Chapter 11

Replacements

Replacements

Introduction

11.1 Replacing, as far as possible, the use of animals for experimental purposes is a highly desirable goal. Progress in reducing animal use, partly but not wholly through developing Replacements, has been made in the UK. Nevertheless over 2.7 million animals were still being used in experiments in 2003. In this chapter we explore the prospects for the Replacement approach. We begin by clarifying the use of the concepts of alternatives and Replacements. We then discuss several different notions within the concept of Replacement and differentiate between different forms (complete and incomplete). We consider the role of non-animal methods as 'advanced' methods, as adjuncts to animal experiments, and as a way of avoiding animal use altogether. We then turn to the potential for Replacement of animals in different areas of research, focusing on toxicity testing required by regulation, and basic research. We describe scientific and non-scientific barriers to further implementation of the approach, and comment on recent initiatives to overcome these. Replacement is only one of the Three Rs. Refinement, Reduction and Replacement are interrelated, and adjusting one can affect one or both of the others. We discuss Reduction and Refinement in Chapter 12.

The current debate

11.2 There is much debate about the potential to replace animals in experiments with alternative methods. Some, often those involved in animal research, point out that the use of alternatives to animals is a legal requirement in the UK; that alternatives are always used if they are available; and that it is simply not possible to avoid the use of animals in most of the experiments that are currently carried out. They argue that large sums of money are spent on the search for alternatives; and that most research on Replacement methods is in fact undertaken by the scientific community.

11.3 Others, often those who work for animal protection organisations, and some scientists, argue that efforts to develop new, alternative methods and use of those already available could be increased substantially; that funding to develop (and validate) alternatives ought to be augmented; and that the search for alternatives requires greater commitment and focus. They argue that much more could be done with political will, greater resources and greater motivation within the scientific community. Some commentators also assert that animal experiments are poorly validated and sometimes misleading, and that alternative methods are therefore 'better science'.[1] The divergence of views on the role of alternatives is also illustrated by the following observations made by respondents to the Consultation:

> 'Far from being a separate activity, research into alternatives happens continuously when researchers seek and introduce new methods as part of normal working practice, and through the application of existing technologies. Replacement of animal use happens when information derived from new technologies allows us to gain knowledge which might otherwise have required animals. However, it is often unclear whether developments in say tissue culture are genuinely "alternatives" to animal use… They may simply be "different" methods which provide different information.'
> *AMRC*

[1] See, for example, Greek CR and Greek JS (2000) *Sacred Cows and Golden Geese* (New York: Continuum). See also New England Anti-Vivisection Society (2004) *Better Science: alternatives to animal research*, available at: http://www.neavs.org/betterscience/Alt-Contents.htm. Accessed on: 6 May 2005.

'Many of the suggestions for alternatives are based on misunderstanding or wilful misreporting of the facts. …the majority of medical research is nowadays on long-term degenerative diseases…, it is very difficult to see how any grossly simplified system (*in vitro*, *in silico*, etc.) can provide anything other than grossly simplified and misleading data.'
Dr Chris Jackson

'Despite British and EU legislation prohibiting the use of animals where a valid alternative exists, there are no centralised, comprehensible and easily accessible sources of information on alternatives for scientists to consult…. The establishment of a national centre of excellence for alternatives that could develop, promote and disseminate information and advice on alternatives to animals could solve this problem.'
The Dr Hadwen Trust for Humane Research

'The sooner the enormous sums of money that fund irrelevant experimentation on animals [are] diverted to relevant human-based, non-invasive methodologies, the sooner the pace of human medical progress will quicken.'
Derek S. Paton, Dundee Animal Rights

11.4 Arguments from both 'sides' of the debate about the potential to replace animals with alternatives are often applied to animal experiments *in general*, which is not particularly helpful or constructive. Animal experiments are used to provide information to try and answer a very wide range of scientific questions. The potential for using alternatives depends on the nature of the specific scientific question being addressed and therefore has to be evaluated on a case by case basis rather than in general terms, if progress in replacing animals is to be made.

Use of the concepts 'Alternatives' and 'Replacements'

11.5 Before we consider these different areas in more detail, we need to be clear what is meant by the term alternative in the context of animal experiments. To the general public, an alternative is likely to mean *an alternative method that does not involve using an animal*. This is the principle encompassed by UK and EU laws, which require that animal experiments can only be carried out if the purpose of the programme of work '…cannot be achieved satisfactorily by any other reasonably practical method not entailing the use of protected animals.'[2] However, in recent years, the term 'Alternative' has been applied to all of the Three Rs as an overarching term referring to any procedure that reduces the harms caused to animals in experiments, not only by replacing them (Replacement), but also by reducing the numbers used (Reduction) or by causing less animal suffering (Refinement). Such a conceptual muddle is unhelpful and in this chapter we focus exclusively *on Replacements* since this is the area in which there is most debate about the potential to improve on current practice.

Definition and scope of Replacements

11.6 Animal experiments are carried out to try to answer scientific questions. The term 'Replacement' is used to encompass methods that permit a given scientific purpose to be achieved without conducting experiments or other scientific procedures on living animals.[3]

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2 See Home Office (2000) *Guidance on the Operation of the A(SP)A 1986* (London: TSO), Chapter 5. Note that animals here means the animals covered by the Act and therefore only vertebrates, and one species of octopus; see Section 5.5 (a) of the A(SP)A, Article 7.2 of Directive 86/609.

3 Balls M (1994) Replacement of animal procedures: alternatives in research, education and testing *Lab Anim* **28**: 193–211; Balls M (2002) Future improvements: replacement *in vitro* methods *ILAR J* **43**, Supplement: 569–73; Gad SC (2000) Alternatives to *in vivo* studies in toxicology, in *General and Applied Toxicology*, Vol. 1, 2nd ed, Ballantyne B, Marrs TC and Syversen T (Editors) (London: Nature), pp401–24.

For *complete* replacement of animals, an alternative method should not require any animal-derived biological material. Examples of such methods or approaches include the use of predictions based on the physical and chemical properties of molecules, mathematical and computer studies of biological processes, analysis of epidemiological data, research involving human participants or research on isolated human cells and tissues in culture (see Box 11.1). However, many methods considered as Replacements also use some biological material obtained from living or humanely killed animals. These include research on cells and tissues derived from living or humanely killed animals for culture *in vitro* and animal-derived growth supplements such as serum derived from fetal or newborn calves. These methods can be called *incomplete* Replacements.[4]

Box 11.1: Complete and incomplete replacements

Computer studies and in vitro methods

Mathematical and computer modelling studies (*in silico* techniques) comprise a variety of approaches. They include the prediction of the biological activity of substances, and the modelling of biochemical, physiological, pharmacological, toxicological and behavioural systems and processes.* *In vitro* techniques are also varied, increasing in complexity from subcellular (cell-free) fractions, through primary cells and cell lines grown in liquid suspension, and three-dimensional cultures, to tissue slices or fragments and even whole perfused organs, all consisting of cells or tissues derived from animals or humans.† Examples of techniques that involve cells, tissues or organs from animals that have been killed humanely include: the use of guinea pig skin to provide information that would previously have been obtained from tests on the skin of living animals, or the use of primary cell cultures to replace neonatal mice as a virus isolation or assay system.

Human studies

In many types of biomedical and toxicological research, animals are used because ethical considerations preclude conducting the experiments on humans. However, a number of approaches have been suggested which, in some cases, might replace the use of animals with studies on humans. These include non-invasive brain scanning to replace some experiments on primates,‡ and studies on ultra-low-dose ADME metabolism in human volunteers in the early stages of selection for potential medicines.∫ Human tissue samples can be used both for direct examination (e.g. histopathology) and in cell culture and other *in vitro* techniques.**

* Using, for example, molecular modelling and the development of quantitative structure-activity relationships; see Combes RD and Judson P (1995) The use of artificial intelligence systems for predicting toxicity *Pest Sci* **45**:179–94; Combes RD and Rodford R (2003) The use of expert systems for toxicity prediction – illustrated with reference to the DEREK program, in *Modelling Environmental Fate and Toxicity* Cronin M and Livingstone D (Editors) (London: Taylor & Francis); Assessing the cumulative effect of mutations: Kirkwood TB & Proctor CJ (2003) Somatic mutations and ageing *in silico Mech Ageing Dev* **124**:85–92.

† The European Collection of Cell Cultures (ECACC) operates a cell bank of peripheral lymphocytes from approximately 40,000 donors. Forty percent are in the form of lymphoblastoid cell lines representing around 450 genetic disorders. These lines are useful for the analysis of the role of genes in disorders that have a genetic component, for example cardiovascular diseases, Alzheimer's disease or depression.

‡ Langley G, Harding G, Hawkins P, *et al.* (2000) Volunteer studies replacing animal experiments in brain research *Alternat Lab Anim* **28**: 315–31.

∫ In this type of research, the absorption, distribution, metabolisation and excretion (ADME) of new medicines is assessed by measuring the effects of administering extremely low doses of candidate compounds. Extrapolations are then made concerning the effects of higher doses. The approach is at the early stages of development and is not yet suited to replace the use of animals in pharmaceutical research. Combes RD, Berridge T, Connelly J *et al.* (2003) Early microdose drug studies in human volunteers can minimise animal testing. Proceedings of a workshop organised by volunteers in research and testing *Eur J Pharm Sci* **19**: 1–11.

** Access to patients and issues of consent are critical factors in the feasibility of human studies, see paragraph 11.26.

11.7 Tests using invertebrates, or early developmental stages of vertebrates (i.e. before they reach the point at which their use in experiments and other scientific procedures is regulated), are also sometimes described as Replacements, even though they do not replace animals *per se*. For example, the horseshoe crab (*Limulus*) can be used to replace

[4] Although this practice does not replace the use of animals *per se,* it replaces the carrying out of procedures on living animals. Sacrificing the life of one animal can save the lives of many other animals, as its organs and tissues can be used in many different experiments. The humane killing of an animal carried out according to methods prescribed in Schedule 1 of the A(SP)A is not counted as a procedure.

the pyrogen test for microbial contamination of biological fluids, which was previously carried out in rabbits.[5]

11.8 The term Replacement can be misleading in that it implies that an animal technique is already in place, and that a non-animal technique can directly and completely replace it. Sometimes, non animal methods may directly replace an established animal test, but they are often simply the best or only method of addressing certain scientific problems, and are used within multi-disciplinary research programmes to reduce overall reliance on animal experiments. In other words they may *displace* or *avoid*, rather than *replace* animal experiments. We take the view that the concept of Replacement is best understood in a broad sense.

Complete Replacement

11.9 The most obvious targets for Replacement are the established animal methods used to comply with testing regulations or standard operating procedures for the toxicity testing of chemicals and biological medicines. Considerable effort has been directed to replacing these tests, such as the Draize eye-irritancy test in rabbits (see Box 11.2). Complete Replacement of these procedures has not yet been achieved, although *in vitro* tests are being increasingly used to identify strongly irritant and corrosive chemicals, so that animal tests are not required to screen out these compounds.[6]

Box 11.2: The Draize test

Developed in 1944, the Draize test, along with the LD_{50} (paragraph 9.14) is an animal test for toxicity. It involves placing the tested substance directly into the eye of a live, conscious animal and observing the results. The test is usually performed using albino rabbits. In 1999, 3500 Draize tests were undertaken. The test has been recently replaced by alternative approaches and in 2003 a total of 33 eye tests, including Draize and other tests, were undertaken.*

Many people are concerned that the Draize test causes suffering and it has received much attention from animal protection groups. Some scientists also claim that the test is invalid because of differences between the human and rabbit eye. Rabbits have a third eyelid, a thinner cornea, a more alkaline eye than the human eye, and produce less tear fluid to wash away irritants.† It is claimed that the Draize test overestimates how irritating a product is to the human because rabbits' eyes are more sensitive. The test is also thought by some to be imprecise because it is purely observational. The toxicity is evaluated by an investigator rather than quantitatively measured.‡

The Draize test is still widely used in the USA. In the UK it is no longer used for the testing of cosmetic products and ingredients, following the ending of animal testing for cosmetics. However, it is still used as a safety test for non-cosmetic products and chemicals, and is recommended for regulatory risk assessments of chemicals and a range of manufactured products that may be deliberately or accidentally brought into contact with the eyes.∫ The Home Office has published guidance for the test. These include the following stipulations: testing should only take place when *in vitro* screening tests have been used to identify, classify and eliminate materials with obvious irritant potential; it should not be carried out with strongly acidic or alkaline substances, nor with substances which are already known to produce severe adverse effects on the skin.** In response to a study which claimed that a variety of valid alternatives existed,†† the Home Office concluded in 2001 that the currently available alternatives to the Draize test had significant limitations and were not suited to replace live animal use.‡‡ Research aiming to develop alternatives to the Draize test continues. This includes, for example, the use of human eye tissue obtained from tissue and organ donors, and protein solutions that can be manufactured to be sensitive to potential irritants.∫∫

* Home Office (2004) *Statistics of Scientific Procedures on Living Animals Great Britain 2003* (London: HMSO).

† Kaufman SR (1989) *Problems with the Draize Test. Perspectives On Animal Research,* Vol. 1, available at: http://www.curedisease.com/Perspectives/vol_1_1989/Problems%20with%20the%20Draize.html. Accessed on: 16 Jun 2004.

‡ The Group for the Education of Animal Related Issues

Continued

5 The pyrogen test is used to determine whether a substance is fever inducing. The test involves injecting a sample of the substance being tested, usually into rabbits. The rabbits must be individually held in a fixed position for a number of hours in a cage. Through temperature probes placed in the rectum of the animal, increased temperature is measured and, if recorded, gives an indication of pyrogen contamination. 3R Research Foundation Switzerland, 3R Training: *Rabbit in vivo pyrogen test*, available at: http://3r-training.tierversuch.ch/content.php?ctool_page_id=134&lang=en. Accessed on: 6 May 2005; Liebsch M (1995) History of the LAL-test: validation and regulatory acceptance *ALTEX* **12**: 76–80.

6 See OECD (2001) *Series On Testing And Assessment, Number 33: Harmonised Integrated Classification System For Human Health And Environmental Hazards Of Chemical Substances And Mixtures: ENV/JM/MONO(2001)6*; Chapters 2.2 (Skin Irritation/Corrosion) and 2.3 (Eye Irritation/Corrosion), available at: http://www.oecd.org/LongAbstract/0,2546,en_2649_34365_2671862_1_1_1_1,00.html. Accessed on: 6 May 2005.

(GEARI), available: http://www.geari.org/faqdraize.html. Accessed on: 16 Jun 2004.

∫ See European Commission *Directive on dangerous substances 67/548/EEC, Directive on plant protection products 91/414/EC and Directive on medicinal products for human use 2001/83/EC* and their UK counterparts.

** Statement by Secretary of State for the Home Department, House of Commons. Hansard Written Answers for 16 Jan 2001 (pt 21), available at: http://www.parliament.the-stationery-office.co.uk/pa/cm200001/cmhansrd/vo010116/text/10116w21.htm. Accessed on: 16 Jun 2004.

†† Wilhelmus KR (2001) The Draize eye test *Surv Ophthalmol* **45**:493-515.

‡‡ Statement by Secretary of State for the Home Department, House of Commons. House of Commons (2001) Hansard Written Answers for 16 Jan 2001 (pt 21), available at: http://www.publications.parliament.uk/pa/cm200001/cmhansrd/vo010116/text/10116w21.htm. Accessed on: 6 May 2005.

∫∫ For example, EpiOcular developed by the Mattek Corporation, available at: http://www.mattek.com/pages/products/epiocular Accessed on: 16 Jun 2004; the Irritection Assay system developed by Invitro International, available at: http://www.invitrointl.com/products/irritect.htm. Accessed on: 16 Jun 2004; the Agarose Diffusion method, Cottine M *et al.* (1993) Critical evaluation of an Agarose Diffusion method for the assessment of eye irritancy *ATLA* **21**: 427–40, available at: http://altweb.jhsph.edu/publications/journals/atla/atla21_4/atla21_4b.htm. Accessed on: 16 Jun 2004; see also Draize FAQ The Group for the Education of Animal Related Issues (GEARI), available at: http://www.geari.org/faqdraize.html. Accessed on: 16 Jun 2004.

11.10 A major success in the use of Replacements in toxicity testing was achieved in 2000, when, following a successful validation led by ECVAM (Box 2.5 and paragraph 11.32), an *in vitro* test for phototoxicity[7] was adopted as a standard test guideline by the EU, and two years later by the OECD.[8] In basic biomedical research there are also examples of where Replacement methods have successfully been applied to established methods or techniques in a particular research field. For example, monoclonal antibodies were usually produced in mice (by the ascites method (see paragraph 5.25) before *in vitro* methods were developed. Here, the deployment of a non-animal alternative method can be seen as complete Replacement.

Non-animal techniques as 'advanced' methods

11.11 Animal experiments are often only one part of a scientific study or programme of research. For example, developing an effective vaccine against West Nile virus, a fatal infection of horses transmitted by mosquitoes, includes the following: molecular studies of the virus, studies of virus growth and development in insect and mammalian cell lines, epidemiological studies of vector populations and disease incidence in the field, mathematical modelling of the transmission and spread of disease, and clinical studies. This work usually involves very little experimental live animal use. Some laboratory infection of horses (or small-animal models) is undertaken to examine the progression of the disease in a controlled manner, to discover the exact means of insect transmission and to develop and test candidate vaccines. In this case, the molecular and epidemiological studies are not *Replacements* for the animal work; they are addressing *different scientific questions* within the research programme.

11.12 The terms 'advanced' or 'complementary' have been applied to many non-animal methods (for example, the molecular biology and mathematical modelling techniques mentioned above) to indicate that these methods have been developed to answer specific scientific questions that animal tests cannot address. They were not developed exclusively to replace animals for ethical reasons, and it is therefore unhelpful to refer to them in claims that all animal research could easily be replaced, if there was only a will to do so.

7 While a medicine by itself may have no toxic effects, this may change in combination with light. Phototoxicity studies test whether the toxic properties of a compound change when exposed to light. This is important if a compound is applied to a specific area of the body that may be exposed to light, in the form of a skin cream for example. A phototoxic compound may enhance the possibility of ultraviolet (UV) light inducing skin cancer. TNO Nutrition and Food Organisation *Phototoxicity: the combined effect of sunlight and pharmaceuticals on skin*, available at: http://www.voeding.tno.nl/ProductSheet.cfm?PNR=ZE_226A. Accessed on: 29 Apr 2005.

8 Animal tests for phototoxicity carried out before this date could in principle have been replaced by the alternative method. The new test did not in fact replace an existing EU or OECD test guideline for an animal test until 2000/2002.

Non-animal methods as adjuncts

11.13 Non-animal methods may act as an adjunct to animal experiments rather than replace them, but in so doing, they may serve to reduce the total number of animals used in a programme of work. A classic example is the screening of anti-cancer drugs in nude[9] mice with human tumours. An initial screen using cell cultures can be used to demonstrate basic tumour cytotoxicity, and only the active toxins are tested *in vivo*. The same principle is used in high-throughput screening of potential medicines (see paragraph 8.6). This approach involves testing a large range of potentially useful candidate chemicals for a particular purpose in non-animal systems (especially computer prediction studies and *in vitro* tissue culture) using techniques that can be carried out very rapidly. Those chemicals with desirable biological activity (efficacy) and devoid of undesirable activity (toxicity) can then be selected for further study. In this way, it is possible to reduce the numbers of animal tests required to assess a given number of chemicals. The severity of animal tests can be minimised by screening out substances that are likely to be toxic at an early stage. In recent years, high-throughput screening has become widely adopted by the pharmaceutical industry (see paragraphs 8.4–8.6).

Alternative approaches

11.14 Another equally important concept is the use of an *alternative approach* to an experimental goal enabling the *avoidance* of animal use. Even where there are no obvious alternatives, any proposed scientific study should consider at an early stage not only whether the animal experiment is the most appropriate and only method of addressing each research question, but also whether the question is worth asking, and whether it justifies causing pain and suffering to a sentient animal. In other words, the first alternative to consider is the option not to carry out the experiment at all. For example, within the REACH testing programme (see Box 9.2) the first consideration might be whether a particular test is actually necessary, regardless of whether there are, for example, adjunct Replacement methods that could be used in research.

The potential for Replacement of animals in different areas of research

Toxicity testing required by regulation as a special case

11.15 There is a tendency for discussion on the potential for replacing animals to focus solely on toxicity testing required by regulation and efficacy testing (which comprises around 16 percent of all animal use in science). Tests for regulatory purposes have received the most obvious and specific attention with respect to the development of Replacements.[10] Two factors have been influential in this respect: first, over the past 30 years public concern about the types of substances tested, such as cosmetics, household products and chemicals, and the type of tests carried out has increased (for example, the LD_{50} and Draize tests; see paragraph 9.14 and Box

9 'Nude mice' are mice born without any T lymphocytes, which means that they effectively have no immune responses.

10 The British Toxicology Society (BTS) produced a report on the use of *in vitro* methods for toxicity testing in 1997, see Fielder R, Atterwill CK, Anderson D *et al*. (1997) British Toxicology Society (BTS) Working Party Report on *in vitro* toxicology *Hum Exp Toxicol* **16**: S1–40. The Third FRAME Toxicity Committee has also published a comprehensive discussion on the development of replacement methods for toxicity testing over the last decade, see Combes R, Schechtman L, Stokes WS and Blakey D (2002) The international symposium on regulatory testing and animal welfare: recommendations on best scientific practices for subchronic/chronic toxicity and carcinogenicity testing *ILAR J* **43**, Supplement: S112–17; see also Salem H and Katz SA (1999) *Toxicity Assessment Alternatives – Methods, issues, opportunities* (Totowa, NJ: Humana Press); Castell JV and Gómez-Lechón MJ (Editors) (1997) *In vitro Methods in Pharmaceutical Research* (London: Academic Press); Knight DJ and Breheny D (2002) Alternatives to animal testing in the safety evaluation of products *Alternat Lab Anim* **30**: 7–22; Combes RD (2002) The ECVAM workshops: a critical assessment of their impact on the development, validation and acceptance of alternative methods *ATLA* **30**, Supplement 2: 151–65.

11.2)[11]. Secondly, the nature of these tests suggests that it is easier to make progress in this field, as toxicity testing required by regulation asks defined questions and tends to involve a limited number of standardised tests, which are repeated (on different chemicals) many times. There is also an established institutional structure for the validation of alternatives (see paragraph 11.32).

11.16 Most toxicity testing required by regulation is carried out by industry which has devoted considerable resources and managed effort to the development and implementation of Replacements. These developments have occurred partly in response to activities by animal protection organisations, and partly because many alternative approaches are developed as 'advanced methods' to solve specific problems (paragraph 8.42). Added impetus has recently been given by the amendment of the EU Cosmetics Directive[12] to impose a marketing ban on cosmetics that have been tested or have had any of their ingredients newly tested on animals.

11.17 Standard test methods are also used in the safety and efficacy assessment of biologicals, including vaccines. The technical problems in replacing these tests are quite different from those encountered in the testing of chemicals. Further efforts are required to develop and validate methods that allow replacement of the use of animals, particularly in highly distressful challenge tests (see paragraph 8.24 and Box 8.5).[13]

Biomedical research

11.18 In contrast to tests for safety and efficacy, the development of Replacements to current uses of animals in biomedical research is generally perceived as more difficult. The scientific questions that are addressed in biomedical research are more diverse and open-ended, with less-predictable outcomes. Moreover, the animal model itself is often the focus of the research (see Chapters 6 and 7). The objectives and designs of biomedical research projects are extremely diverse. It may sometimes be possible to identify certain basic, widely used techniques that would be amenable to replacement of animals. The replacement of the ascites method of production of monoclonal antibodies is one such example (see paragraph 5.26). In general, however, opportunities for replacement or avoidance of animal use in every project need to be explored on a case by case basis, with due regard to the specific objectives and the scientific barriers to the use of non-animal methods.

Barriers to developing Replacements and how these could be overcome

11.19 There are some general principles regarding the constraints on the development of Replacements. These are well documented in the case of toxicity testing required by regulation[14] but many of the same principles apply to biomedical research. We now consider some general features of scientific and non-scientific barriers to developing Replacements. In Chapter 15 (paragraphs 15.61–15.67) we set out recommendations about how they might be overcome.

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11 See The Boyd Group (1998) *The use of animals for testing cosmetics: A discussion paper from the Boyd Group*, available at: http://www.boyd-group.demon.co.uk/cosmetics.htm. Accessed on: 29 Apr 2005; The Boyd Group (2002) The *use of animals in testing household products: A discussion paper and statement of principle*, available at http://www.boyd-group.demon.co.uk/householdproducts.pdf. Accessed on: 29 Apr 2005.

12 European Commission (2003) Directive 2003/15/EC of the European Parliament and the Council *Official Journal of the European Union* 11 March 2003.

13 See Hendriksen CFM, Spires J-M, Akkermans A *et al*. (1998) Validation of Alternative Methods for the Potency Testing of Vaccines: ECVAM Workshop Report 31, *ATLA* **26**: 747–61, and Weissler K and Hechler U (1997) *Animal Welfare Aspects in the Quality Control of Immunobiologicals: A critical evaluation of the animal tests in Pharmacopoeial Monographs* (London: FRAME).

14 European Commission (2004) *Opinion of the Scientific Committee on Toxicity, Ecotoxicity and the Environment on The BUAV-European Coalition to End Animal Experiments Report: The Way Forward – Action to End Animal Toxicity Testing*, available at: http://europa.eu.int/comm/health/ph_risk/committees/sct/documents/out217_en.pdf. Accessed: 26 Apr 2005.

Scientific barriers

11.20 There are scientific obstacles to developing relevant and reliable non-animal methods that can mimic the complex integrated physiological systems of humans and others animals. It is extremely difficult, using computational or *in vitro* systems, to take account of factors such as:

- The diversity of different tissues and cell types that make up a living organism; hundreds of different cell types at various stages of development may function and respond in different ways, or to different degrees.
- The ways in which cells and tissues interact, both locally and via the bloodstream and nervous system; immune reactions, germ cell development, metabolism and many other normal and disease-related processes involve extensive interaction between cells of different types and in various locations in the body.
- The influence of tissue organisation on the cellular environment; oxygen levels, rate of nutrient supply, intercellular communication and barrier formation all affect how cells behave and respond to external stimuli.

11.21 In research involving human volunteers, the scientific constraints are quite different, and usually secondary to ethical considerations. They include problems caused by variability (genetic and lifestyle) in the human population, the difficulty of controlling environmental variables such as diet and health over long periods, and the slow rate of human reproduction. Although human variability is an intrinsic facet of the very subject of medical research, there are occasions when it makes the design of conclusive scientific studies on humans impossible (see paragraph 10.33).

11.22 Scientific barriers to Replacement are likely to be more difficult to overcome in some areas of research than in others, and need to be considered on a case by case basis. To make further progress, there is an obvious need for scientific research to find ways of overcoming obstacles, and to develop non-animal techniques capable of addressing scientific questions about how biological systems work, how they are altered in disease, and how they are affected by chemicals and medicinal products.

Non-scientific barriers

11.23 Scientific obstacles are not the only limiting factors in replacing animal research. There are other possible constraints that may impede the implementation of Replacements. They include: regulatory inertia, insufficient funding, non-availability of human tissue, lack of incentives to explore the potential of Replacements, lack in the availability of information about suitable Replacements, insufficient integration of *in vitro* and *in vivo* research, and the possibility that tradition and conservatism may mean that researchers are reluctant to explore the potential of Replacements.

Regulatory inertia

11.24 Regulatory agencies have the crucial role of ensuring the safe use of products such as industrial chemicals, pharmaceuticals or vaccines. A very complex and intensely bureaucratic regulatory system has evolved to achieve adequate protection of humans, animals and the environment. The introduction of Replacements for established animal tests is therefore not straightforward. Regulatory authorities can be reluctant to depart from methods which they have traditionally relied upon for safety and liability requirements. The international regulatory authorities also need to be convinced that the alternative methods which are available and accepted in particular countries provide an adequate assessment of risk. Intensive efforts are needed to facilitate and accelerate the validation and regulatory acceptance of Replacements through bodies such as the OECD and ICH, as well as ECVAM

and the European Commission (see paragraphs 11.32 and 15.84–15.87).

Funding

11.25 It is difficult to estimate accurately the amount of funding that is spent on research into Replacements. This is partly because funds are more commonly made available for all Three Rs rather than specifically for Replacement. Research is often directed towards developing specific techniques that, although they may have potential as Replacements, are envisaged as advanced methods rather than targeted specifically at replacing animals. There is a small number of charities such as FRAME, the Dr Hadwen Trust, the Lord Dowding Fund and the Humane Research Trust (see Boxes 2.3 and 2.4) that are dedicated to funding research on Replacements, but their budgets are limited.[15] More recently, major research funding bodies, such as the MRC, the Biotechnology and Biological Sciences Research Council (BBSRC), and the newly established NC3Rs, have offered limited funds for research specifically dedicated to the development of Replacements (see Box 11.3). The pharmaceutical and chemical industries have already invested comparatively large sums in research on Replacements, particularly in toxicology, and seem likely to increase that investment.[16] An initiative has also been established by the cosmetics and chemical industries, which seeks to fund development of Replacements in a limited number of specific regulatory tests.[17]

Availability of human tissue

11.26 Controversy surrounding issues of informed consent have highlighted ethical constraints on obtaining human tissue for research. In the UK, concerns about the unauthorised retention of human tissue and organs at a number of hospitals led to the drafting on new legislation to regulate their use.[18] The draft provisions of the Human Tissue Bill were criticised by a range of stakeholders who feared that difficulties in both recruiting volunteers and gaining access to human tissue for use in non-animal research would be increased.[19] However, revisions made in light of the ensuing discussion appear to have met most of these.

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15 See *FRAME* website, available at: http://www.frame.org.uk. Accessed on 29 Apr 2005; *Dr Hadwen Trust* website, available at: http://www.crueltyfreeshop.com/drhadwen/about.htm. Accessed on 29 Apr 2005; *The Lord Dowding Fund for Humane Research* website, available at: http://www.navs.org.uk/research/about/ldf.htm. Accessed on 29 Apr 2005.

16 The ABPI estimates that the UK-based pharmaceutical industry spends in excess of £300 million annually on the development of non-animal methods, see Association of the British Pharmaceutical Industry (2003) *Alternatives to the use of animals in medicines research*, available at: http://www.abpi.org.uk/press/media_briefings_03/2003/Brief_%20Ani.pdf. Accessed on 29 Apr 2005.

17 Major companies of the chemical, pharmaceutical and cosmetic industry are in the process of establishing an International Partnership for Alternatives to Animal Testing (IPAAT). So far, there are three Working Groups focusing on developing Replacements for tests which are currently used in lung (inhalation) toxicity, repeat-dose toxicity/toxicokinetics and risk assessment strategies. The companies directly involved in these Working Groups are BASF, Cognis, DuPont, Henkel, L'Oreal, Novozymes, Pfizer, P&G, TNO and Unilever. In the field of respiratory (immuno)toxicity a first joint project is expected to commence in late 2005. Personal communication Dr Erwin Roggen (Novozymes AS), 27 April 2005.

18 NHS Retained Organs Commission (2002) Retention and use of human tissue and organs (London: DoH); see also Furness P and Sullivan R (2004)The Human Tissue Bill *BMJ* **328**:533-4

19 See also Home Office (2004) *Human Tissue Act 2004*, available at: http://www.legislation.hmso.gov.uk/acts/acts2004/20040030.htm. Accessed on: 29 Apr 2005; Nuffield Council on Bioethics (2004) *Human tissue: ethical and legal issues – Response from the Nuffield Council on Bioethics to the Human Tissue Bill*, available at: http://www.nuffieldbioethics.org/fileLibrary/pdf/ncob_response_-_ht_bill.pdf; European Commission (2004) *Directive 2004/23/EC of the European Parliament and of the Council on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells*, available at: http://europa.eu.int/comm/health/ph_threats/human_substance/tissues_en.htm. Accessed on: 29 Apr 2005.

Incentives

11.27 Biomedical researchers are usually under pressure to achieve results and solve problems quickly. A number of factors are likely to influence this pressure: these include a genuine urgency to understand and alleviate human or animal suffering and a competitive environment that frequently makes research grants dependent on publication activity. In either case, researchers may be reluctant to spend time on developing non-animal alternative methods when it appears that an available animal method will give publishable results. In addition, the development of alternative methods may be perceived as having a lesser status than research. We consider ways of improving the recognition of the development of Replacements from within the academic research community in paragraph 15.61.

Availability of information

11.28 Fundamental to identifying alternative approaches is the availability of adequate information on past and current research in specific fields (see paragraph 11.34). Accessing information about suitable Replacements or alternative approaches to particular scientific questions can be difficult as such information is not always published. Even if it is published, the information is not usually indexed so as to highlight any of the Three Rs (see paragraph 15.58).

Integration of *in vitro* and *in vivo* research

11.29 *In vitro* toxicology, as distinct from *in vivo* toxicology, has become a science in its own right and there may be a risk that the primary goal of replacing animals can be overlooked. Some commentators are concerned that there is insufficient communication between scientists working *in vivo* and *in vitro*. They fear that *in vitro* toxicologists are becoming overly focused on methodological issues and the development and application of new techniques, gradually losing contact with the mainstream *in vivo* research in their original field. Such a shift could mean that valuable information on alternative techniques is not available to those who could apply them, because it is not published in journals relevant to their research interests or presented at the meetings that they attend. Others counter that it is problematic to make generalising statements in this area, asserting that, for example, in the pharmaceutical industry, there is a high degree of coordination and exchange of information.

Tradition and conservatism

11.30 Most scientists whose work involves animals are comfortable with the concept of Reduction and Refinement, although members of the Working Party also reported from personal experience that knowledge about the potential for Refinement varied. They had sometimes experienced hesitancy from other scientists in entering into serious discussion about the potential for replacing animals in their own field of research. If researchers have always used animals and are working in a field that has historically relied substantially on animal research, a change in methodology may not be straightforward, as it is common for scientists to frame research objectives in light of the means available. The creation of opportunities for appropriate lateral thinking is likely to require more than 'better training', and it may be useful to explore ways of achieving structural and institutional change which allow researchers to reconsider ways in which specific research questions can be answered by non-animal methods (see paragraph 15.60). This approach could be especially relevant to research fields such as experimental physiology and experimental biology, which have always depended very substantially on the use of whole, living animals and where the only alternative may be not to do the experiment. Questioning the justification for an entire research programme is, understandably, not something that comes

easily to most researchers. This is particularly so in a climate where technological advances such as biotelemetry are continually pushing the boundaries of what is possible in fundamental physiology, and scientists are under increasing pressure to fully exploit these techniques. Hence, the concept of Replacements might be regarded by some researchers as either completely irrelevant or as a direct attack on their life's work.

Making progress – some national and international activities

11.31 Over the past decades, a number of organisations have been established which seek to coordinate efforts in relation to the promotion of Replacements. We briefly summarise them below.

Coordination of effort

11.32 The ECVAM was established by the European Commission in 1993, for the express purpose of undertaking research into alternative methods and facilitating and organising their validation (see Box 2.4). ECVAM now works with its US counterpart, the Interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM). These organisations have been concerned primarily with validating Replacements in regulatory safety testing. In this regard, ECVAM has been working with the European Directorate for the Quality of Medicines[20] on the development of alternative methods for testing vaccines, and with the Test Guidelines Programme of the OECD for chemicals. The OECD has recently admitted observers from animal protection organisations to its meetings on test methods for chemicals testing via the International Council on Animal Protection in OECD Programmes (ICAPO).

11.33 A number of European countries have national organisations, or platforms, that are coordinated by ECOPA, the European Consensus Platform and which seek to promote the application of alternatives. Some of the member organisations, such as The Netherlands' Centre for Alternatives, are well-established institutions involving government, academia, industry and animal-welfare organisations in a variety of activities including commissioning research and providing information on alternative methods. In ECOPA, the UK is represented by the Boyd Group which, although it has members from the four main sectors mentioned above, is not primarily a centre for alternatives. The UK has recently established a centre dedicated specifically to the Three Rs (see Box 11.3).

Box 11.3: UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)

In July 2002 a House of Lords Select Committee published a Report on Animals in Scientific Procedures which recommended, among other things, the establishment of a national centre for the Three Rs. This was envisaged as a small, administrative hub to coordinate research units embedded in existing centres of scientific excellence. Several stakeholders commented on the recommendation, including the Dr Hadwen Trust and the Lord Dowding Fund, who published a joint proposal, suggesting that the national centre should focus on Replacements only.*

In April 2004, the UK Government announced the establishment of the *UK National Centre for the Replacement, Refinement and Reduction of Animals in Research* (NC3Rs).† The Centre aims to provide a focus for the promotion, development and implementation of the Three Rs in animal research. It replaces and builds upon the Medical Research Council's Centre for Best Practice for Animals in Research (CBPAR). The NC3Rs will fund Three R-related research, develop a range of information resources and guidelines, and organise workshops and symposia to disseminate and advance information about the Three Rs. The Centre's ultimate aim is the Replacement of animals in research, but it recognises that as long as animals continue to be used in research it is essential that every effort is made to reduce numbers of animals used, and to refine as far as possible the procedures in which they are involved.

* Dr Hadwen Trust and Lord Dowding Fund (2002) A national centre for the replacement of animals in experiments. A proposal by Lord Dowding Fund and Dr Hadwen Trust, see http://www.navs.org.uk/download_files/news/NationalCentreProposal.pdf.

† 10 Downing Street (2004) Welfare drive for animal experiments 21 May. Press release available at: http://www.number-10.gov.uk/output/Page5851.asp. Accessed on: 16 Jun 2004. See also: http://www.nc3rs.org.uk/. Accessed on 21 April 2005.

20 This organisation is part of the European Pharmacopoeia, operated by the Council of Europe.

Information on Replacements, education and training

11.34 Many organisations provide information on Replacement methods in research and education. For example, ECVAM provides an online database, the ECVAM Scientific Information System (SIS), which provides details of methods currently undergoing validation[21] and, in Germany, the German Institute of Medical Documentation and Information (DIMDI) Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET) gives access to a database called Animalt-Zebet, which has extensive information on Replacements.[22] A bibliographic database (Altbib) is maintained by the US National Library of Medicine.[23]

11.35 The provision of information in tertiary and postgraduate education has long been promoted by the RSPCA and is now being pursued in conjunction with the Boyd Group. Interniche has produced a comprehensive guidebook to educational alternatives[24] and there are web-based information services, such as the European Union Resource Centre for Alternatives in Higher Education (EURCA)[25] and the Norwegian Reference Centre for Laboratory Animal Science and Alternatives (NORINA).[26]

Summary

11.36 In this chapter we have explored the concept of the Replacement approach, and its current and future applications. We differentiated between *complete* Replacement, which relates to alternative methods that do not involve any use of animals, or animal tissue or organs, and *incomplete* Replacement, where either early developmental stages of animals or animal tissue, for example of humanely killed animals, is used. We argued that the concept of Replacement is best understood in a broad sense. We also discussed several different ways in which non-animal methods can be used: on the one hand, they can *replace* existing tests; on the other they may *displace* or *avoid* animal experiments altogether. Non-animal methods may also function as advanced methods, or as adjuncts to animal experiments.

11.37 The public debate about the potential for replacing animals usually focuses on what is or is not possible with animal experiments in general. This is not particularly helpful or constructive. We observed that the potential for achieving Replacement of animals depends on the nature of the specific scientific question being addressed and therefore has to be evaluated on a case by case basis rather than in general terms. Similarly, claims about whether or not Replacements are more economic, faster or produce more reliable scientific data need to be assessed in the same way. Accordingly, we considered a range of approaches where Replacements are currently being used, including computer studies, *in vitro* methods and human studies.

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21 ECVAM *Scientific Information Service*, available at: http://ecvam-sis.jrc.it/. Accessed on: 6 May 2005.

22 German Institute of Medical Documentation and Information (DIMDI) Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET) *Animalt-Zebet*, available at: http://www.dimdi.de/static/en/db/dbinfo/dbmemo/zt00eng.html. Accessed on: 6 May 2005.

23 National Library of Medicine *ALTBIB: Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing*, available at: http://toxnet.nlm.nih.gov/altbib.html. Accessed on: 6 May 2005.

24 Jukes N and Chiuia M (2003) *From Guinea Pig to Computer Mouse*, 2nd Edition (Leicester: International Network for Humane Education).

25 European Resource Centre for Alternatives in Higher Education (EURCA) website, available at: http://www.eurca.org. Accessed on: 6 May 2005.

26 Norwegian Reference Centre for Laboratory Animal Science & Alternatives (NORINA) website, available at: http://oslovet.veths.no. Accessed on: 6 May 2005.

11.38 There is a tendency for discussion on the potential for replacing animals to focus solely on toxicity testing required by regulation, and it appears that most progress has been made in this area. In order to explore the potential for replacing animals elsewhere, scientific and non-scientific barriers that can influence the implementation of Replacements need to be considered. These include the high degree of complexity of human biological processes, which is relevant where animals are used for the study of human disease; possible reluctance by regulators to accept new alternative methods; access to human tissue; and the scientific standing of research that aims to develop Replacements. We return to ways of overcoming these obstacles in paragraphs 15.57–15.62 and now turn to the current state and future potential of Refinement and Reduction.