use could be achieved by further harmonising toxicity testing regulations, for

example, with respect to group sizes, dose levels and the length of studies.

17. In view of the uncertainties inherent in "extrapolating" to humans, the need for very high precision in data provided by animal experiments should be reconsidered.

18. There is evidence that some non-regulatory animal experiments are poorly designed and incorrectly analysed. As a minimum, all research workers should have adequate training in experimental design and the proper use of statistical methods.

19. The concept of the "named statistician" as an essential part of the regulatory framework of animal experimentation should be explored.

Refinement alternatives

20. An international data bank on refinement alternatives should be developed.

21. The validation process should include the evaluation of refinement alternative procedures, particularly in relation to regulatory testing.

22. There should be internationally agreed guidelines for the categorisation of animal pain, distress and other adverse effects, including agreement on physiological and behavioural signs for the recognition of adverse effects and for their measurement.

23. Working parties should be set up, on an international, collaborative basis, to develop codes of practice and guidelines of best practice for specific animal husbandry (welfare) and research procedures. When such codes and guidelines have been developed and agreed, adherence to them should be mandatory.

24. Individuals and institutions should be responsible to their national authorities for prospective and retrospective assessments of the nature and levels of adverse effects likely to be experienced or actually experienced by animals in each programme of work.

25. Research on refinement and welfare aspects should be encouraged and funded, including studies on the effects of minimising pain and distress on the quality of research data.

26. Journal editors should be encouraged to include a separate consideration of *Animals and Procedures* within the *Materials and Methods* section of the articles they publish.

Replacement alternatives

27. Statistics on animal use should include the numbers of animals killed specifically for the purpose of providing cells and tissues for *in vitro* studies.

28. In the case of regulatory efficacy and toxicity testing, research specifically aimed at providing validated replacement alternatives for the currently accepted animal procedures should be conducted.

29. The development and acceptance of replacement alternatives for both research and testing must be based on an understanding of the molecular and cellular mechanistic basis of the phenomenon being studied or measured. The current trend toward a more mechanistic approach should be welcomed, encouraged and financially supported.

30. Monoclonal antibodies should only be produced by using *in vitro* methods, unless a convincing scientific case can be made for using the mouse ascites technique.

31. New *in vitro* methods for the production of hormones and other biological products, which would result in purer preparations, should be sought. Efforts should be made to determine whether *in vivo* bioassays for the safety and efficacy testing of hormones and related products can be replaced by using a combination of physicochemical and *in vitro* tests.

32. The animal welfare and ethical issues pertaining to the procurement of fetal and neonatal calf sera should be investigated. The development of fully defined substitutes which could replace the use of serum when culturing cells should be encouraged.

33. Since it is unlikely that an animal test could be replaced with a single *in vitro* test, the development, evaluation and

optimisation of testing strategies and integrated testing schemes should be a high priority.

34. Human cells and tissues should be used in preference to those isolated from laboratory animals whenever possible. However, it is recognised that there are ethical, safety, legal and logistical problems which may prevent the widespread use of human tissues.

Education and training

35. A clear distinction needs to be made between *education* which aims to contribute to the development of proper attitudes toward the use of animals, and the *training* of individuals who will be practically involved in animal experimentation itself, be it by conducting experiments, by contracting or permitting experiments, or by caring for the animals used.

36. The responsible authorities should require that all those with any practical involvement should take accredited courses which emphasise the importance of all of the Three Rs, and the legal requirement to use alternatives whenever possible. Emphasis should be placed on "best practice", for the sake of both scientific quality and the welfare of the animals used.

37. Permission to conduct experimental procedures on animals should be based not only on general training, but also on specific training and evidence of competence, which should be reassessed regularly.

38. A Three Rs education and training database should be established, so that all concerned can have easy access to information and advice on the availability of relevant literature.

39. School students and undergraduates should not be forced to conduct procedures on animals as part of their courses, but, should, where necessary, be provided with alternative options.

Informing scientists about the Three Rs concept

40. National, regional and international centres should be established to facilitate and promote research and the implementation of the Three Rs through funding and education. These centres should be networked, to facilitate coordination and information exchange.

41. Government, industrial and academic scientists should be encouraged to become involved to a greater extent in the development, validation and implementation of alternative methods.

42. Funding agencies should allocate funds specifically for research on all of the Three Rs.

43. The concept and availability of the Three Rs, in the context of excellence and humanity in scientific research, should be incorporated into graduate education and into training programmes which also cover experimental design, animal welfare issues and statistics.

44. The editors of appropriate academic journals should be encouraged to introduce regular and specific consideration of progress in the Three Rs in relation to the subject areas covered by their journals.

45. The officers of learned societies should be encouraged to establish subdisciplines within their organisations to consider the Three Rs aspects of their disciplines, thereby encouraging recognition of the scientific and humanitarian importance of the Three Rs concept.

Informing the general public about the Three Rs

46. Organisations concerned in promoting all types of alternative methods should devote some of their energies to informing the general public of the nature and importance of the Three Rs approach.

47. Such organisations should consider joint efforts, as a means of improving quality, avoiding pitfalls, and sharing costs. When appropriate, concerted advertising, mailings and other actions should be devised and implemented, to take advantage of timely, high profile issues, whether to encourage positive developments or to forestall negative developments.

48. Priority should be given to providing materials for teachers.

49. Organisers of workshops, conferences and other meetings on the Three Rs, should, when appropriate, consider publicising the outcome of such meetings. Lay summaries of the proceedings of meetings on alternatives should, when appropriate and feasible, be published.

Special considerations

50. Animal welfare considerations should be an essential part of the evaluation of the acceptability of proposed new regulatory test guidelines, or modifications of existing guidelines.

51. When a new regulatory test guideline has been accepted, which involves fewer animals or less animal suffering, it should not be optional, but should be required in preference to any other method.

52. Wherever possible, quantitative endpoints should be used. Death and other qualitative endpoints are often inhumane, and provide less information than quantitative measurements. It is inexcusable to use lethal endpoint acute toxicity tests (for example, LD50 tests), when a non-lethal endpoint test (for example, the Fixed Dose Procedure) has been formally accepted as an alternative OECD test guideline method.

53. When challenge of experimental animals is unavoidable as, for example, in vaccine potency testing, clear clinical symptoms should be taken as being equivalent to the lethality endpoint, and animals showing such symptoms should then be killed humanely.

54. A proposed alternative approach for estimating vaccine potency should be evaluated, which involves limiting the use of animals to the first few batches, while monitoring the consistency of the quality of subsequent batches by using *in vitro* methods.

55. Since it is of questionable value, the use of the Abnormal Toxicity Test in vaccine safety testing should be discontinued.

56. Urgent consideration should be given to the revision of laws and regulations concerning the use of animals, so that, where necessary, animals with a deleterious phenotype, whether spontaneous or caused by mutagens or transgenic techniques, are afforded the same level of protection throughout their lives as that which would be appropriate had that level of suffering been induced by any other method or technique.

57. There should be a review of the systems currently used for evaluating the ethical acceptability of gene transfer into animals on a case-by-case basis, with particular emphasis on the transfer of human genes into animals.

58. The higher primates, cats, dogs and horses should continue to be regarded as animals deserving special consideration.

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