Chapter 12

Reduction and Refinement

Reduction and Refinement

Introduction

12.1 In the previous chapter we discussed the opportunities and current limitations of the first of Russell and Burch's Three Rs, the Replacement approach. We now turn to the remaining two concepts Refinement and Reduction, which need to be considered whenever the use of animals to achieve a scientific objective is deemed unavoidable. As we have said, the Three Rs are closely interrelated.[1] The relationship between Reduction and Refinement is particularly evident when these principles are applied at the early stage of research projects to improve research strategy as a whole. We first give brief consideration to this relationship and then examine Reduction and Refinement more closely as individual concepts. The role of harmonising international test guidelines for the purpose of reducing animal research is then explored before we focus on the potential of Refinements. We give examples of how to implement Refinements in specific areas of research and also consider possible barriers.

Applying Reduction and Refinement to research strategies

12.2 Animals will suffer needlessly if they are used in research where scientific methodology is poor. In such cases, research does not achieve its scientific objectives, and fails to generate significant knowledge. It is for this reason that the application of the Three Rs should begin with a careful assessment of the initial experimental design and be continued throughout the duration of each and every research project. This process requires a number of basic questions to be addressed at a very early stage. For example, is the chosen animal model sufficiently relevant to the scientific question being asked or health problem under study? Is there a genuine scientific basis for using a particular animal model? Could the scientific question itself be refined? Could the scientific objective of the work be modified to avoid the use of an animal model? The following three general approaches are relevant to the successful implementation of Reduction and Refinement at this stage of research.

- **Background research**: it is essential that a thorough search of the published literature is undertaken to ensure that the proposed experiments have not already been undertaken and the objectives of the research have not already been met by previous well-conducted experiments. Part of this survey of the literature should be an assessment of the validity of the conclusions of previous studies. New experiments should not be based on unsound conclusions drawn from poorly designed experiments.
- **A staged approach**: before embarking on large or complex experiments the project should be broken down into a series of pilot experiments, with defined decision points that inform the transition from one stage to another. A small pilot experiment on animals or *in vitro* research can be very useful in guiding the design of subsequent, larger experiments. For example, an initial pilot study might help define experimental parameters early on, so that fewer animals could be used later. It might also be possible to refine experiments so that suffering and the number of animals used is reduced by carrying out pilot experiments on anaesthetised animals that are not allowed to recover.
- **Teamwork and resources**: the successful completion of an experiment depends on many factors, including the skills and performance of the staff involved, and the availability of suitable equipment and facilities. Optimal experimental design and successful application of the Three Rs requires a multidisciplinary approach with contributions from biomedical

[1] Russell and Burch themselves acknowledged that there was overlap between the Three Rs; see Russell WMS and Burch RL (1959) *The Principles of Humane Experimental Technique* (London: Methuen).

scientists and animal care staff, statisticians, information scientists and other specialists such as biochemists, geneticists and clinicians. Lack of adequate staff training and expertise and the use of unreliable equipment can lead to failed projects and the fruitless use of animals.

12.3 These are just some of the crucial factors that need to be taken into account when considering whether the use of animals can be justified. The goal should be to design each experiment or overall project plan in such a way that it causes the least amount of suffering to the minimum number of animals, at the lowest level of neurological development. As we have said, this goal is an integral part of UK legislation (see paragraph 3.59). We have also emphasised that it is not sufficient to simply follow rules; researchers must strive actively for best practice (paragraph 3.69). The continued application of Refinement and Reduction before, and throughout the duration of a research project is especially relevant in this context. We note that doubt has been expressed in a recent Report by the House of Lords Select Committee about the effort that is put into these approaches.

> 'We are not, however, persuaded that enough effort is always made to avoid the use of animals. We are similarly not persuaded that where this is possible, sufficient effort is always made to minimise the number of animals used, and to minimise the pain and suffering inflicted on each animal.'[2]

We consider next ways in which the application of Refinement and Reduction can be improved.

Reduction

Definition and scope

12.4 Russell and Burch initially defined Reduction as 'reduction in the numbers of animals used to obtain information of a given amount and precision'. More recently, this definition has been developed to state: 'the use of fewer animals in each experiment without compromising scientific output and the quality of biomedical research and testing, and without compromising animal welfare'.[3] The proviso that Reduction should not compromise animal welfare is necessary because reduction in the number of animals used can sometimes be achieved by performing more procedures on each animal. This could cause an undesirable increase in the suffering of individual animals. In addition to improved research strategy, as outlined above, Russell and Burch suggested two additional ways in which animal use could be reduced: better control of variation and better statistical analysis.

- Reducing variation: choice of appropriate animal species and strains

  Many factors need to be considered in choosing the most appropriate animal model for a particular experiment. Thus, for example, the species, strain, sex and age of the animals are all important criteria. The outcome of a project may depend critically on the strain(s) used. A wide range of inbred strains, mutants, outbred stocks and transgenic strains of mice and rats are available. The use of genetically more uniform or inbred stocks, if appropriate to the particular experiment, may reduce variation and therefore allow the use of fewer animals.

- Statistics and experimental design

  A lack of understanding of the basic principles of statistical methods can lead to

2 House of Lords Select Committee (2002) *Animals in Scientific Procedures* (Norwich: TSO).

3 Festing MF (1994) Reduction of animal use: experimental design and quality of experiments *Lab Anim* **28**:212-21; Festing MFW, Baumans V, Combes RD, *et al*. (1998.) Reducing the use of laboratory animals in biomedical research: Problems and possible solutions *ATLA* **26**:283-301.

inappropriate analysis of experimental results and to the conduct of experiments which yield results that are not even amenable to proper statistical analysis. A survey of 78 experiments, described in papers published in two leading toxicology journals between 1989 and 1990, showed that over 60 percent had obvious statistical errors. About one third of the experiments involved far more animals than necessary to achieve the stated aims of the research.[4] Where statistical analysis is crucial to the outcome of a research project, it is vital that careful consideration is given to the design of experiments to take account of the degree of variation to be expected, the required statistical power, and the method of statistical analysis to be used. Ways of improving experimental design by controlling variability and allowing the use of more-sophisticated statistical methods have been suggested.[5] One way of using these methods in practice would be to improve training of scientists; a more practical and reliable option may be to ensure that scientists have the opportunity to consult at an early stage with a statistical expert.

12.5 The two approaches above are of special relevance for ensuring that numbers of animals intended to be used in a specific research project are reduced as far as possible. But Reduction also has another dimension in the sense that it is desirable to reduce the total number of experiments which are undertaken. In this context, data sharing is an important means of avoiding duplication of testing in toxicology as well as pharmaceutical and academic research. In the case of toxicology testing and pharmaceutical research the results of tests are often commercial property, and the need for confidentiality may sometimes lead to duplication of testing. In basic research, duplication[6] may occur when researchers are unaware that a particular experiment or test has already been carried out by other researchers. There have been claims and counter-claims about the extent to which studies are duplicated.[7] Nevertheless, ensuring that results from research are shared as much as possible is a useful way of reducing the total number of animals involved in research. The principal way in which data are currently shared is by publication of research in peer-reviewed journals. However, not all research actually undertaken is published. Some therefore argue that it would be desirable to ensure greater availability of reports of 'negative', or unsuccessful, research results.[8] But there are problems in publishing research findings that are not peer reviewed. The peer-review process helps to ensure that only findings from properly conducted research are published, and publication of poorly conducted research may lead to confusion.

4 See also Festing MFW (1996). Are animal experiments in toxicological research the "right" size? in *Statistics in Toxicology* Morgan BJT (Editor) (Oxford: Clarendon Press), pp3-11; Festing MFW and Lovell DP (1996) Reducing the use of laboratory animals in toxicological research and testing by better experimental design *J R Stat Soc* **58**: 127-140; see also: Editorial: Statistically significant (2005) *Nat Med* **11**: 1.

5 See Festing MF (1990) Use of genetically heterogeneous rats and mice in toxicological research: a personal perspective *Toxicol Appl Pharmacol* **102**:197-204; Festing MF (2001) Guidelines for the design and statistical analysis of experiments in papers submitted to ATLA *Altern Lab Anim* **29**:427-46; Festing MF (2002) The design and statistical analysis of animal experiments *ILAR J* **43**: 191-3; Festing MF and Altman DG (2002) Guidelines for the design and statistical analysis of experiments using laboratory animals *ILAR J* **43** 244-58; Shaw R, Festing MF, Peers I and Furlong L (2002) The design and statistical analysis of animal experiments *ILAR J* **43**:191-3; Howard BR (2002) Control of variability *ILAR J* **43**: 194-201.

6 It is important to distinguish between *duplication* and *replication* of experiments, see paragraph 15.16.

7 See, for example BUAV (2001) BUAV *Submission to the House of Lords Select Committee on Animals in Scientific Procedures*, available at: http://www.buav.org/pdf/BUAV_HOL_Evidence.pdf. Accessed on: 9 May 2005; see also Home Office (2005) *Report by the Animal Procedures Committee (APC) Review of the cost benefit assessment in the use of animals in research: Government Response by Caroline Flint MP, Parliamentary Under-Secretary of State for the Home Department*, available at: http://www.homeoffice.gov.uk/docs4/jw280305flint_banner_report_by_the_animal_procedures_committee.pdf. Accessed on: 9 May 2005.

8 See paragraphs 35-37 of Animal Procedures Committee (2001) *Report on openness*, available at: http://www.apc.gov.uk/reference/openness.pdf. Accessed on: 9 May 2005.

12.6 In the UK, in 2002 the inter-Departmental Group on the 3Rs[9] was formed, as a successor to the Inter-Departmental Data Sharing Group, which produced and published in 2000 the inter-Departmental Data Sharing Condordat. The Concordat is a voluntary scheme which seeks to 'promote opportunities for encouraging agencies, industry and other stakeholders to endorse the principle of data sharing and to extend its scope by looking to overcome the practical, legal, commercial and cultural barriers to its effective implementation.'[10] Among other things, the Concordat encourages minimisation of data requirements for tests as far as possible, and the reviewing of procedural and legal barriers to data sharing. Under the Concordat, 'UK regulatory authorities, as lead agencies, [will] press for agreement on behalf of the UK Government for fullest provisions and procedures which enable data sharing when negotiating, updating and transposing relevant European Directives and when taking part in other international harmonisation processes.' We return to issues raised by the possible duplication of research in different areas in Chapter 15, where we reconsider the national and international context of research (paragraphs 15.68–15.70 and 15.83). We also explore ways in which the avoidance of duplication can be ensured especially in relation to research involving GM animals (paragraphs 15.71-15.75).

Harmonisation of international test guidelines

12.7 We have noted that many tests involving animals are conducted to provide safety or efficacy data for regulatory authorities, in compliance with national or international legislation (see paragraphs 9.4 and 13.48). If different authorities require testing to be carried out using their own specific study designs, a single chemical that is marketed in a number of countries might need to be tested several times for toxic effects. Harmonisation of test guidelines, so that a single study design is acceptable to regulatory authorities in many countries, is a very valuable means of reducing the number of animals used in safety and efficacy testing worldwide. Harmonisation has many advantages: it can reduce the need for repeat testing; eliminate the requirement for redundancy in testing (where more than one test provides the same information); minimise group sizes (e.g. by agreement to use a single sex) and lead to the adoption of shortened protocols, reduced animal numbers and less-severe treatments and procedures.

12.8 A relatively high level of harmonisation of test methods for chemicals has been achieved by the Test Guidelines Programme of the OECD. Similarly, in the area of pharmaceuticals, the ICH has achieved a substantial decrease in the numbers of animals used globally in the pre-clinical safety assessment of new pharmaceuticals (about a 50 percent reduction overall for a typical package of tests).[11] Examples of reduction in regulatory testing include:

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9 The Group is led by Home Office officials and has members from the Department of Health, the Department for the Environment, Food and Rural Affairs, the Department of Trade and Industry, the Office of Science and Technology, the Food Standards Agency, the Health and Safety Executive, the Medicines and Healthcare Products Regulatory Agency and other agencies. Its terms of reference are 'to improve the application of the 3Rs and promote research into alternatives, reducing the need for toxicity testing through better sharing of data, and encouraging the validation and acceptance of alternatives.' See http://www.homeoffice.gov.uk/docs2/interdept3rs.html. Accessed on: 3 May 2005.

10 Home Office (2002) *The Inter-Departmental concordat on data sharing*, available at: http://www.homeoffice.gov.uk/docs/dataconcordat.html. Accessed on: 3 May 2005; see also Animal Procedures Committee (2003) *Review of the cost-benefit assessment in the use of animals in research* (London: HO), p52-6.

11 The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration. The purpose is to make recommendations on ways to achieve greater harmonisation in the interpretation and application of technical guidelines and requirements for product registration, in order to reduce the need to duplicate the testing carried out during the research and development of new medicines; Lumley CE and van Cauteren H (1997) Harmonisation of international toxicity testing guidelines for pharmaceuticals: contribution to refinement and reduction in animal use *EBRA Bulletin* November: 4–9.

- As discussed in Chapter 9, the 'classical' LD_{50} test was used for many years to estimate the oral toxicity of a single dose of a chemical (paragraph 9.14). Although a few LD_{50} tests are still used in some circumstances, widespread and enduring opposition to this test on animal-welfare grounds led to the development of several alternative methods in which fewer animals were dosed, in a stepwise manner.[12]
- Tiered testing strategies have been employed to reduce the use of animals in several areas of toxicity testing. For example, the irritancy of chemicals for the skin and eye was previously assessed by using rabbits, without any prior testing. The currently recommended procedure involves assessing the chemical properties of the test materials and the use of *in vitro* methods as a first stage. Assessment of skin irritancy is undertaken before the eye test. Only if none of the previous assessments indicate irritancy is the eye test performed on live rabbits.[13]
- Vaccines that are produced from living organisms (e.g. viruses and bacterial toxoids) are tested for safety and/or efficacy at a number of points during manufacture, and batches are usually tested more than once to ascertain their efficacy (Box 8.5). These tests involve the use of large numbers of animals, and often involve infecting both vaccinated and unvaccinated animals with the relevant pathogen, leading to severe suffering in some unprotected animals.[14] Some of these 'challenge tests' for vaccine potency can be replaced with serological methods in which the presence of specific antibodies in the blood of immunised animals is used to demonstrate protection against challenge by the pathogen. A major success of this approach has been the development and validation of serological methods for the potency testing of tetanus vaccines, which have reduced the number of animals required.
- Prompt deletion of obsolete or redundant tests from testing requirements is a high priority for avoiding unnecessary use of animals. For example, tests for abnormal toxicity, which were general tests for adverse effects of vaccines, have been deleted from most monographs of the European Pharmacopoeia. Also, two types of animal test that were previously required for toxicity testing of diphtheria and tetanus vaccines have been eliminated. Substantial reductions in the number of animals used per batch have been achieved by modifications to five other tests on diphtheria, tetanus and pertussis vaccines.[15]

Refinement

Definition and scope

12.9 The original definition of Refinement by Russell and Burch was 'any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used [in experiments]'. This definition[16] has been modified to encompass the positive

12 The Fixed Dose Procedure (FDP) uses approximately one quarter of the animals required by the LD_{50} test. FDP avoids the death of the animals as an endpoint, recording signs of "evident toxicity" instead. The Up-and-Down Procedure (UDP) is a stepwise approach where one animal receives the dose thought to be the best estimate of the LD_{50} dose. Depending on the outcome (death/life), the dose for the next animal is adjusted. After reaching the reversal of the initial outcome (i.e. the point where an increasing or decreasing dose pattern is reversed by giving a smaller or higher dose) four additional animals receive the dose, to replicate the finding. UDP requires more time than the previous methods and is more expensive, but uses fewer animals; See *Test Guideline No 420: Fixed Dose Method and Test Guideline No 425: Up-and-Down Procedure* in OECD (2001) *Guidelines for Testing of Chemicals* (Paris: OECD).

13 Guideline 405 Acute eye irritation/corrosion in OECD (2001) *Guidelines for Testing of Chemicals* (Paris: OECD).

14 Associate Parliamentary Group for Animal Welfare (2005) *The use of animals in vaccine testing for humans*, available at: http://www.apgaw.org/userimages/Vaccinetesting.pdf. Accessed on: 9 May 2005.

15 Associate Parliamentary Group for Animal Welfare (2005) *The use of animals in vaccine testing for humans*, available at: http://www.apgaw.org/userimages/Vaccinetesting.pdf. Accessed on: 9 May 2005.

16 Refinement is sometimes referred to incorrectly as 'the refinement of experiments to get more data'. This is clearly an important goal, but it is not an interpretation of Refinement as originally defined in the Three Rs.

concept of improving welfare as well as of reducing suffering, and to encompass husbandry and care as well as procedures. Reducing suffering and improving animal welfare are important for the following four reasons:

- First, as discussed in Chapters 3 and 4, it is clear that animals can suffer and that their suffering needs to be taken seriously.
- Secondly, societal concerns about the use of animals, and acceptance of different uses, appears to depend to a considerable degree on the amount of suffering experienced by animals.
- Thirdly, both the physical and psychological welfare of laboratory animals has a significant effect on the experimental results. For example, sympathetic nervous system (SNS) activity is significantly increased in mice housed in stressful conditions such as social deprivation. The SNS controls many different body systems including the immune and gastrointestinal systems. Any change in SNS function will therefore have widespread effects on the animals and on their physiological responses that will effect experimental data.[17]
- Fourthly, the law controlling experiments on animals requires animal suffering to be minimised.

Thus, aside from the moral and legal requirement to reduce and prevent suffering, good animal welfare is consistent with good science and also ensures the effective use of resources, and animals.

Potential for Refinement

12.10 Of all the Three Rs, Refinement to reduce suffering and improve welfare is probably the easiest to achieve in the short term for all types of animal use, as highlighted in the following response to the Consultation:

> 'It is attractive, and undoubtedly important, to focus a great deal of effort on the development of Replacement methods. However, it is important that expectations about the scope for replacement with non-animal methods should not be unrealistic and that focus on Replacement should not be at the expense of efforts for Refinement. The potential for improvements through Refinement – making animals' lives better through better husbandry, better research techniques, and better veterinary methods to alleviate discomfort and stress – should not be underestimated.'
> *UFAW*

12.11 However, in order to achieve maximum effect, it is essential to be aware of the kind of Refinements available and how best to implement them. Since Refinement concerns the reduction of suffering, a crucial prerequisite is to be able to recognise what causes, or is likely to cause, animals to suffer (see Chapter 4). As we have noted, there are many sources of potential suffering throughout the lifetime of each animal which may need to be considered in addition to those resulting from scientific procedures and their effects (paragraphs 4.49–4.59).

Some specific examples of Refinement

12.12 Further to the discussion in Chapter 4, we now consider examples of four especially important areas in which Refinement can be implemented: housing, husbandry and care, experimental procedures, pain management and humane endpoints.

17 Gentle MJ (2001) Attentional shifts alter pain perception in the chicken *Anim Welfare* **10**: S187–94.

Refining housing, husbandry and care

12.13 Laboratory animals spend most of their time in cages or pens, so their immediate environment, and the care they receive, has a major impact on their well-being. Standards for laboratory housing are defined in the Home Office Codes of Practice for the husbandry and care of animals[18] and corresponding European guidelines.[19] These represent minimum standards only and are mainly concerned with satisfying the physiological rather than the behavioural needs of the animals. For example, rats are social animals that, in the wild, have large home ranges, eat a varied diet and exhibit a range of complex behaviours.[20] Yet according to current guidelines for laboratory animals, two adult rats can be kept for the whole of their life in a cage with a floor area of 700 cm^2 (the size of a large shoe box) containing a few millimetres of sawdust and perhaps a tube to hide in (see also Box 12.1). Unmodified, this is a very confined and barren environment.

12.14 Refinement of laboratory animal husbandry requires the provision of an 'enriched' environment that satisfies not only the physiological, but also the behavioural needs of the animals and these have to be identified for each species and strain. One way of doing this is to use the results of behavioural studies, which measure an animal's preference for, or motivation to obtain, a particular resource, such as a nest box for chickens, access to social companions in rats, and rooting materials for pigs (see Box 4.2).

12.15 It is easier to identify the needs of some species than others. In the case of rats and mice there is a significant scientific literature on their behavioural needs. Similarly, nesting material, facilities for animals to hide, and material for gnawing are fundamental requirements for both rats and mice.[21] It is therefore relatively straightforward to ascertain from the scientific literature what the laboratory environment must provide in order to try and satisfy the basic needs of rats and mice.[22] Important aspects of Refinement for rodent husbandry are listed in Box 12.1.

Box 12.1: Husbandry – needs of mice and rats

A good-quality environment providing for a range of activities would include:

- housing in stable, compatible groups;
- enough space for exercise and to perform normal social behaviour;
- a solid floor with a wood-shaving substrate;
- height to accommodate rearing (up to 30 cm in an adult rat);
- nesting material;
- material to gnaw; and
- refuges.

18 Home Office (1989) *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures* (London: HMSO). See also the supplementary codes which concern a range of more specific research contexts, available at: http://www.homeoffice.gov.uk/comrace/animals/legislation.html#codes. Accessed on: 9 May 2005.

19 European Community (1986) *Council Directive 86/609 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*, OJ L.358. (Luxembourg: EC).

20 See Berdoy M (2003) *The Laboratory Rat: A Natural History*, available at http://www.ratlife.org. Accessed on: 3 May 2005.

21 See Fillman-Holliday D and Landi MS (2002) Animal Care Best Practices for Regulatory Testing *ILAR J* V43 Supplement; Sherwin CM (2002) Comfortable quarters for mice in research institutions, available at: http://www.awionline.org/pubs/cq02/Cq-mice.html. Accessed on: 9 May 2005; Lawlor MM (2002) Comfortable quarters for rats in research institutions, available at: http://www.awionline.org/pubs/cq02/Cq-rats.html. Accessed on: 9 May 2005.

22 Chmiel DJ and Noonan M (1996) Preference of laboratory rats for potentially enriching stimulus objects *Lab Anim* **30**: 97–101; Manser CE, Elliott HE, Morris TH and Broom DM (1996) The use of a novel operant test to determine the strength of preference for flooring in laboratory rats *Lab Anim* **30**: 1–6; Townsend P (1997) Use of in-cage shelters by laboratory rats *Anim Welfare* **6**: 95–103; Patterson-Kane EG, Hunt M and Harper DN (1999) Behavioural indexes of poor welfare in laboratory rats *J Appl Anim Welfare Sci* **2**: 97–110.

12.16 Once species-specific needs have been identified, ways of satisfying the animals' needs in a laboratory setting may be developed. Further to improving the welfare of animals and the quality of scientific results, implementations of Refinements have the benefit that animals housed with a good quality and quantity of space are frequently easier to handle and work with. Research shows that rats group-housed in enriched environments are quicker to learn new tasks, less stressed, more confident, less aggressive and in better general condition than singly housed animals. Such benefits can improve staff morale and encourage further exploration of opportunities to implement all Three Rs.

Refining experimental procedures

12.17 As we have illustrated in Chapters 5-9, a very wide variety of experimental procedures is applied to laboratory animals, from those that are relatively minor, such as blood sampling, through to major surgery. The procedures themselves may cause adverse effects (paragraph 9.28) and there may also be adverse effects as a result of a procedure (paragraph 4.54). For this reason it is crucial to consider what opportunities there are to refine every aspect of each procedure from start to finish. One particular category of procedures where there is great potential for Refinement is the administration of substances to animals.[23] Such procedures are required for many experiments, for example to create a disease in order to study it, to test the effectiveness of a new medicine, or to assess the toxicity of a chemical. There is a variety of techniques employed for such purposes, and in each case it is important to think about Refinement with respect to the animal's immediate experience of the administration method and all that it entails. This assessment should include any distress from necessary handling and restraint (see paragraphs 4.44–4.47), as well as from the administration method itself. The substance administered can also have a profound effect on the animal in the short and long term. For example, it may irritate the animal's nose or stomach, or cause nausea or seizures.

12.18 The potential for Refinement may be understood better in considering a specific example, such as the injection of a substance into an animal's joint to study arthritis (see paragraph 6.7) or to ascertain the efficacy of medicines to treat the disease. This procedure can be very painful and has the potential to cause swelling, inflammation and infection of the joint, and consequent lameness. Refinement of the technique encompasses several elements. The needles used for injection must be the smallest size possible and the volume of the substance given and frequency of dosing should also be kept to a minimum so as not to distend the joint. The animal needs to be kept calm and held very still and the operator has to have a good knowledge of the anatomy of the joint. The procedure should only be done once and to one joint only. If all these Refinement aspects are fully implemented, the animals will suffer far less pain. The guiding principle in this, and in any other aspect of Refinement, is never to assume that current practice is best practice, and to review all the techniques and protocols that are used at regular intervals. One helpful approach in devising possible improvements can be to think about a technique from the animal's point of view and to ask how a specific procedure would feel if it were applied to oneself. While this suggestion is not intended to encourage uncritical anthropomorphism (see paragraph 4.3) it can be a helpful tool in reviewing current practice in view of species-specific needs.

Refining the management of pain

12.19 Reducing any pain associated with experiments is another important aspect of Refinement. Success depends critically on the ability of those dealing with the animals to recognise and

23 Morton DB, Jennings M, Buckwell A *et al.* (2001) Refining procedures for the administration of substances *Lab Anim* **35**: 1–42.

assess pain and suffering (paragraphs 4.18–4.30). Most people involved with animal use are confident of their ability to detect the relevant signs, but some staff are insufficiently trained and lack the relevant expertise.[24] Special training is required because many laboratory animals are adapted to conceal signs of pain or distress (see paragraph 4.12). Most people can recognise and respond to overt clinical signs of moderate to severe pain in laboratory animals, but it can be more difficult to recognise indicators of mild to moderate discomfort, pain or distress, which can be very subtle and hard to detect. For example, audible vocalisation is still often cited as a sign that rats are in pain, yet it is now widely known that rats usually vocalise ultrasonically. For truly effective Refinement, these subtle signs of suffering also need to be identified so that staff can become familiar with them. For example, in the case of rats undergoing abdominal surgery, recent research has shown that behaviours such as flank twitching can be used to identify whether rats require more pain relief.[25] Until this research was carried out, few if any guidelines on pain assessment for rats mentioned this behaviour, yet it occurs regularly and is highly diagnostic. This approach requires rigorous evaluation of animal behaviour and an open mind.

12.20 It is important to appreciate that Refinement is a continuous process and not a static formula that is only applied at one stage. Ideally, research establishments should have a framework in place for regularly reviewing the way in which experiments are conducted, and comparing current practice with new evidence emerging from research on animal behaviour. This can allow for the development of improved methods of managing pain. A proactive establishment would provide any or all of the following measures as appropriate in its pain management programme:

- pre-emptive pain relief as well as post-operative pain relief;
- multi-modal pain therapy using different pain relieving medicines, which work in different ways and therefore achieve improved control of pain;[26]
- husbandry and care in the spirit of critical anthropomorphism, which addresses species-specific needs (paragraph 4.30);
- staffing (of appropriate expertise) at such levels as will enable the need for intervention (whether treatment or euthanasia) to be anticipated.

Refining endpoints

12.21 The vast majority of animals are killed at the end of the experiment, either because their tissues are required as part of the experiment, or because the scientific objectives have been achieved and the animal can no longer be used. However, under UK law there is provision for limited and tightly controlled re-use, or release of animals to the wild, or a home, where this is appropriate for the individual animal.[27] If the experiment leads to an increasing amount of suffering during its course then it is best for the animals to be killed as early as possible. This approach is described as operating 'humane endpoints' and requires indicators of likely suffering to be detected at an early stage. For example, if it is known that particular clinical signs such as decreased body temperature lead to a specific outcome such as death, then animals can be killed as soon as these signs appear. Other markers that can

24 See, for example, Flecknell P and Karas A (2004) Assessing and Managing Pain and Distress for Ethics Committees *ATLA* **32** Supplement 1: 265–6.

25 Roughan JV and Flecknell PA (2001) Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats *Pain* **90**: 65–74.

26 Multimodal pain therapy is the combined administration of opioid and non-opioid pain relief.

27 See A(SP)A Schedule 14 and 15, the options of release of animals to the wild, an abattoir or a home are chosen very rarely.

be used to define humane endpoints include flank twitching (paragraph 12.19) and chemical and haematological changes in the blood.[28]

12.22 But in some cases the only way to determine the clinical signs indicating that animals should be euthanised may be to allow some animals to suffer considerably or even die, carefully recording the clinical signs throughout their lives so that a retrospective analysis can be undertaken. This approach has been used successfully to refine some of the protocols required for toxicity testing of vaccines.[29] Humane endpoints should generally be easier to define within safety-assessment programmes because routine procedures are used and the only variable is, for example, the batch of vaccine.[30]

Barriers to implementing Refinement

12.23 We have observed above that implementation of Refinements is usually more straightforward than Reduction and Replacement and that many Refinements have been developed by scientists during the normal course of their work. There should in fact be fewer scientific barriers to Refinement. Where they do occur, it can be difficult to determine whether they are real or perceived and exactly what the nature of the barrier is. One common concern is that the provision of Refinement in the form of environmental enrichment may add unwanted variables that may reduce the validity of experimental data. For example, in toxicology it might be argued that giving wooden chews to animals affects their metabolism and interfers with the results. This may mean that more animals have to be used to generate statistically significant results. However, there are usually ways around such problems, for example, by using commercially available enrichments that have been fully characterised and standardised.

12.24 Other, non-scientific, barriers to the application of Refinement can result from:

- limited understanding of the concept of Refinement, why it is important and when and how to apply it;
- limited understanding of the species-specific needs of animals, causes of suffering and the impact of laboratory research and housing on the full lifetime experience of an animal;
- lack of specific information and guidance on practical Refinements, relating to what to do and how to do it;
- lack of resources, including time and funds; and
- lack of motivation and training.

12.25 All of these factors can significantly limit the implementation of Refinement, which, in view of its relative ease of application and its great potential for reducing suffering, is regrettable. Nevertheless, many establishments in the UK are very proactive with regard to Refinement and, taking animal husbandry as an example, have good, innovative environmental enrichment programmes. But there is also anecdotal evidence that some researchers argue that animals 'do not do anything', and therefore do not need anything

28 See Hendriksen CFM and Morton DB (Editors) (1999) *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the Int Conference, 22–5 Nov 1998, Zeist, The Netherlands. (London: Royal Society of Medicine).

29 Johannes S, Rosskopf-Streicher U, Hausleithner D *et al*. (1999) Use of clinical signs in efficacy testing of erysipelas vaccines, in *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the Int Conference, 22–5 Nov 1998, Zeist, The Netherlands, Hendriksen CFM and Morton DB (Editors) (London: Royal Society of Medicine), pp102-105; Krug M and Cussler K (1999) Endotoxin in porcine vaccines: clinical signs and safety aspects, in *Humane Endpoints in Animal Experiments for Biomedical Research* Proceedings of the Int Conference, 22–5 Nov 1998, Zeist, The Netherlands, Hendriksen CFM and Morton DB (Editors) (London: Royal Society of Medicine), pp114–17.

30 See also: Hendriksen CFM and Morton DB (Editors) (1999) *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the Int Conference, 22–5 Nov 1998, Zeist, The Netherlands. (London: Royal Society of Medicine).

to do. Such judgements point to limited knowledge of animal behaviour because the converse is often true. Animals that have nothing to do tend not to do anything.

12.26 Some problems in promoting Refinements arise from the fact that species-specific needs are not well characterised. Even when they are, as in our earlier example of rats, available knowledge is not always applied. For example, grid floors instead of solid floors may be used in some establishments without specific scientific justification and important resources such as substrate and nesting material are not universally provided. In a survey published in 2001, up to 25 percent of rats received no nesting material, up to 35 percent were not given anything to gnaw and over 50 percent were not provided with refuges, all of which play a significant role in relation to promoting the well-being of rodents.[31] The effectiveness with which pain is managed is also inconsistent as practice with regard to recognising and alleviating animal pain varies across different establishments and abilities of different people.[32]

12.27 Another significant barrier to the implementation of Refinement is the relative dearth of detailed and accessible information on, and practical examples of, Refinement. Relevant information tends to be found in journals on research techniques and animal welfare which are rarely consulted by researchers whose primary interest is in their own specialised research field. Useful information is provided by the reports of the Joint Working Group on Refinement (JWGR),[33] which provide practical advice on Refinement in husbandry of rabbits,[34] mice,[35] birds[36] and dogs;[37] and procedures including blood sampling,[38] administration of substances,[39] telemetry[40] and the generation and care of GM mice.[41] Lack of time and resources can also have implications for the implementation of enrichments and other Refinements, since such improvements require significant expenditure on staff and materials.

Overcoming constraints

12.28 Thus, overcoming the constraints and improving the implementation of Refinement requires significant commitment to:

- an open-minded, innovative and proactive approach to developing new Refinements;
- seeking out available information on good practice and implementing it;

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31 Mortell N (2001) Practical environmental enrichment for rats and mice (the results of a survey) *Anim Technol* **52**: 1–19.

32 Richardson CA, Flecknell PA (2005) Anaesthesia and post-operative Analgesia Following Experimental Surgery in Laboratory Rodents: Are we making Progress? *ATLA* **33**: 119–127.

33 This group was set up by the BVA Animal Welfare Foundation (BVA/AWF), FRAME, the RSPCA and UFAW.

34 Morton DB, Abbot D, Barclay R *et al*. (1993) Removal of blood from laboratory mammals and birds *Lab Anim* **27**: 1–22; Morton DB, Jennings M, Batchelor GR *et al*. (1993) Refinements in rabbit husbandry *Lab Anim* **27**: 301–29

35 Jennings M, Batchelor GR, Brain PF *et al*. (1998) Refinements in mouse husbandry *Lab Anim* **32**: 233–59.

36 Hawkins P, Morton DB, Cameron D *et al*. (2001) Refinements in husbandry and procedures for laboratory birds *Lab Anim* **35**, Supplement 1: 1–163.

37 Prescott MJ, Morton DB, Anderson D *et al*. (2004) Refining dog husbandry and care: Eighth report of the BVAAWF/FRAME/RSPCA/UFAW Joint working Group on Refinement. *Lab Anim* **38** Suppl 1:1–94

38 Morton DB, Abbot D, Barclay R *et al*. (1993) Removal of blood from laboratory mammals and birds *Lab Anim* **27**: 1–22; Morton DB, Jennings M, Batchelor GR *et al*. (1993) Refinements in rabbit husbandry *Lab Anim* **27**: 301–29.

39 Morton DB, Jennings M, Buckwell A *et al*. (2001) Refining procedures for the administration of substances *Lab Anim* **35**: 1–42.

40 Hawkins P, Morton DB, Bevan R *et al*. (2004) Husbandry refinements for rats, mice, dogs and non-human primates used in telemetry procedures *Lab Anim* **38**: 1–10; Morton DB, Hawkins P, Bevan R *et al*. (2003) Refinements in telemetry procedures *Lab Anim* **37**: 261–99.

41 Robinson V and Jennings M (2004) Refinement and reduction in the production of genetically modified mice: Sixth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement *ATLA* **32** Supplement 1: 373–5.

- knowing the animals' physiological and behavioural needs and being aware of current evidence on how to address these in the laboratory environment;
- anticipating expected *and* unintended adverse effects of all experimental work;
- being familiar with subtle signs of distress or discomfort in the species, strain, phenotype and individual animal, and knowing how to alleviate the cause;
- disseminating specific information on Refinement in an accessible way;
- publishing details of Refinement as an integral part of scientific papers in the mainstream literature; and
- most importantly, not assuming that *existing* practice is necessarily *best* practice.

Summary

12.29 Effective implementation of Refinement and Reduction requires both concepts to be considered at an early stage to improve the general research strategy of a project. A staged approach before embarking on large or complex experiments is useful, starting with thorough background research of the published literature and considering the possibility of conducting a small pilot study. Effective teamwork also plays a significant role, involving staff with a wide range of relevant expertise, in for example *in vitro* technology, experimental design, statistics, and animal care.

12.30 We observed that the concept of *Reduction* is best understood as requiring 'the use of fewer animals in each experiment without compromising scientific output and the quality of biomedical research and testing, and without compromising animal welfare'. To improve its application, the importance of appropriate research strategies, better control of variation among animals, better statistical analysis and the avoidance of duplication need to be recognised. We considered successful examples of Reduction in regulatory testing and noted that harmonisation of international test guidelines can contribute significantly to further reduction.

12.31 *Refinement* is probably the most effective of the Three Rs in achieving immediate reduction of pain and suffering, and improvement of welfare of animals involved in research. The approach is of great relevance since reducing pain, suffering and distress is a crucial aspect of the moral debate about animal research, and a legal requirement. It is also important scientifically since the physical and psychological welfare of laboratory animals can have a significant effect on the scientific validity of experimental results.

12.32 Possibilities for implementing Refinement were considered in four areas: housing, husbandry and care, experimental procedures, pain management and humane endpoints. Refinement of housing conditions is particularly important since the quality of animals' cage or pen environments can have a major impact on their lives. While standards for laboratory housing are defined in the Home Office Codes of Practice for the husbandry and care of animals and relevant European guidelines, the requirements represent minimum standards. There should be relatively few scientific barriers to Refinement, and these should be considered on a case by case basis. A fundamental principle is never to assume that current practice is best practice. All the techniques and protocols that are used at regular intervals should be reviewed and critically assessed throughout the duration of any research programme. We present our conclusions and recommendations about the implementation of the Three Rs in Chapter 15 (paragraphs 15.57–15.62) and now turn to the regulation of animal research.