

Advancing refinement of laboratory animal use

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Whatever view is taken of the morality of using animals in scientific research and safety testing, it can generally be agreed that so long as such use continues, every effort should be made to keep animal suffering to a minimum. This is the thinking behind the 'Three Rs' of refinement, reduction and replacement of laboratory animal use. This paper concerns refinement. We recognize that the Three Rs are taken very seriously in many countries of the world [see for example a recent editorial in the journal *Science* (Goldberg *et al.* 1996)] and, although we have written this paper from our own perspective in the UK, its principles are generally applicable.

Keywords Refinement; laboratory animals; animal husbandry; scientific procedures; scientific quality; education and training; protocol review

1 Introduction

1.1 *Refinement and the Three Rs*

Scientists have moral and, in many countries, legal obligations to avoid causing harm to animals in research wherever possible. Unfortunately, with present knowledge, complete replacement of animals with suitable non-animal alternatives remains a long-term goal. Whilst animals continue to be used in research, reducing their numbers and refining procedures are realistic, imperative goals. Refinement of husbandry and procedures, so as to cause the least possible animal suffering, is of key importance in improving the well-being of laboratory animals.

1.2 *Definition of refinement*

Refined methods in animal research are those which alleviate or minimize the pain, distress

This report is prepared on behalf of the Boyd Group which is a forum for the open exchange of views on issues of concern related to the use of animals in science. Its objectives are to promote dialogue and, where there is consensus, to recommend practical steps towards achieving common goals. Membership includes a representative range of views on the use of animals in science.

or other adverse effects suffered by the animals involved, and/or enhance animal well-being (Russell & Burch 1959, Balls *et al.* 1995).

Refinements may be applied at any stage in the use of a laboratory animal, from its birth to its death. Thus, refinement encompasses all aspects of a procedure: the source, transport, husbandry and environment of the animals involved; the experimental design (e.g. the choice of species, and the group sizes employed—so that 'reduction' of animal use could be considered to be part of refinement of animal procedures); the techniques applied; the care of the animals before, during and after a procedure; the end-points of the procedures; and the method of killing the animals. Morton (1995) describes examples of refinements in most of these areas.

1.3 *Benefits for science*

Whilst its primary objective is to reduce animal suffering, refinement can also enhance the quality of the science and reduce the economic cost of animal research, and the justification for its application can be made on these criteria as well as on welfare grounds.

Refinement should be consistent with scientific objectives. Nevertheless, there may be concern that implementing refinements might hinder research; a change of technique could bring in new variables, which might lead to incompatibility with historical and other data. There is much that can be done, however, without changing the overall scientific protocol. For example, better acclimatization of the animals to novel environments or husbandry conditions, better habituation of the animals to the procedures involved, improved postoperative care and more skilful application of the experimental techniques are all strategies which can reduce animal suffering whilst employing the same basic procedures.

When refinement necessitates a change of experimental technique, any possible negative effects need to be balanced against the possibility of real benefits for the science, as well as for animal welfare (Morton 1995, Poole 1997). Close observation of animals when monitoring for adverse effects can reveal subtle changes; modern anaesthetic drugs not only produce better anaesthesia (e.g. better muscle relaxation, predictable duration of action, less irritation, fewer physiological side effects) but are safer, resulting in loss of fewer animals; and the use of analgesics or humane killing of animals before death can produce more meaningful scientific results. That is, they provide results that measure the animals' physiological responses to the scientific variables, rather than to unwanted 'side effects', such as pain and distress, or pathophysiological changes around the time of death. Furthermore, if refinement also means that fewer animals are used, there will be a financial benefit from reduced animal purchases and savings on animal care and maintenance.

1.4 *Advancing refinement: some concerns*

In spite of these possible benefits, promotion and implementation of refinements in laboratory animal use appear to be patchy. Whilst much progress is being made, commitment to refinement varies between individual animal users, between institutions and between scientific disciplines. Several

factors contribute to this:

- there can be a lack of awareness of the need to refine procedures;
- it can be difficult to obtain information about the possibilities for refinement, and there is a lack of funding for research into refinement; and
- there can be uncertainty about where responsibility for implementing refinement lies.

Each of these areas is explored in detail below and practical ways forward are suggested.

2 **Advancing refinement: problems and potential solutions**

2.1 *Awareness of the duty to refine animal use*

2.1.1 Undergraduate and postgraduate education It seems likely that scientists who are well-acquainted with the wider aspects of laboratory animal use will more readily appreciate the need to refine that use wherever possible. Increasingly, however, biological education is not whole-animal orientated and, at undergraduate level at least, there may be little consideration of the animal methods used to obtain biomedical knowledge.

Recommendation: Consideration of practical and ethical aspects of laboratory animal use should be included in all undergraduate and postgraduate courses in biomedical and related sciences. This is especially important in courses involving animal use or when graduates are likely to enter employment where animals are used.

2.1.2 Training for users of laboratory animals The provision of training for all scientists using laboratory animals might improve awareness of the duty to refine animal use. In Europe, for example, all laboratory animal users are required by law to undertake prescribed training. However, whilst a syllabus is specified, the educational objectives are not, and it is

therefore uncertain whether attitudes towards refinement (and other aspects of animal use) actually are changed by such training. Furthermore, the training does not necessarily embrace the whole of the existing animal research community.

Recommendations: All users of laboratory animals should be required to undertake training, which should include consideration of refinement of animal use. The educational objectives of such mandatory training should be specified, and consideration should be given to means of assessing whether these objectives have been achieved. The provision of continuing professional training and, where appropriate, re-assessment for all animal users should be considered, in order to help them keep abreast of relevant developments in laboratory animal use, including refinement.

2.1.3 Research protocol review In countries which require review of protocols involving animal use, it is not necessarily a specific requirement to consider *refining* animal use. In the UK, for example, applicants for project licences under the Animals (Scientific Procedures) Act 1986 are required to sign a declaration stating that they have considered the possibility of replacing animal use with methods 'not involving regulated procedures on animals protected under the Act', and that no such replacements 'would achieve the objectives' of the project. This declaration, however, makes no reference to refinement of animal procedures. Evidence of refinement of procedures is asked for in the plan of work, but the need to refine procedures is not mentioned in the notes on completing the protocol sheets, where full details of the procedures must be given (Home Office 1991). Furthermore, all references to refinement relate only to scientific procedures, and applicants are not required to show that they have considered refining animal husbandry. *Recommendations:* The possibilities for refining animal use should be considered in the review of all research protocols involving animals. Specifically, in the UK, for example, the declaration about consideration of replacement alternatives should be changed to

encompass refinement (and reduction), and to require licensees to give explicit details of their application of the Three Rs. (For example, a question, such as 'How have you attempted to reduce animal suffering in this project?', requiring a paragraph in answer, could replace the declaration, or could be included in the plan of work.) The guidance on completing the protocol sheets should emphasize the need to refine procedures, and applicants should be required to show that they have considered refining animal husbandry.

2.1.4 Funding agencies' commitment to refinement Some funding agencies do not require applicants to provide evidence in grant proposals that they have refined their proposed animal methods. This apparent lack of official recognition of the duty to refine might lead to complacency amongst some animal users.

Recommendation: Funding agencies should consider making a statement of their commitment to refinement and requiring detailed evidence of refinement of animal methods on their grant application forms. Furthermore, they should consider involving people with expertise in refinement in the review of applications involving animal use.

2.1.5 Continuing awareness of refinement Once funding and, where appropriate, statutory approval are obtained for a project, some animal users may see this as the end of any explicit consideration of the possibilities for refining animal use, but the responsibility is ongoing. Furthermore, animal technicians increasingly carry out many of the more routine procedures, so that it is possible that those responsible for directing the work may become remote from the use of animals. Whilst the technicians might be more practised in the techniques used and more familiar with the animals, so that the severity of any adverse effects might be reduced, it is essential that project directors remain aware of those adverse effects and of the possibilities for refining the use of animals.

Recommendation: Refinement should not be a single event occurring only at the outset of a project; rather it should be an ongoing process which can evolve as the project progresses (for example, as scientists become more familiar with the animal model, and as more animals undergo the procedures). To assist this, project directors should ensure that they visit their animals regularly and the scientists actually carrying out the procedures involving animals should give project directors regular feedback on the effects of the procedures. Furthermore, every institution in which laboratory animals are used should consider putting in place some mechanism to raise awareness of the need to consider refinement (see further comments in 2.3 below).

2.2 Obtaining information about refinement

2.2.1 Refinement research It can be difficult for animal users to obtain information about refinements relevant to their work, sometimes because the information simply is unavailable. The paucity of information in this area is partly due to a lack of adequate funding for research into developing and validating refinements to animal procedures (Balls *et al.* 1995).

Recommendation: Research into refinement should be encouraged and more funding provided. Grant awarding bodies which support animal research have a moral obligation to ensure that animal suffering is kept to a minimum. In order to meet this obligation they should consider setting up funds to support scientific research into the Three Rs.

2.2.2 Publishing information on refinement When relevant information about refinement is available, it is often difficult to access. It can remain 'in house' and unpublished or it might be tucked away in a small part of a paper on some other topic, or it may be published in one of the specialized laboratory animal science journals or professional literature, which are not read by the broader scientific community.

Recommendations: Several strategies could help to make existing information more widely available:

- (i) Articles summarizing advances in refinement, together with key references, should be published not only in laboratory animal science journals but also in discipline-related journals and other publications accessible to professional scientists, and should be made available on the Internet.
- (ii) Learned societies should be encouraged to produce, and regularly update, guidelines on animal procedures common in their work (examples include guidelines produced by the United Kingdom Coordinating Committee on Cancer Research (1988) and Association for Study of Animal Behaviour with the Animal Behavior Society (1996)). The BVA/FRAME/RSPCA/UFAW refinement workshop reports [e.g. on withdrawal of blood and husbandry of rabbits, (BVA/FRAME/RSPCA/UFAW 1993a & b)] are especially useful in this context, and the findings should be widely disseminated.
- (iii) Argued cases should be produced, showing that inadequate refinement (of husbandry or procedures) can have serious adverse effects on any scientific results obtained (see, for example, Poole 1997). Such publications could be very valuable in persuading scientists to consider such factors more seriously.

2.2.3 Reporting details of animal procedures In reports of animal experiments published in scientific journals, refinement of animal procedures is usually given low status alongside other aspects of the science, and the animal procedures are sometimes reported in scant detail (Smith *et al.* 1997). *Recommendation:* Editorial policies on the reporting of animal procedures should be reviewed, and wherever possible full details should be required (or references to where full details may be found), especially about the steps taken to reduce or alleviate any animal suffering (Morton 1992). Journal space should not be a limiting factor, as

details of animal husbandry and procedures are usually of key importance in the science, and the additional information can usually be reported using only a few extra words (viz: the experience of *Laboratory Animals* which has rigorous reporting requirements for animal procedures). At the very least, authors should be required to submit full details of the animal procedures for peer review prior to publication, and such details could be archived by the journal for future reference, as required.

2.2.4 Disseminating information about refinement and best practice Within the animal research community, dissemination of information about refinement usually depends on local arrangements, which may vary greatly in their effectiveness. In most countries, there is no nationwide mechanism for ensuring that information about refinement reaches animal users.

Recommendation: Refinement databases should be established. Then, institutions could ensure that someone within the organization has responsibility both for sending relevant information to the database, and for obtaining information from the database and communicating it to appropriate animal users.

2.3 Responsibility for implementing refinement

2.3.1 Everyone involved with laboratory animal use has a duty to be alert to the possibility of refining that use, and to implement refinements wherever possible. Animal users are likely to require considerable support to enable them to fulfil this duty, especially as most will have other demands on their time. Nevertheless, at the institutional level, there are rarely any established lines of responsibility for *initiating* refinement and supporting animal users in implementing refinement. The provision of such support varies greatly between institutions.

Recommendations: All scientific establishments in which animals are used should have well thought-out and agreed structures for managing laboratory animal use, including specified procedures for taking action when

problems arise. In respect of refinement, whilst everyone involved in animal use has responsibility for refinement, it may be appropriate to identify people who should have *key* responsibility for *initiating* refinement. For example, in the UK context, four distinct aspects of laboratory animal use and corresponding key initiators of refinement might be identified as follows:

Aspects of laboratory animal use

Key initiator

Refinement of animal husbandry	Person with statutory responsibility for care and welfare of experimental or stock animals (Named Animal Care and Welfare Officer)
Refinement of experimental design	Principal investigators (project licence holders)
Refinement of techniques used in scientific procedures	Staff carrying out the techniques and procedures involving animals (personal licensees)
Refinement of peri-procedural care	Institutional veterinary surgeon with statutory responsibility to provide advice on health and welfare of experimental animals (Named Veterinary Surgeon)

Institutions should have responsibility for putting in place mechanisms to support animal users in fulfilling their responsibilities for refinement, including the provision of regular institutional seminars on refinement and other animal use matters. This might be easier where responsibility for providing such support could fall to an institutional ethics committee or an animal care and use committee.

2.3.2 Role of 'animal advocates' Experience suggests that the advisory roles of the animal care staff and laboratory animal veterinarians are of central importance in creating a climate in which all animal users within an

institution take refinement seriously. In order to fulfil this role, these people require adequate resources (including time) and managerial support. Again, there is variation in the levels of support provided.

Recommendation: All institutional management structures should include clear lines of communication and support for animal care staff and laboratory animal veterinarians.

Furthermore, institutional management should provide adequate resources, including sufficient time, to enable these people to be proactive in refinement.

3 Further work

This document includes a series of practical recommendations for advancing refinement of laboratory animal use. The Boyd Group is actively exploring ways of encouraging implementation of these recommendations and would welcome constructive comments.

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