Finding alternatives: an overview of the 3Rs and the use of animals in research

Vicky Robinson

The use of animals in scientific research is a controversial and emotive issue. The principles of the 3Rs, which underpin the humane use of animals in scientific research, are perhaps one area where proponents and opponents can reach common ground.

Animals are used in scientific and medical research to understand a whole range of questions relating to how the body works and what causes diseases in man and animals, and to try to develop treatments that are safe and effective. This use of animals causes many people concern because of the potential to cause pain and suffering to the animals involved. The use of animals in experiments to develop safe medicines to treat humans and animals poses an ethical dilemma. The principles of the 3Rs (that is the replacement, refinement and reduction of the use of animals in research – see Box 1) provide a framework to address this dilemma.

The legal, ethical and scientific basis for the 3Rs

The 3Rs are important from a legal, ethical and scientific standpoint. All research using animals in the UK, for example at universities and pharmaceutical companies, is regulated by the Animals (Scientific Procedures) Act 1986 (ASPA), which is administered by the Home Office. The 3Rs are implicit in the Act and any researcher planning to use animals in their research must first demonstrate why there is no alternative and that the number of animals used and any suffering caused will be kept to a minimum.

The 3Rs are also important from an ethical standpoint, as research using animals has the potential to cause pain, suffering or distress. This can arise from the experiments themselves or from the way that animals are housed. In a humane society there is clearly a moral obligation to ensure that any harm caused is kept to an absolute minimum. Indeed, opinion polls have shown that the public only accepts research using animals where this is the case. Minimising suffering is the responsibility of all those

ABSTRACT
This article describes the techniques and scientific advances that are allowing researchers to replace, refine and reduce the use of animals in research. It outlines the legal, ethical and scientific basis for the principles of the 3Rs – replacement, refinement and reduction, and then describes progress in each area, with examples. Finally challenges for the future are summarised.

Box 1 The 3Rs

Replacement: the use of non-animal methods such as cell cultures, human volunteers and computer modelling instead of animals to achieve a scientific aim.

Refinement: the use of methods that alleviate or minimise potential pain, suffering or distress, and that enhance animal welfare for those animals that cannot be replaced.

Reduction: the use of methods that enable researchers to obtain comparable amounts of information from fewer animals, or more information from the same number of animals.

involved in the use of animals in research, including the research establishments and the people that carry out the studies. Under the ASPA, all research establishments are required to have what is called an 'ethical review process' to consider ethical issues and the 3Rs.

Finally, and very importantly, there are strong scientific reasons for implementing the 3Rs. The way that animals are housed, handled and used in scientific research can affect their physiology, immunology and behaviour. For example, even something as simple as holding an animal can affect its blood pressure and the level of some hormones. This, in turn, can affect the validity and reproducibility of any data obtained. Minimising the impact of the research on the animals is therefore important for science and animal welfare.

Replacement

The vast majority of scientific and medical research does not involve the use of animals. However, the use of animals is an important aspect in some areas of research and, in an ideal world, alternatives would be available. Although difficult, considerable progress on replacement has been made by scientists. Animals have been replaced for example by the use of cell-culture systems, human volunteers, computers and new imaging techniques. There are a number of sophisticated examples of replacements including *in vitro* models of skin, which can be used for drug discovery research as well as for testing new chemicals and products (e.g. for dermal irritation), and computer models to study how the heart works or to select potential new medicines.

Many replacement technologies arise as a result of research that is being carried out for another purpose. Imaging techniques such as magnetic resonance imaging (MRI) were originally developed for clinical use but are now being used to allow human volunteers to replace animals in some studies. Similarly, a technique called transcranial magnetic stimulation, which safely and temporarily disrupts brain function in healthy human volunteers, has been used to replace the use of animals in some brain-function studies.

Replacement also embraces the idea of using non-sentient organisms in research. Many genes and biological processes are conserved across evolution and this means that relatively simple organisms can be used instead of vertebrates in some circumstances. Thus, for example *Caenorhabditis elegans*, a primitive roundworm, can be used in toxicity testing and for research into the process of cell death and the development of the nervous system. Similarly, the fruit fly *Drosophila melanogaster* can be used to understand some aspects of complex disorders such as Parkinson's disease, while single-celled yeast can be used to study cell division and how this is disrupted in cancer cells.

Refinement

Although replacement is the ultimate goal, as long as animals are used in research, refinement is the 'R' with the greatest and widest potential impact. Refinement not only improves the life of every animal used in research, it also improves the quality of the science. This is illustrated by a study of genetically modified mice with similar mutations to those found in people with Huntington's disease – a disease that causes difficulties in movement and memory. If the Huntington's disease mice are provided with a complex cage environment that provides opportunities to hide, nest, gnaw and forage, the disease progresses much more slowly than in mice kept in barren cages, and in fact the mice mimic the progress of the human disease more closely. Thus, by providing a better environment for the mice, researchers have a more realistic 'model' of Huntington's disease that they can use to try to understand how to treat the disease. Of course the mice are happier too.

One obvious way to improve animal welfare it to provide an environment that meets the animals' specific needs (see Barley, 2005, this issue, for more details). Different animals have different behavioural requirements (e.g. mice like to make a nest, primates like wooden perches) and it is important for their wellbeing in captivity that they can express natural behaviours. If behavioural needs are not met, the animals can suffer mental and physical stress. The difficulty is knowing what actually matters to the animals. Researchers are developing ways of assessing this by getting animals to 'tell them' what they want, rather than trying to guess at their needs from a human perspective. For example, rats used to be kept in barren cages with grid floors that were easy to clean out. Nowadays, they are more often kept in cages with solid floors containing places to hide. These refinements are supported by scientific experiments in which, for example, rats are taught to push against a weighted door to gain access to cages with a solid floor or extra space. How much the rats will lift indicates how important these resources are to the rats. Quite amazingly, rats will lift 83 per cent of their body weight to gain access to a cage with a solid floor.

Another important refinement under investigation concerns the frequency of cage cleaning for rodents. In the past the emphasis has been on keeping cages clean and animals were transferred on a regular basis to fresh cages with clean bedding and so on. But animals such as mice use odours in their urine to maintain social hierarchies and mark territories; frequent cage cleaning is likely to disrupt these odour cues and this may be stressful for the mice if they regularly have to re-establish hierarchies and territories. Research is now underway at the University of Oxford to determine the optimal cage-cleaning regimen.

Minimisation of stress is another aim of refinement. One way to achieve this is by training animals by reward (e.g. food treats) to cooperate with procedures such as the dosing of substances or blood-sample collection. Not only does this minimise any potential stress caused to the animals, but it also improves the quality of data obtained because the animals are less stressed, as indicated by changes in blood pressure and heart rate. Training is commonly used with dogs and monkeys.

Provision of pain relief is another mainstay of refinement, but for some commonly used laboratory animal species this can be problematic because it can be difficult to identify whether an animal is in pain. Because rodents are prey animals, they tend to hide signs of suffering that would make them vulnerable to their predators. However, again scientists are using some clever approaches to get animals to tell them when they are in pain. Researchers at the University of Newcastle are videoing rats after surgery to see whether they can identify behaviours that indicate pain in these animals by comparing them with those that have not had surgery. Using this approach they have identified several telltale signs, such as specific stretching movements (rather like the way a cat arches its back to stretch) that the rats only do after surgery. These behaviours disappear if the rats are given painkillers, which strongly suggests that the behaviours are a good indicator of rats in pain. The researchers are now investigating whether the rats can self-administer pain relief through their drinking water.

Technological advances can also be used to refine some studies. For example, radio-telemetry devices can be implanted into animals to measure blood pressure, heart rate and activity levels. Although this requires surgery, subsequent measurements of physiological variables can be done non-invasively and remotely while the animals are in their cage.

Reduction

Keeping the number of animals used to a minimum is extremely important. However, when thinking about ways to reduce the number of animals used, researchers also have to ensure that the design of their experiment is robust. If by reducing animal numbers, researchers end up with data that have no statistical significance, they have wasted animal lives, which would be unacceptable.

The number of individual animals used is important but what really matters is what actually happens to the animals and how much they suffer. At times there can be a conflict between applying the principles of reduction and refinement. For example, some researchers may have to face the ethical dilemma of deciding whether it is better to use 10 rats in an experiment that will involve suffering or to use 100 rats in the same experiment but involving little or no suffering. A specific example is the use of female mice in the production of genetically modified mice. Females are used to provide fertilised eggs for microinjection. In order to reduce the number of females used it is common practice to give hormones to increase the number of eggs released per animal – a process called superovulation. However, superovulation requires that each animal is given an injection into its abdomen and this may cause pain. A choice has to be made between reduction and refinement.

Reduction is sometimes seen as the neglected 'R', but progress is being made here, as with the other 'Rs'. For example, experiments that used to be monitored by doing post-mortems on animals can now be done using non-invasive imaging. Tumours, for example, can be fluorescently labelled and their growth followed over time in the same animal, rather than having to use many animals and killing some at each time point of interest. This greatly reduces the number of animals used and also improves the scientific data gathered because each animal acts as its own 'control'. In addition to allowing a reduction in animal use, such technologies also contribute to refinement because, in this case, the tumour size can be closely monitored to prevent animals suffering unnecessarily.

Challenges and hopes for the future

Progress in the 3Rs has been good over recent years but much more needs to be done. While animals continue to be used in research, it is imperative that we think proactively and creatively about how to replace, reduce and refine this use. This requires increased investment and commitment, as it seems likely that the use of animals in research will continue for many years to come. Indeed, the development of techniques to genetically modify animals as 'models' for human disease, combined with the completion of the human genome sequencing projects, is creating a driving force to use more animals. In addition, the development of biotechnological products, such as vaccines and growth factors, has the potential to increase the need for testing in species such as non-human primates. However, perhaps the greatest challenge for the 3Rs is the proposed new European Union legislation (referred to as REACH), which will require the safety testing of many already widely used household and industrial chemicals and will result in an unprecedented increase in animal use.

With these huge pressures to use animals in research, we must continue to question the justification for that use, to look for alternatives and to apply the 3Rs. Some of the future advances in this area will come from specific organisations dedicated to advancing the 3Rs (e.g. see Box 2). However, most major advances will come from mainstream research programmes, and it is here that we have to raise the profile of the 3Rs.

Box 2 A UK national centre for the 3Rs

In 2002, a House of Lords Select Committee considered the use of animals in scientific procedures. Among their recommendations, they proposed that a national centre for the 3Rs should be established. After further consideration by the Government, Lord Sainsbury (Parliamentary Under-Secretary of State for Science and Innovation) announced in May 2004 that the existing Medical Research Council's Centre for Best Practice for Animals in Research would be replaced by a National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). The new Centre held its first board meeting in September 2004.

The Centre's mission is to advance and promote the 3Rs in research and testing using animals. To do this it will develop a UK strategy for the implementation of the 3Rs; support high-quality research that advances the 3Rs; help to coordinate and disseminate 3Rs research; provide advice and guidance on the 3Rs and animal welfare to the scientific community and support its commitment to best practice in these areas; and work to gain validation of alternative methods.

On 27 September 2004, the NC3Rs announced funding totalling more than £360 000 for two research projects designed to advance the 3Rs. Researchers at the University of Bristol are developing methods to detect changes in the behaviour of genetically modified mice that may identify animals with poor welfare. Researchers at the University of Newcastle are investigating pain in rodents used to study cancer. A further £500 000 of research grants will be awarded in 2005.

Reference

Barley, J. (2005) Balancing the needs of animals and science. *School Science Review*, **87**(319), 00–00.

Websites

More details about the NC3Rs can be found at: http://www.nc3rs.org.uk

The Home Office website lists links to sites describing the use of alternatives to animals in research at: http://www.homeoffice.gov.uk/comrace/animals/furtherinfo.html

The Coalition for Medical Progress describes alternatives to animals at: http://www.medicalprogress.org/alternatives/index.cfm

Vicky Robinson is Chief Executive of the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). She was previously Director of the Medical Research Council's Centre for Best Practice for Animals in Research and has worked for the RSPCA, promoting the implementation of the 3Rs in biotechnology. She has a PhD in molecular biology.
E-mail: enquiries@nc3rs.org.uk