

THE THREE PRINCIPLES FOR ANIMAL RESEARCH

REFINEMENT

The term refinement signifies the modification of any procedures that operate from the time a laboratory animal is born until its death, so as to minimise the pain and distress experienced by the animal and enhance its well-being. Being aware of animal welfare is not only important from the viewpoint of ethics, it is also a matter of good science. The experience of pain and other stress is likely to result in physiological changes which may increase the variability of experimental results.

Therefore, it is in the interests of scientists to ensure that conditions in the animal house are the best possible. The environmental enrichment of laboratory animal housing need not be an expensive exercise. Toilet rolls, egg cartons and PVC tubing can provide rats and mice with places in which to hide. Bales of straw and rubber tyres can be used to create an area for rabbits to interact with others of their species. Dogs can be given toys to play with, and be provided with a raised platform so that they are not forced to stand in their own dirt.

The choice of species also has relevance to the concept of refinement. Some species have a greater capacity to feel pain and distress, and therefore should only be used when absolutely necessary. Some species have more complex husbandry requirements and should not be used if the institution cannot provide appropriate facilities. Moreover, there are environmental, ethical and scientific arguments for giving preference to captive-bred animals rather than those caught in the wild.

Once the study is in progress, it is important that laboratory staff are well trained and competent in the handling of the species that is being used, and that they have the correct attitude towards the animals. Anaesthesia and analgesia should be used whenever appropriate and possible. The injection volumes used should be as small as possible as should the sizes of needles. The endpoints and measurable parameters used in the experiment should be those which are least invasive and which can be assessed as early as possible, so as to minimise both the distress caused to the animals and the duration of the study. Where possible, researchers should aim to reduce the frequency with which samples are taken and also the volume of each sample.

In some cases, it may be possible to use a non-invasive method. For example, high-quality MRI (magnetic resonance images) can provide information on the distribution of water and electrolytes, fat deposition and the development of oedema in an organ tissue, MRS (magnetic resonance spectroscopy) can be used for serial non-invasive measurements of xenobiotics, metabolites and endogenous compounds in cells and tissues, and can enable differential measurement of mitochondrial and cytosolic pH. New advances in MRS raise the possibility of it being used in future to investigate more-complex structures, such as receptor interactions and cell membrane perturbations.

At the end of the experiment, the most humane method of euthanasia should be chosen. Guidelines on the killing of laboratory animals are available, which discuss the problems associated with the use of some methods.

REDUCTION

The concept of reduction covers any strategy that will result in fewer animals being used to obtain the same amount of information, or in maximising the information obtained per animal and thus potentially limiting or avoiding the subsequent use of additional animals. There are several possible approaches that can serve to reduce the use of animals:

(a) Some laboratories alert all their researchers when animals are going to be killed. For example, if one researcher intends to carry out a study on perfused livers, other researchers may be able to make use of the kidneys, brain tissue, serum or other components of the same animals.

(b) Appropriate experimental design and appropriate analysis of the resulting data, with due consideration to statistical principles, can increase the precision of the data and at the same time enable fewer animals to be used for the generation of these data.

(c) The choice of species and strain may influence the numbers of animals that are required. Different strains and species may be more or less sensitive in their reactions to the experimental procedures and this may influence the quality of the data and the ease of distinguishing effects of the procedures. Also, the use of pure inbred strains may result in less variability than seen in the outbred type.

(d) Overall research strategy may also contribute to a reduction in animal use. A small pilot study in a just a few animals can indicate if further work would be appropriate. In some cases, it may be possible to use an *in vitro* system to obtain preliminary data which could provide hints about the validity of the hypothesis, or indicate ways in which the main study might be modified to use fewer animals, earlier endpoints and/or less-invasive procedures.

REPLACEMENT

The range of replacement alternatives includes:

- the collation and use of information already gained;
- the use of physical and chemical analysis techniques;
- the use of mathematical and computer models (including molecular modelling, structure-activity relationship [SAR] approaches, and physiologically based pharmacokinetic [PBPK] modelling);
- the use of *in vitro* systems (including sub-cellular fractions, short-term maintenance cultures, and cells and tissues maintained in culture for longer periods);
- the use of human-oriented post-marketing surveillance and epidemiological approaches, and the ethical use of human volunteers;
- the use of organisms not classed as protected animals; and
- the use of early developmental stages of protected animals species, before the regulations apply to them.

There are two main types of replacement, namely, *direct replacement* (for example, when *in vitro* human skin preparations are used instead of *in vivo* tests on guinea-pigs or rabbits), and *indirect replacement* (for example, when the pyrogen test in rabbits is replaced by the *Limulus* amoebocyte lysate [LAL] assay or a test based on whole human blood).

Replacement can also be total or partial. One kind of *total replacement* involves the decision not to require an animal test any more, since the information it provides does not justify its continued performance. A recent example is the decision of the European Pharmacopoeia not to require the Abnormal Toxicity Test for certain kinds of vaccines. This will reduce laboratory animal use in Europe by about 35,000 animals per year.

Another kind of *total replacement* is where information which is needed can be gained without recourse to the existing animal procedures, as in the case of the batch testing of hormones such as insulin and somatotrophin, and in the replacement of the rabbit pyrogen test.

A third kind of *total replacement* occurs when production via laboratory animal procedures can be replaced by *in vitro* production (for example, monoclonal antibody production *in vitro* in place of the *in vivo* ascites method).

There are also various kinds of *partial replacement*. For example, animal use by the

pharmaceutical industry in the discovery of potential new medicines has been greatly reduced by the use of computer-based studies and *in vitro* systems as screens.

A second kind of *partial replacement* involves the use of physicochemical tests, SAR approaches and/or *in vitro* systems to identify highly toxic substances, so that any subsequent animal studies will be conducted either to confirm lack of toxicity or to identify mild or moderate effects. For example, scientifically validated *in vitro* methods are now available for identifying chemicals likely to be corrosive to the skin, and progress can be expected in the near future with *in vitro* methods for skin irritation.

This kind of *hierarchical* or *stepwise testing strategy* is now widely used by industry as a means of reducing both animal numbers and animal suffering, and is recognised in a number of OECD test guidelines (which form the internationally recognised system for the regulatory testing of chemicals and certain kinds of products). They are a good example of one way in which the Three Rs of Russell & Burch - *reduction*, *refinement* and *replacement* - can be achieved simultaneously as a result of the application of good

Taken from <http://www.frame.org.uk/3rs/3rsintro.htm>