The **Academy** *of* **Medical Sciences**

Animals containing human material

July 2011

The Academy of Medical Sciences

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Contents

Summary

This report considers research that involves the introduction of human DNA sequence into animals, or the mixing of human and animal cells or tissues, to create entities we refer to as 'animals containing human material' (ACHM). Such approaches are long-established, and thousands of different ACHM have been used in biomedical research, yet they have received relatively little public discussion. Technical and scientific advances (such as those in stem cell science) are rapidly increasing the sophistication of ACHM and expanding their utility. This report considers the new opportunities in research and medicine, and the ethical and regulatory issues that emerge.

ACHM are used to study human biological functions or disease that cannot be accurately modelled in cell cultures or through computer simulation; where experiments using humans are infeasible or considered unethical; and where modification of an animal's body makes it more closely represent that of the human. Their use enables more accurate conclusions to be reached about the functions of DNA sequence, aspects of biology and the nature of disease. ACHM are widely used in finding new ways of diagnosing and treating disease, and in the development and even production of therapeutics.

We describe many examples, including: mice genetically altered to acquire susceptibility to diseases which do not normally affect them such as human immunodeficiency virus (HIV) and hepatitis; chimæric mice, engrafted with pieces of human tumour, which have for several decades been an invaluable system in cancer research, and in which radiotherapy and anti-cancer drugs have been tested; monoclonal antibody anti-cancer therapies which have been developed using mice with their immune system 'humanised' by replacement of mouse by human genes; and goats which produce a human substance used to treat a blood clotting disorder. Across the spectrum of ACHM use, the modification of animals to make them more similar to humans, in specific biological or disease characteristics, may improve the utility of the research results and outcomes.

The use of animals in research generally has received intense public discussion, and remains unacceptable in principle to some people. We did not revisit that wider discussion, but started from the current legislative position that animal research is permissible (and acceptable to the majority of the UK population) provided that it is carried out for good reason, where there are no feasible alternatives, and under strict regulation. We then considered what new ethical and regulatory issues might arise that would be specific to the creation and use of ACHM.

At the outset of our study, we commissioned a consortium led by Ipsos MORI to facilitate a public dialogue on ACHM. The findings showed a high degree of public acceptance of ACHM research provided it is well regulated, and justified by the potential gain in understanding or treating medical conditions. Areas of particular sensitivity were identified; however, in general, the dialogue participants did not regard ACHM research as being significantly different from other research involving animals.

Many ACHM models, such as transgenic rodents each containing one (or a few) human genes, and animals with human tissue grafts, have a long history of research use without major ethical or regulatory difficulties. However, technologies are advancing rapidly; more extensive sections of DNA can be manipulated, and methods using human stem cells to replace parts of tissue, or even whole organs, are becoming increasingly refined. By enabling progressively more extensive, and precise, substitution of human material in animals, these approaches may soon enable us to modify animals to an extent that might challenge social, ethical, or regulatory boundaries. Based on the evidence we received, the published literature, our public dialogue, and our own discussions, we identified areas which might merit special consideration, including:

- Extensive modification of the brain of an animal, by implantation of human-derived cells, which might result in altered cognitive capacity approaching human 'consciousness' or 'sentience' or 'human-like' behavioural capabilities.
- Situations where functional human gametes (eggs, sperm) might develop from precursor cell-types in an animal; and where fertilisation between either human (or human-derived) gametes and animal gametes might then occur.
- Cellular or genetic modifications which could result in animals with aspects of human-like appearance (skin type, limb or facial structure) or characteristics, such as speech.

Current scientific knowledge often does not permit precise prediction of the effects that modification of an animal's organs might produce. However, we anticipate some important reasons for possibly undertaking such research in the future. We therefore recommend additional expert scrutiny and regulation of experiments in these sensitive areas.

As researchers seek to create more effective research models and to evaluate potentially important medical interventions, there is a need to ensure a comprehensive system for the regulation of ACHM that protects animal welfare, maintains the highest standards of safety and ethics, and keeps the issues of public acceptability of research to the forefront. Before making recommendations on the regulatory system itself, we considered how each of these aspects applies specifically to ACHM.

We concluded that research involving ACHM does not have a generally increased potential for causing animal suffering, in comparison to other licensed research involving animals, and that the development and use of ACHM could indeed contribute to refining and improving the effectiveness of experiments involving animals. Research involving ACHM should be subject to scrutiny, licensing and advancement from an animal welfare perspective, in the same manner as other animal studies.

We considered whether the creation of ACHM might pose particular safety issues, for example through the close combination of human and animal tissue allowing opportunities for viral reactivation, as well as the potential consequences of accidental or deliberate release of ACHM from containment. We concluded that risks are very low, but not zero, and that scientists, research institutions and regulators should remain alert to these risks and take appropriate precautions.

To consider the distinctive ethical issues raised by ACHM, we drew from broader ethical perspectives: concerns about animal welfare and human dignity, and considerations arising from our stewardship responsibility towards animals. We considered how the portrayal of animal–human entities in literature and culture influences societal values.
While recognising that, as with any research, positive outcomes cannot be predicted, and timescales from research to application may be long, we concluded that, in our view, research involving ACHM can in general be justified by the prospect of facilitating novel insights into human biology, and treatments for serious human disorders.

The principal legislation relevant to the research use of ACHM in the UK is the Animals (Scientific Procedures) Act (1986) (ASPA), which is enforced by the Home Office through a system of licensing and inspection. The Department of Health, Human Fertilisation and Embryology Authority, Human Tissue Authority, the UK Stem Cell Bank and other bodies also regulate aspects of the use of ACHM. In all, the regulatory framework is complex, it involves several different Government departments and agencies, it was not developed specifically in reference to ACHM, and the interface between the different regulators has received little consideration.

The recommendations of this report should ensure that valuable and justifiable research involving ACHM can proceed within a robust, proportionate regulatory system, which is capable of responding to developing scientific knowledge and social attitudes, and which avoids undue bureaucracy and duplication of regulation.

We recommend that ACHM research should be classified in three categories, which would determine the level of regulatory scrutiny required prior to authorisation:

1. The great majority of ACHM experiments pose no novel issues and should continue to be regulated through the same procedures as other research involving animals.
2. A limited number of types of ACHM research should be permitted subject to additional specialist scrutiny by a national expert body. We outline a graded approach that should be considered for research in this category.
3. A very narrow range of ACHM experiments should not currently be undertaken, because they raise very strong ethical concerns and lack sufficient scientific justification.

While indicating the types of experiment that we would currently place within these categories, we emphasise that this classification would necessarily change over time, in response to new scientific understanding, and evolving social attitudes. The regulatory system should be capable of adapting to such changes.

Assessment of research in the second and third categories will require specialist knowledge, and decisions to license such research may be socially sensitive; moreover the number of experiments is likely to be relatively small. Consequently we recommend that the Home Office put in place a single, national expert body with a duty to advise on the use of ACHM, taking social, ethical and scientific considerations into account. This body would regularly review the system of categorisation; advise on the licensing approach to be taken for experiments in the second category; maintain consideration of areas where concerns may arise; and develop guidance for Government and for researchers. We recommend that the national expert body should be multidisciplinary, transparent, and open to public scrutiny. It should engage actively and regularly with the public, the scientific community and with other regulators to maintain a broad coordinated framework for regulating research involving ACHM.

There are clear advantages; in terms of consistency of practice, operational efficiency, and the best use of specialist expertise; that research involving ACHM is considered by the same body that advises Government on other aspects of animal research. Therefore, the national expert body we recommend should be integral to the wider system for the regulation of animal research.

In implementing the European Directive 2010/63/EU by 2012, the Home Office will consult on the requirement to establish a UK 'national committee for the protection of animals use for scientific purposes'. We have placed emphasis on the value of ACHM being considered alongside other animal research, and suggest that every effort is made to ensure that the 'national committee' mandated by the Directive has within its remit and competence, the function of the 'national expert body for ACHM' that we recommend.

We have described the complexity of the current regulatory system as it relates to ACHM, and the involvement of several Government departments and regulatory agencies. There are areas in which the close alignment of various regulators will be essential in securing comprehensive and functionally efficient governance of ACHM. The most striking example is research involving human admixed embryos, which is tightly regulated by the Human Fertilisation and Embryology Authority (HFEA) under the Human Fertilisation and Embryology Act (HFE Act). It is a matter

of expert judgement to distinguish between embryos that are 'predominantly human' and so come under the HFE Act, and embryos that are considered to be narrowly on the other side of the boundary and so 'predominantly animal', and outwith the terms of the HFE Act. These latter embryos are not currently regulated during early gestation (although their mothers are regulated under ASPA). Since such cases will fall at the boundaries of the two regulators, we recommended that the Department of Health and Home Office (and their expert advisory bodies) work closely together to ensure that there are no regulatory gaps, overlaps, or inconsistencies, between their respective regulatory systems. It is essential that a smooth operational interface be established to ensure the timely and appropriate assessment of such research.

As with much biomedical research, ACHM research frequently involves international collaboration. We have noted a paucity of international guidance relating specifically to ACHM. We recommend raising international awareness of ACHM, promoting international consistency in research practice, and the development of international standards and guidance. This is an area in which the UK can lead.

Public dialogue findings

A majority of participants in the public dialogue accepted and were ultimately supportive of research using ACHM, on the condition that such research is conducted to improve human health or to combat disease. Three areas of particular sensitivity to participants were identified: ACHM research involving the brain, reproductive tissues or aspects of human-like appearance. Participants also expressed broader concerns, including those relating to the welfare of the animals involved, safety aspects of research involving ACHM and its regulation.

Categorisation of ACHM

We propose that experiments involving ACHM could be usefully classified into three categories:

Category 1

The great majority of ACHM experiments, which do not present issues beyond those of the general use of animals in research, should be subject to the same oversight and regulation under ASPA as other animal research.

Category 2

A limited number of types of ACHM research (outlined below) should be permissible, subject to additional specialist scrutiny by the national expert body we propose[1]. Although we would expect this list to evolve over time as knowledge advances, the major types of research that we would currently include in this category are:

- Substantial modification of an animal's brain that may make the brain function potentially more 'human-like', particularly in large animals.
- Experiments that may lead to the generation or propagation of functional human germ cells in animals.
- Experiments that could be expected to significantly alter the appearance or behaviour of animals, affecting those characteristics that are perceived to contribute most to distinguishing our species from our close evolutionary relatives.
- Experiments involving the addition of human genes or cells to non-human primates (NHPs). We recognise that research on NHPs is appropriate, and in some types of research probably essential if it is to lead to clinical benefit, but such research should remain under a high degree of regulatory scrutiny.

Category 3

A very narrow range of experiments should not, for now, be licensed because they either lack compelling scientific justification or raise very strong ethical concerns. The list of such experiments should be kept under regular review by the proposed national expert body, but should at present include:

- Allowing the development of an embryo, formed by pre-implantation mixing of NHP and human embryonic or pluripotent stem cells, beyond 14 days of development or the first signs of primitive streak development (whichever occurs first); unless there is persuasive evidence that the fate of the implanted (human) cells will not lead to 'sensitive' phenotypic changes in the developing fetus.[1,2,3]
- Transplantation of sufficient human-derived neural cells into an NHP as to make it possible, in the judgement of the national expert body, that there could be substantial functional modification of the NHP brain, such as to engender 'human-like' behaviour. Assessing the likely phenotypic effect of such experiments will be informed by prior work on other species (possibly including stem cell transfer between NHPs) or by data on the effects of 'graded' transplantation of human cells into NHPs.
- Breeding of animals that have, or may develop, human derived germ cells in their gonads, where this could lead to the production of human embryos or true hybrid embryos within an animal.[4]

1 Such experiments should be approached with caution. Strong scientific justification should be provided to the national expert body, who should closely consider the ethical and any safety issues in addition to the potential value of the research. Authorisation may require studies to adopt an incremental (graduated) approach. Proposed studies should be assessed on a case-by-case basis, at least until experience allows the formulation of guidelines

2 This applies whether the embryo is implanted within an animal uterus or maintained as an intact embryo in vitro. Equivalent statutory restrictions are applicable to human and human admixed embryos under the HFE Act (see 6.2.2).

3 This supplements the 14 day provision applied to human admixed embryos under the HFE Act, so that mixed embryos, which are judged to not quite meet the criteria for being 'predominantly human', should nevertheless be regulated on the basis of the likely phenotypic effect on the embryos created. Currently, any mixed origin embryo judged to be 'predominantly human' is regulated by HFEA and cannot be kept beyond the 14 day stage, whereas an embryo judged to be predominantly animal is unregulated until the mid-point of gestation (likely to be increased to two-thirds on implementation of the European Directive 2010/63/EU) and can in principle be kept indefinitely. As to whether or not an admixed embryo is predominantly 'human' is an expert judgement, including an assessment of likely phenotype, but neither the precise eventual composition of an individual embryo nor the phenotypic effect of the admixture will be easily predictable in the current state of knowledge.

4 Placement of human embryos into animals is prohibited by the HFE Act, which seems likely to be interpreted to include placement of human embryos into animals modified to contain human uterine tissue.

Recommendations

1. We recommend that the Home Office ensures that a national expert body with a duty to advise on the use of ACHM in research is put in place.
2. We recommend that this national expert body should:
   - 2.1 Be multidisciplinary, involving people with knowledge of ethics, the humanities, social sciences, law and the biological sciences as well as people without specific expertise in these fields, and be able to co-opt additional expertise when relevant.[5]
   - 2.2 Be transparent, making its proceedings, deliberations, reasoning, conclusions and recommendations available for public scrutiny.
   - 2.3 Be outward facing so that interested persons are aware of its function and feel able to input into its work programme.
   - 2.4 Be actively involved in public engagement and consultation; and maintain regular forward-looking dialogue with the scientific community.
   - 2.5 Have the power to develop guidelines to promote consistency and transparency in the regulatory process.
3. We recommend that the Home Office ensures that the body that meets the requirement of the 'national committee for the protection of animals used for scientific purposes' in the UK has within its remit and competence the function of the national expert body for ACHM.
4. We recommend that, for those classes of ACHM where it is relevant, a risk assessment should be undertaken and appropriate containment levels specified. The risk assessment is the responsibility of investigators, research institutions and regulators, and should where relevant take the advice of an independent virologist.
5. We recommend that the Home Office and the Department of Health work closely together to ensure that there are no regulatory gaps, overlaps or inconsistencies, between the two regulatory systems. We consider it essential that the Home Office and the Human Fertilisation and Embryology Authority (HFEA) (or, as appropriate, the Department of Health) work together to develop and maintain a smooth, functionally integrated operational interface, at the boundaries of their areas of responsibility. This should be supported by clear guidance to the research community, to ensure the timely and appropriate adjudication of innovative scientific projects without undue bureaucracy. Such an interface may well involve the expert advisory bodies in the two systems, as well as officials acting for the agencies concerned.
6. We recommend raising international awareness of ACHM, promoting international consistency in research practice involving their use, and exploring the development of international standards or guidance. This might be achieved through international collaboration among regulators, policy-makers, national and international bioethics bodies and medical research councils, or initiatives within the research community. This is an area in which the UK should provide leadership.

5 Given the special issues associated with experiments on NHPs, we recommend that the national expert body should include either in its membership or as an advisor, an independent scientist with experience in NHP research who should be present to advise the group when such issues are discussed.

1 Introduction

Animals containing human genetic or cellular material are widely used in laboratories worldwide. There is a long and successful history of their role in advancing our understanding of human and animal physiology and disease, and increasingly in the development of new treatments. Of the thousands of examples of animals containing human material (which we refer to as 'animals containing human material' (ACHM)) developed since the 1960s, the great majority are mice each containing a single human gene, used to study gene function and disease.

The scientific techniques used to transfer genetic or cellular material from one entity to another are becoming increasingly sophisticated. Far greater quantities of genetic sequence can be manipulated, and stem cell technologies have enabled significant percentages of an animal's tissues or organs to be replaced with equivalents derived from human tissues. These techniques are applicable to fields of research as diverse as neuroscience, reproductive biology, cancer research, immunology and many more.

In 2007, the Academy convened a working group to examine the use of embryos combining human and animal material in medical research. To support the revision of UK legislation that was underway at that time, the study was concerned with human embryos incorporating animal material, and focused on one type of these now known as '*human* admixed embryos'.[6] However, the study's report, '*Inter-species embryos*', also mentioned research involving the converse situation i.e. the use of embryonic or adult animals containing human material.[7] The report drew attention to the need to review the regulatory environment in this area in light of the rapidly developing science, and to engage the public in discussion of these issues.

Whilst the UK Human Fertilisation and Embryology Act (2008) (the HFE Act) provided a contemporary legislative framework for research involving human embryos, it was noted that the 'animal end of the spectrum of human–animal mixture' had received relatively little consideration. Having recognised the possibility that this area of science could present future regulatory and ethical challenges in the UK and beyond, and the relatively little public attention that it had received, the Academy committed to undertake further work in this area to inform future public debate.

1.1 Scope and terms of reference

The Academy's study on the use of ACHM in biomedical research was launched in Autumn 2009. The scope of the study was to: examine the scientific, social, ethical, safety and regulatory aspects of research involving non-human embryos and animals containing human material. The study's terms of reference were to:

- Agree definitions for animals, and animal embryos, containing human genetic or cellular material.
- Describe the current use of animals containing human material in medical research, and to anticipate future research directions and challenges for this work.
- Assess future applications of research involving animals containing human material – including potential requirements for preclinical (animal) studies of candidate human stem cell therapies.
- Address safety concerns surrounding the generation and use of animals containing human material in research, and to consider welfare issues which apply specifically to animals containing human material.

6 Academy of Medical Sciences (2007). *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html
7 Academy of Medcal Sciences (2007). *Non-human embryos and animals incorporating human material.* In *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html

- Explore societal and ethical aspects of medical research involving the creation of animals that include significant amounts of human material, and to develop a constructive public dialogue in this area.
- Explore the current and future regulation of the use of animals and embryos containing human material for research purposes, including primary legislation, regulations and guidelines.
- Draw conclusions and make recommendations for action.

To avoid replication of previous work and debates, several wider areas were excluded from the study scope. These are not addressed in any depth:

- Scientific or ethical issues relating to the general use of animals in research. While recognising the debate in this area, and the need to be constantly aware of the importance of minimising the impact of research on experimental animals, this report concerns ACHM, which are a small proportion of animals used in medical research. We therefore start by accepting as given, all legislative and other controls that currently regulate animal experimentation in the UK, and restrict our consideration to specific issues of animal welfare arising from the inter-species nature of ACHM research.
- The use of human admixed embryos in research. These and other closely related issues were subject to full public debate throughout the passage of the HFE Act (2008).
- Broader issues relating to genetic modification in a wider sense and not involving human material, such as the genetic modification of animals, or plants, for agricultural purposes.

1.2 Conduct of the study

The study was conducted by a working group chaired by Professor Martin Bobrow CBE FRS FMedSci, which included expertise in biomedical science, philosophy, ethics, social science and law. Observers from Government and research funding bodies joined working group meetings but not discussion of the study's conclusions and recommendations. (See Annex I for a list of working group members and observers.)

The Academy issued an open call for evidence in November 2009 to which submissions were received from a wide range of organisations and individuals. Additional consultation was achieved through oral evidence sessions and correspondence between the working group and additional experts (Annex II details contributors to the study).

The strength of public opinion around the creation of mixed human–animal entities was evident throughout parliamentary debates around the HFE Act (2008), and in associated media coverage. The Academy's '*Inter-species embryos*' report recognised the importance of public values and judgements in informing the development of law and policy in these areas, but also warned of a gulf between current and future scientific practices, and public awareness of them. A programme of public dialogue was therefore commissioned to inform the current study (see Annex III for the dialogue methodology). Its findings were published in full in 2010 and are also incorporated into this report (see blue boxes).[8] An independent evaluation of the dialogue process has also been published.[9]

8 Ipsos MORI (2010). *Exploring the boundaries: report on a public dialogue into animals containing human material*. http://www.acmedsci.ac.uk/index.php?pid=209

9 Laura Grant Associates (2010). *Exploring the boundaries: a dialogue on animals containing human material. Evaluation report*. http://www.acmedsci.ac.uk/index.php?pid=240

The report was reviewed by a group appointed by the Academy's Council (see Annex I) and has been approved by the Academy's Council.

We thank all those who contributed to this study. We are grateful the Department for Business, Innovation and Skills' Sciencewise Expert Resource Centre, the Department of Health, Medical Research Council, and Wellcome Trust for their financial contribution to the study.

1.3 Overview and terminology

Chapter 2 describes the types of ACHM and briefly illustrates how and why they are used in biomedical research. In Chapter 3 we consider methodological areas in which developments relevant to the creation of ACHM are apparent, and areas in which future research may approach social, ethical or regulatory boundaries. Specific welfare and safety considerations related to ACHM use are discussed in Chapter 4. Social and ethical considerations are described in Chapter 5.

Chapter 6 provides an overview of the regulatory framework governing ACHM use in the UK; a wider international perspective is then outlined in Chapter 7. Chapter 8 sets out our conclusions and recommendations.

Common terminology has as far as possible been used for simplicity, and a glossary of terms is given in Annex IV. Though in correct scientific taxonomy, humans are both primates and animals, in this text 'animal' (rather than 'non-human animal') is used to refer to animals of all species in the animal kingdom *except* humans, whereas humans are referred to as either 'human', or 'man'. Primate species *except* humans are referred to as 'non-human primates', abbreviated as 'NHPs'.

A lay summary of this report is available separately.[10]

10 The lay summary is available at www.acmedsci.ac.uk/publications

2 Research involving inter-species mixtures

2.1 Overview

A broad range of inter-species entities, including both animal–animal and animal–human mixtures, are created and used in biomedical research. This report focuses on animal–human mixtures which involve the incorporation of human genetic or cellular material into animals. We refer to these as 'animals containing human material' (ACHM).

2.1.1 Why are ACHM used in medical research?

Experiments involving ACHM are undertaken for several overlapping reasons:

- Understanding human body function, or malfunction in disease, often requires *in vivo* study carried out in humans or, where that is morally or practically infeasible, in animals. This is because substitutes such as cell culture or computer simulation often do not satisfactorily mimic the complex three-dimensional structures that typify human tissues and organs, or their change over time.
- DNA sequence data from many species is increasingly available, but often the only way to determine the function of a specific piece of DNA is to observe its effect in a living animal. For example, this can reveal whether the function of the DNA in man is the same as in other species, or if it affects development, or causes disease.
- In many cases research is driven by a desire to improve our ability to diagnose and treat disease. Animals containing human DNA or cells provide important methods to study human disease more effectively, to test potential solutions and sometimes to develop or produce therapeutics.[11]

Of course, scientists like everyone else, are also motivated by wider factors (e.g. a desire to understand how things work, career advancement) and this applies to ACHM research in the same way as it does to other areas of science. The outcome of their work may be just as important, irrespective of their motives.

Animals used in the laboratory are sufficiently good models of aspects of human biology that their use can often generate useful information. However, the differences between species mean that experimental findings in animals always need careful consideration before extrapolation to man. Modifying animals to make them more similar to humans, in specific biological or disease characteristics, may improve the utility of results from such experiments. We recognise that, as for other types of animal research, the creation and use of ACHM has the potential to cause pain, suffering or harm to the animals involved. Consideration of these matters is the basis of UK regulation of animal research, which serves to minimise these concerns (see 4.1 and 5.5).

2.1.2 What species of animals are used?

A wide range of animals are used as recipients of human material in research. Mice are the most frequently used due to their small size, short generation time and well-understood biology and genetics; the development of rodents with biology more like that of humans is an important aspect of inter-species research. Some species are used because of their inherent similarity to humans (e.g. the size and physiology of organs such as the heart in pigs; the organisation of the NHP brain), others because aspects of their biology facilitate the techniques used (e.g. human DNA can be easily inserted into the eggs of frogs).[12]

It is difficult to estimate the number of ACHM used in UK research as these data are not systematically collected. But, although ACHM are only a proportion of the animals used in research, their development and use can support animal research welfare principles by contributing to the improvement of research approaches (see 4.1).

11 It is usually a regulatory requirement to test drugs and other therapies in animals before they can be used in humans, to assess both safety and efficacy. Because ACHM are likely to provide more relevant data than normal animals, it is possible that in future fewer animals may need to be used. The use of ACHM may also in some situations replace the use of NHPs.

12 For a broader discussion of the use of animals in research see Nuffield Council on Bioethics (2005). *The ethics of research involving animals*. Section 2, 83–184.

2.1.3 Types of research involving ACHM

ACHM are used in both investigational research (to understand underlying biology) and translational research (to find treatments and diagnostics), although the distinction between these is not clear cut. We consider the research uses of ACHM in two broad groups:

- **Investigating health and disease.** By substituting part of an animal's genetic material or tissues with a human equivalent, animals can be made to more closely replicate aspects of human biology, or to become susceptible to human diseases. These 'animal models' are used in investigational studies to understand human biological processes in health and in disease.
- **Developing and testing therapeutic products.** Animals are increasingly used both to produce humanised substances (e.g. proteins and antibodies) for use as therapeutic agents, and to test drugs and other therapies (including human-derived products such as stem cell therapies).

There are many different research avenues, and thousands of studies, in these overlapping fields. In section 2.3 we give illustrative examples of work across these areas, to give a flavour of the research which is being undertaken. These examples are intended to inform readers about the range and nature of work we are discussing, and not to imply that ACHM research is uniformly successful or that other research avenues are less valuable.

Box 2.1 What do we mean by a 'species'?

To discuss inter-species (between different species) mixtures it is helpful to consider the meaning of the term 'species'. At a simple level, the distinction between animals of different species is intuitively obvious; a cat is easily recognised as different from a dog, and we instinctively think of animals from separate species as different 'kinds'. However, all animals are evolutionarily related, with a clear gradient of relationships from distant (e.g. beetles and fish) to close relations (e.g. gorillas and chimpanzees). Some species are so closely related that they can interbreed, although the resulting offspring are generally sterile: for example a horse and donkey can breed to produce a mule.

A common biological definition of 'species' is *'a group of organisms capable of interbreeding and producing fertile offspring'*. However, this definition has some limitations, e.g. where breeding is not attempted owing to geographical separation, we do not know whether mating would produce fertile offspring.

Since the late 1980s scientists have explored species differences by comparing DNA sequence similarity – which can be quantified at a molecular level. DNA sequences of closely related species are more similar than those of distantly related species, and this principle has enabled the evolutionary relationships between different species to be clarified (an approach known as molecular phylogenetics). Studies are also now underway to identify regions of DNA that are species-specific, including those unique to humans and our ancestors (human-lineage specific sequences: see 3.2).

There must be sequences of DNA that contain the critical variations which set different species apart by determining their unique spectra of physical characteristics and their ability to interbreed, but most of these are still unknown. Species boundaries cannot be adequately defined as percentage variation between DNA sequences, or by the inclusion of currently known specific DNA sequences, and therefore currently continue to depend on distinctions between visible characteristics and the ability to interbreed. Indeed, DNA of closely related species is very similar – and much research involving inter-species mixtures is only possible because sections of DNA moved between even distantly related species can remain functional.

2.2 Types of ACHM

ACHM are a range of 'inter-species' entities in which the animal component predominates over the human (for definitions see Box 2.1 and Annex IV).[13] We consider three types of ACHM: genetically altered animals (including transgenics), chimæras and hybrids.

2.2.1 Genetically altered animals

There are two principal ways in which human DNA sequence can be incorporated into an animal's genome:

1. A section of human DNA sequence can be inserted into the genome of an animal cell. Cells carrying the inserted (human) gene sequence, or animals developed from them, are often referred to as 'transgenic'. This approach is possible in several animal species, using a range of techniques (see Box 2.3).
2. The genome of an animal can be modified so that it has, in part, the same DNA sequence as that found in the human. This can be achieved using 'gene-targeting' techniques, which are well-established in mice and in development for use in other species (including rat and some NHPs) (see Box 2.3). Specific DNA sequences can also be deleted to mimic aspects of the human genome, such as when genes or regulatory regions have been lost during human evolution (see Box 2.2). In such cases the animal's genome can be considered to have been humanised because it is altered to resemble the human, even though no human DNA sequence has been added. The use of such animals in research should therefore be governed by the same principles as ACHM.

These approaches create an animal with a genetic sequence that, in a specific part, resembles that of the human (the animal's DNA is humanised or made 'human-like'). For simplicity we refer to animals created by these methods as 'genetically altered'.

Genetic alterations can range from changes to one or two DNA base pairs (see *FOXP2*, 3.6.2), up to the exchange of extensive regions of animal DNA for human equivalents (see a-globin locus, 3.2), or the addition of an entire human chromosome (see Down's mouse, 3.2). Where 'human' DNA is used to create ACHM, it is very rarely taken directly from a person. DNA may be derived from cultured human cell lines, grown as recombinant DNA in bacteria, or artificially synthesised to produce the exact sequence found in humans.

Usually, almost every cell of a genetically altered animal contains the same DNA.[14] Where genetic alterations are present in the reproductive (germ) cells of the animal, they can be transmitted to offspring. Methods have also been developed to introduce genes into particular somatic tissues (e.g. the lung or eye) of animals. In this case, modifications are not introduced into animals' reproductive cells, and would not be transmitted. These techniques are the basis of 'gene therapy' approaches to treating disease (see 2.3.2).

Sections 2.3.1 and 2.3.2 illustrate research uses of animals humanised by genetic alteration.

2.2.2 Chimæras

Chimæras are formed by mixing together whole cells originating from different organisms. The new organism that results is made up of a 'patchwork' of cells from the two different sources. Each cell of a chimæra contains genes from only one of the organisms from which it is made.[15,16,17] In contrast to transgenics, DNA from different origins is not mixed within individual cells. The 'mixture' of cells found in tissues of a chimæra is not transmitted to future generations.

13 For a discussion of entities in which the *human* element is predominant see Academy of Medical Sciences (2007). *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html

14 With the exception of some unusual cell types e.g. red blood cells that lack DNA, and germ cells after they have undergone meiotic recombination, where the DNA sequence is shuffled.

15 With the exception of certain cell types that naturally undergo cell fusion such as specific cells in the placenta (syncytial trophoblast), and skeletal and cardiac muscle cells.

16 For an example of inter-species fusion involving muscle cells see Gentile A, *et al.* (2011). *Human epicardium-derived cells fuse with high efficiency with skeletal myotubes and differentiate toward the skeletal muscle phenotype: a comparison study with stromal and endothelial cells*. Mol Biol Cell **22**, 581–92.

17 There are also reports of rare cell fusion events, which complicate the interpretation of results of investigation of stem cell potential in chimæras, see Ying QL, *et al*. (2002). *Changing potency by spontaneous fusion*. Nature **416**, 545–8.

Chimæras can occur naturally, including in man. For example, cells from a developing fetus can colonise the mother, maternal cells can colonise a developing fetus, two pre-implantation embryos can combine, and in rare instances, cells can be transferred between siblings during twin pregnancy.[18]

The extent to which cells from different origins become integrated into the body of a chimæra depends on several factors including:

- The kind of cells involved (e.g. cells from the early embryo with broad developmental potential (the potential to develop into many kinds of tissue) may integrate widely; stem cells derived from an adult tissue such as liver or brain with narrower potential may integrate less widely).
- The relative numbers of cells of the two species.
- The developmental stage of the recipient animal (e.g. embryonic, fetal, newborn or adult). Earlier mixing is more likely to lead to widespread integration of the different species' cells, in many organs and tissues (although this also depends on the potential of the donor cells and on species compatibility: for example, slowly dividing human cells may not contribute widely to a rapidly growing animal embryo).

For the purposes of our discussions, we consider two types of chimæra:

- **Primary chimæras** are formed by mixing together two early embryos, or an early embryo with isolated embryonic cell types obtained from a different embryo or cultured stem cell line. The resulting chimæra has cells of different origins, in many tissues.
- **Secondary chimæras** are formed experimentally by transplanting (or grafting) cells or tissues into animals at later stages of development, including late fetal stages, post-natal or even adult animals.[19] The donor cells are only present in a few tissues.[20] The recipient animal is often chosen to be immune-deficient, or immune-suppressed.[21] However, especially with recent developments in imaging techniques, it is possible to introduce cells into an embryo *in utero* (or *in ovo*) and to study the results in live-born animals. This can be done before the development of the host's immune system, such that the grafted cells are recognised as 'self' and not rejected.

In making primary chimæras, various methods can be used to bias the contribution of 'donor' versus 'host' embryo cells. For example, if one pre-implantation embryo is more advanced than the other, the smaller cells of the former preferentially contribute to the inner cell mass (ICM; developing embryo proper) of the resulting blastocyst, whereas the larger cells of the latter tend to give rise to extra-embryonic tissues of the placenta. If chimæras are being made with pluripotent stem cells (embryonic stem (ES) or induced pluripotent stem (iPS) cells; for further information on stem cells see 3.3) combined with cleavage stage embryos, the former will preferentially end up in the ICM. A more rigorous way to alter the contribution of cells from two different sources ('donor' and 'host') to an embryo is to use a method termed 'tetraploid complementation' (see 6.2.2). Some stem cell types, including ES or iPS cells, (at least of the mouse) readily contribute to the embryo proper (the developing body of the organism) but not to extra-embryonic tissues (e.g. placental tissues). In contrast, embryo cells made to have double the normal number of chromosomes ('tetraploid cells') are able to produce extra-embryonic tissues, but contribute poorly to the embryo proper, especially in a chimæra where they are in competition with normal cells. By combining tetraploid host embryos with pluripotent stem cells, the latter can give rise to the entire fetus and thus to the live-born animal while the host embryo cells become confined to the placental tissues. This is an example of cell selection. More sophisticated examples of such approaches using genetic methods can replace a whole organ with cells from another species (see examples in 2.2.3).

18 Boklage CE (2006).*Embryogenesis of chimæras, twins and anterior midline asymmetries.* Hum Reprod **21,** 79–91.
19 There is no distinct boundary between primary and secondary chimæras.
20 The mixture of tissues in a secondary chimæra cannot be transmitted to its offspring.
21 The term 'xenotransplantation' is commonly used to refer to animal-to-human xenotransplantation.

Human cells used to create chimæras can be taken with appropriate consent directly from early embryos (e.g. surplus from IVF treatments), aborted fetuses, or a live-born person (e.g. human liver cells, or a cancer biopsy) or from cultured human cell lines. Sections 2.3.3 and 2.3.4 illustrate the uses of animal–human chimæras in research.

2.2.3 Hybrids

Animals formed by the fertilisation of an egg of one species by the sperm of a different species are called 'true hybrids'.[22] Each cell of the hybrid embryo, and the resulting animal if development occurs, has a complete set of genes from each parent. A small number of true hybrid animals occur in nature, as a consequence of mating between closely related animal species. The offspring are usually infertile (e.g. a mule is the sterile hybrid of horse and donkey). It is now possible to attempt techniques of assisted reproduction, such as intra-cytoplasmic sperm injection (ICSI), using eggs from one species and sperm from another. However, we are not aware of the production of viable offspring between animal species, other than those that are very closely related, in this way.[23]

The use of true hybrid *animals* formed from the combination of human and animal gametes is not currently envisaged in medical research. The fertilisation of animal eggs (hamster or mouse) by human sperm was previously used in sperm fertility testing.[24] It continues to be used in studies of reproductive biology, and has enabled, for example, identification of the roles of ion channels and enzymes found in human sperm in the process of egg activation, and the relationship between factors such as the sperm head shape and successful egg activation.[25,26] This information has been claimed to improve the selection of sperm for clinical use in assisted reproductive techniques.[27]

Although the creation of true hybrids using human cells is permitted in the UK, it is illegal to keep or use the hybrid embryos *in vitro* beyond very early developmental stages, or to implant them into a uterus (of a woman or animal) (see Box 6.5). Such entities would in any case be very unlikely to survive into later stages of development (except perhaps between very closely related species) because of the multiple biochemical and molecular incompatibilities between different species.

In contrast to hybrid *animals,* inter-specific *cell hybrids,* created by the fusion of cells from two different species (e.g. human cells fused with mouse cells) are widely used in research. Fusions are usually made between somatic cells rather than germ cells, and the cell hybrids do not develop into animals. They can, however, be made to grow for long periods of time in cell culture. On fusion, each hybrid cell contains a full set of chromosomes from each species; however, chromosomes are shed during cell culture, resulting in cell lines in which chromosomes from one species often predominate. Thousands of hybrid cell lines have been used over the past 30 years to explore fundamental issues in biology. Many human genes were mapped in the 1970s using this kind of cell hybrid, as a prelude to the human genome project.[28]

22 *True hybrids* are one of five types of *human admixed embryos* described in the UK's Human Fertilisation and Embryology Act (see Box 6.4). For further discussion of their use in research see Academy of Medical Sciences (2007). *Inter-species embryos.* http://www.acmedsci.ac.uk/p48prid51.html

23 Cross-species reproductive cloning methods involve the production of 'cytoplasmic hybrids', with nuclear DNA from one species and cytoplasm (containing mitochondrial DNA) from another. Such techniques have been investigated as a method of 'preserving' endangered species. For example, successful cloning of closely related sub-species has been achieved in the cat and wolf. However, a recent attempt to clone the panda using rabbit eggs was unsuccessful. See Lanza RP, *et al*. (2000). *Cloning of an endangered species (Bos gaurus) using interspecies nuclear transfer.* Cloning **2,** 79–90; Gomez MC, *et al.* (2008). *Nuclear transfer of sand cat cells into enucleated domestic cat oocytes is affected by cryopreservation of donor cells.* Cloning Stem Cells **10**, 469–83; Oh HJ, *et al*. (2008). *Cloning endangered gray wolves (Canis lupus) from somatic cells collected postmortem*. Theriogenology **70,** 638–47; Chen DY, *et al*. (2002). *Interspecies implantation and mitochondria fate of panda–rabbit cloned embryos*. Biol Reprod **67,** 637–42.

24 The 'hamster zona-free ovum test' initially proved to be a promising new test of fertilisation potential but was not found to be of significant clinical use compared with routine semen analysis. See Yanagimachi H, *et al*. (1976). *The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa*. Biology of Reproduction **15** (4), 471–76; Aitken RJ (1985). *Diagnostic value of the zona-free hamster oocyte penetration test and sperm movement characteristics in oligozoospermia*. Int J Androl **8,** 348–56.

25 Li CY, *et al.* (2010). *CFTR is essential for sperm fertilizing capacity and is correlated with sperm quality in humans*. Hum Reprod **25**, 317–27.

26 Heytens E, *et al*. (2009). *Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men.* Hum Reprod **24**, 2417–28.

27 Ito C, *et al*. (2009). *Oocyte activation ability correlates with head flatness and presence of perinuclear theca substance in human and mouse sperm*. Hum Reprod **24**, 2588–95.

28 By creating a range of cell lines with differing human chromosome content, and comparing the chromosome content with the gene expression and function of different cell lines, specific genes could be mapped to specific chromosomes. See Griffiths AJ, *et al.* (2000). *Mapping human genes by using human–rodent somatic cell hybrids*. In: An Introduction to Genetic Analysis. Freeman WH, New York.

Box 2.2 Genes and their function

What is a gene?

Most genes encode proteins that are the molecules that comprise much of our cells and tissues. DNA coding for one protein is seldom found in a single stretch of DNA sequence, but is split into sections (exons) along the DNA molecule. By splicing different parts a single gene together, cells can sometimes make several related proteins from a single section of genetic template. The regulatory elements that function as switches to control gene expression are located adjacent to the protein coding region, or sometimes at considerable distances 'upstream' or 'downstream' and/or within the intervals (called 'introns') between the protein coding parts of genes.

How do genes 'work'?

In simple terms, a length of DNA known as a gene is 'read' (transcribed), by an enzyme in the cell nucleus, creating a matching chemical message (messenger RNA (mRNA)) which passes into the cell body and is translated into the protein encoded by the gene. DNA in many different organisms is remarkably similar, so that some genes can be made to 'work' in this way even when moved between very different organisms. For example, human gene sequences (such as the *cdc2* gene, see 2.3.1) can be read by yeast cells, producing human gene products that can function in conjunction with other yeast cell components. Some genes do not code for proteins, but for active RNA molecules, many of which are involved in regulating genes.

What is a 'disease gene'?

While people often speak loosely of a 'gene for' a disease, genes actually code for functional proteins, and disease is a consequence of an error ('mutation') within the gene or its regulating regions, which means the corresponding protein does not function properly. For example, a 'gene for haemophilia' actually codes for a protein that is needed in blood clotting; patients with the damaged gene lack the functional protein, and the resultant failure of normal clotting is called haemophilia.

What is a 'human' gene?

What do we mean when we use the terms 'human gene' or 'mouse gene'? We are referring to the DNA sequence of a gene found in humans or mice. However, DNA can be made synthetically from its chemical parts, and it is possible to create pieces of DNA identical to the genes found in a human or mouse, that have never been part of a living animal. The 'artificial life form' created in 2010 is an extreme illustration of this; a copy of the full genome of the bacterium *Mycoplasma mycoides* was artificially synthesised and inserted into a cell of another bacterium, producing an organism able to grow and self-replicate under the direction of artificial DNA alone.[29]

The DNA sequence of a particular gene is often very similar in different species. For example the DNA sequence of the *PAX6* gene, which codes for a protein in eye and brain development, is almost identical in human and mouse; the protein coded by the gene has the same amino-acid sequence in both species. There are also large regions, up to 1000 nucleotides long, of *PAX6* regulatory DNA that are completely identical in humans and mice.[30]

What we really mean by a 'human' gene is a section of DNA performing a particular function, which carries the few distinctive bits of sequence (which may only be a few percent of its total length) and which differ between humans and other species. However, there are probably some genes (and perhaps more regulatory regions) that are unique to humans. We can determine their importance and relevance to human evolution by asking how they work in transgenic animals.

29 Gibson DG, *et al.* (2010). *Creation of a bacterial cell controlled by a chemically synthesized genome*. Science **329**, 52-6.
30 van Heyningen V & Williamson KA (2002). *PAX6 in sensory development*. Hum Mol Genet **11**, 1161-7.

2.3 How are ACHM used in research?

2.3.1 Genetically altered animals in investigating health and disease

The DNA sequence of many species is sufficiently similar for sections from one species to retain their function when incorporated into cells of a different species. In a classic experiment, human DNA was inserted into mutant yeast cells defective in a gene (*cdc2*) known to be crucially important in regulating yeast cell division. Remarkably, some pieces of human DNA were able to compensate for the defective yeast gene, allowing the mutant cells to divide normally. Researchers thus identified the human *cdc2* gene, which is so similar that it could compensate for the defective yeast gene.[31] These experiments were important in demonstrating that some genes responsible for controlling basic cell functions like cell division are highly conserved (meaning they have retained the same structure and function throughout evolution). The process of cell division is fundamental to understanding cancer, and variants of the *cdc2* gene are associated with some forms of human cancer. (See Box 2.4 for uses of genetically altered cells.)

It is now almost routine to incorporate human DNA into animal eggs or embryos; the resulting genetically altered animals are used ubiquitously in research to investigate the function of human genes and the proteins they encode. For example, the melanocortin receptor (MC1R) regulates pigmentation in mammals and is necessary for the production of dark melanin pigment in skin and hair. Humans with certain MC1R variants have red hair, pale ultraviolet-sensitive skin and are at increased risk of skin cancer. Mice expressing these human MC1R variants have yellow coats, and have been used to study the activation of MC1R receptors, and to identify the cell signalling pathways through which they work.[32]

Where the genetic basis of a disease in humans is known or suspected, the particular variant of the human gene associated with the disease can be incorporated into an animal to study the disease (see Box 2.3). We received many submissions describing the use of mice expressing human genes to study conditions as varied as migraine, anxiety disorders, osteoporosis, diabetes, heart disease and cancer.[33] However, the use of a wider range of species was also evident, including fruit flies expressing human ion channels used to study neurodegenerative disorders, and pigs expressing human polypeptide receptors in diabetes research.[34,35]

31 Lee MG, *et al*. (1987). *Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2.* Nature **327** (6117), 31–5.
32 Jackson IJ, *et al*. (2007). *Humanized MC1R transgenic mice reveal human specific receptor function*. Hum Mol Genet **16**, 2341–8.
33 Eikermann-Haerter K, *et al*. (2009). *Androgenic suppression of spreading depression in familial hemiplegic migraine type 1 mutant mice*. Ann Neurol **66**, 564–8; Jennings KA, *et al*. (2006). *Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission*. J Neurosci **26**, 8955–64; Daley E, *et al*. (2010). *Variable bone fragility associated with an Amish COL1A2 variant and a knock-in mouse model*. J Bone Miner Res **25**, 247–61; King M, *et al*. (2008). *Humanized mice for the study of type 1 diabetes and beta cell function*. Ann N Y Acad Sci **1150**, 46–53; Su Q, *et al*. (2008). *A DNA transposon-based approach to validate oncogenic mutations in the mouse*. Proc Natl Acad Sci USA **105**, 19904–9.
34 Moffat KG (2008). *Drosophila genetics for the analysis of neurobiological disease*. SEB Exp Biol Ser **60**, 9–24.
35 Renner S, *et al*. (2010). *Glucose intolerance and reduced proliferation of pancreatic ß-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function*. Diabetes **59**, 1228–38.

Box 2.3 Examples of research methods used to make genetically altered animals

1. Transgenesis can be achieved in a wide range of species, using methods including:

- **DNA microinjection.** Copies of a segment of (e.g. human) DNA are directly injected into the nucleus of a fertilised animal egg, which is gestated in a surrogate female.[36] The genomes of the offspring are analysed, and animals in which the injected DNA has integrated are bred for use. DNA insertion occurs at random, and often in multiple copies. Genes within the introduced DNA can be expressed in a manner that is expected, or they can show ectopic (out of place) expression depending on the site of integration. In a minority of cases the integration event can disrupt the activity of an endogenous gene.[37]
- **Retrovirus-mediated gene transfer.** A modified carrier virus (or 'vector') is used to insert a transgene into the cells of a developing embryo, which is gestated in a surrogate female. The resulting offspring are often genetic 'mosaics', developed from a mixture of cells with one or more copies of the inserted sequence at different places in their genomes. Animals where the germ cells have the required integrated DNA are bred to create transgenic animal strains. Recent studies indicate that it may be possible to generate transgenic NHPs in this way.[38]

2. Gene-targeting methods include:

- **Homologous recombination in embryonic stem (ES) cells** is used to engineer precise changes in the mouse genome.[39] ES cells are genetically modified *in vitro,* e.g. to add, remove or exchange a specific genetic sequence at a specific location in the genome. Individual cells can be selected that following rare DNA recombination events, have the intended changes to their DNA.[40,41] These cells are injected into early stage mouse embryos to make chimæras. Mice with germ cells developed from the altered ES cells are bred, to create a line of genetically altered mice.

These methods in the mouse have become very sophisticated. Similar techniques are being developed in other species (see 3.2). In theory it ought to be possible make chimæras with NHP ES cells (which have very similar properties to human ES cells, distinct from those of the mouse) and NHP embryos, though this has not yet been attempted to our knowledge.[42] It is not clear whether human pluripotent cells can contribute to pre-implantation human embryos to make chimæras.[43] (Additional methods of transgenesis and gene targeting see [44])

3. Somatic cell 'gene therapy'.Techniques have been developed to integrate transgenes into particular somatic tissues (such as immune cells, the lung or retina). These methods often use modified viruses as 'vectors' to carry sections of DNA into the cells of adult animals or humans, rather than embryos. These methods generally involve gene addition rather than replacement, with the purpose of restoring the function of an abnormal gene.

36 Gestation in a surrogate is used for research involving mammals; the embryos of other genetically altered species, including chick, frog and fish can develop by themselves.

37 For an overview see Gama Sosa MA, *et al*. (2010). *Animal transgenesis: an overview.* Brain Struct Funct **214**, 91–109.

38 Niu Y, *et al*. (2010). *Transgenic rhesus monkeys produced by gene transfer into early-cleavage-stage embryos using a simian immunodeficiency virus-based vector*. Proc Natl Acad Sci USA **107**, 17663–7.

39 The types of change can include deletions, insertions, or replacement of one DNA sequence with another. These methods rely on the use of DNA sequences, at the ends of the donor DNA that are homologous to (match) the target site in the ES cell genome.

40 While DNA usually integrates at random in mammalian cells, even rare homologous recombination events can be found by screening large numbers of ES cells.

41 Gordon JW, *et al*. (1980). *Genetic transformation of mouse embryos by microinjection of purified DNA*. Proc Natl Acad Sci USA **77**, 7380–4.

42 Wianny F, *et al*. (2011). *Embryonic stem cells in non-human primates: An overview of neural differentiation potential*. Differentiation **81**, 142–52.

43 Although the HFE Act (2008) would allow these experiments to be initiated, it would be illegal to keep such entities intact *in vitro* for more than 14 days or to implant them (see Box 6.5).

44 **a. Sperm-mediated gene transfer.** Can also be used to create transgenics. A sequence of DNA is introduced into the head of a sperm, which is then used for fertilisation. This approach has been used in species including frog, mouse, rat and pig.
b. Genetic alteration of somatic cells combined with nuclear transfer. In species for which ES cells are unavailable (e.g. sheep) gene targeting can be conducted by combining the use of somatic cells (e.g. fibroblasts) genetically modified in culture, with nuclear transfer cloning techniques. See Denning C, *et al*. (2001). *Gene targeting in primary fetal fibroblasts from sheep and pig*. Cloning Stem Cells **3**, 221–31.
c. Zinc-finger nuclease (ZFN) methods. These methods can be used on cells in culture, or after DNA microinjection into fertilised eggs. In principle this method can be used to introduce human DNA into any animal species and in a targeted fashion. See Whyte JJ, *et al.* (2011). *Gene targeting with zinc finger nucleases to produce cloned eGFP knockout pigs*. Mol Reprod Dev **78**, 2.
d. Genetic modification of spermatogonial stem cells. Male germ-line (spermatogonial) stem cells can be genetically modified and transplanted into the testicular tissue of an infertile male animal where they give rise to modified sperm cells. This approach has been developed in the mouse. See Takehashi M, *et al*. (2010). *Generation of genetically modified animals using spermatogonial stem cells*. Dev Growth Differ **52,** 303–10.

Box 2.4 Transgenic and genetically altered cells

Individual animal cells, or cell lines, into which human genes are inserted (or 'transfected') are widely used in investigational research and drug development.

Expression of human DNA in frog eggs has been used to understand the function of some human transporter proteins (molecules that move substances into and out of cells). One of the first demonstrations of the chloride channel function of the cystic fibrosis gene was achieved using this approach.[45] More recently, suggestions arose of an association between variants of the human gene *SLC2A9* with high uric acid levels in gout. Human *SLC2A9* was initially thought to encode a protein used only to transport sugars; however, its expression in frog eggs revealed a new role for the transporter in carrying uric acid, and suggested a rationale for the links between human *SLC2A9* gene variants and gout.[46]

Transfected cells lines expressing human genes are also used in the pharmaceutical industry in screening to identify novel drug molecules, and to express human proteins (marketed products include human erthyropoetin for use in anaemia, and blood clotting factors for use in haemophilia, produced in Chinese hamster ovary cells).[47]

(See also 2.2.3 for the uses of inter-specific cell hybrids.)

Huntington's disease (HD) is a genetic neurodegenerative condition, in which nerve cells in some parts of the brain accumulate granular protein and subsequently die. Animal models of HD have been created in flies, zebrafish, mice and sheep by incorporating the mutant form of the human Huntingtin gene, which causes HD in man, into the animals' genomes.[48,49] A rhesus macaque transgenic model of the disease was also reported in 2008, although the mutant human Huntingtin gene did not transmit to offspring.[50]

Studies using cell cultures and these animal models indicated that the abnormal granular protein product of the mutant Huntingtin gene, which is toxic to brain cells, could be cleared by a process called autophagy. Drugs that induce autophagy were identified, and found to enhance the removal of the protein and thus decrease its toxicity. The consistent effect of this strategy in the animal models of HD suggested that a drug might similarly modify the accumulation of the toxic protein granules in human brain cells. Safety testing of one these drugs is now underway, as a precursor to clinical trials in patients.[51] Autophagy has also been implicated in other diseases including Parkinson's, Alzheimer's, and forms of cancer – some of the evidence for this association comes from comparable studies in transgenic mice expressing the human proteins mutated in these diseases.

The study of Duchenne muscular dystrophy (DMD), a condition that causes progressive muscle wasting in boys leading to death in early adulthood, has been facilitated by genetically altered animals expressing human gene variants. A mouse was first discovered that carried a dystrophin gene mutation similar to that causing DMD in humans.[52] Although the mouse had some biochemical and physical features of DMD, it lacked the characteristic

45 Bear CE, *et al*. (1991). *Cl- channel activity in Xenopus oocytes expressing the cystic fibrosis gene*. J Biol Chem **266**, 19142-5.
46 Vitart V, *et al*. (2008). *SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout*. Nat Genet **40**, 437-42.
47 See the European Medicines Agency http://www.ema.europa.eu/; EMEA/H/C/000726 epoetin alfa for the treatment of anaemia; EU/3/09/655: Human recombinant octocog alfa for the treatment of haemophilia A.
48 Williams A, *et al*. (2008). *Novel targets for Huntington's disease in an mTOR-independent autophagy pathway*. Nat Chem Biol **4**, 295–305.
49 Jacobsen JC, *et al*. (2010). *An ovine transgenic Huntington's disease model*. Hum Mol Genet **19**, 1873–82.
50 Yang SH, *et al*. (2008). *Towards a transgenic model of Huntington's disease in a non-human primate*. Nature **453**, 921–4.
51 Rose C, *et al*. (2010). *Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease*. Hum Mol Genet **19**, 2144–53.
52 Bulfield G, *et al*. (1984). *X chromosome-linked muscular dystrophy (mdx) in the mouse.* Proc Natl Acad Sci USA **81**, 1189–92.

severe early onset, and so did not fully mimic human DMD. A second gene, utrophin, was later identified and found to have a very similar function to the dystrophin gene. Although the utrophin gene is inactivated early in embryonic life in humans, mice can partially re-activate this gene in adulthood, compensate for an absence of dystrophin, and ameliorate the effects of DMD. Mice genetically altered to lack the function of both genes show severe disease and more closely model human DMD. Research using mouse models has since led to the development of several putative DMD treatments, including an approach which partially corrects the genetic defect in many cases of DMD, now in clinical trial.[53]

A strain of dog with a spontaneous dystrophin mutation has also been used in DMD research.[54] Large animal models are not always needed in disease research, and pre-clinical research in such species including dogs is not necessarily a pre-requisite for drug development. However, conditions such as heart disease and cognitive dysfunction may require large animal models because of the significant biological differences between man and mouse; humanised animal models may in future be of use in the development of therapies for such diseases.

While many human diseases (e.g. HD, DMD) are caused by mutations in protein coding regions of DNA, disease-causing mutations also occur in DNA regulatory regions (which do not encode protein but regulate gene expression). Regulatory regions are often located at a considerable distance from the genes they control, and the creation of accurate animal disease models involving mutations in these regions therefore requires the transfer of extensive sections of DNA (see the modification of α-globin gene locus used to model the blood disorder α-thalassaemia in the mouse in 3.2). The study of human gene regulatory regions in transgenic animals (mice, chick, embryos, frogs and fish), combined with detailed sequence comparisons, has also led to basic understanding of how these function normally, or are defective in genetic disease, and how they and the gene regulatory mechanisms have evolved.[55,56,57] We anticipate that it will become increasingly possible to accurately manipulate large sections of human DNA.

2.3.2 Genetically altered animals used in developing and testing therapeutics

Animals containing human genetic sequence can be developed to produce humanised substances (e.g. proteins and antibodies) for use as 'biological therapeutics' in people with deficiency of a particular substance, or in other forms of novel treatment.[58]

In an approach sometimes called 'pharming', transgenic animals have been created which carry a human gene, and secrete the associated human protein e.g. as a component of their milk. The protein is extracted, purified and used for treatment. Such 'therapeutic proteins' have been produced in sheep, goats, cattle, and rabbits; chickens have been developed which produce human proteins in their egg white.[59] In 2009, *ATryn*, a human anti-thrombin protein made by transgenic goats was licensed for use during surgery in patients with a congenital blood clotting disorder.[60] Similar products in development include human α-1 antitrypsin for emphysema treatment, and blood clotting factors for haemophilia treatment. In these approaches the genetically altered animals are, in effect, used to manufacture often large amounts of fully functional proteins, which cannot be produced in cell lines.

53 Kinali M, *et al*. (2009). *Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study*. Lancet Neurol **8**, 918–28.
54 To date these have not been used extensively in therapeutic drug development.
55 For an example of an early transgenic experiment see Koopman P, *et al*. (1989). *Widespread expression of human alpha 1-antitrypsin in transgenic mice revealed by in situ hybridization*. Genes Dev **3**, 16–25.
56 For an example of a recent paper involving a systematic study of regulatory sequences see Schmidt D, *et al*. (2010). *Five-vertebrate ChIP-seq reveals the evolutionary dynamics of transcription factor binding*. Science **328**, 1036–40.
57 For an example of a recent work considering loss of regulatory sequences in human evolution see McLean CY, *et al*. (2011). *Human-specific loss of regulatory DNA and the evolution of human-specific traits*. Nature **471**, 216–9.
58 Biological therapies are treatments for diseases that involve the use of biological materials or biological response modifiers, such as genes, cells, tissues, organs, serum, vaccines, antibodies or humoral agents. In contrast, pharmacological or chemical therapies are those which use small drug molecules.
59 Written evidence from the Biotechnology and Biological Sciences Research Council (BBSRC), and see for example Lillico SG, *et al*. (2007). *Oviduct-specific expression of two therapeutic proteins in transgenic hens*. Proc Natl Acad Sci USA **104**, 1771–6.
60 The European Public Assessment Report (EPAR), produced by the European Medicines Agency for ATryn is available at http://www.ema.europa.eu/humandocs/Humans/EPAR/atryn/atryn.htm

Humanised antibodies produced in animals are increasingly used as biological therapeutics. Animals produce a huge range of different antibodies which underpin the immune recognition and rejection of 'foreign' proteins ('the adaptive immune response'). Each antibody interacts highly specifically with a particular protein. This ability has been used to develop 'therapeutic antibodies' in which an antibody can act directly as a 'biological drug' by blocking some cellular function or killing the cell type targeted (e.g. cancer cells); or can be coupled to a drug which the antibody delivers to a specific target. This field is fast-growing; in mid-2009, there were close to 50 approved therapeutic antibodies on the market, and over 150 applications for new antibody products under consideration in the USA.[61] Antibodies are large, complex proteins, which are difficult to produce synthetically, but they can be obtained from animals or certain cell lines. However, animal antibodies injected into humans would be recognised as 'foreign protein' and eliminated by the human immune system. Recently, mice with 'humanised immune systems' have been engineered to produce antibodies that are not rejected by the human body, and so can be used in therapy.[62] This has been achieved using mice with antibody genes replaced by human equivalents (e.g. XenoMouse, see also 3.2).[63] In response to immunisation the mouse humanised immune systems respond by producing humanised antibodies, which can be selected and manufactured in cell lines. The human antibody Panitumumab, licensed for colorectal cancer treatment, was developed in this way. It targets a growth factor receptor, and inhibits tumour growth and vascularisation.[64]

The concept of 'gene replacement therapy' was first discussed in the early 1970s, but safe, effective procedures have proved difficult to develop. Gene therapy is based on the concept of inserting a functional copy of a gene into tissues where the gene is dysfunctional or absent (see Box 2.2). The aim is to perform human–human gene transfer; however, animal models are necessary to develop and refine the required reagents and techniques.

Leber congenital amaurosis (LCA) is a set of genetic eye diseases which often lead to complete blindness. One form of LCA is caused by a mutation in the *RPE65* gene, which encodes a protein needed for the recycling of visual pigment in the eye's light-sensing cells. Gene therapy aims to carry functional copies of the *RPE65* gene into the retina using a modified viral carrier introduced into the eye.[65] These methods have been developed in transgenic mice with a defective *RPE65* gene and in the Briard dog which naturally lacks the *RPE65* gene.[66] Both the mouse and dog models have early, severe visual impairment similar to that in human LCA; however, the dog eye is more similar to the human eye in size and structure. The effectiveness of this therapy in these animals enabled the approach to be taken forward into clinical trials; initial results suggest that it can be effective in humans, though further refinement will be required to produce a licensed treatment.[67,68] This approach may in future also turn out to be applicable to other eye diseases. There are particular sensitivities in using 'companion' animals such as dogs and cats for experimental purposes, but there are some unusual situations where they have clear advantages (either because of some aspect of

61 Nelson AL, *et al*. (2010). *Development trends for human monoclonal antibody therapeutics*. Nat Rev Drug Discov **9**, 767–74.
62 Kyowa Hakko Kirin California, Inc. have developed the TransChromo Mouse (TC Mouse™) that is capable of producing a variety of fully human monoclonal antibodies. They are also developing the TransChromo Cow (TC Cow™) for the production of polyclonal antibodies. See: http://kyowa-kirin-ca.com/tc_pubs.cfm
63 Jakobovits A, *et al*. (2007). *From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice*. Nat Biotechnol **25**, 1134–43; Written evidence from the NC3Rs.
64 Giusti RM, *et al*. (2007). *FDA drug approval summary: panitumumab (Vectibix).* Oncologist **12**, 577–83.
65 Acland GM, *et al*. (2001). *Gene therapy restores vision in a canine model of childhood blindness*. Nat Genet **28**, 92–5. See also http://www.ucl.ac.uk/ioo/research/patients/clinical_trials.html
66 Bemelmans AP, *et al*. (2006). *Lentiviral gene transfer of RPE65 rescues survival and function of cones in a mouse model of Leber congenital amaurosis.* PLoS Med **3**, e347.
67 Bainbridge JW, *et al*. (2008). *Effect of gene therapy on visual function in Leber's congenital amaurosis*. N Engl J Med **358**, 2231–9.
68 Maguire AM, *et al*. (2008). *Safety and efficacy of gene transfer for Leber's congenital amaurosis*. N Engl J Med **358**, 2240–8.

their normal function or, as here, because of the presence of a naturally occurring disease which closely resembles a human disorder) as to outweigh this aversion. Animal models are also contributing to attempts to develop gene therapies for conditions including spinal muscular atrophy and β-thalassemia.[69,70]

Owing to a shortage of human donor organs, tissue from animals, particularly pigs, has for many years been investigated as a source of tissue for transplant, although safety concerns hampered the development of the field. Another major barrier to the xenotransplantation of organs from pigs to humans is the 'hyperacute immune response' in which the recipient's immune system destroys the lining of blood vessels in the engrafted tissue. Such rejection occurs in part because an antigen (alpha-Gal), which is not made by humans, is expressed on the surface of pig cells. Attempts are under way to develop pigs which do not express alpha-Gal.[71] An alternative approach is the development of transgenic pigs expressing critical human proteins which inhibit the human immune response, and whose organs are therefore less likely to be rejected. Evidence from pre-clinical studies has indicated the potential of this approach, for example hearts from transgenic pigs have been found to function following transplant into NHPs treated with immunosuppressive drugs.[72]

Transgenic mice may, in future, be used in drug-toxicity testing and in testing biological products such as live vaccines. These are avenues in which the use of humanised animals may reduce, or ultimately replace, the use of larger animal species. However, the development of such methods can take several decades, not only for the necessary scientific development, but in subsequently gaining acceptance from regulatory agencies.[73]

2.3.3 Chimæras in investigating health and disease

Primary chimæras

Chimæras are formed by combining whole cells from different origins (see 2.2.2). Primary chimæras, created by mixing together early embryos, or embryos and cells, have been used in the study of developmental biology for several decades. Embryonic cells (including ES cells, see Box 3.3) that are identifiably marked, are isolated from specific regions or at different embryonic stages, combined with normal embryos, and traced throughout subsequent development, revealing the origins of the different types of cells, organs and tissues in the developing animal.[74] Such research was fundamental to understanding early vertebrate development.[75] Usually such chimæras are constructed using embryonic cells from the same species, although a variety of inter-specific combinations have been tried. The latter usually only work at early embryonic stages when the two species are very close in evolutionary terms, otherwise incompatibilities, for example in growth rates or cell adhesion, lead to abnormalities and to early embryo failure. The recent availability of human ES and iPS cells (see Box 3.3) opens the way for an expanding amount of work of this sort, though we are aware of relatively few studies involving the introduction of human ES pluripotent cells into animal embryos. In 3.2 we consider situations in which the introduction of human stem cells into animals might require particularly careful consideration.

Secondary chimæras

Although human biology and disease pathology can often be studied directly in volunteers or patients, this approach is sometimes infeasible or unethical. Secondary chimæras, made by transplanting human cells or tissues into adult animals (see 2.2.2) are therefore used to:

69 Chiara F, *et al*. (2010). *Systemic Delivery of scAAV9 Expressing SMN Prolongs Survival in a Model of Spinal Muscular Atrophy*. Science Translational Medicine **2**, 35ra42.
70 Sadelain M (2006). *Recent advances in globin gene transfer for the treatment of beta-thalassemia and sickle cell anemia*. Curr Opin Hematol **13**, 142–8.
71 Cooper DK, *et al*. (2007). *Alpha1,3-galactosyltransferase gene-knockout pigs for xenotransplantation: where do we go from here?* Transplantation **84**, 1–7.
72 Ekser B, *et al*. (2009). *Xenotransplantation of solid organs in the pig-to-primate model*. Transpl Immunol **21**, 87–92. See also 4.2.4.
73 Since 2004, the European Medicines Agency have recognised a role for a some specific transgenic mice carrying human genes in the carcinogenicity testing of new drugs. See *Addendum to ICH S6: preclinical safety evaluation of biotechnology-derived pharmaceuticals* at http://www.ema.europa.eu/
74 These are sometimes known as cell 'potential' and 'lineage' experiments.
75 Le Douarin N & McLaren A (1984). *Chimæras in Developmental Biology.* Harcourt Brace Jovanovich, London.

- Maintain human cells and tissues, enabling their study *in vivo* (e.g. cancer biopsies).
- Model human organs or organ systems, by substituting an animal's cells or tissues with human equivalents. These approaches use human cell types which replicate and colonise in the recipient (e.g. human blood stem cells used to humanise the immune system of mice).
- Study infectious diseases which are normally human-specific (e.g. HIV) by introducing human cells which confer disease-susceptibility to the animal.

Engraftment of human cells into animals is complicated by the recipient's immune system, which often rejects foreign tissue. Immuno-compromised mice, which lack the ability to mount an adaptive immune response, and can therefore accept xenografts, have greatly facilitated such research.[76]

Studies, particularly in mice, have played a fundamental role in research over the past 50 years to understand the complex processes underpinning cancer. In these studies, excised pieces of human cancers, cancer cells or human cancer cell lines, are grafted into immune-deficient animals. These models have enabled investigation of the mechanisms of cancer tumour initiation and spread and facilitated the development of therapies including chemo- and radiotherapy.

For example, a recent use of cancer xenograft models has been to investigate the roles of certain cancer cell types in leukaemia (blood cancer). Studies in mice engrafted with human blood stem cells or leukaemic cells led to the identification of 'self-renewing' or 'cancer stem' cells. Evidence indicates that these cells can be responsible for the creation or relapse of tumours, and that they are resistant to chemotherapy and radiation therapy. The significance of these cells was a major conceptual change in the field, which is now being investigated in carcinomas (solid tumour types). Primary xenograft models (using tissue taken directly from patients) are becomingly increasingly used in preclinical drug development as they can show closer similarity to human cancer, including a better representation of cancer pathways and variation in therapeutic response, than earlier cell culture methods. Biopsied human cells can also be genetically modified before implantation, to investigate the specific mechanisms involved in particular cancers. These same models can be used to test therapeutics *in vivo*.[77]

Type 1 diabetes results from destruction of the insulin-producing β-cells in parts of the pancreas called islets, by the person's own immune system. Mice implanted with human islets have been used to study this condition. Recently, combined models have been made by engrafting human blood stem cells into immune-deficient mice (these cells colonise and humanise the mouse immune system) and subsequently implanting human islets. This approach is being used to refine techniques for transplanting islets between humans in the clinic. A long-term research goal is to develop treatments to restore human β-cells in diabetics (e.g. using stem cell therapy). The combined mouse model can be used in the development of these treatments, to study how human β-cells, derived from stem cells, colonise and function in human islets in the presence of a humanised immune system.[78]

A humanised mouse model has been used to study *Salmonella enterica serovar Typhi*, the bacterium that causes typhoid and usually only infects humans. Mice lacking their own lymphatic system, but engrafted with human leukocytes (a form of white blood cell), were found to be susceptible to the bacterial infection and after inoculation displayed symptoms

76 Immune-deficient mice are widely used in medical research. Their lack of immune response means that they do not reject foreign tissue and can be used to 'incubate' cells or tissue from mice or other species, typically as grafts under the skin on the back. The first mice to be used in this way were the 'nude mice' in which a mutation in the *FOXN1* gene results in the lack of the thymus organ (and so immune deficiency) together with a hairless appearance.

77 Dick JE (2008). *Stem cell concepts renew cancer research*. Blood **112**, 4793–807.

78 Brehm MA, *et al*. (2010). *Human immune system development and rejection of human islet allografts in spontaneously diabetic NOD-Rag1null IL2rgammanull Ins2Akita mice*. Diabetes **59**, 2265–70.

similar to the human disease. The mice have been used to study the mechanisms of typhoid disease progression (and to correlate these to the four stages of untreated typhoid in humans), and to investigate therapeutic strategies.[79]

The Epstein-Barr virus (EBV) is associated with lymphatic system cancers (lymphomas); the same virus, in adolescence, causes glandular fever. EBV is a human-specific pathogen; however, 'BLT' mice, humanised by transplantation of human fetal blood stem cells, liver and thymus tissues are susceptible to the virus.[80] Studies in these mice using modified viruses have clarified the way that EBV establishes lytic (cell killing) rather than latent (delayed) infection. Findings indicate that the outcome of EBV infection can be moderated by immune system responses, and that the lytic functions of EBV are important in lymphoma formation.[81]

Mammalian liver is capable of restoring its own damaged cells because liver cells (hepatocytes) have the ability re-enter the cell cycle and replicate. Isolated human hepatocytes can be introduced into surgically reduced, or genetically compromised livers of immune-deficient mice which they colonise, resulting in organs made up of cells of both species, which partially resemble human liver. Up to 95% of mouse liver cells can be replaced by human hepatocytes in this way.[82] Mice with such humanised livers are used to study liver diseases including hepatitis B and C (viruses that usually only infect humans and chimpanzees), and to test antiviral drugs.[83] Mice with humanised livers of this kind should also be useful for drug toxicity testing, as they should predict the metabolism of drugs by the human liver more effectively than tests on 'ordinary' mice.

Chimæric mice with humanised immune systems have been important in studying many aspects of HIV infection. For example 'BLT mice'[84] have been used to investigate how HIV infection causes depletion of a form of white blood cell important in the immune response ('T cells', which express a protein called CD4), leaving patients vulnerable to other infections. Studies in these mice have provided evidence that HIV causes this effect by directly infecting CD4-expressing cells, rather than by acting on other cell types. Humanised mouse models have also been used in studies to determine the mechanism of viral spread within the female reproductive tract, and to investigate putative prophylactics.[85]

2.3.4 Chimæras in developing and testing therapeutics

Stem cell treatments are a form of biological therapy (see footnote 55) ultimately intended to treat human patients with human stem cells. However, chimæric animal models are used to develop and to establish the methodologies involved.

Parkinson's disease (PD) is a degenerative disorder of the central nervous system, which involves the loss of multiple populations of nerve cells. Since the early 1980s human fetal tissues have been experimentally transplanted into the brain of patients to replace these lost neurons. These clinical studies have been supported by research in an NHP model, in which grafts of human fetal cells were shown to reverse Parkinsonian-like movement deficits induced by treatment with a neurotoxin. Although early clinical studies showed benefit, subsequent studies indicated a more variable outcome with some patients also experiencing adverse effects caused possibly by the abnormal innervation of the brain by different populations

79 Firoz Mian M, *et al*. (2010). *Humanized mice are susceptible to Salmonella typhi infection*. Cell Mol Immunol **8**, 83–7.
80 'BLT' is an abbreviation for blood, liver and thymus.
81 Ma SD, *et al.* (2011). *A new model of Epstein-Barr virus infection reveals an important role for early lytic viral protein expression in the development of lymphomas*. J Virol **85**, 165–77.
82 Bissig KD, *et al*. (2010). *Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment*. J Clin Invest **120**, 924–30.
83 Lupberger J, *et al.* (2011). *EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy*. Nat Med **17**, 589–95.
84 Immune-deficient mice in which the immune system is humanised through implantation of human bone marrow stem cells, and the tissues of the fetal thymus and liver: see footnote 76.
85 Olesen R, *et al.* (2011). *Immune reconstitution of the female reproductive tract of humanized BLT mice and their susceptibility to human immunodeficiency virus infection*. J Reprod Immunol **88**, 195–203.

of cells within the graft (see 3.3.5). However, the preclinical studies in NHP plus the clinical studies have provided important proof-of-concept for the stem cell therapy and clinical trials that are currently envisaged, and so have been pivotal in the development of this field. Studies aimed at refining treatments involving both NHP and rodent models of PD are underway, and include the improvement of the preparation of the tissue and the way it is implanted in the brain.[86]

Rats engrafted with stem cells have been used to study the potential for repairing damage to the brain caused by stroke. A rat model of stroke has been developed in which the middle cerebral (brain) artery is transiently blocked, causing a loss of blood supply to brain tissue, as occurs in the commonest form of human stroke. The rats subsequently have human stem cells engrafted into the brain. Human neural stem cells derived from a human fetal tissue sample and grown *in vitro*, mesodermal or haematopoietic stem cells derived from bone marrow, or cord blood have been tested.

Typically, a few hundred thousand cells are injected, so that less than 0.001% of the rat's cells are replaced by the human cells. Evidence suggests that some stem cells become integrated in the rat brain, but this may not be necessary to achieve therapeutic effect. The effect of the stem cell treatment is usually evaluated by assessing the rats' behaviour, in tests of sensory or motor performance.[87] Following the evidence gathered in preclinical studies of this kind, stem cell therapies are now being clinically trialled in stroke patients.

Approval for the first trial of a human neural stem-cell-based product in the UK was granted in 2009. A trial in Glasgow is continuing following a positive review of the first patient's progress in December 2010.

In these animal studies, a relatively small proportion of the rat or NHP brain cells are replaced with human-derived cells. Extensions of these methods might involve a greater proportion of cells. We consider the implications of these approaches in Chapter 3.

2.4 Summary

A wide range of genetically altered and chimæric ACHM are in current use in investigational research, as models of disease and in the development, production and testing of therapeutic products. Although there is little public awareness of ACHM (see Box 2.5) their use is long-standing and has made significant contributions across many fields of research. However, the development of animal models of human function and disease is often a gradual process, with models requiring refinement for particular purposes. This can involve iterative research processes spanning several decades. The likelihood of success, and timescales, are difficult to predict. For example the development of humanised monoclonal antibody therapies is one result of over 30 years of intensive research; the development of animals to provide tissue for transplants has not yet yielded clinical benefits after some decades of work.

Box 2.5 Public awareness of research involving ACHM

At the outset of the public dialogue (see Annex III), most participants had little specific knowledge of research involving ACHM, or of the kinds of research that might be possible in the future. However, many participants related such research to other, more familiar approaches (for example the use of animal heart valves transplanted into humans) and were not greatly surprised to learn that such research is taking place.

86 Redmond DE, Jr., *et al.* (2010). *Cellular repair in the parkinsonian nonhuman primate brain*. Rejuvenation Res **13**, 188–94.
87 Pollock K, et al. (2006). *A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke*. Exp Neurol 199, 143–55.

3 Future science and implications

3.1 Introduction

The previous chapter described animals containing human genetic or cellular material (ACHM) and illustrated their use in biomedical research. Techniques that enable the transfer of human DNA sequence and the engraftment of human cells into animals or animal cells are well-established. However, continuing advances in the power of the techniques involved are rapidly extending the range and complexity of animal models that can be created. We anticipate that the use of ACHM will continue to expand, as more sophisticated models of human health and disease are developed.

In this section, we consider selected examples to illustrate possible future research directions. We describe two methodological areas in which developments relevant to the creation of animal–human models are apparent.

1. Genetic engineering methods.
2. Stem cell methods.

We also consider three areas in which future research may be particularly sensitive or approach current social, ethical or regulatory boundaries.

1. Research involving the brain.
2. Research involving the reproductive system.
3. Research involving aspects of human appearance or behavioural traits.

These reflect areas highlighted in the public dialogue (Box 3.1).

Box 3.1 Areas of public interest and concern

Overall, a majority of participants in the public dialogue accepted and were ultimately supportive of research using animals containing human material, on the condition that such research is conducted to improve human health or to combat disease. The considerations taken into account by the public when giving their conditional support will be discussed in more detail in Chapter 5 (see Box 5.1).

For the majority of public dialogue participants, *in vitro* experiments such as the creation of animal–human hybrid cells did not cause concern. However, a very small minority of participants objected to this type of *in vitro* research on animal welfare or religious grounds. Some participants raised concerns around the source and disposal of the human tissues, and the risk of unintended release of material, in *in vitro* experiments.

Participants showed greater concern for *in vivo* experiments, and some found such research unacceptable (see Box 5.2). Participants tended to focus on the overall outcome for the research animal involved, in terms of the animal's welfare, capability, and physical appearance, rather than either the proportion of human and animal cells in the resulting animal or its genetic make-up. Internal manipulations, such as the addition of human liver cells to animals, or the development of humanised organs in animals, were generally accepted. However, three areas of particular sensitivity to participants were identified. These were research involving the brain, reproductive tissues or external features (see Boxes 3.9–3.11).

3.2 Genetic alteration of animals

It is now commonplace to genetically alter animals so that their genomes contain up to a few thousand bases of human DNA sequence (see Box 2.2). As genetic technology advances it is becoming possible to manipulate increasingly large sections of DNA, and to modify DNA sequences with greater accuracy. This ability is markedly increasing the range of transgenic models that can be created.[88]

The development of mice generating humanised monoclonal antibodies (see 2.3.2) is underpinned by the ability to transfer extensive sections of DNA which encode the antibody-producing components of the human immune system. In the *Kymouse*™ model around 3 million base pairs of human sequence (approximately 0.1% of the human genome) including coding regions and other DNA sequences essential for B-lymphocyte (antibody producing white blood cell) function will be transferred.[89] The extent of this substitution means that the *Kymouse*™ more closely models the human immune system than previous models, increasing the diversity of human antibodies which the mouse can produce, from which the most specific can be selected for therapeutic development.[90]

A mouse model of Down's syndrome was developed using a chromosome engineering approach and has the largest addition of human DNA of which we are aware.[91] Cells within these mice contain almost all of human chromosome 21 (around 42 million bases of DNA) replicating the 'trisomy' (additional copy) of this chromosome found in human Down's syndrome. The mouse has been developed to study aspects of Down's syndrome which may be treatable (e.g. early-onset Alzheimer's disease). The abnormal development of the mouse's heart (its 'cardiac phenotype') resembles that of humans with Down's syndrome.[92] The mice have been found to have defective blood vessel growth, which is thought to be important in explaining why both the mouse model and people with Down's syndrome have a low risk of some cancers.[93] These phenotypes are probably caused by the imbalance of multiple genes, and may not have been discovered without the transfer of a very large amount of genetic material.

DNA 'regulatory sequences' control the activity of the protein-coding parts of genes, and influence key aspects of gene function, including when and in which tissues a gene is activated, and how much of its product is made (see Box 2.3). Many diseases are caused by mutations in these sequences. There is substantial evidence that changes in regulatory sequences during evolution can underlie species divergence (see 2.3.1). The study of regulatory function will be a major focus over the next decade. Regulatory sequences are often located at a long distance (tens or hundreds of kilobases away) from the protein-coding part of the gene. However, the ability to move extensive stretches of DNA means that the coding sections of human genes can now be transferred together with their corresponding regulatory sequences. This can result in an animal model in which the human gene under investigation is expressed in a human-specific way (only in relevant tissues and at specific times) meaning that the biological function

88 A range of different techniques have been established to transport DNA from one cell into another. For larger amounts of DNA these include the use of vectors, such as bacterial artificial chromosomes (BACs) (which usually carry DNA constructs of 150–350 kilobases (kb)); yeast artificial chromosomes (YACs) (used to clone DNA fragments of 100–3000 kb, and to express proteins that require post-translational modification); mammalian artificial chromosomes (MACSs) (which can carry tens of megabases of DNA). 'Chromosome engineering' includes a range of techniques used to create modifications of DNA at a whole chromosome level including chromosomal duplications, deletions, inversions, or translocations. These rearrangements can span many megabases of DNA and hundreds of genes.

89 Oral evidence from Bradley, A. For information on the *Kymouse*™ see http://www.kymab.com/index.php?option=com_content&view=article&id=52&Itemid=54.

90 Therapeutic products, such as human antibodies or proteins (see 2.3.2) developed in transgenic animals and intended for human application would be subject to pre-clinical safety testing as 'Biotechnology-derived therapeutic products'. See Guidance from the European Medicines Agency http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002828.pdf

91 O'Doherty A, *et al*. (2005). *An aneuploid mouse strain carrying human chromosome 21 with Down's syndrome phenotypes*. Science **309**, 2033–7.

92 Dunlevy L, *et al*. (2010). *Down's syndrome-like cardiac developmental defects in embryos of the transchromosomic Tc1 mouse*. Cardiovasc Res **88**, 287–95.

93 Reynolds LE, *et al*. (2010). *Tumour angiogenesis is reduced in the Tc1 mouse model of Down's syndrome*. Nature **465**, 813–7.

of the gene is more accurately modelled. The haemoglobin genes and their corresponding regulatory sequences have been intensively studied over recent decades. In one model a 120-kilobase fragment corresponding to the human α-globin region and all its regulators replaced the homologous mouse region so that the mice expressed human α-globin. A regulatory mutation that causes the human blood disorder α-thalassaemia was then recreated in these mice and shown to model the severe human disease accurately.[94,95]

We anticipate that methodological developments will continue to extend the quantity of DNA that can be manipulated.[96] Ultimately, studies may be limited, not by technical challenges, but by the effect of the genetic modifications on the animals involved. For some genes, too much protein product (or its activity) can cause severe defects.[97,98] At a cellular level, when genes from two different species are made within the same cell, as occurs in transgenic animals with human genes, the proteins produced by the different genes need to work together. At very high degrees of transgenesis it seems likely that certain critical human and animal proteins would not interact properly and so compromise the animal's viability.[99] It is known that chromosomes need to 'pair' during meiosis (the special cell divisions that occur in reproductive cell precursors). The presence of a large amount of unpaired DNA, such as a whole extra chromosome, can lead to the failure of meiosis and thus infertility; in the Down's syndrome mouse model the added human chromosome is only transmitted along the female germ line and the male mice are infertile, as are most men with Down's syndrome.[100]

New techniques are enabling models to be created in which the human DNA functions in a more biologically accurate manner. Future developments, for which the α-globin experiment is a forerunner, might include new approaches to:

- Replace (rather than add) genetic material in an animal's genome.
- Control the location in the genome at which copies of transgenes are integrated.
- Precisely control gene expression levels.
- Understand and modify regulatory regions to allow control of temporal and spatial expression of transgenes.
- Translocate sections of human chromosomes onto animal chromosomes.[101]
- Enable germ-line transmission of transgenes (this is currently difficult in some species).[102]

Most transgenic animals carrying human genes are mice; however, gene-targeting methods are now being developed in additional species including the rat and some NHPs, and can in principle be used to introduce human DNA sequence into any animal species (see Box 2.2). There are very few published studies involving transgenic NHPs to date. Early studies reported the creation of a rhesus macaque monkey which expressed a mutant form of the human gene responsible for Huntington's disease, and a marmoset which over-expressed

94 Wallace HA, *et al*. (2007). *Manipulating the mouse genome to engineer precise functional syntenic replacements with human sequence*. Cell **128**, 197–209.
95 Vernimmen D, *et al*. (2009). *Chromosome looping at the human alpha-globin locus is mediated via the major upstream regulatory element (HS-40).* Blood **114**, 4253–60.
96 Written evidence from Wellcome Trust Sanger Institute.
97 Woods KS, *et al*. (2005). *Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism*. Am J Hum Genet **76**, 833–49.
98 Alatzoglou KS, *et al*. (2011). *Increased transactivation associated with SOX3 polyalanine tract deletion in a patient with hypopituitarism*. J Clin Endocrinol Metab **96**, E685–90.
99 If the proteins produced are very similar then too much protein could lead to abnormal phenotypes. Alternatively, even subtle species differences could result in one protein interfering with the function of the other. Gene products, whether proteins or RNA, also function via interactions with other molecules, which can be different proteins, RNA or DNA sequences. A human protein may fail to interact properly with its mouse partner protein or target DNA sequence. It is therefore likely that, if very large amounts of human DNA are incorporated into an animal's genome, one or more of the many human gene products may lead to a deleterious or even lethal phenotype, preventing the establishment of viable transgenic animals.
100 Correspondence from Fisher, E.
101 This approach might enable the development of mouse models containing large sections of human chromosomes with greater viability and stability (e.g. avoiding factors such as the loss of the added chromosome in some tissues over time – creating mosaics). Cell death due to the triggering of an unpaired chromosome in cell division (meiosis) might also be avoided, permitting male germ line transmission of the manipulation.
102 Coors ME, *et al*. (2010). *The ethics of using transgenic non-human primates to study what makes us human*. Nat Rev Genet **11**, 658–62.

(made too much of) the protein 'α-synuclein' to model human Parkinson's disease, although in these models the transgenes did not transmit between NHP generations.[103,104] The introduction of the gene for a protein derived from jellyfish called 'green fluorescent protein' (GFP) into a common marmoset, with germ-line transmission was reported in 2009, and a study in 2010 used viral transfer methods to produce two rhesus monkeys expressing GFP.[105,106,107,108,109] These few reports indicate the imminent possibility of developing transgenic NHP models of human disease, which together with NHP chimæras (see 2.3.4), might be particularly important in studying neurological disorders.

Following recent elucidation of the full genome sequences of many animal species, research is underway to identify sections of the genome that are unique to humans or to our near ancestors. When compared between humans and NHPs, these sections (sometimes called 'human-lineage-specific' sequences) show increases or decreases in the number of copies of a gene, changes in gene sequence (ranging from one or two base pairs, to much larger differences), or altered gene expression patterns. They include genes important in brain development which have been suggested to have a role in the evolutionary enlargement of the human brain.[110,111,112,113]

To fully understand the function of some of these sequences, it is likely to be necessary to insert them into (or delete them from) animals during development, while recognising that this may pose some difficult societal questions. We suggest that manipulation of 'human-lineage-specific' sequences in animals to increase resemblance to the human form, particularly in NHPs, would require particularly careful consideration (see 8.2.2).

3.3 Stem cell research

3.3.1 ACHM and stem cells

The previous section describes modification of animals' genomes to resemble the human, usually by addition of human gene sequence. Creation of chimæras, by mixing human and animal cells, is the second approach that can be used to make ACHM. Many chimæric ACHM are developing using the unique properties of stem cells. These cells can produce specialised (or 'differentiated') cells as well as renewing the stem cell population. These properties enable stem cells to 'colonise' or reconstitute a tissue or organ in a recipient animal.[114] For example, human haematopoietic (blood) stem cells can be grafted into mice, where they replace the mouse immune system with a human–derived (humanised) equivalent (see 2.3.3).[115,116] The rapid recent growth of knowledge about human stem cells is opening many new research

103 Yang SH, *et al*. (2008). *Towards a transgenic model of Huntington's disease in a non-human primate*. Nature **453**, 921–4.
104 Kirik D, *et al*. (2003). *Nigrostriatal α-synucleinopathy induced by viral vector-mediated overexpression of human α-synuclein: a new primate model of Parkinson's disease*. Proc Natl Acad Sci USA **100**, 2884–9.
105 Under certain light, GFP glows and so can be used to 'mark' the cells into which it is integrated, without affecting their function.
106 Chan AW (2004). *Transgenic nonhuman primates for neurodegenerative diseases*. Reprod Biol Endocrinol **2**, 39.
107 Wolfgang MJ, *et al*. (2001). *Rhesus monkey placental transgene expression after lentiviral gene transfer into preimplantation embryos*. Proc Natl Acad Sci USA **98**, 10728–32.
108 Sasaki E, *et al*. (2009). *Generation of transgenic non-human primates with germline transmission*. Nature **459**, 523–7.
109 Niu Y, *et al*. (2010). *Transgenic rhesus monkeys produced by gene transfer into early-cleavage-stage embryos using a simian immunodeficiency virus-based vector*. Proc Natl Acad Sci USA **107**, 17663–7.
110 Evans PD, *et al*. (2004). *Adaptive evolution of ASPM, a major determinant of cerebral cortical size in humans*. Hum Mol Genet **13**, 489–94.
111 Coors ME, *et al*. (2010). *The ethics of using transgenic non-human primates to study what makes us human*. Nat Rev Genet **11**, 658–62.
112 Sikela JM (2006). *The jewels of our genome: the search for the genomic changes underlying the evolutionarily unique capacities of the human brain*. PLoS Genet **2**, e80.
113 Evans PD, *et al*. (2006). *Evidence that the adaptive allele of the brain size gene microcephalin introgressed into Homo sapiens from an archaic Homo lineage*. Proc Natl Acad Sci USA **103**, 18178–83.
114 Unlike many differentiated cell types, such as nerve cells, stem cells retain the ability to divide, producing further stem cells (a process known as self-renewal) as well as cells that go on to specialise.
115 Much of our discussion is focused on stem cells, but we are often using this term loosely also to encompass other progenitor cell types, notably those present in the embryo or fetus, that do not strictly self-renew under normal circumstances. However, their capacity for proliferation and the generation of many differentiated cell types means that they are very similar to stem cells. In addition, their role in promoting growth and development of the embryo can be harnessed in chimæras to substitute tissues in the same way as with stem cells.
116 Haematopoietic (blood) stem cells (HSCs) are found in bone marrow. They are able to self-renew, and to give rise to cells that differentiate into the different forms of blood cells; these include erythroid (red blood) cells and myeloid (white blood) cells such as lymphocytes, which are the key cellular components of the adaptive immune system. Engraftment of human HSCs can therefore be used to reconstitute the immune system of an immune-deficient mouse; these cells colonise the animal giving rise to a 'humanised' immune system.

avenues, based on chimæric animals containing human stem cells.

The same essential properties of stem cells underpin their roles in 'regenerative medicine': the use of cellular therapies to replace damaged or dysfunctional cells in humans (e.g. bone marrow transplants to treat leukaemias or the use of human neural stem cells to repair brain tissue after stroke). The rapidly increasing understanding of stem cell biology is opening up many potential avenues for their use. However, advancement of stem-cell-based treatments is dependent on knowledge of human stem cell biology, and refinement of techniques, which often require prior animal studies.

Stem cell potential

Although much has been learned of the conditions required for the differentiation of several cell types *in vitro*, to fully understand stem cell potential it is still necessary to study them *in vivo* (for further detail on stem cell potential see Box 3.2). Stem cell potential can be assessed to determine either the range of cells a stem cell *normally* gives rise to, or those that it *can* give rise to. The former requires marking a stem cell in its normal location *in vivo* (its 'niche') and following the fate of its progeny over time. The latter can often be explored *in vitro* by varying culture conditions, or *in vivo*, for example by grafting marked stem cells into ectopic sites in an embryo or animal.[117]

It is clearly difficult to conduct such *in vivo* experiments in humans although some information is available, for example after therapeutic grafts of bone marrow cells from a male (XY) donor into a female (XX) host and using Y chromosome DNA as a marker. An alternative is to use animal hosts, although care has to be taken when interpreting results as species differences could affect cell survival or differentiation.

For the *mouse* (and recently for other animals) three techniques have been adopted for testing the potency of stem cell lines such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells in order to classify them as pluripotent:

1. Growth *in vitro*: by changing the culture conditions ES and iPS cells *can* give rise to a wide range of cell types.
2. Growth *in vivo* in ectopic sites: for example when implanted under the skin, the kidney capsule or into the testis of genetically matched or immuno-compromised mice implants grow into tumours called 'teratomas' or 'teratocarcinomas' which *can* contain a wide range of cell types, and can include some organisation into discrete tissue types (see 3.6.1).
3. Growth and ability to contribute to *normal* embryonic development after reintroduction into an early stage embryo, which is implanted into the uterus of a surrogate mother. This method provides a much stricter test of potential, as it is possible to determine whether the cells contribute to all the tissues of the resulting animal, including the germ line. The ultimate test (which is not used routinely) is tetraploid complementation (see 2.2.2), as this shows whether the stem cells are able to give rise to an entire animal.

Stem cell lines

Embryonic stem cells (ES cells, obtained from an animal or human embryo) can be grown in culture and induced to proliferate indefinitely. It is also possible to derive cell lines from certain tissue specific stem cells (e.g. neural stem cells), although this can be difficult. Unlike ES or iPS cells, differentiated cells and tissue-specific stem cells are usually non-tumorigenic. With prolonged culture, cells (whether stem cells or specialised cells) can pick up mutations, which can make them tumorigenic. For clinical purposes, it is important to avoid tumour formation, so the majority of stem-cell-based treatments use either primary cells (e.g. bone marrow, fetal midbrain cells, limbic cells, skin grafts), or cell lines that have been rigorously tested and shown not to lead to tumours in

117 For stem cells that may be in sites that are difficult to access physically, a range of genetic tools exist, especially in the mouse (which rely on cell-type-specific and conditionally activated reporter transgenes), which can be used in the intact animal.

animals.[118] Development of stem cell therapies is thus dependent on ACHM experiments.

Human stem cells

Human stem cells used in research or for cell-based treatments can be obtained from early-stage human embryos, aborted fetuses, cord blood and some adult tissues.[119] Many cell lines have been derived from human tissue-specific stem cells, and around 400 human ES cell lines have also been derived, mainly from embryos donated by IVF patients. There are now also many human iPS cell lines (see Box 3.3) from both normal individuals and patients carrying a genetic disease. It is illegal to perform the strictest test of pluripotentiality using human embryos (the third test of pluripotency, see above) so the term 'pluripotent', when associated with human ES and human iPS cell lines, should be used with the caveat that it is currently only possible to test this by *in vitro* differentiation (the first test of pluripotency, see above) and/or by the ability to make many tissues in teratomas after grafting the human cells into mice (the second test of pluripotency, see above). However, human ES and human iPS cells are thought to have the potential to give rise to all the tissues of a human embryo, and their creation and use are therefore carefully regulated (see 6.2.7). Realising the potential of human stem cells will thus require ACHM experiments.

Box 3.2 Stem cell potential

Different types of stem cell are found in particular tissues and at different stages of development, and these vary in the range of specialised cells they produce. This property (stem cell 'potential' or 'potency'), is often used to group stem cells, as:

- **Unipotent**: able to give rise to a single specialised cell type.
- **Multipotent**: able to give rise to more than one, or many, specialised cell types.
- **Pluripotent**: able to give rise to all cell types of the developing embryo (e.g. ES and iPS cells, see Box 3.3).[120]

However, assessment of stem cell potential is complicated by several factors, including that:

- Stem cells in adult tissues can be largely 'quiescent' (non-dividing).
- The normal potential of a stem cell type in its 'niche' can differ substantially from its behaviour *in vitro* or in an ectopic location.[121]
- Rather than giving rise immediately to specialised cell types, several stem cell types give rise to 'transit amplifying cells' which divide rapidly and often still have several possible fates. These are not 'true' stem cells because they are set on a path to differentiate and therefore do not strictly self-renew. However, the distinction is often blurred and such transit amplifying cells may revert to quiescence and/or a true stem cell state *in vitro* and in some circumstances *in vivo*. Indeed, current thinking is that it may be a question of probability – the further the cell is from its native tissue environment *in vivo* (its 'niche'), the less likely it is to self-renew and the more committed it becomes.

118 To facilitate the growth of certain stem cells, scientists often 'conditionally-immortalise' them using an oncogene (a growth-promoting gene). Such cells could conceivably be tumorigenic unless the activity of the oncogene were turned off. Thus in these lines the gene activity is tightly controlled by a molecular switch. Some such lines are being approved for clinical trials.

119 With appropriate consent.

120 The additional term 'totipotent' is sometimes used in relation to stem cells. For further discussion see Academy of Medical Sciences (2007). *Inter-species embryos*. http://www.acmedsci.ac.uk/p48prid51.html

121 Or marking it and then transplanting a marked cell back to its niche. The 'niche' for adult stem cells is often complex, requiring several cell types, secreted molecules and perhaps even three-dimensional organisation; consequently we do not yet know how to culture many adult stem cell types in a manner that supports their 'normal' differentiation. Nevertheless, several adult stem cell types are successfully grown *in vitro*, and these are already the basis for several human therapies, such as skin cells for burns victims and limbic stem cells to repair corneal damage.

3.3.2 Stem cells in pre-clinical research, and in the development of therapeutics

In previous sections (see 2.2.3) we have outlined how stem cell methodology has contributed to the development of chimæric humanised animals, which are used for a range of research purposes, for example:

- Engraftment of human haematopoietic cells into immune-deficient mice, used to produce mice with humanised immune systems, susceptible to human-specific diseases including HIV and hepatitis (see 2.3.3).
- To test possible treatments for Parkinson's disease (PD), for example, showing that neurons derived from human iPS cells can reverse symptoms in a rat model of PD.[122]

Stem cell technology is opening up new avenues in regenerative medicine. For several decades, bone marrow (and more recently human cord blood) stem cells have been successfully used to replace the bone marrow after treatment for leukaemia, and skin stem cells grown *in vitro* are used to treat burns victims. Limbic stem cells are being used to treat corneal damage, while the replacement or restoration of damaged tissue using human stem cell lines is now being tested for a much wider range of conditions (e.g. stroke). Both human tissue-specific and human ES cells are current candidates for cell-based clinical therapies. Clinical trials using cells derived from human ES cells are currently underway for spinal cord repair and for macular degeneration.[123] Ultimately, it may prove possible to derive iPS cell treatments from a patient's own somatic cells, so avoiding the problems of immune rejection.

Although the eventual aim of such techniques is to introduce human stem cells into human tissues, animal models will increasingly be required to develop the relevant methodologies (potential, dosage, stem cell handling techniques) and to test human stem cell therapies for their efficacy and safety. For example:

- Human neural stem cells, human mesodermal stem cells, or human haematopoietic stem cells have been investigated for efficacy in rat models of stroke (see 2.3.4).
- Human neural stem cells have been investigated in the NHP brain as a prelude to attempts to correct human developmental disorders such as Batten disease.[124]
- Human enteric nervous system stem cells have been investigated in the fetal gut for Hirschprungs disease.[125,126]
- Studies in NHPs have investigated the potential of human neural stem cells in Parkinson's disease.[127,128]

3.3.3 Current boundaries of research involving human–animal stem cell chimæras

ACHM involving human tissue-specific stem cells

Proper understanding of human stem cell biology, especially stem cell potency, can only be obtained through studying human stem cell types *in vivo*.

Whilst the majority of this research has involved adult animals, there are limited reports in which human tissue-specific stem cells have been introduced into animals at early stages of gestation. For example, human haematopoietic stem cells were introduced into fetal goats, and human mesenchymal stem cells (see Box 3.3) into fetal sheep.[129] The outcomes of such

122 Hargus G, *et al*. (2010). *Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats*. Proc Natl Acad Sci USA **107**, 15921–6.
123 Carr AJ, *et al*. (2009). *Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat.* PLoS One **4**, e8152.
124 See Hayden EC (2008). *California institute to help stem-cell biotechs*. Nature **455**, 436–7.
125 Heanue TA & Pachnis V (2011). *Prospective identification and isolation of enteric nervous system progenitors using Sox2*. Stem Cells **29**, 128–40.
126 Schafer KH, *et al*. (2009). *Neural stem cell transplantation in the enteric nervous system: roadmaps and roadblocks*. Neurogastroenterol Motil **21**, 103–12.
127 Muramatsu S, *et al*. (2009). *Multitracer assessment of dopamine function after transplantation of embryonic stem cell-derived neural stem cells in a primate model of Parkinson's disease*. Synapse **63**, 541–8.
128 Emborg ME, *et al*. (2008). *GDNF-secreting human neural progenitor cells increase tyrosine hydroxylase and VMAT2 expression in MPTP-treated cynomolgus monkeys*. Cell Transplant **17**, 383–95.
129 In 2006, Chinese researchers transferred human haematopoietic (blood) stem cells, extracted from cord blood, into fetal goats during gestation. Analysis at 2 years showed that the stem cells were integrated into the goats' tissues (including blood, bone marrow, spleen, liver, kidney, muscle, lung) and were expressing human genes and proteins. The chimæric goats provide an *in vivo* model to study human blood stem cell differentiation. Similar research has been conducted using another form of human stem cells injected into foetal sheep. See Zeng F, *et al*. (2006). *Multiorgan engraftment and differentiation of human cord blood CD34+ Lin– cells in goats assessed by gene expression profiling*. Proc Natl Acad Sci USA **103**, 7801–6.

Box 3.3 Stem cell types

- **Tissue-specific (or adult) stem cells.** Most adult tissues need a supply of new cells to replace those damaged through normal processes of wear. These new cells are derived from 'tissue-specific' stem cells, which usually contribute only to cells of one tissue type (e.g. blood cells, or skin cells, not both). Some are unipotent (e.g. spermatogonial stem cells usually give rise only to sperm), whereas others are multipotent (e.g. haematopoietic (blood) stem cells in the bone marrow give rise to all the cell types of the blood including red and white cells).
- **Mesenchymal stem cells.** MSCs; sometimes called 'marrow stromal cells' are multipotent stem cells that can differentiate into a variety of cell types, including bone, cartilage, and fat. They can be isolated from several tissues, including fat, and bone marrow. They are the most widely used stem cell types in clinical trials.[130]
- **Fetal stem cells.** The developing embryo contains 'fetal stem cells', which can produce specialised cell types during fetal development. Fetal stem cells tend to have broad potential which becomes reduced ('restricted') as development proceeds, and to change their potential over time. The conditions for culturing some fetal stem cells (e.g. neural stem cells from the developing brain) *in vitro* have been determined. Under these artificial conditions, the fetal stem cells can grow essentially indefinitely (for far longer than they exist *in vivo*) while retaining the ability to differentiate.
- **Embryonic stem (ES) cells.** ES cells correspond to cells in the very early embryo, before any restriction has been made to tissue type within the embryo proper. Research, originally in the mouse, demonstrated that ES cells can give rise to all the cell types of the developing embryo and adult mouse; they are therefore considered 'pluripotent'. They can be maintained essentially indefinitely as a self-renewing cell line *in vitro*; however, any such cell type in the embryo must be very short-lived (if they exist there at all), as this corresponds to a period of very rapid development and ES cells cannot be isolated from an embryo once it begins the process of gastrulation.
- **Extra-embryonic stem cell types.** In the mouse, it is possible to derive stem cells that correspond to the two extra-embryonic stem cell types of the late blastocyst, trophoblast stem cells and extra-embryonic endoderm stem cells. These are able to differentiate into cell types of the placenta and yolk sac respectively, but not to cells of the embryo proper.

Other stem cell types with broad potential.

- **Embryonic germ (EG) cells** can be derived from primordial germ cells (which are normally fated to give eggs or sperm) isolated from embryonic gonadal precursors. EG cells are very similar to ES cells in their potential. Those from the mouse can contribute to normal development after injection into host embryos, and give rise to teratocarcinomas after injection into ectopic sites.[131]
- **Spermatogonial stem cells** (male germline stem cells) are tissue-specific stem cells present from early postnatal stages in the testis. Their self-renewal and differentiation in adulthood enable continuous production of sperm. When grown in specific culture conditions, a minority of spermatogonial stem cells transform into ES-like cells (in a process that may mimic the origin of spontaneous testicular teratocarcinomas).
- **Amniotic stem cells,** obtained by amniocentesis, have a broad potential, variously described as multipotential or pluripotential (although they do not fulfil all the criteria for this as outlined above). They are being investigated as a source of cells for therapies.

130 For examples of clinical trials involving mesenchymal stem cells see: http://www.osiris.com/clinical.php and http://www.nature.com/stemcells/2008/0804/080410/full/stemcells.2008.55.html

131 Because genomic imprinting is erased in the germ line, both of these germ cell-derived stem cell types may not be useful for obtaining certain functional specialised cell types.

- **Cord blood stem cells** are found in umbilical cord blood. Their potency is not yet fully understood. Although they are similar to haematopoietic (blood) stem cells (HSCs), several reports suggest they may be able to give rise to a wider range of cell types, and they probably include a population of MSC-like cells. They have even been reported to give rise to some neurons *in vitro*, although claims that they can do so *in vivo* are controversial.[132] They have been used in treatment to replace bone marrow and blood cells in conditions such as leukaemia since the 1990s.
- **Induced pluripotent stem cells** (iPS cells), do not occur naturally, but are created artificially by 'reprogramming' other cell types, such as adult body somatic cells (e.g. skin cells). For example, iPS cells have been derived by transfection (adding in) of certain genes into adult fibroblasts.[133] iPS cells were first produced in 2006 from mouse cells and from human cells in 2007. Their properties are broadly similar to ES cells; however, individual lines vary in their properties, which may reflect incomplete reprogramming, and some genetic or chromosomal damage. It is not known how relevant these differences will be to their clinical use. For the time being, ES cells are viewed as the 'gold standard' to which iPS cells should be compared. However, iPS cells are very important for research into genetic diseases, in cell culture or after introduction into animals, because they can be derived from specific patients. They are already being used in screens for drugs.

experiments are currently unpredictable. As the human stem cells are merged into the animals at an early stage, there is greater potential for the stem cells to contribute to a wider range of tissues, and there is little control over the types of tissue likely to incorporate the human stem cells. Although the stem cell types involved (haematopoeitic, mesenchymal) were thought to be tissue specific, the actual potential of the stem cells could not be taken for granted before these studies were undertaken.

ACHM involving human embryonic stem cells

It is now technically possible to make animal–human chimæras involving the engraftment of human ES cells into animal embryos. We are aware of only a small amount of such research to date (and this is largely unpublished); however, the development of human ES cell lines, and new approaches to create human pluripotential stem cells, open the way for more work of this kind.

In 2006, researchers claimed that human ES cells could engraft into mouse blastocysts, where they proliferated and differentiated for a few days when these embryos were maintained *in vitro*.[134] However, very few human cells were found within post-gastrulation stage embryos after transfer into surrogate mice, suggesting that the human cells were at a disadvantage compared with the surrounding mouse cells.[135,136] If such chimæras were allowed to be born, it is highly likely that they would have very few or no surviving human cells in most of their tissues. However, because the earlier in development human cells are introduced, the less predictable is the outcome, it remains possible that human cells may not be at a disadvantage in all tissues, so human cells could make a significant contribution to a few cell types in a live-born animal.[137] This might be even more likely if the specific mouse cell types were themselves compromised or eliminated (e.g. similar to the way that mice

132 Bicknese AR, *et al*. (2002). *Human umbilical cord blood cells can be induced to express markers for neurons and glia*. Cell Transplant **11**, 261–4; Lim JY, *et al*. (2011). *Neural differentiation of brain-derived neurotrophic factor-expressing human umbilical cord blood-derived mesenchymal stem cells in culture via TrkB-mediated ERK and β-catenin phosphorylation and following transplantation into the developing brain.* Cell Transplant. *In press*.

133 Notably *Oct4*, *SOX2, K1f4* and *cMyc,* all transcription factors characteristic of pluripotent cells.

134 James D, *et al*. (2006). *Contribution of human embryonic stem cells to mouse blastocysts*. Dev Biol **295**, 90–102.

135 Gastrulation is a phase early in the embryonic development of most animals, during which the single layer of cells called the blastula (or in higher vertebrates the epiblast), is reorganised into a three-layered patterned structure that will go on to form the three primary tissues of the embryo proper (ectoderm, mesoderm, endoderm). In human embryonic development it begins at around 14 days after fertilisation, in the mouse at about 7 days.

136 NHP ES cells have recently been shown also to contribute poorly to early mouse embryos; see Simerly C, *et al*. (2011). *Interspecies chimæra between primate embryonic stem cells and mouse embryos: Monkey ESCs engraft into mouse embryos, but not post-implantation fetuses.* Stem Cell Res **7(1)**, 28-40.

137 See discussion on mice with human immune system or liver 2.3.3.

with human livers or a human immune system are made, or as demonstrated in mice carrying ES-cell-derived rat pancreas (see 3.3.4)).

One concern associated with such studies is that human ES cells may contribute to the germ-line cells in the chimæric mouse, resulting in a mouse with human-derived reproductive cells (see 3.4). In theory, if such an animal were to be bred its offspring could be 'true hybrids'; or if two such animals were to breed, this could result in the fertilisation of a human egg with human sperm. Specific regulation of such experiments is recommended (see 8.2.3).

The evolutionary distance between mouse and humans, and the significant difference in rates of cell division between most human and mouse cell types (human cells are generally significantly slower, which puts them at a competitive disadvantage in a rapidly growing embryo) reduces the chance of human cells surviving in the chimæras. However, if the animal component is one where human cells are less disadvantaged (e.g. as perhaps evidenced by the experiments involving human stem cells introduced into fetal goats), and particularly if NHPs are used, then the concern may increase significantly (see 8.2.2).

3.3.4 Future directions in stem cell research

Several new sources of stem cells are being investigated. iPS cells can be derived and grown essentially indefinitely, from any individual. They provide a novel way to study human genetic disease, where the iPS cells are directed (*in vitro* or in animals) to differentiate into the affected cell type. These can then be used to study the detailed pathology of the disease and to search for treatments. Extending the idea behind iPS cells, several groups are exploring the possibility of direct cell reprogramming, to go from one adult cell type to another.[138,139,140,141]

New imaging techniques are being applied to stem cell biology that will allow increasingly sophisticated observation of cell behaviour *in vivo*. Ultrasound imaging can guide instruments to introduce cells (or DNA) into precise locations within embryos developing *in utero*.[142,143,144,145] This can be done, for example, at early stages of mouse embryos when the developing organs are first seen.[146] Cells to be introduced can be labelled such that that their fate can be followed *in vivo*, using MRI, bioluminescence, fluorescence, positron-emission tomography (PET) scans and X-rays.[147,148,149,150,151,152] Fluorescence imaging allows single cells (or subcellular components, such as nuclei, chromosomes, cell membranes), to be followed after labelling with variously coloured fluorescent proteins.[153,154]

Gene activity can now be manipulated, even within single cells (transplanted or host) within an animal. This can be achieved by, for example, using gene-targeting methods (see Box 2.2) to allow genes to be switched on or off by, for example, a drug, temperature

138 Zhou Q, *et al*. (2008). *In vivo reprogramming of adult pancreatic exocrine cells to β-cells*. Nature **455**, 627–32.
139 Ieda M, *et al*. (2010). *Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors*. Cell **142**, 375–86.
140 Efe JA, *et al*. (2011). *Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy*. Nat Cell Biol **13**, 215–22.
141 Kim J, *et al*. (2011). *Direct reprogramming of mouse fibroblasts to neural progenitors*. Proc Natl Acad Sci USA **108**, 7838–43.
142 Nieman BJ & Turnbull DH (2010). *Ultrasound and magnetic resonance microimaging of mouse development*. Methods Enzymol **476**, 379–400.
143 Pierfelice TJ & Gaiano N (2010). *Ultrasound-guided microinjection into the mouse forebrain in utero at E9.5*. J Vis Exp **13**, 45.
144 Olsson M, *et al*. (1997). *Specification of mouse telencephalic and mid–hindbrain progenitors following heterotopic ultrasound-guided embryonic transplantation.* Neuron **19**, 761–72.
145 Wichterle H, *et al*. (2001). *In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain*. Development **128**, 3759–71.
146 These are known as organ primordia.
147 Modo M (2008). *Noninvasive imaging of transplanted cells*. Curr Opin Organ Transplant **13**, 654–8.
148 Daadi MM, *et al*. (2009). *Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain*. Mol Ther **17**, 1282–91.
149 Bible E, *et al*. (2009). *The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles*. Biomaterials **30**, 2985–94.
150 Srinivas M, *et al*. (2010). (19)F MRI for quantitative in vivo cell tracking. Trends Biotechnol **28**, 363–70.
151 Daadi MM, *et al*. (2010). *Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic–ischemic brain injury*. Stroke **41**, 516–23.
152 Seiler MJ, *et al*. (2010). *Three-dimensional optical coherence tomography imaging of retinal sheet implants in live rats*. J Neurosci Methods **188**, 250–7.
153 Udan RS & Dickinson ME (2010). *Imaging mouse embryonic development*. Methods Enzymol **476**, 329–49.
154 Vermot J, *et al*. (2008). *Fast fluorescence microscopy for imaging the dynamics of embryonic development*. HFSP J **2**, 143–55.

or even light.[155] These methods allow the function of endogenous genes to be assessed, but they can also be used to manipulate cell behaviour, including migration, proliferation and cell death. Many of these techniques are still technically challenging, and most have been applied only in the mouse, but the availability of stem cells will allow much of this to be applied to other species. Indeed this is already occurring with NHP and human stem cells and their differentiated derivatives, and their use in ACHM is likely to increase rapidly.

A recent study showed that rat iPS cells injected into mouse blastocysts lacking the *Pdx1* gene required for pancreas formation, were able to form a fully functional (rat) pancreas in the resulting mice. This is similar in concept to the methods used to derive mice with a human immune system or liver (see 2.3.3), but shows that it can be done with tissues that do not normally regenerate, if the donor cells are introduced at a sufficiently early stage. The availability of human stem cells and sophisticated ways to genetically manipulate host embryos and animals may eventually make it possible to humanise any specific tissue or body system. This could even include parts of the brain, although the challenge of generating functional circuits in rodents from human cells is formidable.

Tissue engineering is also a rapidly expanding discipline, where artificial material or tissue-derived matrices are used to support cells *in vitro* or *in vivo*. Sophisticated chemistry and optical 'etching' techniques can be used to pattern artificial matrices, such as 'Matrigel™', to create three-dimensional substrates that can then be seeded with cells, including stem cells. These can be made to form tissue-like structures, with cells in the correct arrangement, including blood vessels or other structures.[156] These entirely artificial structures could perhaps in future be used to replace lost or damaged tissue or perhaps to decrease dependence on animal models for research.[157]

Decellularised matrix (the extracellular protein and other molecules that comprise the support for cells within a tissue) has been found to have patterning information, such that when re-seeded with a mixture of the appropriate cells (or stem cells) for the tissue from which they were obtained, they can reconstitute a functional tissue or organ. These are already being used clinically to replace small sections of tissue lost through trauma or cancer, e.g. of bladder, ureter and trachea.[158,159,160,161] It may become possible to use such techniques to rebuild more complex organs and tissues, such as the heart, or parts of the brain.[162] To show that these engineered human structures are functional and safe will require testing in animals.

3.3.5 Current boundaries and controversies in stem cell research and application

We discuss below several areas currently under intense investigation in the development of potential therapies based on stem cells. The resolution of almost all of these issues will most likely involve testing of human cells in animals.

Sources of stem cells

There is considerable debate about how 'good' each stem cell type (e.g. ES, fetal, cord blood, adult, iPS) is with respect to research and therapeutic potential (including safety). It

155 Recently developed 'optogenetic' techniques use light to trigger genetic or molecular changes, and are increasingly being applied to study neural function and connectivity because they can be used not only to mark cells, but also to induce activity or inactivity of ion channels, nerve conductance and synaptic function. See Kravitz AV & Kreitzer AC (2011). *Optogenetic manipulation of neural circuitry in vivo*. Curr Opin Neurobiol; Tonnesen J, *et al*. (2011). *Functional integration of grafted neural stem cell-derived dopaminergic neurons monitored by optogenetics in an in vitro Parkinson model*. PLoS One **6**, e17560; Carter ME & de Lecea L (2011). *Optogenetic investigation of neural circuits in vivo*. Trends Mol Med **17**, 197–206.

156 Moon JJ, *et al*. (2010). *Biomimetic hydrogels with pro-angiogenic properties*. Biomaterials **31**, 3840–7.

157 Bible E, *et al*. (2009). *The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles*. Biomaterials **30**, 2985–94.

158 Macchiarini P, *et al*. (2008). *Clinical transplantation of a tissue-engineered airway*. Lancet **372**, 2023–30.

159 Orlando G, *et al*. (2010). *Regenerative medicine applied to solid organ transplantation: where do we stand?* Transplant Proc **42**, 1011–3.

160 Tian H, *et al*. (2010). *Differentiation of human bone marrow mesenchymal stem cells into bladder cells: potential for urological tissue engineering*. Tissue Eng Part A **16**, 1769–79.

161 Badylak SF, *et al*. (2010). *Whole-Organ Tissue Engineering: Decellularization and Recellularization of Three-Dimensional Matrix Scaffolds*. Annu Rev Biomed Eng. *In press*.

162 Iyer RK, *et al*. (2011). *Engineered cardiac tissues*. Curr Opin Biotechnol. *In press*.

seems likely that each source of cells will find specific applications depending on the tissue and the problem to be solved.

In developing cell based therapies it is important to consider whether it is more effective to transplant stem cells, or a form of cell derived from them (e.g. transit amplifying cells, committed progenitors or fully differentiated cells). The most effective approach is likely to be specific to the tissue requiring repair. For example, tissue-specific stem cells are likely to be better where production of many new cells is required over an extended period (e.g. to make new skin), while in other cases, such as the retina, there is evidence that post-mitotic (i.e. no longer dividing) cells are necessary.[163,164]

Selection and derivation of cells

It can be difficult to obtain specific cell types from some stem cells. Certain differentiation protocols (e.g. specific growth factors or inhibitors added to the cultures) can be used that favour the production or survival of one cell type over another. Antibodies that recognise molecules on the cell surface can be used to select for or against specific cell types.[165]

With the mouse (and increasingly for other animals) it is often possible to genetically engineer ES cells (or the animal from which the stem cells are to be derived) to introduce a marker gene (e.g. encoding a protein that is fluorescent or confers drug-resistance), to allow purification of the relevant cell type *in vitro*. Clearly it is not an option to genetically engineer humans for this purpose, and introducing marker genes directly into stem cell types including human ES and iPS cells, is often difficult. In addition, regulatory authorities are concerned about the use of modified cells as each alteration carries a risk of damaging an endogenous gene, perhaps promoting cancer. Demonstrating the safety of a cell line is costly and time-consuming, and though this might be justifiable where a single cell line could treat many patients, in other cases, especially for 'personalised' treatment, it may prove a barrier.

Compared with other stem cell types, pluripotent stem cells grow well in culture and have greater potential, allowing many different cell types to be derived from a single source. While an advantage in many respects, and essential if the cell type in question is specified relatively early in the embryo (e.g. motor neurons), this can cause difficulty in separating out the required cell type. It has been difficult to use *in vitro* differentiation of pluripotent cells to obtain fully mature functional cells, even if these are grafted into an appropriate *in vivo* site, but because we know that mouse pluripotent stem cells can form functional tissue in chimæras or even give rise to entire adult mice, any inability to obtain mature cell types possibly reflects our current lack of knowledge, rather than an intrinsic problem of the pluripotent stem cells.[166,167,168] In contrast, adult stem cells are thought to be better able to give mature cell types, but such stem cells are often difficult to isolate and grow *in vitro*. This may again reflect limitations in our understanding, but in some cases it could be due to an intrinsic property of the adult stem cells, which are often largely quiescent in their niche *in vivo.*

Risks of therapeutic uses of stem cells

The risk of having abnormal cell types (especially cancer-causing cells) present within a stem cell line, varies according to stem cell type. Even a single ES cell is able to give rise to a teratocarcinoma, so any protocol to derive cells for transplant has to be very efficient at removing these.[169,170] Various protocols have been established for trials based on ES cell-derived cell types (notably oligodendrocyte precursors

163 Lapouge G & Blanpain C (2008). *Medical applications of epidermal stem cells.*
164 West EL, *et al*. (2009). *Cell transplantation strategies for retinal repair*. Prog Brain Res **175**, 3–21.
165 This can be done with techniques such as fluorescence-activated cell sorting, magnetic bead separation or complement-mediated cell killing.
166 Mignone JL, *et al*. (2010). *Cardiogenesis from human embryonic stem cells*. Circ J **74**, 2517–26.
167 Vidarsson H, *et al*. (2010). *Differentiation of human embryonic stem cells to cardiomyocytes for in vitro and in vivo applications*. Stem Cell Rev **6**, 108–20.
168 We know that certain cell types have fetal and adult forms, where the latter only arise postnatally from an undifferentiated precursor (e.g. blood stem cells and Leydig cells), and protocols developed to date may favour isolation of the fetal rather than the adult cell type.
169 Blum B & Benvenisty N (2009). *The tumorigenicity of diploid and aneuploid human pluripotent stem cells*. Cell Cycle **8**, 3822–30.
170 Lindgren AG, *et al*. (2011). *Loss of Pten causes tumor initiation following differentiation of murine pluripotent stem cells due to failed repression of Nanog*. PLoS One **6**, e16478.

for acute spinal cord repair and pigmented retina cells for macular degeneration). With the notable exception of spermatogonial stem cells, there is little or no risk of teratocarcinomas from tissue-specific stem cells, especially when these are obtained from adults; however, these may show signs of ageing (such as short telomeres and somatic mutations), and therefore have an increased risk of carrying tumour-promoting genetic abnormalities compared with an embryonic cell type.

Potential risks associated with iPS cells are even greater. If derived from an adult cell, they could carry mutations. The iPS cells are as efficient as ES cells at making teratocarcinomas. Moreover, there are some concerns about incorrect reprogramming and genetic damage in iPS cells.[171,172] Incomplete reprogramming appears to be common, and can result in the iPS cells retaining a 'memory' of the starting cell type, which might compromise their ability to differentiate into the desired cells.[173] The factors added to reprogramme the cells are often oncogenic (tumour-promoting); moreover, they turn cells that may be relatively quiescent into ones that divide rapidly, which can lead to 'replicative stress' and to chromosome abnormalities and other mutations. The original methods to obtain iPS cells relied on the integration of retroviral vectors carrying the four reprogramming genes[174], and this could also lead to mutation of endogenous genes. New methods to induce reprogramming without integration of vectors will overcome this problem, but not necessarily others associated with the process.[175,176,177]

Regardless of the source of stem cells, there are issues related to the quantities of cells required for therapies. Problems of 'scale-up' include risks of contamination and the appearance of mutations giving a replicative advantage, where the latter may be associated with a loss of function and increased cancer risk.[178]

Another important issue with respect to source of stem cells to be used for transplants is how to avoid immune rejection. Some organs such as the central nervous system are thought to be sufficiently hidden from the immune system ('privileged' sites) that tissue (human leukocyte antigen (HLA)) matching is not essential. However, this may not be entirely true and immune damage may confuse the interpretation of results.[179] For other organs and tissue types, immune rejection is a clear problem. To overcome this a variety of options is being explored including immune suppressants, inducing tolerance or use of closely HLA-matched or even autologous cell sources (derived from the patient to be treated).[180,181] The last option can include tissue-specific stem cells (assuming that there are sufficient remaining in the patient to be useful), direct reprogramming or iPS cells, even though personalised treatments are costly and a regulatory challenge. There are efforts to derive a minimal set of ES and iPS cells that would allow at least majority of patients to be treated with closely matched cells; however, many hundreds of lines would still not cover more than 90% of people.

How should potential stem cell therapies be tested?

Rigorous assays of quality, safety and efficacy are a regulatory requirement for all treatments, including those based on cell lines. Until there are validated *in vitro* surrogates, it is likely that human stem cells and their differentiated derivatives will need to be tested in appropriate

171 Hussein SM, *et al*. (2011). *Copy number variation and selection during reprogramming to pluripotency*. Nature **471**, 58–62.
172 Howden SE, *et al*. (2011). *Genetic correction and analysis of induced pluripotent stem cells from a patient with gyrate atrophy*. Proc Natl Acad Sci USA **108**, 6537–42.
173 Barrero MJ & Izpisua Belmonte JC (2011). *iPS cells forgive but do not forget*. Nat Cell Biol **13**, 523–5.
174 See Footnote 125
175 Chen G, *et al*. (2011). *Chemically defined conditions for human iPSC derivation and culture*. Nat Methods **8**, 424–9.
176 Okita K, *et al*. (2011). *A more efficient method to generate integration-free human iPS cells*. Nat Methods **8**, 409–12.
177 Anokye-Danso F, *et al*. (2011). *Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency*. Cell Stem Cell **8**, 376–88.
178 Olariu V, *et al*. (2010). *Modeling the evolution of culture-adapted human embryonic stem cells*. Stem Cell Res **4**, 50–6.
179 Chen Z, *et al*. (2011). *MHC mismatch inhibits neurogenesis and neuron maturation in stem cell allografts*. PLoS One **6**, e14787.
180 Lui KO, *et al*. (2009). *Embryonic stem cells: overcoming the immunological barriers to cell replacement therapy*. Curr Stem Cell Res Ther **4**, 70–80.
181 Autologous transfer refers to the movement of cells or tissue from one part of the body to another in the same individual.

animal models. As outlined in 3.3.4, there are now many ways to follow transplanted cells in live animals. Consideration of the type of animal model is appropriate: rodents, large animals, such as pigs or sheep, or NHPs? This may depend on the body system to be treated, where the animal model is chosen according to the similarity in physiology and/or size and complexity of the relevant organ to that of humans. However, this might seem to imply that NHPs should always be the model of choice for assessing treatments for brain disease or trauma. Most studies so far have made use of rodent models, and these seem appropriate to give at least general answers – such as can the cells engraft, do they promote any functional repair? However, they may not predict what will happen in a more complex brain.

How should clinical trials be conducted?
There are still relatively few clinical applications for stem cell transplants. Protocols have been established for conducting trials of applications relying on bone marrow or cord blood stem cells, which can be introduced into the circulatory system, as well as some that involve grafts to surface epithelia (skin and cornea). For many other tissues it is less clear how to introduce cells, and how to design clinical trials. The problem is perhaps most acute for the central nervous system. Grafts of fetal brain cells to people with Parkinson's disease provide an interesting case history, where extensive preclinical data in animal models led to some promising first-in-man experiments, but then larger trials gave results that were conflicting and hard to interpret, in part because of significant variation in the protocols used (see also 2.3.4).[182,183,184]

Some of the questions that need to be considered are:

- Should the trials be double-blind? If so, what treatment should control patients receive?
- How should patients be chosen: likelihood of benefit, age or whether terminally ill?
- How will transplanted cells be followed in the patients: through short term labels, through genetic engineering to introduce markers for *in vivo* imaging, or post-mortem?
- Should the stem cells be engineered to enable them to be destroyed in some way, in case something goes wrong?

Ethical and societal problems
There are several ethical and social issues that affect work in this area, such as the question of the ownership of stem cells and patent rights to procedures involving them; questions about the proper use of these cells, not merely to cure disease or trauma but also to extend life span and as a route to genetic enhancement; and, fundamentally, questions about the acceptability of research that uses human embryonic stem cells. Because these issues are not specific to work involving ACHM and have been much debated elsewhere, we have chosen not to pursue them here. For further discussion of these issues, see reports from the Hinxton Group (see 7.4.2).[185]

3.4 Research involving the brain

Many animal models of human diseases involving the brain have been developed. These include transgenic mice used to study prion diseases and dementias.[186] A few transgenic NHP models have also been developed (see 3.2). Attitudes to research involving the brain expressed in the public dialogue are summarised in Box 3.9.

Chimæric models that involve the implantation of human neural stem cells into an animal's brain are already used in research. For example, rats engrafted with human neural stem cells are used to study the potential of these cells for repairing damage caused by stroke (2.3.4).[187] In research to develop treatments for

182 Brundin P, *et al*. (2010). *Neural grafting in Parkinson's disease. Problems and possibilities*. Prog Brain Res **184**, 265–94.
183 Dunnett SB, (2010). *Neural transplantation. Handbook of Clinical Neurology* **95**, 885–912.
184 Loewenbruck K & Storch A (2011). *Stem cell-based therapies in Parkinson's disease: future hope or current treatment option?* J Neurol **258**, S346–53.
185 See www.hinxton group.org and Caulfield T, *et al*. (2010). *Stem cell research policy and iPS cells*. Nat Methods **7**, 28–33.
186 Prion diseases include conditions such as variant Creutzfeldt–Jakob disease (vCJD), a human neurodegenerative condition.
187 Pollock K, *et al*. (2006). *A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke*. Exp Neurol **199**, 143–55.

Parkinson's disease, NHPs with Parkinsonian-like brain lesions have had human neural stem cells implanted in their brains. These studies have provided understanding of the ways in which stem cells migrate towards the sites of damage in the primate brain.[188] We are not aware of evidence that the addition of human-derived cells into an animal's brain in studies of this kind has resulted in any obvious changes in the cognitive abilities of the animals involved.

We have described methods whereby cells within an animal's organ, such as the liver, can be replaced by human cells (see 2.3.3). Equivalent studies involving the brain have been the subject of considerable ethical discussion. The predominant question is whether populating an animal's brain with human-derived cells could result in the production of an animal with human 'cognitive capacity' (i.e. some aspect of 'consciousness', 'awareness' or 'sentience') or 'human-like' behavioural capabilities.[189]

In 2000, Dr Irving Weissman (at Stanford University, USA) proposed an experiment to create what has become known as the 'human neuron mouse', which would involve a far greater degree of substitution of the mouse brain with human-derived cells. The proposal was to use mice with a condition causing death several days before birth owing to the loss of most or all of the developing neurons in the fetal mouse brain. Weissman suggested transplanting human brain stem cells into the fetal mice, just as their own neurons were dying, with the intention of producing a mouse with a functional brain made up of mouse glial (supporting) cells and human neurons, to enable the study of human neurons *in vivo*. The proposed experiment was voluntarily subjected to ethical analysis by an independent study group, (led by Professor Greely, Stanford University, USA) which recommended that the experiment could be performed ethically.[190] However, the experiment has not as yet been performed.[191]

The balance of opinion on the working group is that, even if an experiment of this type produced a functional brain, it would be very unlikely to result in a mouse with human cognitive characteristics, as a mouse brain is much smaller and could not develop the complex interconnections that occur in human brains. It would lack much of the sensory input (e.g. through the visual system) received by the human brain and the distinctive motor outputs that characterise human motor behaviour. As one submission to our study indicated, *'If these cells do make effective connections then the signals that pass through them will be the signals of the host. Thus human nerve cells within a mouse would receive signals from the mouse's sensory organs (e.g. auditory signals about high frequency sound, vision adapted to dim illumination but not colour, touch from whiskers and olfactory input from the mouse's sensory world). Conversely, these cells would link to cells controlling the movement of four legs, and not to human hands or facial movement (speech).'* [192] The extent to which mouse glial cells could support normal human neural function is also undetermined. The development of human capacities of sentience and cognition are also crucially dependent on developmental pathways, from conception to adulthood, which would obviously be fundamentally different in a rodent model. However, the precise effects of this modification on the animal's phenotype cannot be fully

188 Bjugstad KB, *et al*. (2008). *Human neural stem cells migrate along the nigrostriatal pathway in a primate model of Parkinson's disease*. Exp Neurol **211**, 362–9.

189 In its broadest sense, human 'cognition' can be defined as the 'faculty of knowing', to include aspects such as knowledge, reason, intelligence, understanding, sensation, perception and conception (as distinguished from feeling and volition). In 3.4.1 we describe how experimental measures could act as a proxy for assessment of human 'cognition' in animals.

190 The original ethical analysis is unpublished; however, its findings were summarised in Greely HT, *et al*. (2007). *Thinking about the human neuron mouse*. Am J Bioeth **7**, 27–40. The group examined the potential costs or risks of the experiment, considered factors to mitigate these, and weighed risks against the possible benefits. Risks included the source of the human brain stem cells from aborted human fetuses; potential for pain and suffering to the mice; propriety of this use of human tissues; risks of conferring some degree of humanity on another species; risks to public support of science. Benefits focused on the potential uses of animal containing human neurons for basic science and for clinical applications. The group concluded that the experiments could proceed ethically, subject to careful staging and monitoring. Recommendations included that human brain stem cells only be used with appropriate consent; the experiments should be performed in stages and should be carefully monitored; the experiments should be done in an open manner with appropriate information conveyed to the press; the mice should be disposed of appropriately and should not be allowed to breed.

191 Greely HT, *et al*. (2007). *Response to open peer commentaries on "Thinking about the human neuron mouse"*. Am J Bioeth **7**, W4–6.

192 Written evidence from Parker, A.

predicted without more experimental evidence.

The potential consequences of a similar experiment conducted in a larger animal, for example a sheep or pig are more debatable; even more so in an NHP, which has sensory and motor capabilities more similar to the human. If an NHP modified in some such way came to approximate the cognitive capacity of a Great Ape (common chimpanzee, bonobo, orang-utan and gorilla), would it no longer be deemed appropriate for use in experimentation, given that research on Great Apes is not currently permitted in the UK (see 5.6 and Box 6.1)?

In 2005, a multi-disciplinary working group considered ethical issues arising from the transplantation of human neural stem cells into the brains of NHPs.[193] This group concluded that such research should minimise the risk that an animal would develop human-like cognitive capacities, and it set out a series of factors that should be considered in reviewing proposals for such research (Box 3.4). Analogous questions could be asked about transplantation of NHP neural stem cells into other animals.

3.4.1 Approaches to assessing alteration in cognition

It is difficult to predict confidently the outcomes of experiments such as those described above, until further evidence is available. However, we can begin to consider which aspects of brain function might be considered particularly 'human', and how these could be monitored. Measures of this kind could perhaps act as a proxy for human 'sentience' and provide a practical basis for assessing change within such chimæric brains.

Neuroscience has made important advances in defining aspects of brain function and in developing methods to assess these functions in humans and other species. Although we intuitively think of human brain function and 'thought' as unique to humans, studies indicate that human and animal brain function have much in common. Some relatively sophisticated aspects of brain function are evident in a range of mammalian species (see Box 3.5).

Box 3.4 'Moral issues of human-non-human primate neural grafting' [194]

In relation to the introduction/integration of human neural stem cells into NHP brain, Greene *et al*. concluded *'we support the National Academy's recommendation that human-NHP neural grafting experiments be subject to special review'* and recommended that *'experiments involving human-NHP neural grafting be required, wherever possible, to look for and report changes in cognitive function. Explicit data collection on cognition and behavior will help to ensure that ethical guidelines can be developed appropriately as the field advances.'*

The group proposed *'six factors that research oversight committees and other review groups should use as a starting framework'.* These were:

1. The proportion of engrafted human cells.
2. The stage of neural development.[195]
3. NHP species.
4. Brain size.
5. Site of integration.
6. Brain pathology.[196]

193 Greene M, *et al*. (2005). *Ethics: moral issues of human–non-human primate neural grafting*. Science **309**, 385–6.
194 *Ibid.*
195 Higher proportions of engrafted cells were considered likely to be achieved by implantation early in neural development; such cells were also considered likely to have greater functional influence.
196 The condition of the recipient brain might affect the influence of the graft – for example damage to neural structures in adult animals, intended to model neurological disease, might give greater scope for engrafted human cells to colonise and in turn effect cognitive capacities. However, such models would also be impaired, and so perhaps less likely to acquire human-like function.

In limited areas, cognitive abilities of some animals approach, or arguably even exceed, those of humans. Macaque monkeys are the most commonly used experimental NHPs. Visual memory (identification of objects that they have, or have not, seen previously) is highly developed in macaques, and they can out-perform people with Alzheimer's disease and even many healthy adults in some tests.[197] This kind of memory in macaques can also be enhanced, for example by cognitive-enhancing drugs such as AMPA-kines. Enhancement of other functions can be achieved through behavioural approaches (e.g. Japanese monkeys have been shown to acquire the ability to use sensory tools such as endoscopes, through training).[198,199] We distinguish between this type of *quantitative shift* in existing animal cognitive capacities and *qualitative change* towards 'uniquely human' capacities. Merely demonstrating quantitative enhancement of one aspect of an animal's cognitive function does not imply its cognitive capacity is approaching that of the human. Conferring an increase in cognitive capacity on an animal through the addition of human cells or DNA would not necessarily hold any greater significance than equivalent effects obtained through drug or behavioural manipulation.

Certain aspects of brain function are, however, only evident in humans and others are mainly present in humans and marginally in the Great Apes. In these areas, we can begin to identify the types of brain function that may distinguish humans from other species (see Box 3.6).

Box 3.5 Set shifting in humans, primates and rodents

The Wisconsin card-sorting test is used in human neuropsychology. It measures the subject's ability to sort cards according to given rules (e.g. by the colour, shape or number of objects on the card) on the basis of feedback – and importantly to *adapt* as the rules are changed. The test has been described as an assessment of 'set-shifting' ability, which may be considered a form of 'executive function' (higher brain processes associated with planning and abstract thinking). Normal human subjects adapt quickly, but people with brain disorders are slower to identify and adapt to new rules.

The CANTAB ID-ED test has been developed as an equivalent test for monkeys, based on a screen touch system. When presented with a series of paired shapes and lines, marmoset monkeys show the ability to learn to respond to particular shapes, as well as the ability to shift from responding to shapes, to lines (i.e. they have the ability to learn the concept of 'classes' of shape and the capacity to set-shift). Rhesus monkeys are superior to marmosets in performance on this task.[200]

Studies using olfactory or textual cues have demonstrated that mice and rats can also set-shift. The brain regions that underpin this ability in rodents may be equivalent to those used in humans.

197 Basile BM & Hampton RR (2011). *Monkeys recall and reproduce simple shapes from memory*. Curr Biol **21**, 774–8.
198 Yamazaki Y, *et al*. (2009). *Acquisition of an externalized eye by Japanese monkeys*. Exp Brain Res **194**, 131–42.
199 Sensory tools are those used to acquire sensory information or to augment sensory function, including tools such as endoscopes.
200 Weed MR, *et al*. (1999). *Performance norms for a rhesus monkey neuropsychological testing battery: acquisition and long-term performance.* Brain Res Cogn Brain Res **8**, 185–201.

Box 3.6 Aspects of brain function that may distinguish humans and the Great Apes from other species

1. **Episodic memory**. This is sometimes called 'autobiographical memory' or memory of events. Operational aspects of episodic memory (recall of what, where and when) have been demonstrated in species such as corvids (crows) and apes.[201] However, it is suggested that the 'subjective component' of episodic memory (an awareness of personal involvement in previous events) is a uniquely human function.
2. **Planning**. Humans have the capacity for 'planning', the ability to recognise and address future needs (sometimes even when these conflict with immediate need). Apes and chimpanzees are believed to be capable of selecting tools for future use.
3. **Numerosity**. The ability to work with numbers greater than 5 and to represent large numbers is extremely limited even in apes. Studies suggest that other monkeys (including macaques) can only work with small numbers.
4. **Language**. The capacity for language in NHPs is a classical controversy. Case studies in chimpanzees, including 'Washoe (1965–2007)' and 'Nim Chimpsky (1973–2000)', are inconclusive.
5. **Theory of mind**. Evidence for this function (the ability to identify and attribute mental states, e.g. beliefs, intents, desires, pretending, knowledge of yourself and others, and the capacity to recognise that the mental states of others can differ from your own) in NHPs is controversial.[202]
6. **Social cognition**. A task known as the 'ultimatum game' has been used to explore aspects of social cognition such as the willingness to accept injustice and social inequality in humans. In a variation of this task, chimpanzees have been found to lack a sense of 'fairness'.[203]

Further insight into the cognitive qualities that differ between humans and other primates has come from studies comparing the abilities of 2-year-old (pre-speech) human children, chimpanzees and orang-utans.[204] Although the human children only slightly out-perform chimpanzees and orang-utans on 'physical domain' tests (e.g. spatial memory and tool use), they significantly out-perform apes in 'social domain tests' (e.g. social learning and comprehension).

Comparative psychologists and ethologists have developed 'test batteries' to assess primate cognition, grouped into physical and social 'domains' (see Box 3.7). Test batteries of this kind could, in principle, be used to assess experimental animals for aspects of cognition that are indicators of relevant alterations in cognitive capacity.

Neuroanatomical correlates

Study of neuroanatomical and imaging correlates of brain function is now beginning to identify brain regions involved in aspects of social learning in NHPs. For example, research using neural recording techniques in monkeys has indicated a role for a region called the medial pre-frontal cortex in capturing a representation of the actions of another animal.[205,206] Studies in humans, sheep and macaques indicate a role for the medial frontal lobes and temporal lobes in tasks such as face perception.[207] Further developments may eventually provide useful diagnostic markers of altered cognitive capacity in experimental animals.

201 Martin-Ordas G, *et al*. (2010). *Keeping track of time: evidence for episodic-like memory in Great Apes*. Anim Cogn **13**, 331–40.
202 Penn DC & Povinelli DJ (2007). *On the lack of evidence that non-human animals possess anything remotely resembling a 'theory of mind'*. Philos Trans R Soc B **362**, 731–44.
203 For further detail see Jensen K, *et al*. (2007). *Chimpanzees are rational maximizers in an ultimatum game*. Science **318**, 107–9.
204 Herrmann E, *et al*. (2007). *Humans have evolved specialised skills of social cognition: the cultural intelligence hypothesis*. Science **317**, 1360–6.
205 See Quallo MM, *et al*. (2009). *Gray and white matter changes associated with tool-use learning in macaque monkeys*. Proc Natl Acad Sci USA **106**, 18379–84; this study shows use of magnetic resonance imaging and additional techniques to reveal brain region changes during learning of rake tool use in macaques.
206 See Yoshida K, *et al*. (2011). *Representation of others' action by neurons in monkey medial frontal cortex*. Curr Biol **21**, 249-53; this study uses neural recording in monkeys to identify where in the brain the action of others is represented.
207 Peirce JW, *et al*. (2001). *Human face recognition in sheep: lack of configurational coding and right hemisphere advantage*. Behav Processes **55**, 13–26.

Box 3.7 Test batteries for assessing aspects of primate cognition

Humans out-perform NHPs on social domain tests, whereas differences in abilities between humans and apes are less distinct in physical domains.[208]

Physical domains and tests	Social domains and tests
• Space – spatial memory	• Social learning
• Space – object permanence	• Communication – comprehension
• Space – rotation	• Communication – pointing
• Space – transposition	• Communication – attentional state
• Quantities – numerosity	• Theory of mind – gaze following
• Quantities – addition	• Theory of mind – intention

3.4.2 Adopting an incremental approach

Because of difficulty in predicting the outcome of human–animal chimæric brain experiments, particularly in larger animals, some might suggest that such experiments should not be pursued. However, there are important reasons for seeking to determine how neurons derived from human neural stem cells, or other cell types, can potentially integrate into, and function in, a damaged brain. Before transplanting such cells into the brains of humans suffering from brain disorders, it is essential to investigate possible safety issues, and to have good evidence of likely efficacy; both these are likely to involve some testing on animals. Authorisation of research of this type should (at least for some time) be based on careful, case-by-case evaluation to ensure that, as in all research, the use of animals can be justified by the potential benefit and the lack of satisfactory alternative research strategies. Many experiments will involve such a low level of engraftment of human cells into the animal brain that they will cause little concern, and can confidently be regulated under ASPA with no additional oversight (see 8.2.1).

We suggest that experiments where there is doubt as to the potential functional effect of modification of the brain, particularly in larger animals and NHP's, should be subject to additional oversight by an expert national body (see 8.2.2), and may need to be carried out on an incremental basis (see Box 3.8).

208 A domain is a specialised sphere of activity or knowledge. See Herrmann E, *et al*. (2007). *Humans have evolved specialised skills of social cognition: the cultural intelligence hypothesis*. Science **317**, 1360–6.

Box 3.8 Expert assessment/incremental approach

- For some forms of experiment (as set out in 8.2.2) an incremental research approach should be agreed at the outset between researchers, inspectors and the national expert body.
- Initial experiments should usually be undertaken using 'lowest' feasible species not previously studied, in small numbers. Where possible there should be a graduated approach to the amount/proportion of human material added.[209]
- Each animal should be tested according to a pre-agreed protocol with clear end-points. Tests appropriate to the different research situations and species should be used to detect any modification/loss of the animal's usual cognitive capacities and behaviours. Close monitoring of the animals should take place, with due regard to minimising observer bias.[210]
- Once experience is gained, studies involving larger numbers of animals, a greater proportion of cellular replacement, and more advanced species could be undertaken.
- For example, research intended to study the effect of incorporating human neurons into an NHP brain, could start with evidence based on modest neuronal incorporation into rodents, and proceed by degrees to experiments involving larger scale replacement in NHPs. In this case, the monitored effects might relate to the development of human-like cognitive capacities. A range of tests, from which a protocol could be developed are set out in Box 3.7.
- Unusual and 'first of a kind' experiments will need to be judged on an individual basis; but as experience is gained, guidance could be developed so that some classes of experiment may be undertaken with lower levels of regulatory scrutiny (see Chapter 8).

Box 3.9 Two ways of viewing the brain

Some participants in the public dialogue appeared to adopt a dual conceptualisation of the brain, in which it was seen as both a purely physical organ, as simply 'tissue', and secondly as the source of consciousness and thought 'greater than the sum of its parts'. When considering scientific research, participants often tended to think about the brain in the first way, and few people appeared to believe that small changes to an animal's brain at the cellular level would have a discernable impact on its cognitive function: *' … a mouse brain is so much smaller, I don't think a little brain will be able to sit there and "think therefore I am" …'*

However, in considering the possible implications of manipulating the brain as a whole, the second view tended to be adopted. From this viewpoint, some participants expressed a clear sense of unease around research involving the brain, and its potential outcomes. Some participants suggested that research that might make an animal's brain more similar to a human brain would be unacceptable: *'I don't have a problem with it until it gets to the brain … but bits to do with memories, that would be too far – it's a human thing to have a memory.'*

209 The basis of the incremental approach should be carefully considered in each situation, and should not mandate additional studies where clear scientific justification is not evident. For example, work on lower species should not be required where previous evidence is already adequate (e.g. from cell-based studies). Good evidence from previous work should always be taken into account in planning and licensing experiments.

210 For example, the use of double blinding, and or automated observation techniques.

3.5 Research involving the reproductive system

There are important differences in reproductive biology between mammalian species. The relatively high frequency of infertility, details of placental development, and menopause are largely specific to humans.[211] Compared with most animal species, the reproductive system of humans is prone to problems such as premature ovarian failure, endometriosis, and cancers of the ovary, testis, cervix and breast. The exploration of inter-species differences has often been very illuminating; however, because of these differences, unmodified animals are frequently unsuitable models for the human. Despite this, animal research has contributed significantly to knowledge in this area, and to the development of treatments for reproductive disorders. For example, many human-assisted reproductive techniques, including *in vitro* fertilisation (IVF), were initially developed in other mammalian species, particularly the rabbit and mouse.[212]

Models involving ACHM have been, and are likely to continue to be, particularly important for research in this field. The fertilisation of animal eggs by human sperm was an important test of male fertility and these are still used to explore mechanisms associated with fertilisation (see 2.2.3). Animals containing human DNA are used to explore the role of specific human genes (and their regulatory sequences) on many human-specific aspects of reproductive function, at any stage from gamete development to parturition (the process of giving birth). Chimæric animals carrying human germ cells (sperm or eggs) or other reproductive system tissues (e.g. endometrium (womb lining)) can provide important investigative models in reproductive research, though they appear to be contentious (see Box 3.10 and 5.7.2).

3.5.1 Germ cell development and function

Male germ cells

Abnormalities of fetal testis development and function can predispose men to disorders that become evident in adulthood, such as testicular germ cell cancers and low sperm counts, disorders that are increasing in incidence for unknown reasons. The fetal origins of these conditions cannot be investigated in adult patients, and it would be unethical and impractical to conduct studies on live human fetuses. To study these conditions, small pieces of testicular tissue, taken with permission from legally aborted fetuses are implanted under the skin of immune-deficient mice. The implants grow and develop normally, and provide a way of dynamically studying the developing human testis allowing investigation of the effects of chemical exposures or other interventions.[213,214] This model is used in investigating the fetal origins of testicular germ cell cancers (the commonest cancer of young men) and in assessing the effects of exposure to environmental chemicals (such as phthalates, used in plastics).[215] Investigations of this type may also yield new insights into the mechanisms regulating human male germ cell proliferation and differentiation, which could be used both for fertility treatments and the development of male contraceptives.[216]

Research is underway to develop procedures to preserve testicular germ cells from boys who are being treated for cancer with therapies that may cause sterility.[217] One approach under consideration is to graft tissue or cells from human testis biopsies, collected before therapy, into mice and to allow the human cells to survive and/or proliferate, with the aim of either

211 Other factors largely unique to human reproduction include poor rates of early embryo development and implantation, and a short gestation period relative to neonatal size.
212 Fauser BC & Edwards RG (2005). *The early days of IVF*. Hum Reprod Update **11**, 437–8.
213 Mitchell RT, *et al*. (2008). *Germ cell differentiation in the marmoset (Callithrix jacchus) during fetal and neonatal life closely parallels that in the human*. Hum Reprod **23**, 2755–65.
214 Scott HM, *et al*. (2009). *Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds*. Endocr Rev **30**, 883–925.
215 Mitchell RT, *et al.* (2010). *Xenografting of human fetal testis tissue: a new approach to study fetal testis development and germ cell differentiation*. Hum Reprod **25**, 2405–14.
216 Mitchell RT, *et al*. (2009). *Male fertility and strategies for fertility preservation following childhood cancer treatment*. Endocr Dev **15**, 101–34.
217 Although it has recently been shown possible to obtain functional mouse sperm by culturing pieces of neonatal testis *in vitro*, the technique has not yet been developed for human testis; moreover, it is not clear that it will work with the relatively early stages of fetal testes that can be obtained from aborted embryos. If it does work, it could replace some of the ACHM experiments of the type described here. See Sato T, *et al*. (2011). *In vitro production of functional sperm in cultured neonatal mouse testes*. Nature **471**, 504–7.

transplanting the cells back into the donor after the patient's recovery, or to use mature germ cells (if these can be grown in the xenografts) for *in vitro* fertilisation.[218] Various studies, including one using childhood tissue, have shown the potential utility of this approach but further development is needed to develop a clinical treatment.[219] Clinical application would also require the development of methods to prevent animal–human disease transmission (see 4.2). Further policy and ethical consideration would also be appropriate. Under the HFE Act (2008), human sperm (or eggs) derived in animals or *in vitro* may be classified as 'non-permitted gametes'; if so, it would be possible to use them for *in vitro* tests of fertilisation and early embryo development (under licence) but not for clinical purposes (see Box 6.4).

Female germ cells

Factors affecting the development and maturation of human egg cells have, similarly, been studied in immune-deficient mice engrafted with human ovarian tissues. Initial studies demonstrating that frozen human ovarian follicles (egg precursor cells) were able to continue development were first made by re-implanting these tissues into immune-deficient mice.[220,221,222] These studies found that the human grafts were able to resume apparently normal follicle growth and maturation, in the mice.[223,224] With such a model it was recently found that when an inhibitor of PTEN (part of a molecular pathway known to block oocyte development) was given to the mice, human primordial follicles (the earliest egg stage) present in the graft can develop all the way to mature pre-ovulatory stages, and contained oocytes able to undergo maturation (and perhaps fertilisation). This process took 6 months (something that would be difficult to achieve *in vitro*), but it is a potentially important way to generate large numbers of human oocytes for research or even potentially for fertility treatments (subject to the possible legal restrictions described above).

Studies using engrafted human ovary played an important role in improving cryo-preservation (freezing) techniques used to store ovarian tissue from people at risk of losing their fertility (e.g. due to cancer therapy).[225,226,227,228] Ovarian tissue banking has since been offered in many oncology centres, and although few transplants of thawed tissue have been reported, there have been successful live-births following this procedure.[229] Xenograft models have also been used to investigate the effects of anti-cancer drugs on follicles within the ovarian tissue, e.g. to determine the treatment least likely to lead to infertility as a side-effect.[230]

Reproductive disorder with genetic origins

Chromosome abnormalities arising during germ cell development leading to extra (or missing) chromosomes are thought to be one of the causes of the high rates of early embryo loss seen in humans, for example as early miscarriages, and are responsible for several syndromes affecting liveborn individuals, including Down's and Turner syndromes. The incidence of some of these abnormalities

218 Mitchell RT, *et al*. (2009). *Male fertility and strategies for fertility preservation following childhood cancer treatment*. Endocr Dev **15**, 101–34.
219 Mitchell RT, *et al*. (2010). *Xenografting of human fetal testis tissue: a new approach to study fetal testis development and germ cell differentiation*. Hum Reprod **25**, 2405–14.
220 Gook DA, *et al*. (2001). *Development of antral follicles in human cryopreserved ovarian tissue following xenografting*. Hum Reprod **16**, 417–22.
221 Oktay K, *et al*. (2000). *Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice.* Fertil Steril **73**, 599–603.
222 Gosden RG, *et al*. (1994). *Follicular development from ovarian xenografts in SCID mice*. J Reprod Fertil **101**, 619-23.
223 Oktay K, *et al*. (2000). *Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice*. Fertil Steril **73**, 599–603.
224 Gook DA, *et al*. (2003). *Oocyte maturation, follicle rupture and luteinization in human cryopreserved ovarian tissue following xenografting*. Hum Reprod **18**, 1772–81.
225 Newton H, *et al*. (1996). *Low temperature storage and grafting of human ovarian tissue*. Hum Reprod **11**, 1487–91.
226 Soleimani R, *et al*. (2010). *Xenotransplantation of cryopreserved human ovarian tissue into murine back muscle*. Hum Reprod **25**, 1458–70.
227 Rahimi G, *et al*. (2010). *Re-vascularisation in human ovarian tissue after conventional freezing or vitrification and xenotransplantation*. Eur J Obstet Gynecol Reprod Biol **149**, 63–7.
228 Van Eyck AS, *et al*. (2010). *Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model*. Fertil Steril **93**, 1676–85.
229 Donnez J, *et al*. (2004). *Livebirth after orthotopic transplantation of cryopreserved ovarian tissue*. Lancet **364**, 1405–10.
230 Oktem O & Oktay K (2007). *A novel ovarian xenografting model to characterize the impact of chemotherapy agents on human primordial follicle reserve*. Cancer Res **67**, 10159–62.

increases significantly with age.[231,232,233,234] It can be difficult to study factors that may predispose to these abnormal cells, especially during the first meiotic division in oocytes as this begins during fetal ovary development. Progress is being made on *in vitro* growth and maturation of primary follicles, which could be used to study later stages of egg cell division, but these methods are not yet sufficiently robust.[235] Animals carrying grafts of human ovarian (or testicular) tissue may allow detailed, longitudinal studies on factors (hormonal, toxic or age-related) affecting chromosome segregation.

3.5.2 Endometrial development and pathology

Experimental approaches involving ACHM are currently being established to study the normal physiology of human endometrial tissue, as well as its malfunction in conditions such as endometriosis, a condition that may affect more than 10% of women.[236, 237] These studies involve the engraftment (usually into the peritoneal cavity) of small sections of endometrial tissue, taken from a healthy human donor, or a patient with endometriosis, into ovariectomised, immune-deficient mice.[238] The mice are treated with a course of endocrine hormones which imitate the human female menstrual cycle. The engrafted human tissue allows study of factors such as tissue morphology and gene expression. Bioluminescent or other markers can also be introduced into the endometrial cells to allow their growth to be monitored *in vivo*.[239] The effects of repeated hormone cycles could be studied. These models are being used to screen for molecules that might reduce endometrial cell proliferation or might prevent attachment and spread of endometriosis.[240,241] Recently, using such mouse models, at least three types of drug have been claimed to reduce growth of human endometrial tissue.[242,243,244,245,246] Apart from giving promising leads towards therapy, these mouse studies replace the use of other animals, notably baboons, that are also used in research on endometriosis.[247]

3.5.3 Implantation and placenta development

Two important areas where ACHM may allow future research are embryo implantation into the lining of the uterus and placental development.

There is a significant loss of human embryos (perhaps as high as 70%) during pre-implantation development and around implantation. This very high rate appears specific to humans, and although chromosomal abnormalities account for a significant proportion (see 3.5.1), most causes are unknown. A failure of interaction between the embryo and the endometrium into which it implants is probably also a common cause. The underlying defect could be intrinsic to the embryo, the endometrium or the hormone system that makes the endometrium receptive.[248] It is, however, very difficult to carry out relevant experiments on human material. Some research

231 Hunt P & Hassold T (2010). *Female meiosis: coming unglued with age*. Curr Biol **20**, R699–702.
232 Thomas NS, *et al*. (2010). *De novo apparently balanced translocations in man are predominantly paternal in origin and associated with a significant increase in paternal age*. J Med Genet **47**, 112–5.
233 Thomas NS, *et al*. (2001). *Maternal sex chromosome non-disjunction: evidence for X chromosome-specific risk factors*. Hum Mol Genet **10**, 243–50.
234 Muhlhauser A, *et al*. (2009). *Bisphenol A effects on the growing mouse oocyte are influenced by diet*. Biol Reprod **80**, 1066–71235.
235 Jin SY, *et al.* (2010). *A novel two-step strategy for in vitro culture of early-stage ovarian follicles in the mouse.* Fertil Steril **93**, 2633–9.
236 Olive DL & Schwartz LB (1993). *Endometriosis*. N Engl J Med **328**, 1759–69.
237 Giudice LC & Kao LC (2004). *Endometriosis*. Lancet **364**, 1789–99.
238 Ovariectomy (removal of the ovaries) is used to remove the mouse's own secretion of endocrine hormones. See Masuda H, *et al*. (2007). *Noninvasive and real-time assessment of reconstructed functional human endometrium in NOD/SCID/gamma c(null) immunodeficient mice*. Proc Natl Acad Sci USA **104**, 1925–30.
239 Defrere S, *et al*. (2009). *Review: luminescence as a tool to assess pelvic endometriosis development in murine models*. Reprod Sci **16**, 1117–24.
240 Hull ML, *et al*. (2008). *Endometrial–peritoneal interactions during endometriotic lesion establishment*. Am J Pathol **173**, 700–15.
241 Collins NH, *et al*. (2009). *Characterization of antiestrogenic activity of the Chinese herb, prunella vulgaris, using in vitro and in vivo (Mouse Xenograft) models*. Biol Reprod **80**, 375–83.
242 The drugs are: simvastatin, a cannabinoid agonist which inhibits the Akt signalling pathway; Raloxifene, a selective estrogen receptor modulator; and an antibody based protein, 'icon', that inactivates a growth factor.
243 Bruner-Tran KL, *et a*l. (2009). *Simvastatin protects against the development of endometriosis in a nude mouse model*. J Clin Endocrinol Metab **94**, 2489–94.
244 Leconte M, *et al*. (2010). *Antiproliferative effects of cannabinoid agonists on deep infiltrating endometriosis*. Am J Pathol **177**, 2963–70.
245 Chen YJ, *et al*. (2010). *Oestrogen-induced epithelial–mesenchymal transition of endometrial epithelial cells contributes to the development of adenomyosis*. J Pathol **222**, 261–70.
246 Krikun G, *et al*. (2010). *The immunoconjugate "icon" targets aberrantly expressed endothelial tissue factor causing regression of endometriosis*. Am J Pathol **176**, 1050–6.
247 Tirado-Gonzalez I, *et al*. (2010). *Endometriosis research: animal models for the study of a complex disease*. J Reprod Immunol **86**, 141–7.
248 Singh M, *et al.* (2011). *Bridging endometrial receptivity and implantation: Network of hormones, cytokines, and growth factors*. J Endocrinol. *In press.*

on implantation is currently conducted in animals such as the baboon, where mechanisms of implantation are thought to be fairly similar to those in humans. There are attempts to derive *in vitro* systems to look at implantation using cultures of endometrium, but these do not replicate the complex three-dimensional architecture or physiology of the womb.

ACHM experiments involving grafts of human uterus or endometrium into animals, or, if the identities of the human genes required for receptivity are known, transgenic animals expressing such genes within their uterus, might allow human embryo implantation to be studied. This would not be permitted under the HFE Act (2008) (see 6.5); however, it might be possible to use disabled embryos, such as trophoblast vesicles or tetraploid embryos.

Different mammals often have very different types of placenta. Some studies can be done in rodents on placental cell types that appear similar to those in the human placenta, and some molecular and genetic pathways are conserved, but to understand many details of human placental development and physiology fully requires studies in humans or closely related species.[249] Although it would be difficult to study entire human placental development in animals, it is possible to study the role of specific human genes in transgenic animals, or to introduce specific human placental cell types into the placenta of animals *in utero* and to determine their effects on placenta function and physiology.

3.5.4 Other studies involving reproductive tissues and general concerns

ACHM may be appropriate to study a wide range of questions about human reproduction, from eclampsia and birth timing to menopause. In such studies, human reproductive tissues are usually implanted into the recipient animal 'ectopically' (e.g. under the skin of a mouse rather than into its own reproductive system), and there is very little possibility of the eggs or sperm contacting another germ cell and being fertilised. However, some experiments of this type do result in the presence of functional human sperm and/or egg cells in animals, which raises the possibility that fertilisation between human and animal germ cells (or even between human eggs and sperm) may inadvertently occur within an animal.

At least one study has reported grafting pieces of human ovary under the membrane of mice ovaries, creating the possibility that human oocytes might enter the reproductive tract of female mice.[250] The females were not allowed to mate, but if they had (with male mice), there would be very little chance of hybrid embryo development or implantation. Human sperm do not normally penetrate the mouse zona pellucida (a type of protective shell around the egg), as species-specific molecules are required. Transgenic mice expressing human zona pellucida proteins are being used to search for the relevant molecules.[251,252] To achieve cross-species fertilisation, for example in tests of human male fertility using hamster or mouse eggs, it is necessary to remove the zona pellucida or to use ICSI.[253] Such tests are usually terminated at the two-cell embryo stage, although 'true-hybrid' embryos may be allowed to develop for 14 days (see Box 6.6).[254] We have briefly discussed the problems of studying human embryo implantation and human placental development (3.5.3). Would it be possible to transplant a human uterus into an animal and then use this to implant human embryos? Given that uterus transplants are being considered

249 For example, at least two retroviral elements, HERV-W and HERV-FRD, both of which lead to the expression of viral envelope proteins (termed Syncytin and Syncytin2, respectively) in the human placenta, are specific to the primate lineage. The Syncytin proteins lead to cell fusion and to the formation of a specialised tissue, termed syncytiotrophoblast, which plays an important role in the maternal–fetal interchange of nutrients. Although some rodents also have syncytiotrophoblast, this appears to be due to the activity of rodent-specific retroviral genes. Other retroviral integrations are thought to have affected the expression of endogenous genes involved in human placental development, such as the Insulin-like 4 gene *(INSL4)*.

250 Dath C, *et al*. (2010). *Xenotransplantation of human ovarian tissue to nude mice: comparison between four grafting sites*. Hum Reprod **25**, 1734–43.

251 Xu YN, *et al*. (2010). *DNA synthesis and epigenetic modification during mouse oocyte fertilization by human or hamster sperm injection*. J Assist Reprod Genet. *In press.*

252 Yauger B, *et al*. (2011). *Human ZP4 is not sufficient for taxon-specific sperm recognition of the zona pellucida in transgenic mice*. Reproduction **141**, 313–9.

253 Intra-cytoplasmic sperm injection (ICSI) involves injecting a single sperm directly into an egg in order to fertilise it.

254 However, subsequent development is unlikely to occur owing to epigenetic defects, aneuploidy, and species-specific factors controlling implantation.

as an alternative to surrogacy in humans, this is not necessarily a remote possibility.[255] Careful thought would need to be given about such experiments from scientific and ethical perspectives. Licensing of any animal experiment where there is a chance of human embryo or true hybrid development should address the precautions taken to avoid this (see 8.2.2).

3.6 Research involving human appearance or behavioural traits

Current ACHM do not show overt human-like appearance or behaviours; the alterations are seen at a biochemical or pathological level. Transgenic mice have the appearance of ordinary mice; chimæric goats engrafted with human stem cells look like 'ordinary' goats. Even the most extensive of current genetic modifications, such as the addition of a human chromosome to mice in the Down's syndrome model (see 3.2.), do not markedly alter the appearance of the animals to a casual human observer.[256]

Participants in the public dialogue expressed particular concern that the incorporation of human material into experimental animals might result in the creation of animals with 'human-like' appearance or characteristics (see Box 3.11). There are some cardinal phenotypic features that are intuitively recognised as essentially human, such as facial appearance and skin texture, and behaviours including speech. Experiments that confer these properties on animals may be expected to attract public interest (see for example the reaction to the Vacanti Earmouse, Box 3.12). The societal and ethical bases of such concern are discussed in Chapter 5.

Box 3.10 Public views on research involving human reproductive tissues

The creation of animal models including human reproductive tissue was a very sensitive area for public participants. Compared with other human tissues, the use of animal models involving human reproductive cells was regarded as acceptable by the fewest number of participants in the quantitative survey (42%).

'*… that is so far out there, just awful. Perhaps if there was no sperm left on earth, but otherwise no way.'*

Dialogue discussions identified several possible explanations for these responses, including:

- The cultural significance of reproductive cells (through associations with sex, the production of children, birth experiences and development, and familial characteristics).
- A suggestion that even small changes to a single reproductive cell might produce profound effects (reproductive cells were seen as easy to 'abuse', and contrasted with the brain, where 'changing a few cells might not matter').
- A view that the consequences of research involving human reproductive cells might be experienced not only by the animal involved, but potentially by resulting human offspring.

Box 3.11 Public views on research involving human-like appearance

Research involving external body parts, such as the use of human hair, skin, or the possible development of human-like limbs on animals, was often met with distaste by dialogue participants. This type of response was attributed to participants' ability to imagine and visualise the resulting animal as 'unnatural'. The physical appearance of animals was found to be an important way in which participants identified animals as different 'kinds', and changes to external features might be seen to blur these well-recognised visible distinctions between species.

255 Grynberg M, *et al*. (2011). *Uterine transplantation: a promising surrogate to surrogacy?* Ann N Y Acad Sci **1221**, 47–53.
256 Correspondence from Fisher, E.

3.6.1 Human external appearance

Studies involving the transplantation of human skin onto animals are undertaken for several research purposes. Exposure of human skin to radiation (such as ultraviolet B in sunlight) can lead to DNA damage and skin cancer, and mouse models have been developed for use in research to understand the mechanistic basis of cancers of this kind. Human skin with different pigmentation types, and skin from cancer-prone patients with the disease *Xeroderma pigmentosum*, have been transplanted onto immune-deficient mice, allowing the effects of radiation exposure to be investigated, and potential therapeutics tested.[257]

Mice are used in the study of psoriasis, a human skin condition that results in the development of scaly, red patches on the skin. Some forms of psoriasis result from disorders of the immune system, and mice transplanted with skin grafts from psoriatic patients have been used to understand the malfunctioning relationship between the epidermal (skin) cells and the immune system.[258] Mice with human skin grafts have also been used to improve grafting techniques (e.g. for use with burns patients) and to investigate approaches to reduce the immune rejection of skin grafts.[259] In such studies only a small area of human skin is grafted onto the recipient mice.

Transgenic animal models are contributing to understanding of the genetic basis of limb development. For example, the *Prx1* gene codes for a DNA-regulating protein important for the growth of limb bones. The regulatory sequences affecting *Prx1* expression are now known in several species, including the mouse and bat. In studies to investigate their function, the mouse regulatory sequences were exchanged for the equivalent regulatory regions from the bat. The resulting transgenic mice had elongated forelimbs.[260] Mutation of a human gene regulatory sequence results in the development of extra digits ('pre-axial polydactyly'). Mice carrying the same genetic mutation are also born with extra digits. The mice have contributed significantly to the identification of the developmental basis of this condition, which is now known to result from extra expression of a protein in a small patch on one side of the developing limb.[261] Animal and human limbs are composed of similar cell types making similar proteins; the different shapes of human and animal limbs presumably reflect differences in gene regulation. Testing this could in theory lead to transgenic animals with human-like hands or feet. This could give basic understanding that has clinical importance, but it may cause some disquiet and it would, of course, have consequences for the animals, including mismatch between hard-wired behavioural patterns and what the new limbs can do.

In future, genes underlying the development of other body parts, perhaps including facial features, may also be studied in animals. Such experiments may require consideration from both socio-ethical and animal welfare perspectives. An animal may be distressed by an unusual body part, may suffer rejection by its own species, or elicit unusual response from those charged with its care (see 4.1.2). Attempts should be made to anticipate such effects, in the design and licensing of the work.

Recognisable fragments of teeth, hair and other tissues can sometimes arise in naturally occurring tumours, known as 'teratomas', which are occasionally found in humans and other species. They arise from remnants of very early stem cells, capable of differentiating into different body tissues. Similar teratomas are often created in stem cell research (see 3.3.1), when human or other embryonic stem cells are implanted into mice, for example to test the cells' developmental potential, resulting in the presence of, for example, fragments of human tooth or hair (within the tumour) in the mouse.[262]

257 Sun XZ, *et al*. (2008). *Animal models of xeroderma pigmentosum*. Adv Exp Med Biol **637**, 152–60.
258 Guerrero-Aspizua S, *et al*. (2010). *Development of a bioengineered skin-humanized mouse model for psoriasis: dissecting epidermal-lymphocyte interacting pathways*. Am J Pathol **177**, 3112–24.
259 Issa F, *et al*. (2010). *Ex vivo-expanded human regulatory T cells prevent the rejection of skin allografts in a humanized mouse model*. Transplantation **90**, 1321–7.
260 Cretekos CJ, *et al*. (2008). *Regulatory divergence modifies limb length between mammals*. Genes Dev **22**, 141–51.
261 Lettice LA, *et al*. (2008). *Point mutations in a distant sonic hedgehog cis-regulator generate a variable regulatory output responsible for preaxial polydactyly*. Hum Mol Genet **17**, 978–85.

Box 3.12 The Vacanti ear-mouse

The 'Vacanti ear-mouse', which *appeared* to have a human ear grown on its back, was created in 1997. The mouse was created to demonstrate a method of fabricating cartilage structures for transplantation into human patients. The 'ear' was actually a cartilage structure, grown by seeding cow cartilage cells into a biodegradable, ear-shaped, polyester fabric mould, which was then implanted under the mouse's skin. Although the Vacanti mouse did not contain any human tissue, and was not functional, its human-like appearance evoked a strong public interest and is still widely remembered today (erroneously) as an example of an animal containing a human organ. [262,263]

3.6.2 Human behavioural traits

It is hard to argue that many behavioural traits are individually unique to humans, although large brains and manual dexterity allow us to generate sophisticated tools, which then influence aspects of our behaviour, such as writing and reading, playing and appreciating music, and playing sports.

Recent research to investigate language-related disorders identified a mutation in a gene (known as *FOXP2*), which was found to be associated with an inherited form of speech and language disorder in humans. The *FOXP2* sequence was found to be different between humans and Great Apes (and other mammals), leading to the suggestion that these changes may be partly responsible for the acquisition of speech during human evolution.[263] Furthermore, when the human equivalent sequences were introduced into mice, they developed vocalisations different from those of non-modified mice.[264,265] These studies provide some evidence to suggest roles for genes such as *FOXP2* in the processes underpinning speech and language development.

However, it is important to distinguish vocalisation (making sound) from speech and language (the complex human system of communication). Parrots are capable of complex vocalisation and 'mimickry'. The capacity to make sounds is not the same as the possession of language, which involves many cognitive processes (e.g. memory symbolisation, a shared communicative structure of signs and a process of learning in interaction with adults at crucial developmental stages). Evidence for true language acquisition, even in higher NHP species such as the chimpanzee, is controversial and inconclusive (see Box 3.6). It is likely that more genes underpinning speech development will be identified in future. However, even if all the genes underlying these processes could be introduced into an NHP, it remains a matter of speculation whether the brain of the modified animal would then be capable of language acquisition. Although in some studies carefully trained NHPs have developed some aspects of communication (see Box 3.6), is it not clear that even a modified NHP brain would have the capacity for complex human communication in its true sense.[266]

Creating characteristics such as speech and behaviour in animals would be very complex, probably requiring manipulation of environmental as well as biological factors. Authorisation of such work would need to be justified by considerable potential benefit and the lack of satisfactory alternative research strategies. Measures to determine and respond to public sensitivity should be considered before licensing such research.

262 Cao Y et al. (1997). *Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear.* Plast Reconstr Surg **100(2)**, 297-302.
263 Hardin J (1998). *Producing tissue-engineered cartilage in the shape of a human ear.* Plast Reconstr Surg **101(6)**, 1745.
264 Reimers-Kipping S, *et al.* (2011). *Humanized Foxp2 specifically affects cortico-basal ganglia circuits*. Neuroscience **175**, 75–84.
265 Enard W (2011). *FOXP2 and the role of cortico-basal ganglia circuits in speech and language evolution*. Curr Opin Neurobiol. *In press.*
266 Human communication conveys meaning and intent, which requires a concept of the mental state of others to whom you are communicating. There are reported instances of human children who have not developed language. Such findings suggest that, although the capacity for human language might have a biological basis, its realisation depends on immersion in complex human communities from birth. For example,

see 'Genie' – http://en.wikipedia.org/wiki/Genie_(feral_child) and 'Oxana' http://www.mymultiplesclerosis.co.uk/misc/feral-children.html.

4 Welfare and safety aspects of ACHM

4.1 Welfare

The protection of animals is central to the operation of the UK's Animals (Scientific Procedures) Act 1986 (ASPA), which is intended to ensure that animals used in research are not subject to unnecessary pain, suffering, distress or lasting harm (see 6.2.1, and for a wider international perspective see 7.3.1).[267] Under ASPA, all experiments involving 'protected' animals must be licensed, and they can be licensed only if there are no scientifically suitable alternatives that *replace* animal use, *reduce* the number of animals needed or *refine* the procedures used to cause less suffering (principles known as the '3Rs' see 6.2.1).[268] Decisions to license research must take into account the likely benefits (to humans, other animals or the environment), weighed against the likely welfare costs to the animals involved.[269] Additional requirements apply to particular research, such as that involving genetically altered animals or species including NHPs (see Box 6.1).[270] This long-standing framework underpins the close governance of animal research in the UK, which is more carefully scrutinised than other uses of animals such as in agriculture, or as companion animals (pets). (See Box 4.1 for public views on animal welfare.)

Application of animal welfare principles is an obligation on individuals and institutions under the Home Office licensing system, and is monitored both locally, for example by 'named animal care and welfare officers', and by the Home Office inspectorate. Further improvements are encouraged and taken forward in the UK through the work of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), and other bodies.[271] An important aspect of such work is the development of guidelines for best practice, in areas such as welfare assessment of genetically modified rodents, and in defining the welfare needs of particular animal species.[272] **We emphasise that research involving ACHM should be subject to scrutiny, and advancement from the perspective of animal welfare, in a manner no different from other animal research.**

Here we introduce two aspects of animal welfare relating *specifically* to ACHM:

- The possibility that the creation or use of ACHM raises specific welfare concerns.
- The potential of ACHM research to contribute to advancement of the 3Rs (replacement, reduction and refinement, see above).

Further consideration is included in Chapter 5 (5.5).

4.1.1 ACHM and animal welfare

In principle, the use of ACHM that closely model human biology increases the likely benefit of the research and so contributes to the refinement of experimental techniques. ACHM use can support animal welfare principles by enabling researchers to use species likely to experience less pain, suffering or harm, or to reduce the numbers of animals used in some experimental situations.[273]

267 New legislation, intended in part to bring harmonisation in animal welfare practices across Europe, has recently been adopted (see 7.3.1).
268 See Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, Section 2.3. http://www.archive.official-documents.co.uk/document/hoc/321/321.htm
269 Animal Procedures Committee (2003). *Review of the cost-benefit assessment in the use of animals in research.* http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/key-reports/
270 Research involving NHPs is only permissible where there is strong scientific justification, and where no other species are suitable for the purposes of the programme of work, or where it is not practicable to obtain animals of any other species that are suitable for those purposes. See Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, Section 5.22.
271 The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is an independent scientific organisation, tasked by Government with supporting the UK science base through the application of the 3Rs. See www.nc3rs.org.uk
272 For example see, 'Mouse Welfare Terms' http://www.mousewelfareterms.org/doku.php?id=home developed by the Medical Research Council Harwell and Wellcome Trust Sanger Institute Cambridge; Wells *et al. (2006) Full report of GA mouse welfare assessment working group.* Lab Animals **40,**111–114; Ellegaard L, *et al.* (2010). *Welfare of the minipig with special reference to use in regulatory toxicology studies.* J Pharmacol Toxicol Methods **62,** 167–83; RETHINK http://www.rethink-eu.dk/index.php?page=one&id=8
273 Use of a lower species (phylogenetic reduction) is often considered to be refinement, but such a judgement can only be made if assessment of the available scientific evidence suggests that the lower species is less sentient/likely to suffer less. See http://www.nc3rs.org.uk/category.asp?catID=78

Research involving ACHM, particularly mice with humanised organs such as the liver, or the immune system, can be used as an alternative to NHPs in investigating infectious diseases. For example, they have facilitated studies of HIV and hepatitis, to which unmodified mice are not susceptible, and for which NHPs have previously been used (see 2.3.3). ACHM approaches also have potential application in drug development and testing; for example the use of transgenic mice susceptible to polio virus through incorporation of the human *CD155* gene has been approved as an alternative to NHP use in polio vaccine testing.[274] A protocol developed using a chimæric mouse model which recapitulates forms of human cancer facilitates a significant reduction in the number of mice used compared with previous approaches. This is due to the more faithful development of cancer in the chimæric model, and elimination of the need for breeding programmes.[275] The recent increase in the development of antibody therapies (see 2.3.2) has resulted in an increase of the use of NHPs in toxicity testing of these therapeutics. This is an important avenue for future study in which mice with humanised immune systems may reduce (though not fully replace) NHP use. For other types of human condition, for example those affecting cognitive abilities, it may be that NHP models incorporating human material are so much better than similar rodent models that they will allow an overall reduction and refinement, and lead more rapidly to treatments. An example where this might be the case (although no treatments are yet available) is Huntington's disease.[276]

We anticipate that the use of animals containing human material is likely to present further avenues for advancement of the 3Rs. We support their development and use, while emphasising the view put forward in evidence that '*… the development of an 'improved' model needs to be followed by a rigorous and critical appraisal of the value of existing models by research funders, scientists and regulators.*'[277] However, we do not anticipate that research involving ACHM would decrease the *overall use* of animals in medical research in the short term, in part because the development of ACHM will open up new research avenues.

4.1.2 Specific welfare concerns

We have considered whether the incorporation of human genetic or cellular material into an animal might in itself have the potential to cause a distinct dimension of 'pain, suffering or lasting harm' to the animal involved. Our general conclusion is that, although individual experiments may give rise to particular types of animal suffering, the techniques in themselves do not raise distinct types of animal welfare concern.

Social aspects of animal welfare are increasingly taken into account, and research animals are housed in appropriate and species-specific environments, which often involve 'group-housing' (e.g. of NHPs). We considered whether, by conferring a human characteristic onto an animal (such as appearance, e.g. through engraftment of human skin (see 3.6), or a behavioural trait) an animal might suffer distress or harm, resulting from the actions of others of its own species, or those responsible for its care. Although the potential for suffering brought about in this way is plausible, it does not represent a 'unique' dimension of suffering that is specific to the creation of ACHM, because similar situations can arise (and need to be taken into account in assessing welfare issues) in other types of research. Evidence submitted to the study indicated that there is: '*no rationale,* specifically on animal welfare grounds, *for moving to regulate this type of research differently from other animal research*' and that '*research involving ACHM is not significantly different to other areas of animal research from an animal*

274 Humans and primates express a protein (CD155) on their neurons which confers susceptibility to infection by the polio virus. Batches of live polio vaccine for human use cannot be tested to determine their activity (virulence) on species that lack the CD155 protein (including mice) and are therefore tested on NHPs. See Shultz LD, *et al.* (2007). *Humanized mice in translational biomedical research.* Nat Rev Immunol **7**, 118–30; Mendelsohn CL, *et al.* (1989). *Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily.* Cell **56**, 855–65; Dragunsky EM, *et al.* (2006). *Further development of a new transgenic mouse test for the evaluation of the immunogenicity and protective properties of inactivated poliovirus vaccine.* J Infect Dis **194**, 804–7.

275 Zhou Y, *et al.* (2010). *Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas.* Nat Biotechnol **28**, 71–8.

276 Yang SH & Chan AW (2011). *Transgenic Animal Models of Huntington's Disease.* Curr Top Behav Neurosci **7**, 61–85.

277 Written evidence from the Royal Society for the Protection of Cruelty to Animals (RSPCA).

welfare perspective.'[278,279] Although we do not currently see any reason for this aspect of animal welfare to be treated differently in ACHM experiments compared with other animal experimentation, this matter should be kept under review as techniques evolve.

We considered whether, through incorporation of human neurons into its brain, an animal might in some way be made more 'self-aware' and therefore capable of experiencing a greater degree of suffering (see 3.4.1 and 5.6.2). The same issues would potentially apply to any situation where neural cells from a more self-aware species are introduced into one that is less self-aware, such as chimpanzee into macaque, or macaque into marmoset. However, as humans are probably the most self-aware species (at least we like to think so), then ACHM pose the greatest risk of this happening. We are not aware of any evidence that self-awareness has been altered in such experiments, but researchers and regulators should be aware of the possibilities.

The effect of animal experimentation on those directly responsible for the day-to-day care of research animals is often underestimated.[280,281] Although ACHM in general are unlikely to pose additional concerns in this respect, it is conceivable that some individual carers might react differently to animals containing large amounts of human material, or with altered appearance or behaviour, whether or not the animals were actually more 'human-like'. There could be positive or negative effects on either the animals or their carers. This is a topic that could be explored, especially as there is an increasing tendency for animal technicians to become more directly involved in the design and interpretation of experiments.

Box 4.1 Public concern for animal welfare

The views of participants in the public dialogue on animal welfare emerged in several ways. Although the dialogue was not intended to explore attitudes to the general use of animals in research, animal welfare concerns were consistently expressed, and participants often transferred broad concerns for the welfare of research animals directly onto research using ACHM.

Overall, as described in Box 3.1, participants expressed conditional support for ACHM. Animal welfare was one of the considerations which they took into account when thinking about whether such research would be justified. (See Box 5.1 for more discussion of these considerations.)

In the quantitative survey, animal welfare was the reason most often given by those who found introducing human material into animals unacceptable. When participants were asked about the welfare aspects relating specifically to ACHM, there were a few suggestions that a new kind of suffering might result from the creation of ACHM. These included concerns that modifying an animal's external organs to cause them to appear human in some way might cause the animal distress, or that research involving the brain might alter an animal's perception of its own circumstances and so increase its suffering. However, for the most part, participants did not feel that the creation of ACHM would produce greater suffering than other types of research involving animals.

'It's a great deal of suffering. The fact that it has human material makes no difference really.'

This concerned but fundamentally supportive view of animal experimentation, if carried out for medical advancement, is in agreement with recent trends in public polling on the topic – see *The 2010 Ipsos MORI Report on Public Attitudes towards Animal Experimentation.*[282]

278 Oral evidence from the Royal Society for the Protection of Cruelty to Animals (RSPCA).
279 Oral evidence from Robinson V., National Centre for the Replacement, Refinement and Reduction of Animals in Research
280 Herzog H (2002). *Ethical aspects of relationships between humans and research animals.* ILAR J **43**, 27–32.
281 Coleman K (2011). *Caring for nonhuman primates in biomedical research facilities: scientific, moral and emotional considerations.* Am J Primatol 73, 220–5.
282 Ipsos MORI (2011). *Views on Animal Experimentation (BIS research) Alternatives to Animal Experimentation (NC3R research).* http://www.ipsos-mori.com/researchspecialisms/socialresearch/specareas/nhspublichealth/attitudestowardsanimalexperimentation.aspx

4.2 Safety

4.2.1 Introduction

Research involving ACHM is subject to safety controls that apply to all biomedical research. These include practices set out in legislation and guidance to protect against hazards to human health and to the environment (e.g. principles of occupational safety and hygiene, and good laboratory practice).[283] Further precautions apply to studies involving genetically altered animals, relating to their containment or deliberate release into the environment (see 6.2.4).

We are grateful for the advice of experts outside the working group, which has aided us in this consideration.[284]

4.2.2 Safety issues considered

Some ACHM experiments will raise safety issues related to the individual experiments being proposed, for example those where animals are made susceptible to infectious agents normally confined to humans, including viruses, bacteria, parasites and prions (see 2.3.3).[285] Such hazards must be considered and managed in order to protect those handling the animals, the animals themselves (from inadvertent infection) and the public, notably from infection or the escape of animals which might act as a reservoir of infection. Neither the issues nor the methods of managing them are different in ACHM experiments from those regularly encountered when dealing with other types of experiment involving infectious agents or other biohazards in animals or cell cultures. For example, ferrets are susceptible to human influenza viruses, but they are routinely used to study the viruses in facilities with a high level of containment. Similarly, how work with human cells or tissue *in vitro* is conducted will depend on the nature of any hazards that might be generated by the experiments proposed, but the minimum conditions used are those that protect both the cells and the researchers from adventitious infection. All such experiments should be assessed in advance for potential risk by researchers and regulators, and managed accordingly; exactly the same considerations apply to ACHM work.

We considered whether there are additional, generic safety issues applicable to research involving ACHM. The major potential issues identified arise from the fact that complex genomes (both human and animal) carry within them integrated viral genomes (endogenous retroviruses or proviruses). These may be quiescent and only able to replicate under certain conditions; indeed many are inactivated because during evolution either the viruses have lost an essential component that enables them to replicate satisfactorily, or the host has lost cell-surface receptors or intracellular factors essential for viral entry and infection of new cells. Specific intracellular host factors (known as restriction factors) can also produce resistance to infectious agents such as viruses; these are species specific and can confer species resistance to particular infectious agents such as HIV.[286] This is a further barrier to cross-species transmission of infectious agents. It is, however, known that infectious agents can, occasionally, change their host specificity (e.g. owing to mutation and/or recombination with other related viruses) such that they become able to infect species previously not liable to infection. Such events are likely to have been involved in influenza strains moving from birds to humans, or from pigs to humans (swine 'flu). HIV might have moved into humans from NHP hosts by similar mechanisms in the wild. We therefore considered whether ACHM experiments could lead to an increased likelihood of reactivation of quiescent viruses or to changes of host specificity of infectious agents.

283 See Health and Safety Executive. *The Scientific Advisory Committee on Genetic Modification (SACGM) Compendium of guidance,* Part 3 (Containment and control of activities involving genetically modified microorganisms). http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm; Organisation for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en

284 Correspondence from Lever A., Stoye J., Bradley A., Weiss R., and Weissmann C.

285 A prion is an infectious agent composed of protein in a misfolded form.

286 Lever AM & Jeang KT (2011). *Insights into cellular factors that regulate HIV-1 replication in human cells.* Biochemistry **50**, 920–31.

4.2.3 Inter-species chimæras

Making chimæras involves mixing cells or tissues containing whole genomes (including their integrated viruses) of different species. We considered whether such an intimate admixture of human and animal cells and tissues might lead to reactivation of infectious particles, such as retroviruses or other pathogens; or to alter their host specificity so that they become infectious to humans. Very similar issues have been extensively discussed in the debate around the transplanting of animal tissues into humans, referred to as xenotransplantation.

Humans and animals have lived in close proximity for a long time, and although examples of viral transfer between human and other species are well known, they are relatively infrequent.[287] Animal tissues have been introduced into people (e.g. pig heart valve transplants, baboon hearts), although in small numbers. What limited evidence is available on humans who have received living pig cells indicates that no infection by porcine viruses has taken place.[288,289,290] A number of studies have, however, shown transient appearances of foreign virus in humans or animals who had received cellular material from other species, suggesting that this issue must be kept under careful review.[291,292,293,294] Any move from experimental into clinical systems will, as with any new therapy, need very careful assessment of safety including infectious risk.

There have been years of experience, and large numbers of experiments, grafting human tissues such as tumours into other species. Human tumour tissue transplanted into immunodeficient mice is known to become infected by endogenous mouse retrovirus. We know of no proven incidents of transmitted infectious events hazardous to man.[295] Mice with human immune systems or mostly human livers have been produced for studying specific infections, and have therefore been closely monitored. To our knowledge there have been no reports that these animals have developed any problems due to activation of proviruses or to novel infections.

Inter-specific cell hybrids involve an even closer association of cells than is generally the case in chimæras because they involve the fusion of whole cells, which can be from a range of species including animal–human combinations. These have been cultured in laboratories for decades, without any generic safety issues of this sort arising.

Chimæras that comprise a mixture of many different cell types, both human and animal, may possibly pose a slightly greater risk than the examples above. This is partly because specific molecules on the cell surface (referred to as receptors) to which viruses and other pathogens attach are often cell-type specific (e.g. influenza viruses tend to infect cells lining the upper respiratory tract, other pathogens target cells in the gut). The greater the range of cell types present from the two species, the greater the chance of any virus finding its appropriate receptor. Moreover, cell fusion does occur naturally in some tissues, such as placenta and muscle, so that inter-species chimæras may also contain inter-specific cell hybrids, increasing the chance of viral recombination events.

Factors relating to the animal host may affect the probability of an adverse event occurring. For example, the longer cell types from two species co-exist the more opportunity there may be for rare events to occur, so that chimæras with a long life span may deserve

287 For example, see Shukla P, *et al.* (2011). *Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus–host recombinant.* Proc Natl Acad Sci USA **108**, 2438–43.
288 Ekser B, *et al.* (2009). *Xenotransplantation of solid organs in the pig-to-primate model.* Transpl Immunol **21**, 87–92.
289 Paradis K, *et al.* (1999). *Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. The XEN 111 Study Group.* Science **285**, 1236–41.
290 Weiss RA (1998). *Transgenic pigs and virus adaptation.* Nature **391**, 327–8.
291 Teotia SS, *et al.* (2005). *Prevention, detection, and management of early bacterial and fungal infections in a preclinical cardiac xenotransplantation model that achieves prolonged survival.* Xenotransplantation **12**, 127–33.
292 Michaels MG, *et al.* (2004). *Baboon bone-marrow xenotransplant in a patient with advanced HIV disease: case report and 8-year follow-up.* Transplantation **78**, 1582–9.
293 Michaels MG, *et al.* (2001). *Detection of infectious baboon cytomegalovirus after baboon-to-human liver xenotransplantation.* J Virol **75**, 2825–8.
294 Stoye JP & Coffin JM (1995). *The dangers of xenotransplantation.* Nat Med **1**, 1100.
295 We are aware of claims that some cases of prostate cancer and myalgic encephalopathy have been associated with murine derived retrovirus. These claims remain scientifically contentious and it is not clear that, even if true, they are related to ACHM. See Urisman A, *et al.* (2006). *Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant.* PLoS Pathog **2**, e25; Hue S, *et al.* (2010). *Disease-associated XMRV sequences are consistent with laboratory contamination.* Retrovirology **7**, 111.

closer attention, especially as aged animals can show reduced immune function. Such events might be more likely to occur in animals that are immune deficient.

It is conceivable that human cells isolated from animal–human chimæras and grown in the laboratory might have acquired replication-competent retroviruses from the animal host. Such animal viruses do not usually cause problems to humans when they are made by animal cells, because they have animal-type coat modifications (alpha-gal epitopes) that would lead to them being detected and destroyed or severely damaged by anti-alpha-gal antibodies that are present in humans. However, if the viruses had moved from the animal to the human cells within a chimæric animal and these human cells were then isolated and grown in culture, the viruses could be competent to infect human cells.[296,297,298]

Any future attempts to use material derived from chimæric animals for therapeutic purposes would need to be very carefully assessed for safety (as is the case with any proposed new therapeutic) and particularly for risk of viral transmission.

Researchers studying pathogens are more likely to consider the infection risks than those who do not. It follows that there needs to be some general awareness of potential infection risks when chimæric animals have been modified in a way that may make them susceptible to human pathogens, but where the study of the latter is not the primary purpose. For example, human respiratory tract cells introduced into animals to study disease such as cystic fibrosis may be susceptible to strains of influenza that could be passed to them by humans, and subsequently passed back. Transmission directly between humans during an epidemic is more likely, but the animals would also need protection.

On balance, we consider the overall risk of an event of this type to be small, though not zero. The types of risk are, however, not unique to ACHM and there are well established methods for risk management. It is important that researchers and regulators bear these risks in mind, particularly when contemplating novel classes of experiment, and act appropriately to manage any possible hazards.

4.2.4 Transgenic and genetically altered animals

Transgenic experiments in which unusually large amounts of genomic material (such as a whole chromosome) are transferred between species (see 3.2) raise similar issues as chimaeras, as it will be difficult to know *a priori* whether the sequences contain proviruses that could be activated or genes that are critical for pathogen infection. However, the great majority of transgenic experiments do not raise these issues because the transfer of one or a few specific known gene sequences should not lead to transfer of viral sequences into an unusual environment, unless it is part of the experimental design.

Modification of cell surfaces can produce or modify viral or other pathogen receptors, leading animal (or human) cells to alter their 'tropism' (ability to be infected by the pathogen).[299] This approach has been used deliberately to develop animals expressing specific human receptors, to study human-specific viruses and infectious agents (see 2.3.3). For example, transgenic mice have been made that express the human cell-surface receptor for polio virus, so that the modified mice become susceptible.[300] Mice susceptible to hepatitis virus have also been developed (see 3.3), and a similar approach for the study of HIV is under investigation.[301]

296 Hara K, *et al.* (2008). *Neural progenitor NT2N cell lines from teratocarcinoma for transplantation therapy in stroke.* Prog Neurobiol **85**, 318–34.
297 Newman MB, *et al.* (2005). *Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells.* Stem Cells Dev **14**, 29–43.
298 Nelson PT, *et al.* (2002). *Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation.* Am J Pathol **160**, 1201–6.
299 Tissue tropism is a term used in virology to define the cells and tissues of a host which support growth of a particular virus. Bacteria and other parasites may also be referred to as having a tissue tropism.
300 Ren RB, *et al.* (1990). *Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis.* Cell **63**, 353–62; Koike S, *et al.* (1994). *Characterization of three different transgenic mouse lines that carry human poliovirus receptor gene-–—influence of the transgene expression on pathogenesis.* Arch Virol **139**, 351–63; Dragunsky E, *et al.* (2003). *Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study.* Bull World Health Organ **81**, 251–60.
301 Shultz LD, *et al.* (2007). *Humanized mice in translational biomedical research.* Nat Rev Immunol **7**, 118–30.

We have described the generation of strains of mice with humanised immune systems (2.3.3) and have considered whether these systems may allow rodent viruses to become selected for the ability to escape human immune systems, and so encourage their ability to cross species barriers. Expert consensus is that this is an extremely unlikely scenario. All such mouse strains should in any event be kept in appropriate containment.

Virus inactivation can occur by the same mechanism as the hyperacute rejection of xenografts.[302] Lysis of animal retroviruses is triggered by the binding of human anti-alpha-gal antibodies to alpha-gal epitopes expressed on the viral envelope (outer shell of the virus). Virus grown *in vitro* in non-primate cells is inactivated by human blood serum, but the same virus cultured in human cells is not. This is because the virus makes its envelope by budding out from the cells it grows in – only when alpha-gal is present on the host cells is the viral envelope sensitive to antibody-dependent, complement-mediated lysis by components of human serum. It follows that modifications to the alpha-gal system to make pig xenografts resistant to hyperacute rejection may make enveloped pig viruses resistant to destruction by humans.[303,304] Two of the three complement regulatory proteins are also receptors for human viral pathogens: CD46 is a cell-surface receptor for measles virus, and CD55 can serve as a binding receptor for Echo and Coxsackie B picornaviruses.[305] Transgenic animals expressing human CD46 and CD55 would therefore be vulnerable to infection from humans with these viruses (this is a welfare concern for the animals), but a greater concern is that such transgenic animals may increase the opportunities for animal viruses to adapt to a human host range. For example, in transgenic pigs that express both pig and human forms of the CD55, picornaviruses that use the porcine CD55 equivalent might readily adapt to recognise human CD55. These viruses would be pre-adapted for transmission to a xenograft recipient, and for human–human transmission.

Where the genes under manipulation carry any risk of modifying viral receptors or aspects of the intracellular environment in a way that risks affecting endogenous pathogens, the same precautions are required as in other experiments involving potentially infectious agents: prior risk assessment and appropriate risk management, including containment strategies. In our view, provided proper vigilance is exercised in the design and licensing of relevant ACHM experiments, current knowledge makes it unlikely that important safety issues of this sort would arise accidentally. These considerations only apply to a small minority of ACHM experiments, but it is very important that proper vigilance is maintained in the design and regulation of these experiments.

In summary, although the use of humanised animals could theoretically lead to adaptation or recombination of viruses, we concur with broader guidance that such risk is low:

'*… if an animal line was produced which was modified to contain a receptor for a human virus, these animals may act as a novel reservoir for human disease. Although the possibility of such additional hazards to humans must always be considered, it is recognised that, in most cases, the activities will not pose any extra hazards to humans.*'[306]

We also consider that any risk to other animals (especially those outside any research facility) is very low.

Any manipulation that is known to, or could, alter viral or other pathogen recognition sites, or in any other way affect susceptibility to pathogens or

302 Magre S, *et al.* (2004). *Reduced sensitivity to human serum inactivation of enveloped viruses produced by pig cells transgenic for human CD55 or deficient for the galactosyl-alpha(1-3) galactosyl epitope.* J Virol **78**, 5812–9. In this study amphotropic murine leukaemia virus, porcine endogenous retrovirus, and vesicular stomatitis virus were tested.
303 Destruction in this context refers specifically to antibody-dependent, complement-mediated lysis of enveloped virus particles.
304 Weiss RA (1998). *Transgenic pigs and virus adaptation.* Nature **391**, 327–8.
305 *Ibid.*
306 See Health and Safety Executive. *The Scientific Advisory Committee on Genetic Modification (SACGM) Compendium of guidance,* Part 5 (Genetic modification of animals), Clause 38. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm

that deliberately involves the activation of human and animal proviruses within the same ACHM (such that they could recombine) should be carefully risk-assessed by researchers and regulators and appropriate control mechanisms should be put in place (see 8.5).[307]

4.2.5 Accidental or deliberate release

We have considered potential issues relating to the accidental or deliberate release of ACHM into the environment. Accidental release would be mainly relevant to animals that are less easily contained, such as rodents, those with small free-living eggs or larval forms, or those with flight.[308] The release of large or non-endemic animals would be more apparent and recapture more likely.

Chimæric animals containing human cells are very unlikely to pose any specific hazard, unless they are also infected with an animal or human pathogen as part of a research programme or are very likely to pick up such a pathogen in the wild. We do not consider such ACHM to pose risks different from conventionally infected animals used in research.

Animals containing human DNA sequence may transmit these modifications to offspring. However, there are well-established protocols for containing genetically modified animals, which would equally apply to ACHM (see 6.2.4). Competition to breed outside a contained environment is usually high and evidence suggests that laboratory strains are less able to compete and breed in the wild.[309] If there was concern around a specific human DNA alteration, and a risk of interbreeding in the wild, then inclusion of a genetic alteration to prevent survival or fertility should also be considered in designing and reviewing the experimental protocol.[310]

Good practice requires that ACHM should be kept under appropriate containment, and any deliberate release should only be contemplated after full risk assessment, and with appropriate regulatory permission (see 6.2.4).

4.2.6 Other considerations

ACHM and the food chain

We have considered whether it is feasible that ACHM may be consumed by other organisms (by intention, or accident) and whether there may be safety concerns associated with ACHM entering the animal or human food chain. For example, the possibility of genetically engineering cows to express human milk proteins has been considered and some progress reported.[311,312]

There are general arguments related to the use of genetically altered animals in agriculture, beyond the scope of the current study (see 1.1), which we do not replicate here. As a specific subset of such animals, it is not evident that the consumption of animals (e.g. sheep or goats) carrying human DNA would merit concern from a safety perspective above that of genetically modified animals in general, unless the particular genetic modification itself created a hazard. We therefore see no additional considerations that should be applied to such animals, except in limited cases that relate to the specific modifications involved.[313]

Although we have considered only safety issues in this section, we stress that deliberate introduction of any such materials into the human food chain could only be contemplated after full public discussion of all the issues involved; and with appropriate evaluation and authorisation under the relevant European frameworks for genetically modified and novel foods. These are administered in the UK by the Food Standards Agency and enforced by local authorities.[314]

307 It is critical that the provenance of human material to be used clinically is known and considered during the risk assessment.
308 Such as aquatic species including *Ciona* (sea squirt), fish and frogs, insects (e.g. *Drosophila*) and birds.
309 See Meagher S, *et al.* (2000). *Male-male competition magnifies inbreeding depression in wild house mice.* Proc Natl Acad Sci USA **97**, 3324–9; Jimenez JA, *et al.* (1994). *An experimental study of inbreeding depression in a natural habitat.* Science **266**, 271–3.
310 For example, the animal could be modified to become dependent on administration of a drug.
311 Wang J, *et al.* (2008). *Expression and characterization of bioactive recombinant human alpha-lactalbumin in the milk of transgenic cloned cows.* J Dairy Sci **91**, 4466–76.
312 Yang B, *et al.* (2011). *Characterization of bioactive recombinant human lysozyme expressed in milk of cloned transgenic cattle.* PLoS One **6**, e17593.
313 For example, animals that have been modified to render them susceptible to carry human pathogens, or human prions, would require very stringent control.
314 There are two pieces of relevant European legislation in this area: Regulation (EC) No 1829/2003 on genetically modified food and feed, which would apply to genetically altered animals, and Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, which would apply to chimæras.

Biological weapons
ACHM could, in theory, be applied to the development of biological weapons or to development of antidotes or countermeasures, but it is not obvious that it creates important novel hazards, nor do we see that it raises concepts that have not already been covered elsewhere in this report.

Concerns about the safety of ACHM raised by participants in the public dialogue are set out in Box 4.2.

Box 4.2 Public concerns about the safety of the use of ACHM

Participants' safety concerns around ACHM fell into two categories: immediate and future risks.

Immediate risks related to unintended release of modified animals and the consequences for humans, animals or the environment. Concerns included:

- Triggering disease epidemics (some participants related this to the origin of HIV through human-primate contact).
- 'Contamination' of the food chain.
- Permanent alteration or loss of existing species due to breeding with released animals.
- Unpredicted impacts of modified animals on existing flora, fauna and the ecosystem.

Future risks concerned events such as the creation of species for terrorism or warfare, which participants felt might ultimately result from the decision to permit certain types of research now (sometimes described as the 'slippery slope' argument).

5 Ethical and social concerns

5.1 Ethical principles and biomedical research

Biomedical research seeks to determine the normal processes of life, to advance the understanding of health, and to identify and develop new methods of promoting health and preventing illness. This research deals with conditions which affect humans and therefore at some stage entails investigations of human subjects; ideally, the research ultimately leads to new interventions that need to be tested on human subjects before they can enter clinical practice. So the involvement of human subjects in medical research is inescapable. But it is also constrained by the rights and interests of the human subjects, and where medical research poses serious risks to humans it is important to minimise these risks by undertaking other kinds of research before research involving human subjects is undertaken.

5.1.1 The contested domain of animal research and our working assumption

The way of pursuing this objective which is under examination here, involves the use of animals which have been modified to contain human genetic or cellular material. It may be objected at once that the acceptability of such research can be challenged on the grounds that all research involving the use of animals is unethical, except where the research involves procedures which benefit the animals involved. We do not attempt to enter directly into these arguments here; for a recent survey of the issues and arguments in this highly contested area, we commend the 2005 report by the Nuffield Council on Bioethics on '*The ethics of research involving animals*'[315] and two previous reports led by the Academy of Medical Sciences '*The use of non-human primates in research'* (2006)[316] and '*Inter-species embryos*' (2007).[317,318] But in Chapter 6 we describe current legislation and practice in the UK under which some types of animal research (such as the use of Great Apes) are not undertaken, and in which use of animals is licensed only where principles such as the 3Rs (see 6.2.1) are followed and it is judged that the potential benefits of the research outweigh the harm done to the animals involved; and we assume here that these practices are broadly acceptable. We recognise that not everyone will agree with this assumption (see Box 5.2); but our aim is to focus specifically on the issues raised by the use of animals which include human genetic or cellular material (ACHM) and these issues are best addressed in the context of present practices.

5.1.2 Three ethical perspectives: utilitarianism, deontology and virtue ethics

Although we start here from the assumption that the use of animals in the course of medical research is morally acceptable where its benefits outweigh the harm done, and thus from a position that in this respect addresses moral questions from a broadly utilitarian perspective, we accept that moral thought often includes 'deontological' duties to others whose basis lies in their status and our relationships with them rather than in the relative value of the consequences of action. So the approach taken here is to be understood to allow for consideration of similar duties to animals which would place limits on the ways in which animals may be used in medical research.[319] We also recognise the importance of the ethical perspective characteristic of virtue ethics, which invites us to reflect on the kind of person we aim to be, in addition to considering the justifications for and defences of the actions we undertake. This perspective is manifest in the ways in which we think about other people; for we do not just evaluate the acceptability of their actions – we also care about their character, their motivations, dispositions and aspirations. This, then, is an ethical perspective which approaches

315 Nuffield Council on Bioethics (2005). *The ethics of research involving animals.* Nuffield Council on Bioethics, London.
316 Weatherall D (2006). *The use of non-human primates in research.* http://www.acmedsci.ac.uk/images/project/nhpdownl.pdf
317 Academy of Medical Sciences (2007). *Inter species embryos.* http://www.acmedsci.ac.uk/p48prid51.html
318 See also Box 5.2 for the views obtained through our public dialogue.
319 For further discussion of 'deontological' considerations of this kind, see Fiester A (2009). *Ethical issues in transgenesis.* In Taupitz J. & Neschka M., Chimbrids. Springer, Berlin.

the moral questions raised by the use of animals in research, not only by reference to the rights and wrongs of the research, but also by reference to what it shows about the character and relationships of those involved in it and of the societies which practice it.

5.2 The significance of the distinction between animals and humans

We begin by reflecting briefly on traditional attitudes to the distinction between humans and animals. Over the last two million years of human history people have been profoundly affected by their encounters and relationships with animals, especially those on which they have come to depend for their way of life and those which threaten it. The distinctions that have been drawn between humans and animals, and among groups of animals, have been central to the values and culture of almost all human societies of which we have records. Some of these distinctions may be arbitrary, such as that between animals which we eat and those we refuse to eat; many just reflect human interests, such as the categorisation of some animals as pets and others as vermin. But the understanding of the relationship between humans and animals always has a special status: in many cultures it defines what it is to be human, informing social rituals and taboos, shaping what humans may do, and determining those to whom special responsibilities are owed by defining the limits of those who are considered human. The fact that the ways this distinction is made may sometimes seem to us to be irrational, unstable or hard to define does not rob it of importance, though it indicates that its significance is often a matter of social practice, and hence of cultural and historical specificity.[320]

5.2.1 The special 'dignity' of man

We use palaeontology and molecular genetics to distinguish between the species to which we belong, *Homo sapiens sapiens,* and other hominid apes; with continuing debate about the status of Neanderthal man and other earlier creatures we see as significantly near-relations of ours. The ethical and symbolic significance of this distinction, and that between humans and animals generally, is normally explained by reference to capacities which are central to our sense of what gives special value to human life, such as the capacity for rationality and self-consciousness, for free will and moral sensibility, or for language and culture. And one term, 'dignity', has come to symbolise the thought that human life has a special value. Kant famously maintained that only humans have the kind of self-conscious rationality which gives them dignity as 'ends-in-themselves' and entitles them to respect from others;[321] and following Kant, 'human dignity' is regularly invoked in declarations and charters of human rights (see 7.4.1).[322]

5.2.2 Challenging the moral boundary between animals and humans

But these explanations, and the boundaries that come with them, can be challenged. In the 19th century Jeremy Bentham argued that it is the capacity for suffering that is of fundamental ethical significance, and that once this is recognised the moral boundary between humans and animals should be erased:

'The day may come, when the rest of the animal creation may acquire those rights which never could have been withholden from them but by the hand of tyranny. … It may come one day to be recognized, that the number of the legs, the villosity of the skin, or the termination of the os sacrum, are reasons equally insufficient for abandoning a sensitive being to the same fate. What else is it that should trace the insuperable line? Is it the faculty of reason, or, perhaps, the faculty of discourse? But a full-grown horse or dog is beyond comparison a more rational, as well as a more conversable animal, than an infant of a day, or a week, or even a month, old. But suppose the case were otherwise, what would it avail? the question is not, Can they reason? nor, Can they talk? but, Can they suffer?'[323]

320 The classic account of this topic is Douglas M (1966). *Purity and danger: an analysis of concepts of pollution and taboo.* Routledge Classics, London.
321 Kant I (1785). *Groundwork of the metaphysics of morals.*
322 See, for example, articles 1 and 2 of the 1997 UNESCO Declaration on the Human Genome and Human Rights. http://www.unesco.org/new/en/social-and-human-sciences/themes/bioethics/human-genome-and-human-rights/
323 See Chapter 17, section IV, note 122 of Bentham J (1823). *Introduction to the principles of morals and legislation.*

Bentham did not convert his contemporaries to his radical point of view. But in our own time Bentham's challenge has been renewed by philosophers such as Peter Singer and Tom Regan, and there is no doubt that through their writings they have managed to broaden support for a Benthamite animal-rights movement.[324] Many important issues are raised here concerning the ways in which animals are viewed and treated in contemporary life, in agriculture, in domestic contexts, in protected natural habitats as well as in the course of medical research; and we recognise the importance of the continuing debates on these issues. As we have indicated earlier in this report we do not seek to enter into these broad debates, our focus is on the question of whether the use of ACHM makes a significant difference to the acceptability of research involving them. But there is one project championed by Singer which merits some attention here, – his 'Great Ape project' which aims to secure a legal status for Great Apes comparable to that of humans.[325] For it is an explicit aim of Singer's project to establish a ban on the use of Great Apes in medical research.

Research involving Great Apes has not in fact been undertaken in the UK in the past 50 years (unlike research on human subjects); nonetheless the issue of a complete ban remains controversial. Opponents of a complete ban such as Colin Blakemore argue that the use of Great Apes for research needs to be retained as an option for cases where there is a pressing medical need involving a serious disease whose control requires research that cannot be carried out in any other way.[326] In this report we accept that there are powerful moral reasons for being very reluctant to use Great Apes for medical research; but we argue that it is reasonable to hope that the issue of a complete ban can be set to one side by the use of other transgenic animals containing human materials (see 4.1). Nonetheless the fundamental issue between animal-rights advocates and their opponents is whether there is a moral boundary between humans and (other) Great Apes. Where Singer's Great Ape Project is explicitly founded on the claim that there is no such boundary, Blakemore took the opposite position: '*I worry about the principle of where the moral boundaries lie. There is only one very secure definition that can be made and that is between our species and others.*'[327] In our discussion below of the use of primates in medical research, we too find ourselves drawn into this debate.

5.3 Humanised animals in fiction

The phrase 'humanised animal' is often used in scientific literature to describe transgenic animals or chimæras in which human genetic material or cells have been incorporated. For those who know the origin of the phrase 'humanised animal' the use of this description will be disconcerting. It was coined by H G Wells to describe the results of the cruel activities of the fictional vivisectionist Dr. Moreau whose project of creating 'humanised animals' is described in *The Island of Dr. Moreau.*[328] But because the 'humanisation' inherent in the work of today's researchers is not at all like that attempted by H G Wells' Dr. Moreau, who sought to turn animals of other species into quasi-humans, any direct association between the two would be misguided and unfair.

5.3.1 Frankenstein and his 'monster'

Wells's book is not well-known these days. But popular discussions often allude to Mary Shelley's *Frankenstein.* Unlike Wells's Dr Moreau, Shelley's Victor Frankenstein is not represented as engaged in a deliberately vicious project – instead he is carried along by a thoughtless, obsessive wish to bring life back to a human corpse, or rather to a creature assembled from several human corpses.

324 Singer's most famous work in this area is Singer P (1976). *Animal liberation.* Cape, London. Tom Regan's writings include Regan T (1983). *The case for animal rights.* University of California Press, Berkeley.
325 Singer P & Cavalieri P eds (1994). *The Great Ape project: equality beyond humanity.* Fourth Estate, London.
326 For a recent statement to this effect, see the transcript of Blakemore's ARZone discussion (19 February 2011): http://arzone.ning.com/profiles/blogs/transcript-of-prof-colin
327 Owen J & Lean G (2006). *Leave our apes alone.* The Independent. http://www.independent.co.uk/environment/leave-our-apes-alone-481035.html
328 Wells H G (1962). *The island of Dr. Moreau.* Penguin Books, Harmondsworth.

The horrendous consequences of his success are then the substance of Mary Shelley's extraordinary story. Although Frankenstein's 'monster' is not a humanised animal, Shelley's depiction of the monster's thoughts and feelings, and of the attitudes of the humans whom the monster encounters to his advances, brilliantly captures a natural fear concerning humanised animals, especially humanised primates: the fear that although through their humanisation they become so close subjectively to humans to merit treatment as humans, their appearance and behaviour gives rise to revulsion and horror as a result of which they turn against their human creators.

5.3.2 Children's fiction

These stories by Wells and Shelley are of course just the tip of the iceberg when it comes to fictional explorations of variations of the boundary between humans and animals. From Aesop's Fables to Maurice Sendak's '*Where the Wild Things Are*', stories for children have been populated with animals, familiar or imaginary, which take on human capacities for thought and feeling and also human virtues and vices.[329] Quite why stories about animals are so well-suited as ways of introducing children to human characters and situations is a deep question for child psychology which we do not attempt to investigate here.[330] But there is no doubt that our attitudes to animals and sympathies for them are affected by these stories, even when we recognise that they are fanciful and that we are prone to the 'pathetic fallacy'[331] of projecting human sentiments into animals that are not capable of them.

The temptation when faced with fictional and mythical explorations of humanised animals is to regard them as intriguing exercises of the imagination, often charming though sometimes frightening, but not especially revealing when it comes to a serious understanding of animals, which requires instead more austere scientific research. But that may be too quick. It is often through our relationships with the animals with which we share our homes, our 'pets', that we learn to appreciate something of their subjectivity even when we recognise the truth of Montaigne's famous remark '*When I play with my cat, who knows if I am not a pastime to her more than she is to me*'.[332] Our capacity to understand and engage with each other draws upon an intuitive 'theory of mind' which is more a matter of empathetic simulation than of overt reasoning,[333] and there is every reason to suppose that a similar capacity is engaged in our direct relationships with animals.[334] So although fiction no doubt exaggerates the empathetic projection of human sentiments into animals, it draws on a capacity which is fundamental to our understanding of each other.

5.3.3 Kafka's animals

Two stories by Kafka exemplify these types of fiction.[335] In his well-known story, 'Metamorphosis', the hero, Gregor, is mysteriously transformed into a cockroach; and the story then imaginatively explores Gregor's terrifying predicament and the attitudes of his family to the giant cockroach who shares their small apartment. Wonderful though this story is, it tells us nothing about cockroaches. But Kafka wrote another short story, whose title, 'A Report to the Academy' is nicely appropriate for this report. In this story Kafka writes from the point of view of a humanised chimpanzee about the life of a circus ape who has learnt to speak. It is not a comforting story and Kafka clearly writes to make one wonder what 'it might be like' for a chimpanzee to be in this situation.[336] So there is a 'Kafkaesque concern' which we need to take seriously, alongside what one might call the 'Frankenstein fear' that the medical research which creates 'humanised' animals is going to generate 'monsters'.

329 Sendak M (1963). *Where the wild things are.* Harper & Row, New York.
330 Bruno Bettelheim developed an influential Freudian approach to this issue in Bettelheim B (1976). *The uses of enchantment.* Knopf, New York. For a critical discussion of Bettelheim's position see Zipes J (1979). *Breaking the magic spell: radical theories of folk and fairy tales.* University of Texas Press, Austin.
331 The phrase is Ruskin's; see volume 3, part 4, of Ruskin J (1856). *Modern painters.* Smith, Elder, London.
332 Montaigne M (1580). *An apology for Raymond Sebond* (Essays Book 2, Chapter 12).
333 For discussion of the issues here see Carruthers P & Smith PK (eds) (1996). *Theories of theories of mind.* Cambridge University Press, Cambridge.
334 The issues here are explored in Haraway D (2008). *When species meet.* University of Minnesota Press, Minnesota.
335 Both stories are included in Kafka F (1996). *The metamorphosis and other stories.* Dover, New York.
336 *ibid*.

5.4 'Playing God'

'*God made all the animals and then he made man to be in charge of animals and take charge of the world. We have the ability to do that.*'
Public dialogue participant, London.

One of the themes of Mary Shelley's novel is that the terrible consequences of Frankenstein's success in acquiring a God-like power to overcome death show the need for humility in the exercise of power gained through scientific research. In a similar way, it might be said that by creating animals with significant human genetic and cellular components contemporary scientists are 'playing God'. This is not a specifically religious objection, although some may make it on religious grounds; the phrase carries a more general sense that scientists are possessed of a certain hubris, a false belief in their own powers and their own rights to exercise them in pursuit of their own projects, hence abusing their capacities without proper consideration of the consequences, in this case the transgression of the boundaries between humans and other animals.

5.4.1 Humanity's stewardship responsibility

There are two ways in which this complaint can be made more specific. From one direction, it might be said that by creating humanised animals scientists threaten the distinctive dignity of man; from the other direction, it might be argued that the process of humanising an animal undermines the integrity of the animal's inherent life-form. We discuss the first point in this section and come back to the second in the next section. But in both cases we start from the thought that humans have a general ethical responsibility to act as 'stewards' of the natural world, valuing and caring for the environment, including plants, fish and animals, instead of just treating them as a resource to be exploited for the benefit of one species, mankind. We take it that the exercise of this stewardship responsibility can be thought of as a virtue which should inform our relationships with the natural world, bringing with it duties that are appropriate to these relationships.

This report is not the place for a detailed exploration of these duties whose exercise enters into a great number of activities, but we take it that they do not preclude research which leads to the creation of animals which cross the boundaries between species, as long as the research is conducted in a way which attends to the interests of the animals involved and to the health of the broader environment. However, when one of the species is man, an extra deontological moral claim comes into play, the 'dignity' of humans (see 5.2.1); and the first claim above was that by humanising animals and thus blurring the distinction between animals and humans, scientists threaten the special dignity of man.

5.4.2 Humanised animals and human dignity

We have already observed that the presumption that humans have this distinctive status can be questioned by comparing humans with other animals, especially Great Apes; and we return to this point below. But setting it aside for the moment, it has long been accepted that the dignity of man does not rule out many ways in which animal and human materials are combined. After all, most humans eat meat or drink milk. Of course, some people are vegans on moral grounds, but these grounds are not that the very idea of combining human and animal materials is wrong, but that it is wrong to kill animals for human consumption, that dairy farming is exploitative and so on. Again, humans are not demeaned by the incorporation of parts of non-human animals (such as heart valves from pigs) through xenotransplantation, though it is possible to object to this practice on other grounds.[337] Similarly, therefore, the creation by another form of xenotransplantation of animals which include significant human elements cannot be held to threaten human dignity just because it humanises the animals involved. In particular, the creation of reliable animal models for human disease poses no threat to human dignity. Perhaps this practice imposes unacceptable harm on the animals involved; but that is a different argument which will be considered in the next section.

337 For a further recent work about human–animal xenotransplantation, see Blackman M (1997). *Pig heart boy.* Doubleday, London.

5.4.3 Extending human dignity

But what, one might suggest more speculatively (and this is the Kafkaesque concern of the previous section), about the creation of animals, especially primates, with the types of capacity that are more central to human dignity, such as a capacity for practical reasoning, a sense of their own identity and the ability to understand and engage with others? On reflection, however, what this possibility would undermine is not the dignity of human life, but its supposed *distinctive* dignity, in a way that extends the central claim of Singer's Great Ape project that there is no moral boundary between humans and Great Apes (see 5.2.2 above). For the more such enhanced primates come to have the capacities that have been regarded as characteristically human, the more unacceptable it would be to maintain a firm moral boundary between them and ourselves.[338]

In the present context, this conclusion cuts two ways. It refutes the complaint that it is an insult to human dignity to create animals which include significant human materials. But it also suggests that it would be right to hold that such enhanced primates should be accorded much the same moral status that we take ourselves to have, and thus that there are deontological grounds for opposing their use for research, at least in any way in which we would not use humans for research. In section 5.6 we return to these difficult issues.

5.5 Animal welfare

We now turn to the second point raised earlier, that the process of humanising an animal interferes with it in a way which is destructive of its integrity. In Chapters 2 and 3 we reviewed the ways in which current medical research involves the use of animals which include significant amounts of human material. Much of this research is directed to the development of animal models for human disorders to make it possible to undertake fundamental research into the causes of these conditions and possible treatments for them which cannot be properly carried out on human subjects. In the course of this research, therefore, animals such as mice are modified in such a way that they become susceptible to disorders such as variant Creutzfeldt–Jakob disease, Huntington's disease, Parkinson's, diabetes, Down's syndrome, β-thalassaemia, human cancers and so on. While this list shows the potential of this approach to medical research, from the point of view of animal welfare it is depressing: for the research precisely involves finding ways of transmitting the worst of human disorders to animals that are not normally afflicted by them. While no doubt the animals are treated 'humanely' (a strange word in this context), the whole process is intended to transform these animals into living laboratories for research into these human disorders.

In thinking further about this, there are two questions which one can raise. The first question arises from a utilitarian ethical perspective and looks both to the interests of the animals involved and to the interest of the humans who might benefit; it asks whether medical research, which involves ACHM, makes things distinctively worse for the animals involved as compared with other forms of medical research which use animals and compares this with the benefits that might accrue to humans. The second question arises from the 'stewardship' virtue ethics perspective described earlier (see 5.4.1) and looks to the relationship between humans and animals implicit in this kind of medical research; it asks whether humanising animals, so that they can be used as models for human disorders, introduces a new level of exploitation into the relationship between humans and animals which is unjustified by the correlated benefits to humans.

5.5.1 Comparing welfare

'*A mouse feels the same pain. I'm not saying protect the millions of them. But I feel pain is pain to be honest*' Public Dialogue, London.

338 See Harris J (2011). *Taking the "human" out of human rights.* The Cambridge Quarterly of Healthcare Ethics **20**(1), 9–20.

'*It's a great deal of suffering. The fact that it has human material makes no difference*'. Public Dialogue, Newcastle.

The familiar way of answering the first question is to apply the approach which is characteristic of the existing rules which govern the use of animals in medical research and concentrate primarily on the levels of suffering to which the animals are exposed. Thinking about this requires comparisons which cannot be precise, but the salient points appear to be the following:

1. The specific techniques involved in creating transgenic and chimæric animals involving human material do not themselves bring any great suffering to the animals involved, nor is their quality of life seriously compromised by these transformations, at least as compared with that which is normal for experimental animals (see 4.1.2).
2. But, the use of these animals for research which could not otherwise be conducted into human disorders, including in principle the worst that we experience, does often impose significant suffering on the animals.
3. Equally some current animal research necessarily involves the infliction of suffering on animals, and a minority of research very great suffering, including that mandated by our human safety regulations.
4. In fact (see 4.1.1), research indicates that it should be possible to undertake some types of research and testing (including some toxicity testing) on transgenic mice rather than on species such as primates whose suffering is of more concern to us because of their greater cognitive capacity, but which are currently the best indicators of human reactions.

The last two points here are significant, for from the point of view of animal welfare, it is extreme suffering that is most objectionable, and if this new research makes it possible to limit the need for tests which involve it, or to mitigate the suffering involved in them, then that is an important animal welfare benefit (we return to this point in the next section). The second point above should then be set against this benefit, but it is hard to see that it implies that this work significantly increases the level of suffering experienced by the animals involved as compared with that experienced by animals in other kinds of medical research.

In considering the impact of this research on the animals involved, however, it is not sufficient to take account of the familiar question about the level of suffering involved, since further questions about animal welfare are raised by the process of humanisation itself. But as long as the condition mentioned in the first point above is met, namely that the quality of life of these humanised animals, for example that of breeding colonies of transgenic mice, is not seriously compromised by their humanisation, at least as compared with that which is normal for experimental animals, this kind of research does not appear to bring with it any new animal welfare consideration. What it does open up instead is the challenge inherent in the second question above, namely that humanising animals so that they can be used as models for human disorders introduces a new level of exploitation into the relationship between humans and animals which runs contrary to the values inherent in our stewardship responsibility to animals.

5.5.2 Stewardship, humanisation and exploitation

The process of humanising an animal is not necessarily harmful to it: it could be part of a process of enhancement which endows the animal with greater physical abilities or resistance to disease. Yet although there are no doubt possibilities of this kind, especially where primates are concerned (to which we return in the next section), it would be disingenuous to pretend that a significant part of the work described in Chapters 2 and 3 is of this kind. The type of humanisation of animals we are considering here is undertaken primarily to facilitate medical research for the benefit of the human species.

The issue which this challenge throws into relief is that of the ethical significance of the 'human' dimension of the process of humanisation when it is considered in the context of the assumption that the use of animals for medical research is in principle acceptable under certain conditions (see 5.1.1). Is it the introduction of significant human materials into animals which is thought to make the process especially exploitative? Or is it just the fact that the process is undertaken primarily for the benefit of humans? If the former claim is made, then it needs to be explained why the presence of the human materials (cells or genes) is by itself of decisive significance. Suppose that it is discovered that there are ways of genetically modifying mice which do not involve the insertion of human genes but which provide equally valuable models for human disorders, and that all the animal welfare issues are much as they are for humanised mice: would that kind of practice be ethically preferable to that which we are considering here?[339] We find it hard to see what reason one could have for such a preference beyond the symbolic absence of human materials from the animals in the hypothetical case; yet given that the animal welfare issues are supposed to be the same, it is hard to see why this justifies a moral distinction between the two cases (and if one thinks that it does, suppose that the hypothetical procedure leads to a greater cost in animal welfare; which procedure is then preferable?). If, alternatively, it is just the fact that the primary goal of this research is the promotion of human welfare that is supposed to make it exploitative, then there is no reason to hold that this kind of research is ethically more problematic than other types of medical research which use animals for the benefit of research into human disorders.

There is another way in which the ethical significance of the 'human' dimension of the process of humanisation might be elucidated, namely by supposing that where it involves neuronal cells, it transfers significant human psychological capacities and abilities to the animals involved. But we set that aside for now since we shall discuss the issue to which this hypothesis gives rise in the next section.

5.5.3 A preliminary conclusion

The conclusion that we have arrived at so far is that the practice of humanising animals for the purpose of medical research does not bring significant new ethical problems as compared with other kinds of medical research which use animals. As we have explained, as far as animal welfare is concerned, there are in fact grounds to hope that the new practice will make it possible to decrease the amount of suffering required for some tests (and we say more about this in the next section). The further charge was that humanising animals specifically to benefit humans introduces a new level of exploitation into the relationship between humans and animals. On examination, however, this charge does not stand up: once the symbolic value of the introduction of human materials into animals is set aside, the basis of the charge is that the whole practice is undertaken for the benefit of humans. That should indeed be admitted, but in this respect the new kind of research is not different from others which use animals for medical research without humanising them.

5.5.4 Our conflicting responsibilities

One might respond that the conclusion to be drawn from this argument is that the whole practice of using animals for medical research whose primary goal is the treatment of human disorders is exploitative and runs counter to the stewardship responsibility which ideally guides man's dealings with animals. But that response opens up the general issue of justifying this practice, an issue which we have here set to one side. Our basic presumption is that alongside our stewardship responsibility to animals there is a general social responsibility to facilitate medical research. Thus we face in this area a conflict of responsibilities where the use of animals for medical research provides the best, and perhaps the only, acceptable way of attempting to understand, diagnose and treat some terrible human disorders. It is,

339 In fact, as is noted in 2.2.1, in some cases ACHM-type animals contain no human cells or DNA even though their genome has been modified to mimic relevant parts of the human genome.

we think, reasonable to believe that success in this endeavour would bring a very great benefit, just as withholding or postponing that benefit would risk bringing significant suffering and premature death to very large numbers of people; and our working assumption is that this benefit is sufficient to justify the harm done to the animals involved. We recognise that not everyone shares this assumption, and we ourselves accept that it would be wonderful to be able to make progress in medical research without harming either animals or human subjects. But in our judgement that is not the world we inhabit.

5.6 Non-human primates

'*I don't have a problem with it until it gets to the brain – liver, heart, etc. are all fine. It's the brain which makes people humans*' Public Dialogue, Newcastle.

We are ourselves primates. For this reason the use in medical research of NHPs as substitutes for humans gives rise to a dilemma. Their biological proximity to us implies that they generally provide more reliable models for human disorders and reactions than other animals, which makes them especially suitable for use in medical research; yet it also implies that their capacities and abilities are more similar to ours than those of other animals, and as a result some of the deontological considerations we have for not conducting medical experiments on unconsenting humans apply also to them. It is not our task to explore and debate this dilemma, though we commend the discussion of it in the Academy's 2006 report on '*The use of non-human primates in research*', undertaken by a working group chaired by Sir David Weatherall.[340] For us the question is just what difference is made by the development and use of animals containing significant amounts of human material, which is not a question directly addressed in that report.

5.6.1 Substitutes for NHPs

One striking fact highlighted in the Weatherall report is that the great majority (about 75%) of the NHPs currently used in medical research in the UK are used for the purpose of testing the toxicity of drugs.[341] The explanation for this is that testing drugs on primates has been a much more reliable guide to the effects of drugs on humans than testing the drugs on other animals, such as mice.[342] But, as we mentioned above, the situation is now changing, and it is reasonable to hope that suitable humanised mice, or similar animals, could be developed as effective substitutes for NHPs for the purpose of many toxicity tests. Such a change could therefore eventually lead to a reduction in the number of NHPs used for this type of medical research, which we take to be an important potential change for the better because the primate's greater cognitive abilities imply that it is likely to experience greater suffering and distress in toxicity tests than a mouse.

Similar reasons apply to the potential substitution of transgenic humanised mice for NHPs in research concerning diseases such as HIV, tuberculosis and hepatitis. And here the benefit of substitution is especially important, since in some cases (e.g. hepatitis) the dilemma of primate research applies especially sharply: on the one hand, it is only the primates biologically closest to humans, chimpanzees, which provide a naturally effective model for the human disease; but just because they are so close to humans, with highly developed cognitive abilities and affective sensibilities, their use for medical research is morally very problematic and has not been undertaken in the UK for the last 50 years.[343] Hence the possibility of carrying out research with mice and other similar animals containing human material should make it possible to take forward research concerning these devastating diseases without incurring the moral injury of inflicting them on NHPs.

340 Weatherall D (2006). *The use of non-human primates in research* http://www.acmedsci.ac.uk/images/project/nhpdownl.pdf.
341 *Ibid* Chapter 8.
342 It is important to recall that the value of pre-clinical testing is limited by differences between species. In March 2006, a study of the antibody TGN1412, which had been pre-clinically tested in species including NHPs, caused severe adverse reactions in six trial participants. An expert inquiry into the trial concluded '… the pre-clinical development studies that were performed … did not predict a safe dose for use in humans, even though current regulatory requirements were met.' http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_073165.pdf
343 See 3.4 for more detailed discussion and comparison of the abilities of humans, Great Apes and other primates.

5.6.2 The challenge of using NHPs for research into neurodegenerative disorders

But there are areas of medical research where substitution of this kind is not likely to be helpful, especially that concerning neurodegenerative disorders. Because the brains of mice are very much simpler than those of primates, it is judged very unlikely that they will provide satisfactory models for these human disorders. In this area, therefore, medical research is beginning to use monkeys such as marmosets and macaques (evolutionarily further from humans than the Great Apes) both for fundamental research and as models for human disorders such as Parkinson's and Alzheimer's, and in some cases this research has involved the introduction of human neural stem cells into NHPs.[344] A related development has been work which showed the possibility of germline inheritance of genetic modifications introduced into marmosets, thus holding out the possibility of creating a breeding colony of transgenic humanised monkeys.[345] The issue which this kind of research now raises is whether this kind of 'neural humanisation' of an NHP endows it with added cognitive abilities or affective sensibilities which make it improper to use it for potentially distressing medical research, such as that into Parkinson's or Alzheimer's disease.[346]

As ever, the dilemma of primate research opens up: by humanising these monkeys to make them useful as models of human neurodegenerative disorders, one may endow them with capacities and abilities which make it even more problematic to carry out the research. It is not possible to resolve this dilemma at present. To be confident about a judgement, one needs answers to the following questions concerning this proposed neural humanisation of NHPs:

1. Will it be possible to create useful models for human disorders such as Parkinson's and thereby facilitate research which cannot now be undertaken?
2. Could an NHP, once modified as a model for a disorder such as Parkinson's, lead a life whose quality is acceptable when assessed by the normal standards for experimental animals?
3. But (assuming that the answer to (2) is positive) will the neural humanisation of an NHP enhance its cognitive and affective abilities in such a way that these become comparable to those of Great Apes?

Despite some early work in which transgenic rhesus macaques were developed to model Huntington's disease, it is too early to answer the first question.[347] But it ought to be possible to answer the second question once this work has progressed. If it turned out that the monkeys were seriously impaired by their neural adaptation, or that the quality of life of breeding colonies of transgenic humanised monkeys were significantly impaired by their humanisation (perhaps by their becoming more aware of their confinement), then these would be powerful reasons for halting the research. But assuming that the answer to the second question is positive, we are led to the third, speculative question; and if the answer to this turned out to be positive, then, from the other direction, there would also be reason for halting the research, since it would imply that the reasons we have for not licensing medical research which uses chimpanzees and other Great Apes apply also to research which uses these genetically enhanced monkeys.

It is not straightforward to envisage how this third question is to be settled. One can be confident that the introduction of some human neural stem cells would not endow a monkey[348] with a human-type self-consciousness, since that requires a capacity for higher-order thoughts associated with language, and it is fanciful to suppose that this capacity might be produced in a monkey simply by the introduction of some human neural stem cells into its brain. But once one recognises that

344 Redmond DE *et al.* (2010). *Cellular repair in the parkinsonian nonhuman primate brain.* Rejuvenation Res **13**, 188–94.
345 Sasaki E et al. (2009). *Generation of transgenic non-human primates with germline transmission.* Nature **459**, 523–27. As critics have observed, Sasaki's research was not without costs to the animals involved: to get a single case of germline transmission he used eighty modified marmoset embryos (the modification was the inclusion of the enhanced green fluorescent protein transgene).
346 For a preliminary discussion of these issues, see Olsson I & Sandøe P (2010). *What's wrong with my monkey? Ethical perspectives on germline transgenesis in marmosets.* Transgen Res **19**(2), 181–6.
347 Yang S *et al.* (2008). *Towards a transgenic model of Huntingdon's disease in a non-human primate.* Nature **453**, 921–4.
348 In this section, the term 'monkey' is used to refer to primates other than both man and the Great Apes.

the important comparison here is with Great Apes, then the uncertainties that affect our understanding of their cognitive abilities also affect procedures for comparing their abilities to those of enhanced monkeys.[349] Hence if work of this kind with monkeys proceeds it would be important to study some neurally humanised monkeys before potentially damaging medical research on them is undertaken so that an informed assessment of their abilities can be undertaken.[350]

5.7 Public concerns

The public dialogue we carried out brought out several areas of concern (see Boxes 5.1–5.3 in this chapter and others throughout the report).[351] One was that this research should be carried out in a way which advances the public good and not primarily the interests of business enterprises which have invested in it. This point is indeed implicit in our discussion: given that the practice of using animals for medical research is justified (insofar as it is) by its benefits for human health, the practice clearly needs to be organised in a way which ensures that these benefits are available to the public without excessive cost. Another area of concern was research involving the brain, especially those of monkeys; some participants expressed the kind of unease concerning the transfer of human capabilities to monkeys which we have just discussed here. But there were two further areas of concern which we have not addressed.

5.7.1 Humanising the appearance of an animal

One concern arose from the possibility of humanising the external appearance of an animal in such a way that it strongly resembled some aspect of a human being, an example would be endowing a primate with human-type skin in order to learn something about human skin disorders that could not be investigated in any other way.[352]

Many participants expressed strong distaste concerning possibilities of this kind, even when they were content with experiments which humanised the internal organs of animals of the same kind (see 3.6 and Box 3.11). Hence the issue here is whether this reaction itself provides a strong reason for not permitting the research in question in a situation in which the research is potentially important and it has been established that the condition (e.g. the humanised skin), including its appearance, is not distressing to the primate itself or to others with which it is living.

In thinking about this, the issue is what significance one should attach to the distaste at the visible appearance of a humanised animal. One suggestion might be that this distaste, or repugnance, reveals an ethical truth, the profound error of blurring the boundary between humans and animals.[353] The objection to this suggestion, however, is that once it is acknowledged that the same distaste is not manifested towards substantial internal humanisations of an animal, the reaction appears to be irrational. Instead one can compare this distaste at the humanised appearance of an animal with the common reaction of unease at the sight of human disfigurement. This is a primitive reaction which has no inherent 'wisdom'. Nonetheless, given the likely hostility to research which endows animals such as primates with a humanised appearance, there are pragmatic reasons of public policy for requiring that special consideration be given to proposals for research of this kind.[354]

349 The discussion of this issue in 3.4 makes these uncertainties very clear.

350 These considerations connect with those discussed by Greely and others in their paper: Greely HT *et al.* (2007). *Thinking about the human neuron mouse.* Am J Bioeth **7**, 27–40 in connection with the speculative 'Mouse Neuron Project' first proposed in 2000 by Dr Irving Weissman (see Box 3.4). Whereas working with mice was never likely to yield a useful model for human neurodegenerative disorders, it is quite possible that monkeys will provide useful models; so it is important to begin 'thinking about the human neuron monkey'. Some preliminary considerations were discussed in Greene M *et al.* (2005). *Moral issues of human-non-human primate neural grafting.* Science **309**, 385–6.

351 Ipsos MORI (2010). *Exploring the boundaries: public dialogue on animals containing human material* http://www.acmedsci.ac.uk/index.php?pid=209.

352 *Ibid* p29.

353 This suggestion takes its lead from Leon Kass's thesis of 'the wisdom of repugnance'; Kass L (2002). *Life, liberty, and the defense of dignity.* Encounter Books, San Francisco.

354 For further discussion of animal welfare issues of this kind, see Coors ME *et al.* (2010). *The ethics of using transgenic non-human primates to study what makes us human.* Nature Rev Genet **11**, 658–62.

5.7.2 Research involving reproductive cells

The other area of public concern arose from research which involves introducing human reproductive tissues and cells into animals (see 3.5 and Box 3.10).[355] Although it was not clear quite what kinds of research gave rise to this concern, it is easy to understand anxieties about the possibility of creating human–animal hybrid embryos. In fact the main area of research here involves the grafting of human reproductive tissues such as ovarian tissue into mice or other animals in order to understand reproductive biology, the causes of infertility, and to develop methods for preserving the reproductive potential of young people, for example those whose therapeutic treatment poses a threat to the viability of their reproductive system (see 3.5).[356] By itself this technique is not ethically problematic: on the contrary the research aims to provide a way of enabling those who are undergoing an invasive treatment to recover their reproductive ability once the treatment is over and the tissues in question are replaced in their own bodies. So the issue here is whether there is a significant chance that while these human reproductive tissues are lodged within a mouse or similar animal, some human germ cells might migrate within the host animal to that animal's own reproductive system and then lead to the creation of a hybrid human–animal embryo. In principle it appears that an event of this kind could occur, albeit unlikely. So far as we know, no such event has occurred in the context of current research; but we share the public's concern that this should not happen. There will be many ways of rationalising opposition to the creation of such an embryo, but for us it is sufficient to observe that it could never lead to the birth of a biologically coherent animal. So research that involves placing human reproductive tissues in non-human animals needs to be conducted in a way which avoids the risk of fertilisation inside the animal.

5.8 Conclusion

'*Going into the discussion I think I was very against any kind of animal research, but having heard about what it is and what it is for, I have completely reversed my position*'. Public dialogue – interview with Newcastle respondent.

We accept that the use of animals for medical research remains controversial, and we have not attempted here to justify the practice. Our attention has been directed at the distinctive ethical issues raised by the use of animals which include human genetic or cellular material. In discussing these we have addressed a variety of concerns – including utilitarian concerns about animal welfare, deontological concerns arising from the capacities which underlie human dignity, and considerations arising from our stewardship responsibility towards animals. We have not prioritised any one of these ethical perspectives in our attempt to capture the complexity of the cross-cutting ethical considerations that are in play in this issue. Our conclusion is that this work does not give rise to principled new concerns which provide reasons for curtailing it, and indeed that it offers the prospect of reducing the use of primates and similar animals in damaging experiments such as toxicity tests. Nonetheless, this work does have some troubling features which can be justified only by the prospect of facilitating the development of effective treatments for serious human disorders. In the few areas we have highlighted, such as neural experimentation with monkeys in order to advance the understanding and treatment of neurodegenerative disorders, such work needs to be accompanied by a careful assessment of the abilities of any humanised NHPs and of the ways in which their involvement in research affects their quality of life.

355 See page 31 of Ipsos MORI (2010) '*Exploring the boundaries*'. *Public dialogue on animals containing human material.* http://www.acmedsci.ac.uk/index.php?pid=209

356 For a survey of some recent work, see Dath C *et al.* (2010). *Xenotransplantation of human ovarian tissue to nude mice: comparison between four grafting sites.* Hum Reprod **25**(7), 1734–43.

Box 5.1 Conditional support for research involving ACHM

The majority of participants in the public dialogue accepted and were supportive of research using animals containing human materials (see Box 3.1). However, this support came with conditions attached – the majority of participants gave their support on the understanding that it is conducted to improve human health or combat disease.

In considering examples of research, participants were found to 'trade-off' the anticipated benefits or purpose of the research against concerns about the process.

The purpose of the research was judged on its perceived value against two main factors:

- Tangibility: research with more immediate or certain benefits received most support.
- Severity of the health issue: research addressing common terminal, debilitating or painful diseases found greatest acceptance, followed by research into conditions causing disfigurement or impacting on quality of life.

Key concerns that participants set against the value of the research included:

- Novelty: animal modifications that were seen as extensions of existing techniques were generally more accepted than new approaches, or the creation of new entities.
- The type of entity created: *in vitro* research caused fewer concerns than research involving whole animals (see Box 3.1).
- Tissue type: human-like modifications of an animal's brain, reproductive system, or external features were less accepted than modification of internal organs (see Box 3.1).
- Experimental species: particular concerns were expressed in relation to the use of pigs and monkeys (and especially chimpanzees).
- Animal welfare concerns were important for many participants (see Box 4.1).
- Safety: perceived current and future risks were both a concern (see Box 4.2).
- Animal–human boundaries: some examples raised ethical concerns, such as how partly human experimental remains should be treated, and whether animals with elements of human capacity (particularly cognition) should gain human rights.
- Who would benefit: it was important to some participants that research benefits would be distributed equitably.

Box 5.2 Opposition to research involving ACHM

The dialogue identified a group of participants who did not find research involving animals containing human material acceptable, even to address human health problems. Survey data indicated that this view is held by around 15% of the British population. Around two-thirds of this group in the survey also opposed any form of animal research, and a similar proportion did not trust UK regulation of research involving animals containing human material. Workshop participants who opposed research involving animals containing human material expressed doubt whether such research would deliver benefits, or would achieve its aims.

In the qualitative survey the most frequent reasons for finding such research unacceptable among this group were concerns for animal welfare, that it was against their personal views or that it was unnatural.

Box 5.3 Focus group findings and demographics

Three groups whose views were anticipated to be more distinct than those of the wider public were included in the dialogue:

- Patients and carers of those with serious illness (potential beneficiaries of medical research). Although concerned for animal welfare, this group welcomed all research with clear medical objectives and strongly supported the continuation of research using ACHM.
- Those who indicated religious faith played an important role in their daily life. An underlying view that human life has a pre-eminent value strongly influenced this group. Participants were highly supportive of research seen to enhance human life, and did not voice specific theological objections to research involving ACHM.
- Those with strong concern for animal welfare. This group broadly opposed research involving ACHM. Besides welfare concerns and a belief that animal experiments are unethical, the group expressed wider concerns including that research benefits would not be fairly distributed. Alternative priorities, including addressing poverty, global warming and causes of disease, were suggested.

The dialogue did not find sufficient evidence to indicate that views varied between participants of different ethnicities, or from different regions of the UK. However, there were some differences in views on animals containing human material research across demographics:

- Gender: survey data indicated men were more likely to find research acceptable than women.
- Age: older people were slightly more supportive of the research than younger people.
- Educational level: participants with higher education were more likely to express strongly polarised views, either in favour of or opposing the research. Survey data indicated that those with higher qualifications were more likely to find such research acceptable.

6 Legal and regulatory considerations

6.1 Introduction

No single piece of legislation specifically governs the creation or use of ACHM in medical research within the UK. However, several pieces of UK law are relevant to particular aspects of this research.

The most significant is the Animals (Scientific Procedures) Act 1986 (ASPA) which regulates the use of animals in research. Also relevant are the Human Fertilisation and Embryology Act 1990 (as amended in 2008) (HFE Act), which governs research involving human gametes, human embryos and human admixed embryos, and the Human Tissue Act 2004 (HT Act), which governs the use of human tissue containing cells and human DNA.

Research involving ACHM will generally fall under one or more of these pieces of legislation, and therefore be within the remit of one or more UK regulatory body, depending on the specific nature of the experiments involved. It may also be subject to other UK laws in some instances, including regulations relating to the use of genetically modified organisms, property and intellectual property (patent) law, and the Data Protection Act (DPA). In addition to rules, standards and procedures defined in law, research involving ACHM is also governed by professional guidelines or codes of conduct.

The complexity of the regulatory background is mirrored in the number of Government Departments with some function related to research using ACHM. The Department of Health supports health research and its translation into better healthcare. Its role as sponsor for the independent bodies that regulate the use of human embryos and human tissue sits alongside this broader function.

Responsibility for ensuring a sustainable science base rests with the Department for Business, Innovation and Skills. In contrast to Department of Health and Department for Business, Innovation and Skills, facilitating biomedical research is not a core objective of the Home Office but it has a specific role in the regulation of the research use of animals so its activities impact on the work of many researchers in the biomedical field. Other Government departments also have a role in relation to safety issues (see 4.2.5, 4.2.6).

Some consideration of the UK regulation of research involving ACHM was undertaken in the context of a wider review of the regulation of transgenic and cloned animals, by a working group of the Animal Procedures Committee (APC) in 2001.[357]

This chapter reviews the current UK regulatory environment for the creation and use of ACHM, and considers the interfaces between the relevant legislative instruments.[358] The factors that the public involved in the dialogue felt were important for the regulation of ACHM are outlined in Box 6.8.

6.2 Overview of the current UK legal and regulatory environment

6.2.1 Animals (Scientific Procedures) Act 1986

Scope and purpose

Scientific experimentation conducted in the UK using 'protected animals' is regulated by the Animals (Scientific Procedures) Act 1986 (ASPA), the principal purpose of which is to ensure that animals used in research are not subject to unnecessary pain, suffering, distress or lasting harm.[359,360] ASPA operates a

357 Animal Procedures Committee (2001). *Report on Biotechnology*. http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/key-reports/biotechnology?view=Binary

358 The pathway of regulation and governance of research involving human participants, their tissue or data is addressed in a report from the Academy of Medical Sciences (2011). *A new pathway for the regulation and governance of health research*. http://www.acmedsci.ac.uk/p47prid88.html

359 The Animals (Scientific Procedures) Act 1986 is available at http://www.legislation.gov.uk/ukpga/1986/14/contents. For the associated Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 see http://www.archive.official-documents.co.uk/document/hoc/321/321.htm

360 'Pain, suffering, distress and lasting harm' encompass any material disturbance to normal health (defined as the physical, mental and social well-being of the animal). They include disease, injury and physiological or psychological discomfort, whether immediately (such as at the time of an injection) or in the longer term (such as the consequences of the application of a carcinogen). Guidance on the operation of ASPA, Section 2.14.

licensing and inspection system, which governs experimental or other scientific procedures applied to 'protected animals'.[361]

'Protected animals' are defined as 'any living vertebrate, other than man', and '*Octopus vulgaris*' (the common octopus).[362] The Act applies to these types of animal if they are used, or survive into, any of the following stages of their development:

- Mammals, birds and reptiles: from half-way through the gestation or incubation period.
- Fish, amphibia and *Octopus vulgaris*: from the time at which they become capable of independent feeding.[363]

Vertebrates and *Octopus vulgaris* that do not survive beyond these developmental stages, and all other invertebrates, are not 'protected animals' under ASPA. Use of these life forms in research is not specifically regulated beyond the Genetically Modified (GM) (contained use) regulations, the GM (deliberate release) regulations, and other general health and safety requirements (see 6.2.4).

Application of ASPA to ACHM research

Although research involving ACHM is not explicitly described within ASPA or its associated guidance, in practice, almost all such research is governed by ASPA because it involves 'regulated procedures' applied to 'protected animals'. Moreover, the regulatory safeguards established under ASPA apply to animals genetically altered for the purposes of research and their progeny, howsoever produced, throughout their lives.[364,365]

ASPA licensing system

ASPA operates through a three-part licensing system.[366] The Act sets out an exhaustive list of the purposes for which project licences may be granted (Box 6.1).

The decision to license research is based on an analysis in which the potential benefits (to human welfare or knowledge, to the welfare of other animals or to the environment) are weighed against the likely welfare costs to the animals involved.[367] Research can only be authorised if there are no scientifically suitable alternatives that replace animal use, reduce the number of animals needed or refine the procedures used to cause less suffering – principles known as the 3Rs. Additional conditions apply for research involving particular species or purposes (Box 6.1).

The focus of ASPA and its implementation is on animal welfare and the 3Rs. The legislation was designed and is principally intended to ensure the protection of animals rather than to examine ethics, societal issues, or emerging research. Although these wider issues are considered in the weighing process described above, ASPA was not designed with the complex ethics and societal issues described in Chapter 5 in mind. As respondents to our call for evidence indicated, all animals used in research under ASPA are treated in a manner appropriate to their welfare needs, whether or not they contain human material: '*animal technicians … and researchers will assess the health of animals in their care equally, regardless of whether human materials have been incorporated into the animals' bodies or not'*.[368]

361 These are a defined as 'regulated procedures'. Guidance on the operation of ASPA, Sections 2.13–2.23.
362 The term 'man' is not defined in this context, but could be considered to include certain predominantly human human–animal entities. For discussion see 6.2.2.
363 For example, licences are required for research involving embryonated bird eggs if the embryo is allowed to survive into the second half of the incubation period. Guidance on the operation of ASPA, Section 2.8.
364 Animal Scientific Procedures Committee (2007): *Consideration for the discharge of GA animals from Animal (Scientific Procedures) Act 1986*. A genetically altered animal is defined as an animal in which the heritable DNA has been intentionally altered, or which carries a genetic mutation recognised as harmful, or the progeny of such an animal. This includes animals produced by genetic modification (as defined in the Genetically Modified Organisms (Contained Use) Regulations 2000); animals produced by induced mutagenesis; animals created by nuclear transfer procedures; animals created by the use of certain selective breeding strategies; harmful mutant lines arising from spontaneous mutations. It excludes animals with changes that are not heritable, such as somatic gene therapy or DNA immunisation.
365 It is in theory possible for such animals to be released from the requirements of ASPA once the research has been completed if the Home Office is satisfied this is appropriate on animal welfare grounds and has satisfied itself on any environmental or health and safety issues. In practice this has never happened, though it has been discussed by the APC (see 6.2.4 and Guidance on the operation of ASPA, Section 8.14). The approval of Defra would also be required to release such animals from the controls of the GM regulations.
366 Those carrying out any regulated procedure must hold a *personal licence*, all procedures must be part of a programme of research specified in a *project licence*, and research must be carried out at a designated scientific procedure *establishment*. See Guidance on the operation of ASPA, Section 2.36.
367 See Guidance on the operation of ASPA, Sections 5.10–5.12.
368 Written evidence from Wellcome Trust Sanger Institute.

Enforcement of ASPA and the role of the APC

Enforcement of ASPA, including the issue of licences, is the direct responsibility of the Secretary of State for the Home Office. The Animal and Scientific Procedures Division of the Home Office operates the licensing system on the Secretary of State's behalf, as well as providing the primary source of policy advice. The Animals (Scientific Procedures) Inspectorate provides advice to the Secretary of State as to whether, and on what terms, licences should be granted, and provides the primary assessment of licence applications.[369] The Animal Procedures Committee (APC) is an advisory non-departmental public body, set up to provide strategic advice to the Secretary of State on policy, practice, ethics, science and animal welfare related to ASPA.[370] Neither the Inspectorate nor the APC have executive powers. Their advice to the Secretary of State is not legally binding, though failure of the Secretary of State to have regard to it may be subject to judicial review.

The APC and the ASPA system more broadly operate on a case-by-case basis rather than through the development and application of policy. Typically, the Committee considers fewer than ten applications per year. The Committee reviews any applications referred to it by the Inspectorate and can review further applications on request. It automatically reviews all applications that fall within four categories agreed with the Home Secretary (see Box 6.2). These four categories are principally based around animal welfare issues of particular sensitivity or concern, although the fourth ('applications of any kind raising novel or contentious issues, or giving rise to serious societal concerns'), which is not defined, may be interpreted more broadly.

In conducting a review, the APC must have 'regard both to the legitimate requirements of science and industry and to the protection of animals against avoidable suffering and unnecessary use in scientific procedures'.[371] The Committee does not have any of the broader functions conferred on some of the statutory regulators (for example the functions of issuing guidance, monitoring new developments and engaging with external stakeholders of the Human Fertilisation and Embryology Authority and the Human Tissue Authority).

Some consideration was given to ACHM by the APC's Biotechnology working group in 2001, in the context of a wider review.[372] Their report highlighted concerns that emerged through consultation about experiments involving the humanisation of animals. It recommended that research involving some chimæric and hybrid forms should not be licensed. '*The true worry is about the creation of creatures with overtly human properties, or conversely the production of human-born entities with 'animal' properties. … Concern may be partly for the fate of such hybrids. But there may be a deeper repugnance at the thought of chimæras and hybrids: the wrong may not be in how we would treat them if they did exist but in their existing at all …*'[373] However, these recommendations have not been developed into specific rules and advice by the APC and decisions by the Home Secretary about such research continues to be issued on an *ad hoc* basis.

Local ethical review processes

In addition to the licensing system, ASPA requires every designated user and breeding/supplying establishment involved in animal research to have a local ethical review process. The purposes of the ethical review process are to ensure that all use of animals in an establishment is 'carefully considered and justified, that proper account is taken of the 3Rs, and that high standards of accommodation

369 The Inspectorate is also responsible for conducting inspections of premises where regulated procedures are performed, and where animals are bred or kept, to monitor standards and compliance with the Act. See Guidance on the Operation on ASPA, Sections 2.90–2.92.
370 See http://www.homeoffice.gov.uk/agencies-public-bodies/apc/
371 See Guidance on the Operation on ASPA, Section 2.93.
372 Animal Procedures Committee (2001). *Report on Biotechnology*. http://www.homeoffice.gov.uk/publications/agencies-public-bodies/apc/key-reports/biotechnology?view=Binary. The working group was established to consider 'the adequacy and appropriateness of the present regulatory regime under ASPA in regard to transgenic and cloned animals' in the light of current and likely scientific developments at that time.
373 *Ibid*. The report recommended that 'no licences should be issued for the production of embryo aggregation chimæras especially not cross-species chimæras between humans and other animals, nor of hybrids which involve a significant degree of hybridisation between animals of very dissimilar kinds.'

and care are achieved'.[374] Whilst the ethical review process is intended to be specific and appropriate to each individual establishment, common aims and functions are defined in Home Office guidance (see Box 6.3).

Responsibility for operation of the ethical review process rests with a named 'certificate holder' at each establishment. Membership of any ethical review group should, where practicable, include a veterinary surgeon, representatives from those who provide day-to-day animal care, project and personal licence holders, and one or more lay persons, and involve both establishment staff and others.[375]

Although the local ethical review process considers some ethical matters, we understand that there is variability between ethical review processes. Concern was expressed to us that some focus more on ensuring the practicalities of conducting proposed research within an establishment (e.g. funding and capacity), than considering societal or ethical implications in their broadest context. In this case, it will mainly fall to the Inspectorate to identify broad societal or ethical concerns relating to a particular research project, and to bring these to the attention of the APC or the Secretary of State.

Implementation of the European Directive on the protection of animals

The revised European Directive (2010/63/EU) on the protection of animals used for scientific purposes was adopted in October 2010 and is to be transposed into the national legislation of all Member States by 2013.[376] The influence of the Directive on the UK's current legislation (ASPA) and regulatory system will be explored during Government consultation. We anticipate three areas, of relevance to the current study, which may give rise to discussion and could potentially result in changes to ASPA:

- Regulation of fetal mammals. The Directive applies to 'fetal forms of mammals as from the last third of their normal development', whereas ASPA applies to 'mammals … from halfway through the gestation or incubation period'.[377]
- 'Animal welfare bodies'. The Directive requires that each breeder, supplier and user of research animals sets up an animal welfare body.[378]
- 'National committee for the protection of animals' used for scientific purposes. The Directive requires that each Member State establishes such a committee 'for the protection of animals used for scientific purposes'. Such committees should (among other things) provide advice and ensure the sharing of best practice both nationally and internationally.[379] The development of this committee was discussed in the 2009/10 review of the APC.[380] Proposed aspects of the roles of this committee are not part of the functions of the APC in its current form.[381]

374 See Guidance on the Operation of ASPA, Appendix J, 2.
375 See Guidance on the Operation of ASPA, Appendix J, 5.
376 Directive 2010/63/EU on the protection of animals used for scientific purposes is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF
377 *Ibid* Article 1, 3a (ii).
378 *Ibid* Articles 26 and 27.
379 *Ibid* Article 49.
380 Omand D., (2010). *Report of the 2009/10 NDPB Review of the Animal Procedures Committee*. http://www.homeoffice.gov.uk/publications/apc/publications-2010/review-apc-0910?view=Binary Recommendation 22.
381 Functions of the 'national committee for the protection of animals' perhaps not clearly covered by the current APC include advising animal-welfare bodies on matters dealing with the acquisition, breeding, accommodation, care and use of animals in procedures and ensuring sharing of best practice; exchanging information on the operation of animal-welfare bodies and project evaluation; and sharing best practice within the European Union.

Box 6.1 Permitted purposes of research under ASPA and additional restrictions

A project licence will only be granted for one or more of the following scientific or experimental purposes:

- The prevention (whether by the testing of any product or otherwise) or the diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- The assessment, detection, regulation or modification of physiological conditions in man, animals or plants.
- The protection of the natural environment in the interests of the health or welfare of man or animals.
- The advancement of knowledge in biological or behavioural sciences.
- Education or training other than in primary or secondary schools.
- Forensic enquiries.
- The breeding of animals for experimental or other scientific use. This generally refers to genetically modified animals or animals with harmful mutations.[382]

In line with guidance from the Home Secretary, licences will not be issued for programmes of work involving:

- The use of Great Apes (that is, chimpanzee, pygmy chimpanzee, gorilla and orang-utan).
- The use of protected animals for testing finished cosmetics products and substances intended primarily for use as cosmetics ingredients.
- The use of protected animals for the development or testing of alcohol or tobacco products (the use of tobacco or alcohol as research tools may, however, still be considered and licensed in the context of investigating disease or novel treatments).
- The use of protected animals for the development or testing of offensive weapons (licences may still be granted for the testing and development of means for protecting or treating UK servicemen and women, or the wider population).[383]

Box 6.2 Remit of APC in reviewing research licence applications[384]

By agreement with Ministers, the APC sees all applications for project licences that involve:

- The proposed use of wild-caught non-human primates.
- The proposed use of cats, dogs, equidae[385], or non-human primates in procedures of substantial severity.
- A substantial severity banding or major animal welfare or ethical implications, involving (a) xenotransplantation of whole organs or (b) chronic pain models or (c) study of the central nervous system.[386, 387]
- Applications of any kind raising novel or contentious issues, or giving rise to serious societal concerns.

'*Approximately 1% of applications for licences (e.g. those in the categories described … above) go to the APC for consideration. In practice therefore the APC is examining only the most substantial severity applications (usually involving non-human primates) … In the last three years the APC has given advice on 9 applications …*'[388]

382 See ASPA (1986) Section 5 (3).
383 See Guidance on the operation of ASPA, Section 5.23.
384 Omand D., (2010). *Report of the 2009/10 NDPB Review of the Animal Procedures Committee*. http://www.homeoffice.gov.uk/publications/apc/publications-2010/review-apc-0910?view=Binary (paragraph 8).
385 The Equidae family includes horses, asses and zebras.
386 Xenotransplantation is defined as the transplantation of cells, tissues or organs from one species to an animal of a different species.
387 For detail on the severity limits of experiments see Guidance on the operation of ASPA, Sections 5.40–5.49.
388 Omand D., (2010). *Report of the 2009/10 NDPB Review of the Animal Procedures Committee*. http://www.homeoffice.gov.uk/publications/apc/publications-2010/review-apc-0910?view=Binary. Para 15.

Box 6.3 The ethical review process under ASPA

The function of the (local) ethical review process is described in the guidance on the operation of ASPA.[389,390] The stated aims of ethical review process are:

- To provide independent ethical advice to the certificate holder, particularly with respect to project licence applications and standards of animal care and welfare.
- To provide support to named people and advice to licensees regarding animal welfare and ethical issues arising from their work.
- To promote the use of ethical analysis to increase awareness of animal welfare issues and to develop initiatives leading to the widest possible application of the 3Rs.

6.2.2 Human Fertilisation and Embryology Act 1990 (as amended)

Scope and purpose

The Human Fertilisation and Embryology Act 1990 (as amended by the Human Fertilisation and Embryology Act 2008) (HFE Act) regulates the creation, keeping and use of human embryos outside the human body, the storage and use of human gametes to create embryos, and the creation and use of human admixed embryos (see Box 6.4). The HFE Act defines, and places clear limits on the use of, human gametes, human embryos and human admixed embryos (see Boxes 6.5 and 6.6). Certain activities are prohibited other than when conducted under licence from the statutory regulator set up under the HFE Act, the Human Fertilisation and Embryology Authority (HFEA, see below).[391,392]

The creation and use of human embryos, and human admixed embryos *per se*, are the principal focus of the HFE Act, and outside the scope of this report (see 1.1). However, the Act is relevant to ACHM research in the following situations:

Application of the HFE Act to ACHM research involving human gametes

Animal models have been developed which involve the implantation of human oocytes and sperm, or immature germ-line cells, into animals (see 3.5). Technically, such research falls within the ambit of the HFE Act as it involves the use of human gametes outside the body. However, a research licence is not required from the HFEA to conduct such studies as they would not result in the production of a human or a human admixed embryo.[393] Research would require a licence under ASPA if it involved the use of a protected animal.

Application of HFE Act to ACHM research resulting in human admixed embryos: the predominance of human material and 'evolving' embryos

The HFE Act applies to embryos that are either entirely or predominantly human or equally human and animal. Human admixed embryos are mainly defined by reference to the scientific processes through which they are created (see Box 6.4).[394] However, there is a 5th sub-section of the definition, in which such embryos are defined by reference to the resulting creation (in which the human DNA predominates). It is easy to imagine situations in which it is far from clear whether a given embryo is more human or more animal, when the amounts of genetic mixture are extensive. Interpretation is complicated by lack of current knowledge of exactly which DNA sequences determine phenotypically critical features of species

389 Guidance on the operation of ASPA, Appendix J.
390 See also RSPCA/LASA (2010). *Guiding principles on good practice* for Ethical Review Processes. http://content.www.rspca.org.uk/cmsprd/Satellite?blobcol=urldata&blobheader=application%2Fpdf&blobkey=id&blobnocache=false&blobtable=MungoBlobs&blobwhere=1232992110664&ssbinary=true
391 The Human Fertilisation and Embryology Act (2008) Act is available at http://www.legislation.gov.uk/ukpga/2008/22/contents. For information on the HFEA see http://www.hfea.gov.uk/
392 At the time of publication, the structure and functions of several public bodies, including the HFEA and the HTA were subject to review under the provisions of the UK Public Bodies Bill [HL] 2010-11. See http://services.parliament.uk/bills/2010-11/publicbodieshl/documents.html
393 The HFE (Special Exemptions) Regulations (2009) provide an exemption from the requirement under the HFE Act for a licence to store gametes for research purposes. See http://www.legislation.gov.uk/uksi/2009/1918/contents/made
394 See HFE Act 1990 as amended, sub section 4A(6).

identity; and by the fact that the cellular composition of an embryo may change over time as some cell types expand faster than others – either by chance or by experimental design (as in the case of tetraploid complementation, see 2.2.2).[395]

These issues were discussed during the passage of the HFE Bill through parliament in 2008. Consideration was given to whether the concept 'predominantly human' in the 5th sub-section (sub-section (e)) implied dominance in purely quantitative terms, or whether the functional significance of the human contribution to the human admixed embryo should be determinative. The response elicited from the Minister was that the latter was the proper interpretation.[396] This clarification is helpful, but the difficulties of the assessment should not be underestimated given the current state of the science in this area (it will become easier as scientific knowledge increases).

What if a predominantly animal embryo containing human material were, during the course of an experiment, to alter in some way leading to human functionality becoming predominant? Under the current legislative framework, if such an outcome was possible it would be necessary to either:

- Hold licences for the research from both the Home Office under ASPA and the HFEA from the outset of the experiment.
- If the outcome was unexpected and the experiment was being conducted solely under a Home Office licence under ASPA, to ensure through close monitoring that the experiment was immediately halted once it became evident the threshold had been reached and to seek authorisation from the HFEA before resuming it.[397]

The difficulty of setting down a precise definition of when the HFE Act applies to embryos containing extensive mixtures of animal and human DNA inevitably means that some potential experiments may need consideration under both pieces of legislation. Part of the reason for the current study is to draw attention to the need to ensure that this process is as smooth and clear as possible, with a minimum of bureaucratic uncertainty and duplication in process while avoiding any chance that contentious experiments might escape suitable scrutiny.

Application of the HFE Act to ACHM research conducted using material from human embryos or human admixed embryos

Animal chimæras can be created by the engraftment of human embryonic cells, or embryonic cell lines into animals. For example, these approaches are used in pre-clinical studies to develop the methodologies for cell replacement therapies (see 3.3.2). A HFEA licence would only be required for the *in vitro* creation of a human embryo, or human admixed embryo, intended either as a source of cells for use in research, or for the subsequent derivation of cell lines.[398]

Human Fertilisation and Embryology Authority (HFEA)

The HFEA, which is constituted under the HFE Act, has responsibility for reviewing applications and issuing licences for licensable activities (including research involving human embryos and human admixed embryos). The HFEA also has responsibility for issuing both policy and clinical guidance within the scope of its remit, and monitoring scientific developments in the field. In contrast to ASPA (see above), the HFEA is fully empowered to make licensing decisions under the HFE Act, acting independently of its

395 Tetraploid complementation involves introducing cells from a donor organism into a recipient embryo at an early embryonic stage. Conditions are manipulated to give the donor cells a competitive advantage – donor cells then generate all the embryonic tissues, while the less favoured recipient cells produce only extra-embryonic (e.g. placental) tissues. The potential of such techniques is important, it illustrates that the proportion of cells and DNA from different origins within an organism can change through embryonic development; and secondly that embryos containing cells entirely derived from one organism could feasibly be generated within a recipient embryo (and maternal host) of another species.

396 See House of Lords Hansard (2008). 29 October, Column 1626. http://www.publications.parliament.uk/pa/ld200708/ldhansrd/text/81029-0009.htm

397 If the experiment was judged to involve the placement of a human admixed embryo into an animal, it would not be authorised by the HFEA.

398 In contrast HFE licences are not required for the research use of cells derived from ES cell lines or human ES cells derived from pre-implantation embryos (though the use of these to create chimæras should be reported to the UK Stem Cell Bank Steering Committee); or disaggregated human embryonic cells. Cells isolated from aborted human fetuses have also been investigated as the basis for cellular therapies; these are not subject to HFEA licensing.

sponsoring Government department (see Box 6.7).

The HFEA's code of practice provides guidance in relation to several aspects of the research use of human, and human admixed, embryos, including general requirements, information to be provided to embryo donors, consent and storage requirements.[399] Cell lines generated from human embryos created under an HFEA licence must be deposited in the UK Stem Cell Bank, at which point, the requirements of codes of practice of the bank will apply to the future use of the cell line. However, it is unlikely that a similar requirement would apply to a human admixed embryo or that the UK Stem Cell Bank would store cell lines from such embryos, or by extension apply its codes of practice to the use of such lines (see 6.2.7).

Box 6.4 Definitions within the HFE Act 1990 (as amended 2008)

Principal definitions

The HFE Act defines human gametes as including human germ-line cells at all stages of development, and human embryos as including human eggs in the process of fertilisation. The principal definitions are:

- '**Embryo**': refers to a live human embryo, and includes an egg that is in the process of fertilisation or is undergoing any other process capable of resulting in an embryo, but does not include a human admixed embryo.
- '**Gamete**': refers to a live human egg, including cells of the female germ line at any stage of maturity, but not including eggs that are in the process of fertilisation or are undergoing any other process capable of resulting in an embryo; or to a live human sperm, including cells of the male germ line at any stage of maturity.
- '**Permitted egg**': refers to an egg which has been produced by or extracted from the ovaries of a woman, and whose nuclear or mitochondrial DNA has not been altered.
- '**Permitted sperm**': refers to sperm which have been produced by or extracted from the testes of a man, and whose nuclear or mitochondrial DNA has not been altered.
- '**Permitted embryo**': refers to an embryo created by the fertilisation of a permitted egg by permitted sperm, where no nuclear or mitochondrial DNA of any cell of the embryo has been altered, and no cell has been added to it other than by division of the embryo's own cells.[400]

Definitions: human admixed embryos

The HFE Act defines five types of human admixed embryo each of which contains human and animal material in equal proportion or with human material in predominance. They can be summarised as:

- '**Cytoplasmic hybrids**': embryos created by techniques used in cloning, using human gametes or cells, and animal eggs. Such embryos are mostly human except for the presence of animal mitochondria.
- **Human–animal hybrid embryos**: embryos created using a human egg and the sperm of an animal, or an animal egg and a human sperm; or by combining a pronucleus of an animal with a human pronucleus.
- **Human transgenic embryos**: embryos created by introducing animal DNA into one or more cells of a human embryo.
- **Human–animal chimæras**: human embryos altered by the addition of one of more cells from an animal.
- Any embryo which does not fall within any of the categories above and which contains both human nuclear or mitochondrial DNA and nuclear or mitochondrial DNA of an animal, but where the animal DNA is not predominant.[401]

399 The HFEA Code of Practice is available at http://www.hfea.gov.uk/code.html
400 See HFE Act 1990 as amended, section 1; section 32A
401 See HFE Act 1990 as amended, sub-section 4A(6). See also Explanatory notes on the HFE Act (2008), Section 4, 31. http://www.legislation.gov.uk/ukpga/2008/22/notes/division/6/1/4

Box 6.5 Activities proscribed by the HFE Act 1990 (as amended 2008)

The following activities are specifically prohibited by the HFE Act:

- Placing any embryo or gametes, other than permitted embryos or gametes, into a woman.
- Placing a human embryo in any animal (where 'animal' means any animal other than man).
- Placing a human admixed embryo in an animal.
- Keeping or using a human embryo, or a human admixed embryo, after either the appearance of the primitive streak or 14 days of development.[402]

Box 6.6 Research involving human admixed embryos in the HFE Act 1990 (as amended 2008)

Research involving human admixed embryos

Licences for research may authorise:

- Mixing sperm with the egg of a hamster, or other animal specified in directions, for the purpose of developing more effective techniques for determining the fertility or normality of sperm, but only where anything which forms is destroyed when the research is complete and, in any event, no later than the two-cell stage.[403]
- Creation, keeping or using human admixed embryos *in vitro*, for the purposes of a project of research specified in the licence.

The principal purposes for which a research licence may be granted:

- Increasing knowledge about serious disease or other serious medical conditions.
- Developing treatments for serious disease or other serious medical conditions.
- Increasing knowledge about the causes of any congenital disease or congenital medical condition (that does not fall within paragraph (1).)
- Promoting advances in the treatment of infertility.
- Increasing knowledge about the causes of miscarriage.
- Developing more effective techniques of contraception.
- Developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation.
- Increasing knowledge about the development of embryos.[404]

402 See HFE Act 1990 as amended sub-section 3(2); sub-section 3(3)b; sub-section 4A(4); sub-section 4A(3)
403 There is a limit of 14 days for research use of all human admixed embryos. In HFE Act (2008) Schedule 2(6), a two-cell limit applies to forms created during the human sperm/hamster egg fertility test.
404 See HFE Act (2008). Schedule 2 (6).

Box 6.7 Comparison of regulatory mechanisms under HFE Act and ASPA

Regulator	HFE Authority	Secretary of State for Home Office (advised by Home Office Inspectorate and APC under ASPA)
What is regulated?	**Human embryos, human admixed embryos and human gametes**	**Protected animals**
Status	Independent authority with statutory licensing powers (independent of Government; of individuals with a professional interest (who under the requirements of the Act must not be in a majority on the HFEA)).	Office of Government with statutory powers licensing powers (and civil servants as agents for the Secretary of State); APC advisory only.
Composition	Group of individuals appointed to time-limited terms of office following an open process. Some rules about composition of authority in statute.	Office of state permanently appointed (Secretary of State acting through civil servants). Rules about composition in statute apply to APC only.
Statute	Set up under governing statute solely for purposes set out in the statute; powers and duties of regulator fully set out in the governing statute.	Regulatory powers under governing statute conferred on Secretary of State that exists separately from ASPA and has much broader functions; powers and duties in relation to its regulatory function under ASPA not fully set out in the governing statute. APC's limited powers and duties set out in statute.
Duty and power	Explicit duty to consider applications and issue licences that meet requirements.	Secretary of State has power but no explicit duty to consider applications and issue licences that meet requirements. APC has no decision making or licensing powers.
Guidance	Explicit duty and power to issue guidance under the Act.	No explicit power or duty on either Secretary of State or APC to issue guidance under the Act.

The HFEA is an independent decision-making body, whose members are appointed by the Health Secretary by an open process for time-limited terms of office. Its composition is governed by the HFE Act itself, which provides that while the Chair cannot be a medical practitioner, or involved in commissioning, or undertaking research related to keeping or using gametes or embryos, this expertise must be represented in the HFEA membership. The APC is an independent advisory body, whose members are appointed by the Home Secretary for time-limited terms of office. The APC's composition is governed by ASPA, which provides that at least two-thirds of the membership must be a veterinary surgeon, medical practitioner or have expertise in a relevant biological science and at least one member must be a lawyer.[405]

405 See ASPA (1986), Section 19.

6.2.3 Human Tissue Act 2004

Scope and purpose

The Human Tissue Act 2004 (the HT Act) is the legal framework in England, Wales and Northern Ireland regulating the storage and use of human organs and tissue from the living, and the removal, storage and use of tissue and organs from the deceased, for health-related purposes and public display.[406] The Act is principally intended to ensure that appropriate consent is in place to enable the lawful retention and use of body parts, organs and tissue, for 'scheduled purposes', which include medical research. The Act also prohibits certain forms of DNA analysis without consent throughout the UK.

The HT Act applies to human bodies and human tissue that consist of, or contain, human cells *other than*: hair and nails from living people; human gametes and embryos; and other human material created outside the human body (e.g. human cell lines).[407] It prohibits the possession of 'bodily material' (from a living or deceased human body, consisting of or including human cells, including hair, nails and gametes) with the intention of analysing its DNA without consent.[408] Except to the extent of the prohibition above, DNA itself (extracted human DNA, where no whole cells remain) is not regulated by the Act.

Application of the HT Act to ACHM research

The requirements of the HT Act apply to the creation of *chimæric* animals using human tissue in some circumstances. For example, where human tissue is removed directly from the body of an identifiable living person, and inserted into an animal the HT Act requirements concerning consent and licences for any storage of such tissue would apply.[409,410] The HT Act would not apply to the creation of *transgenic* animals using 'human-like DNA sequence' (since extracted or artificially synthesised human DNA is not regulated by the HT Act), nor would it apply to the creation of chimæras using human cell lines (since cell lines are outside the scope of the Act).

Human Tissue Authority

The Human Tissue Authority (HTA), regulates and licences the use and storage of human tissue under the HT Act.[411] The HTA's remit does not include ethical approval, which is necessary for research involving human tissue in some circumstances and governed by the National Research Ethics Service (NRES).[412]

6.2.4 Health and safety law, including GM Regulations

ACHM research is subject to general health and safety requirements including the Health and Safety at Work Act (1974) and Carriage of Dangerous Goods legislation. Some types of ACHM research are also subject to the controls set out in the Genetically Modified Organisms (GMO) (Contained Use) Regulations and the GMO (Deliberate Release) Regulations, regulated by the Health and Safety Executive (HSE) and the Department for Environment Food and Rural Affairs (Defra) respectively.[413] The GM regulations are designed to control risks from GMOs to human health (both the contained use and deliberate release regulations) and the environment (the deliberate release regulations only). They apply to biological organisms, cellular (including animal cells in culture) and non-cellular material, other than humans and human embryos, which have been genetically altered other than as a result of a naturally occurring process and which are capable of replicating

406 The Human Tissue Act (2004) is available at http://www.legislation.gov.uk/ukpga/2004/30/contents. Removal of material from the living is regulated separately. The equivalent legislation in Scotland is the Human Tissue (Scotland) Act 2006 (which only applies to post not ante mortem tissue) in Scotland. http://www.legislation.gov.uk/asp/2006/4/contents

407 There is an exception in that the HT Act (2004) applies to stem cell lines intended for human application.

408 Unlike the rest of the HT Act, this provision extends to the whole of the UK, including Scotland.

409 In addition to any requirements under ASPA (1986).

410 Though there are various exceptions to requirements that may be relevant, including (a) a storage licence is not required (1) for tissue stored incidentally to transportation for less than a week or (2) for tissue stored solely for use in a NHS research ethics committee ('REC') approved project; (b) consent is not required (1) for use of tissue imported into England, Wales and Northern Ireland or (2) for use of tissue taken from a living person used in anonymised (to the researcher) form for a REC approved project.

411 For detail on the wider remit of the HTA see http://www.hta.gov.uk/

412 For detail on NRES see http://www.nres.npsa.nhs.uk/

413 For detail on the GMO regulations see http://www.hse.gov.uk/biosafety/gmo/law.htm and http://www.defra.gov.uk/environment/quality/gm/; Certain decisions are reserved to Scottish Ministers in Scotland.

or transferring genetic material. Thus, the regulations apply to transgenic ACHM.[414] We presume the application of the regulations to any particular chimæra will depend on whether their genetic material can be said to have been altered other than as a result of a naturally occurring process (since each cell in a chimæra contains an unmodified genome of one of the precursor animals) and whether any change is capable of transmission, (since the chimærism may not involve the germ cells).

Users of GM animals in contained facilities must notify their facilities to the Health and Safety Executive (HSE), carry out risk assessments addressing both risks to human health and to the wider environment, ensure necessary controls are in place to minimise such risks and notify or seek the consent of (depending on the risk level) the HSE in relation to GM activities. GMOs cannot be released from containment without the approval of Defra following assessment by official assessors to ensure there are no risks to human health or the environment. Deliberate release of GM animals governed by both the GM regulations and ASPA also requires the approval of the Home Secretary (though release of GM animals has never been so authorised). Accidental release must be notified to the HSE.[415]

6.2.5 Intellectual property rights

Intellectual property law does not regulate the conduct of research involving animals containing human material, but can strongly influence whether research takes place, and may impede (or create the conditions to enable) research and development activity. For example, a pharmaceutical company making a transgenic animal expressing a human protein is likely to seek to patent the animal. UK legislation makes provision for biological materials to be patented, including: 'inventions which concern plants or animals' [416] and 'an element isolated from the human body … including the sequence or partial sequence of a gene, even if the structure of that element is identical to that of a natural element'. However 'processes for modifying the genetic identity of animals which are likely to cause them suffering *without any substantial medical benefit* to man or animal', and also animals resulting from such processes cannot be patented. [417]

6.2.6 Data Protection Act 1998

The Data Protection Act 1998 (DPA) is the principal legislation relevant to the use of medical information in research in the UK.[418] It regulates the use of 'personal data', that is, data relating to an individual who can be uniquely 'identified from those data, or from a combination of those data and other information which is in the possession of, or is likely to come into the possession of a data controller'. Although the DPA is generally unlikely to apply to ACHM research, it would apply if a particular individual could be uniquely identified from a section of genetic code/ sequence, obtained through the sequencing of human DNA, when combined with other data in the possession of the same researcher. It seems likely that this could be the case in some circumstances, in which event the provisions of the Act including the requirements relating to consent, fair processing and right of access (by the individual concerned) would apply.

6.2.7 Non-legislative requirements in the UK

In addition to statutory legislation, scientific and medical research is subject to, and guided by, a complex raft of non-legislative guidance, which varies in some cases between the four different administrations within the UK.[419] Some touches on the creation of human admixed embryos, but beyond

414 See the Scientific Advisory Committee on Genetic Modification (SACGM) compendium of guidance. *Part 5 Genetic modification of animals*. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm

415 As at March 2011, the HSE were not aware of any incidents of accidental release of GM animals posing any risk to human health.

416 If the technical feasibility of the invention is not confined to a particular plant or animal variety.

417 The relevant UK legislation is the Patents Act (1977), as amended by the Patents Regulations 2000 (SI 2000/2037) – which implemented the provisions of Articles 1 to 11 of the European Directive 98/44/EC on the legal protection of biotechnological inventions. Patent law is overseen by the Intellectual Property Office, an Executive Agency of the Department for Business Innovation and Skills. See http://www.ipo.gov.uk/

418 The Data Protection Act (1998) is available at http://www.legislation.gov.uk/ukpga/1998/29/contents

419 The pathway of regulation and governance of research involving human participants, their tissue or data is addressed in Academy of Medical Sciences (2008). *A new pathway for the regulation and governance of health research*. http://www.acmedsci.ac.uk/p47prid88.html

that, to our knowledge no guidance relates specifically to the creation or use of ACHM as such.[420] Guidance and other non-legislative requirements that have particular impact on ACHM research include the NHS research governance framework (RGF, below) and its equivalent in Scotland, HFEA and HTA codes of practice, professional codes, stem cell banks' codes of practice, guidance from funding bodies (both public and charitable), grant conditions, and publishing requirements. This guidance supports the formal legislation in the development and maintenance of good practice among the research community, including in relation to ACHM research. In many cases it is sufficiently flexible to enable ethical, societal and other issues relating to ACHM research to be identified and considered, notwithstanding that ACHM was not in the contemplation of the draftsmen.

NHS Research Governance Framework (RGF)
The RGF outlines the principles of good governance for research carried out in the NHS, including the different permissions required (e.g. those of the HFEA or the HTA), and more generic requirements which can apply to health research involving NHS facilities, patients, their tissue or data. Although the RGF does not apply to animal research as such, it does set out a regulatory framework, including the requirement for NHS research ethics approval, that is applicable to ACHM research insofar as it involves the use of human tissue or patient data.[421] A similar framework applies in Scotland.

Stem cell guidance
ACHM research that involves the introduction of human stem cells into an animal is guided by the general requirements of the Department of Health Code of Practice for the use of human stem cell lines and the codes of practice of the UK Stem Cell Bank. If the research involves the use of human embryonic stem cells generated under an HFEA licence and supplied by the UK Stem Cell Bank, the Stem Cell Bank Steering Committee must approve the release of the cell line from the bank, and the owner of the line would be required to license its use subject to the HFEA and UK Stem Cell Bank Codes of Practice, which set out general requirements concerning the use of human ESCs (though any resulting animal cell lines could not be deposited in the Stem Cell Bank) (see also 6.2.2). [422]

420 For example, see the HFEA code of practice http://www.hfea.gov.uk/3468.html
421 Department of Health (2005). *Research governance framework for health and social care: Second edition* http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4108962; the equivalent in Scotland is the research governance framework for health and community care. http://www.cso.scot.nhs.uk/publications/ResGov/Framework/RGFEdTwo.pdf
422 For details on the UK Stem Cell Bank see http://www.ukstemcellbank.org.uk/; their Code of Practice is available at http://www.ukstemcellbank.org.uk/codesofpractice/codeofpracticefortheuseofhumanstemcelllines.cfm

Box 6.8 Public views on research regulation

Most dialogue participants were aware that medical research is regulated in the UK, though they had little knowledge of how regulation is brought about or the organisations involved. A majority of the workshop participants expressed confidence that, in the UK, the regulation of research involving animals containing human material would be adequate, properly enforced, and reflective of their concerns and principles. This finding was echoed in the survey data, in which 44% of participants agreed that they would trust regulation of such research in the UK (29% said they would distrust such regulation, the remainder were neutral or unsure). Participants' main concerns about the research regulation related to:

- The possibility that permitting some research of this type might lead scientists to seek to conduct unacceptable research in future (a 'slippery slope' argument).
- Knowledge of situations where regulatory errors were thought to have occurred (participants cited the release of foot-and-mouth disease at Pirbright in 2007).
- A suggestion that 'rogue' scientists would evade authorities and regulation.
- The view that research of this kind would not be adequately regulated beyond the UK and so 'malpractice' would take place elsewhere.

'You trust your doctor and your scientists. Not in other countries but the UK is fine'.

Participants indicated several factors which they felt were important for future regulation of research involving animals containing human material, these included:

- A general principle of transparency should be applied, in that information on research of this type should be available in the public domain.
- Regulation should be conducted by independent/impartial people, and a mixture of different interests should be represented (e.g. public members, independent scientists, specifically appointed regulators).
- Regulation should focus on animal welfare, ensuring that animal suffering and the numbers of animals used are minimised.
- Regulation should aim to eliminate risks, including the unintended release of environmental contaminants or disease-causing factors.
- Regulation should be enforced in a manner that prevents evasion by 'rogue' scientists.
- Regulation should be appropriate to the type of animal created and the human tissue and organs involved.

6.3 Summary

As we have noted, ACHM research conducted within the UK is principally regulated by ASPA, though the focus of ASPA and its implementation is on animal welfare and 3Rs, rather than wider ethical considerations.

Although there is some grading within the ASPA system in the form of the four categories of applications that have been identified as requiring review by the APC, the categorisation is principally designed around animal welfare issues rather than broader considerations, and in the case of the fourth category, which potentially addresses broader issues, lacks definition (see Box 6.2). Given the evolving nature of the science associated with ACHM research, **we see considerable benefit in further developing a graded approach to licensing and regulatory oversight, which is principle based and transparent, seeks to define different levels of sensitivity and differentiates the degrees of scrutiny required accordingly**. We propose a possible approach in Chapter 8 (see 8.2).

Recognising the specialist knowledge required to evaluate likely (and sometimes uncertain) outcomes in this complex field of science, as well as the socially sensitive nature of the judgements to be made, we would also consider that a national expert body, which includes the relevant expertise, is needed to advise on ACHM research (see 8.3). In order to build and maintain trust and ensure accountability to the public, the body needs to operate transparently, be outward facing and engage with the public and the scientific community. To ensure consistency and transparency, it needs to have the power to develop guidelines. There would also be considerable importance and value in it playing a broader function, including the role of sharing knowledge and best practice attributed to the national committee required under the 2010 EU Directive.

As we have set out, the regulatory environment is complex. There are several pieces of UK legislation relevant to the regulation of ACHM. In some cases, more than one regulatory regime applies to a specific piece of ACHM research, or the research is at the borders of specific regimes. Aside from complexity, this also creates the possibility of inconsistency between regulatory regimes. To manage this effectively, a key feature of the UK regulatory environment in the future needs to be that **all relevant stakeholders (Home Office, HFEA, HTA and others) develop a coordinated, consistent approach to regulating the field of research, work together under an agreed framework of operation to continue to monitor scientific developments and consider jointly how to address borderline cases (see 8.6)**. Borderline cases include experiments that involve animal embryos containing human cells or genes that are close to the boundary of human admixed embryos under the HFE Act as well as certain ACHM experiments that involve a degree of uncertainty as to outcome. Regulatory guidance is likely to be particularly helpful in such cases.

Table 6.1 Regulators and regulatory approvals relevant to research involving ACHM in the UK[423]

Regulated research activity	Scope	Regulatory approval required	Legislation	Jurisdiction	Regulator	Sponsor Dept
Use of 'protected' animals which may cause the animal pain, suffering, distress or lasting harm[424]	'Protected' animals include live vertebrates and octopuses and embryonic/ fetal/ larval forms from mid-point of gestation/ incubation/ from point of independent feeding	1. Personal licence 2. Project Licence 3. Certificate of Designation for premises 4. Local ethical review process	ASPA 1986	UK	Secretary of State supported by: ASPD&I, APC, local ethics panel	HO
Use of human gametes	–	None	HFE Act 1990 as amended	UK	Human Fertilisation and Embryology Authority (HFEA)	DH
Use of human embryos	Human embryos (including human eggs in the process of fertilisation)	1. Research licence				
Use of human admixed embryos	Human admixed embryos as defined in HFE Act	1. Research licence				
Storage of human tissue for research	Cellular material from the human body other than embryos, gametes, and hair and nail from the living	1. Storage licence 2. REC approval or use (within limits) of human tissue from licensed tissue bank	HT Act 2004	England Wales and & NI	Human Tissue Authority (HTA)	DH
Use of NHS patients, non-NHS patients and healthy volunteers, their tissue or their data		REC approval		UK	NHS Research Ethics Committees (RECS)	DH
Deliberate release of genetically modified organisms	Genetically modified (other than naturally) organisms capable of replicating or transferring genetic material		GM (Deliberate Release) Regulations		Defra	Defra
Contained use and accidental release of genetically modified organisms	Genetically modified (other than naturally) organisms, including animal cells in culture but excluding humans and human embryos, capable of replicating or transferring genetic material		GM (Contained Use) Regulations		Health and Safety Executive	DWP
Clinical trials	Medicines Devices Products	Marketing authorisation/ regulatory approval	Medicines for Human Use (Clinical Trials) Regulations 2004	UK	Medicines and Healthcare products Regulatory Agency (MHRA) (also EMEA)	DH

423 Abbreviations included in Table 6.1: HO, Home Office; DH, Department of Health; DWP, Department for Work and Pensions.
424 Disregarding the effect of any anaesthetic/other process rendering the animal insentient. Some ACHM research requires regulatory approval from more than one body – the approvals are not mutually exclusive.

7 International perspective

7.1 Introduction

In Chapter 6 we described the law and regulation applicable to the creation and use of ACHM in biomedical research in the UK. However, like much biomedical research, research involving ACHM is an international activity. It frequently involves international collaboration, takes place across national boundaries, and involves funders or researchers who are often free to choose the location in which their research is conducted. In this chapter, we outline the regulation of this research from an international perspective and consider some of the challenges this poses.

As far as we are aware, very few countries have specifically considered the regulation of research involving ACHM. As in the UK, to our knowledge there are no specific national laws, regulation or guidance documents addressing ACHM research, though a range of laws and regulatory frameworks, particularly those governing the use of animals, cover different aspects of this research.

A similar pattern of legislation is evident at European Union level; whilst there is no specific European legislation on ACHM, there are European equivalents of many (though not all) UK and other national European laws which are of relevance. Internationally, and within European states, broad principles that may be applied to ACHM research are addressed in legal instruments (largely in the context of human cloning).

Guidelines developed by international groups address aspects of ACHM research, particularly the use of human stem cells to create inter-species chimæras. Adoption of these guidelines, though largely voluntary, provides a basis for the development of international best practice in this field, which would be of particular value given the degree of diversity in national laws and regulation.

7.2 National regulation and international research

Research involving ACHM, like other forms of medical research, is principally governed by national law and regulation. Although these may derive from international instruments (such as European Directives), legislation relevant to research is, in the main, implemented and enforced at national level, and research is therefore predominantly governed solely by the laws of the country where it takes place. Occasionally, research is regulated extra-territorially; for example in some cases, researchers are subject to the laws of the country of which they are citizens, even when they conduct research elsewhere. However, this is relatively unusual (for example, none of the regulations discussed in Chapter 6 apply to research conducted solely outside the UK, even where conducted by UK citizens). It is considerably more likely that the conditions and requirements imposed by funding and professional bodies operate extra-territorially.[425]

Research involving ACHM conducted across different national locations needs to be designed to take into account legal and regulatory divergence, with research in each national area potentially being subject to different legal and regulatory limits and controls. Although, as this chapter will show, there is some harmonisation (for example, the recent European Directive was intended to promote greater standardisation around the use of animals in research) this is relatively limited. There remains considerable diversity across nations beyond the European Union, both in regulation of the research use of animals, and other aspects of ACHM research (e.g. the use of human tissue).

Guidelines developed by international groups can encourage common standards and aid researchers working across national borders in navigating divergent governance systems.

425 For example, research involving stem cells funded by the US National Institutes of Health (NIH) must accord with their guidance even if conducted in the UK (see http://stemcells.nih.gov/policy/2009guidelines.htm). The UK's Medical Research Council has supplementary terms and conditions for research that it funds that involves human stem cells (see AC24 in http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC001898).

However, these are currently limited in relation to ACHM research, and only address specific aspects or types of ACHM.[426]

National divergence poses a risk of researchers locating their research in certain countries in order to avoid particular national restrictions. The development of international standards backed by collaboration between national and international policymakers may help to reduce this risk, as well as facilitate cross-border research. It would also be beneficial for national regulators to collaborate, and to encourage international data-sharing, where evidence from incremental research studies has been acquired (see Box 3.8).

7.3 Europe

7.3.1 European Union

Some of the UK legislation governing research involving ACHM originates from European Union law (two notable exceptions are the Human Fertilisation and Embryology Act, which has no EU equivalent, and the Human Tissue Act, which only reflects EU law in certain limited respects).[427] The application of EU Directives on the protection of animals used for scientific purposes, the use of genetically modified organisms, data protection and patents to ACHM research, is summarised below.

EU legislation for the protection of animals

Directive 86/609/EEC on the protection of animals used for scientific purposes was revised in September 2010 by Directive 2010/63/EU, which is due to be implemented in all EU member states by 2013 (see also 6.2.1).[428,429] The revised Directive is the principal European legal framework relating to ACHM research. It applies to regulated scientific procedures involving non-human vertebrates, including larval forms capable of independent feeding and fetal forms of mammals in the last third of their development, and to cephalopods (a class of molluscs including octopi and squid). The Directive places clear limits on the scientific procedures that can be carried out using such animals, and places emphasis on the welfare principles of the 3Rs (reduction, refinement and replacement, see 4.1.1). A minority of ACHM research may be outside the scope of the Directive, as it does not apply to research involving vertebrate animals at less than two-thirds of gestation or invertebrate animals (see 6.2.1).[430]

Creation and use of genetically modified entities

ACHM research may also be within the remit of European Directives intended to safeguard against environmental, and health and safety risks associated with the use, storage and containment, and disposal or release of genetically modified organisms and microorganisms. Directive 2009/41/EC lays down measures for the contained use of genetically modified microorganisms (microbiological entities both cellular and non-cellular, in which genetic material has been altered other than naturally) (GMMs).[431] Directive 2001/18/EC (as amended in 2008) lays down requirements concerning the deliberate release into the environment of genetically modified organisms (biological entities other than human beings capable of replicating or transferring genetic material, in which the genetic material has been altered other than naturally) (GMOs).[432] In particular, it requires users to conduct risk assessments and

426 Examples of international guidelines include CURE Report China (http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC006303); Swiss Commission for Research Partnership with Developing Countries KFPE (1998) (http://www.kfpe.ch/download/Guidelines_e.pdf); and further examples in 'The ethics of research related to healthcare in developing countries: a follow-up discussion paper' available at http://www.nuffieldbioethics.org/research-developing-countries-follow)

427 The European Tissue Directive 2004/23/EC applies only to human tissue for human application, and is not relevant to the use of human tissue for research (http://eur-lex.europa.eu/LexUriServ/site/en/oj/2004/l_102/l_10220040407en00480058.pdf).

428 Council Directive 86/609/EEC of 24 November 1986 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 is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31986L0609:en:HTML

429 Directive 2010/63/EU on the protection of animals used for scientific purposes is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF

430 See 6.2.1 for a discussion of the implementation of Directive 2010/63/EU in the UK.

431 Directive 2009/41/EC on the contained use of genetically modified microorganisms is available at http://www.bmwf.gv.at/fileadmin/user_upload/forschung/gentechnik/2009-41-EC.pdf

432 Directive 2001/18/EC 2001 on the deliberate release into the environment of genetically modified organisms is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001L0018:EN:HTML

to notify and seek the consent of the competent national authority prior to GMO release.[433]

Patent law

Directive 98/44/EC on the legal protection of biotechnological interventions limits the legal protection of inventions including biological material, and may therefore affect some ACHM research (see 6.2.5).[434] In addition to generic requirements, the Directive sets limits to patentability on moral grounds, specifically outlawing the patenting of inventions the exploitation of which would be contrary to *'ordre public or morality'* and *'processes, the use of which offend against human dignity …'.*[435,436] This definition includes processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and animals resulting from such processes.[437] Objections to patents on the grounds of these provisions have been raised in a number of cases. The issue was explored in relation to a challenge to the oncomouse patent for example, though the arguments were ultimately rejected by the European Patent Office and the patent upheld.[438,439]

Protection of personal data

ACHM research conducted within the European Union which involves human tissue or data from which a living individual can be identified, is subject to the requirements of the Data Protection Directive (Directive 95/46/EC).[440] In particular, the Directive includes a requirement that the individual concerned is made aware of and has, subject to limited exceptions, given their consent for the use of their tissue or data.

7.3.2 Council of Europe

Convention on Human Rights and Biomedicine (1999)

The Council of Europe Convention on Human Rights and Biomedicine sets out international standards concerning the protection of human rights in relation to biology and medicine.[441] The Convention does not specifically address ACHM research, but includes principles that may be considered of relevance. Notably, it places emphasis on the protection of '*the dignity and identity of all human beings*' and the importance of '*the need to respect the human being both as an individual and as a member of the human species*', recognising '*the importance of ensuring the dignity of the human being*' and '*that the misuse of biology and medicine may lead to acts endangering human dignity*'.[442] The absence of any definition of 'human being' has enabled considerable diversity of interpretation of the Convention across Europe.[443]

7.4 International

7.4.1 International legal instruments

A range of international legal instruments are of broad relevance to medical research, including that involving the use of human

433 In England and Wales the competent authority enforcing jurisdiction over the GMO (Contained Use Regulations) includes the Health and Safety Executive (HSE), the Secretary of State and the Department for Environment Food and Rural Affairs (Defra). In Scotland the competent authorities includes HSE, the Scottish Executive Environment and Rural Affairs Department and the Scottish Ministers. Northern Ireland has its own separate competent authority. The UK Government and Devolved Administrations have established joint arrangements for assessing applications for the deliberate release of GMOs. This involves consultation with the Advisory Committee on Releases to the Environment (ACRE), the HSE, the Food Standards Agency (FSA), and as appropriate, the statutory nature conservation bodies, such as English Nature. For the HSE's guidance see http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part5.pdf

434 Directive 98/44/EC on the legal protection of biotechnological inventions is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:213:0013:0021:EN:PDF

435 Reference to 'ordre public' is made in Article 6 of Directive 98/44/EC (which is implemented through Article 53(a) of the European Patent Convention regulations).

436 Recital 38 of Directive 98/44/EC continues '…such as processes to produce chimæras from germ cells or totipotent cells of humans and animals, are obviously also excluded from patentability'.

437 Article 6 of Directive 98/44/EC defines as unpatentable processes for cloning human beings, processes for modifying the germ line genetic identity of human beings and uses of human embryos for industrial or commercial purposes.

438 EPO Case No. T0315/03 (transgenic animals/HARVARD); 6 July 2004 http://www.epo.org/law-practice/case-law-appeals/pdf/t030315ex1.pdf

439 A second case (Brüstle vs. Greenpeace EV, (Case no. C34/10) which had yet to be considered by the European Court of Justice at the time of writing) was to be the first case before the Court to involve consideration of Article 6(2)(c) of EU Directive 98/44 (non-patentability of use of human embryos for industrial or commercial purposes as being contrary to *ordre public*). The 'opinion of the attorney general' in the case was published on 10 March 2011.

440 Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data is available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31995L0046:EN:NOT

441 The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine is available at http://conventions.coe.int/Treaty/en/Treaties/html/164.htm. The UK has not acceded to this convention.

442 *Ibid* Preamble and Article 1.

443 For example, it leaves open whether a human embryo or fetus could be considered a human being and a rights holder under the Convention, and by extension the application of the Convention in relation to assisted reproduction and use of human embryos in research.

genetic or cellular material.[444] However, the only international legal instruments that might be applied to ACHM research of which we are aware are non-binding instruments, intended principally to address human cloning.

This UNESCO Universal Declaration on the human genome and human rights (1977) was the first international legal text to address the relationship of biotechnological development and human rights with the human genome.[445] The non-binding declaration states that '*No research, or its applications, should prevail over the respect for human rights, fundamental freedoms, and human dignity of individuals or groups of people*' and that '*Practices contrary to human dignity, such as the reproductive cloning of human beings, shall not be permitted.*'[446]

A second non-binding declaration, the UN Declaration on human cloning (2005), was adopted by a weak majority vote in which UN states were called on to '*adopt the measures necessary to prohibit the application of genetic engineering techniques that may be contrary to human dignity*'.[447] The application of these Declarations to the creation or use of ACHM is a matter of interpretation (e.g. it is disputable whether the creation of ACHM would be considered 'contrary to human dignity'). However, as UN declarations, they are likely to influence some states' national laws, policies and practices.

7.4.2 International guidance

At an international level, the instruments that are of most direct relevance to ACHM research are guidelines developed by funding bodies, scientists and other groups. These include guidance on the use of human ES cells, and other human stem cell types. In these areas the development of international guidelines is relatively mature.

International Society for Stem Cell Research (ISSCR) Guidelines for the Conduct of Human Embryonic Stem Cell Research (2006)

The ISSCR guidelines specify rigorous ethical standards for scientists working with human ES cells and seek to promote responsible, transparent and uniform practices worldwide.[448] They set out a categorisation of research involving stem cells and prescribe the required nature of regulatory review and oversight for each category of research (see Box 7.1). Research involving the incorporation of human ES cells and other human stem cells into animals (i.e. the creation of human–animal chimæras) is addressed, and specific forms of research which should not be pursued at present are identified (see Box 7.1). The guidelines also:

- Encourage the deposition of derived human stem cell lines in national or international depositories that allow open distribution, to facilitate the wider dissemination of these valuable research tools.
- Set out guidance for procurement of tissue for human ES cell research, and specify minimum requirements for obtaining informed consent of donors.
- Indicate that funding organisations should pledge to comply with the guidelines, and that publishers should require a statement of compliance with them.

Hinxton Group

In early 2004, members of the Stem Cell Policy and Ethics Program (SCOPE) at the Johns Hopkins Berman Institute of Bioethics brought together an international and interdisciplinary group to explore the ethical and policy challenges of transnational scientific collaboration raised by variations in national regulations governing embryo research and stem cell science. Drawn from delegates at an

444 For example, the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects, adopted in 1964 (as amended), the International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organizations of Medical Sciences, adopted in 1982 (as amended) and the UNESCO Universal Declaration on Bioethics and Human Rights adopted in 2005.

445 The Universal Declaration on the Human Genome and Human Rights is available at http://portal.unesco.org/en/ev.php-URL_ID=13177&URL_DO=DO_TOPIC&URL_SECTION=201.html

446 *Ibid* Articles 10–11.

447 The United Nations Declaration on Human Cloning (2005) is available at http://daccess-dds-ny.un.org/doc/UNDOC/GEN/N04/493/06/PDF/N0449306.pdf?OpenElement. For the associated press release, see http://www.un.org/News/Press/docs/2005/ga10333.doc.htm

448 International Society for Stem Cell Research Guidelines (2006) are available at http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf

Box 7.1 Categorisation of experiments, from the ISSCR guidelines for the conduct of human embryonic stem cell research[450]

Category 1: Experiments that are permissible after review under existing mandates and by existing local committees, and are determined to be exempt from full Stem Cell Research Oversight (SCRO) review.[451]

Category 2: Forms of research that are permissible only after additional and comprehensive review by a specialised mechanism or body established to address the issues pertinent to stem cell research (i.e. the SCRO function). This category includes:

- Forms of research that generate chimæric animals using human cells. Examples of such forms of research include, but are not limited to introducing totipotent or pluripotent human stem cells into non-human animals at any stage of post-fertilisation, fetal, or postnatal development.
- In general, chimærism of the cerebral cortex or the germ-line are of greatest concern.[452]

Category 3: Research that should not be pursued at this time because of broad international consensus that such experiments lack a compelling scientific rationale or raise strong ethical concerns. Such forms of research include:

- Research in which any products of research involving human totipotent or pluripotent cells are implanted into a human or NHP uterus.
- Research in which animal chimæras incorporating human cells with the potential to form gametes are bred to each other.[453]

initial meeting in 2004, the Hinxton Group is an informal collection of individuals interested in ethical and well-regulated science, coordinated by a US/UK steering committee.[449]

National Institutes of Health Guidelines on the use of Human Stem Cells (2009)

These guidelines apply to research involving human embryonic stem cells and certain uses of human induced pluripotent stem cells.[454] Although designed in relation to research funded by the US National Institutes of Health (NIH), they have wider influence and place clear practical limits on certain categories of ACHM research. In the US, the guidelines limit the use of NIH funding for research involving human ES cell lines to those approved lines listed on the NIH Registry and prohibit NIH funding of:

- Reserach in which human ES cells or human iPS cells are introduced into NHP blastocysts.
- Research involving the breeding of animals where the introduction of human ES cells or human iPS cells may contribute to the germ line.[455]

Final Report of The National Academies' Human Embryonic Stem Cell Research Advisory Committee and 2010 Amendments to The National Academies' Guidelines for Human Embryonic Stem Cell Research.

Guidance from the US National Academies intercalates with, and extends, the NIH

449 The three meetings of the Hinxton Group (www.hinxtongroup.org) have dealt with: 'Transnational cooperation in stem cell research', 'Science, ethics and policy challenges of pluripotent stem cell-derived gametes' and 'Policies and practices governing data and materials sharing and intellectual property in stem cell science'.
450 The International Society for Stem Cell Research Guidelines (2006) are available at http://www.isscr.org/guidelines/ISSCRhESCguidelines2006.pdf
451 *Ibid* Extracts: Section10.1. In accordance with the ISSCR guidelines, 'Each institution, academic or commercial, that engages in human stem cell research shall determine an appropriate Stem Cell Research Oversight (SCRO) procedure, either internal or external, by which their researchers will be subject to review, approval, and monitoring of their human stem cell research activities.' The requirements for this procedure are detailed in the guidelines (Sections 8–9).
452 *Ibid* Section 10.2e.
453 *Ibid* Section 10.3b and 10.3c.
454 National Institutes of Health Guidelines on Human Stem Cell Research (2009) are available at http://stemcells.nih.gov/policy/2009guidelines.htm
455 *Ibid* Section IV.

guidelines to provide guidelines for non-federally funded research involving human ES cells and other human stem cell types.[456] The National Academy of Sciences (NAS) guidance acts as the principal reference on the limits of permissible research uses of embryonic stem cell lines (only briefly addressed in the NIH guidelines) and sets out specific recommendations applicable to research using inter-species chimæras involving human embryonic stem cells (Box 7.2) and other stem cell types (Box 7.3). These guidelines established a categorisation for certain types of ACHM; we suggest a similar approach would be of value in the UK (see 8.2).

A number of other reports have considered aspects of research involving ACHM at national or European level (Box 7.4).

Box 7.2 Extracts from NAS guidance on the research use of human ES cells

The US National Academy of Sciences (NAS) guidance sets out requirements in relation to particular uses of human ES cells. These include:

- All protocols involving the combination of human ES cells with non-human embryos, fetuses, or adult vertebrate animals must be submitted to the local Institutional Animal Care and Use Committee (IACUC) for review of animal welfare issues and to the Embryonic Stem Cell Research Oversight (ESCRO) committee for consideration of the consequences of the human contributions to the resulting chimæras.
- Transplantation of differentiated derivatives of human ES cells or even human ES cells themselves into adult animals will not require extensive ESCRO committee review. If there is a possibility that the human cells could contribute in a major organised way to the brain of the recipient animal, however, the scientific justification for the experiments must be strong, and proof of principle using non-human (preferably primate) cells, is desirable.
- Experiments in which human ES cells, their derivatives, or other pluripotent cells are introduced into non-human fetuses and allowed to develop into adult chimæras need more careful consideration because the extent of human contribution to the resulting animal may be higher. Consideration of any major functional contributions to the brain should be a main focus of review.
- Introduction of human ES cells into non-human mammalian blastocysts should be considered only under circumstances in which no other experiment can provide the information needed.[457]

Defined categories of human ES cell research, include:

- **Permissible after ESCRO committee review:**
  - Research involving the introduction of human ES cells into non-human animals other than humans or primates at any stage of embryonic, fetal, or postnatal development.
  - Research involving the introduction of human ES cell into NHPs at any stage of fetal or postnatal development.
- **Currently prohibited:**
  - Research in which human ES cells are introduced into NHP blastocysts or in which any embryonic stem cells are introduced into human blastocysts.
  - No animal into which human ES cells have been introduced such that they could contribute to the germ line should be allowed to breed.

Guidance indicates that particular attention should be paid to at least three factors: the extent to which the implanted cells colonise and integrate into the animal tissue; the degree of differentiation of the implanted cells; and the possible effects of the implanted cells on the function of the animal tissue.[458]

456 National Academies (2010). *Final report of the National Academies' human embryonic stem cell research advisory committee and 2010 amendments to the National Academies' guidelines for human embryonic stem cell research. Appendix C: National Academies' guidelines for human embryonic stem cell research amended as of May 2010.* Available at http://books.nap.edu/openbook.php?record_id=12923&page=19

457 *Ibid* Sections 6.4–6.7.

458 *Ibid* Sections 1.3a–1.3c.

Box 7.3 NAS guidance on the use of non-embryo-derived human pluripotent stem cells and multipotent neural stem cells

Proposals for use of human pluripotent stem cells in animals should be considered in one of the following categories:

- **Permissible after currently mandated reviews and proper documentation.**
  Experiments that involve only transplantation into postnatal animals with no likelihood of contributing to the central nervous system or germ line.
- **Permissible after additional review by an ESCRO committee.**
  Experiments in which there is a significant possibility that the implanted human pluripotent stem cells could give rise to neural or gametic cells and tissues. Such experiments would include generation of all preimplantation chimæras as well as neural transplantation into embryos or perinatal animals.
- **Should not be conducted at this time:**
  (1) Experiments that involve transplantation of human pluripotent stem cells into human blastocysts.
  (2) Research in which human pluripotent stem cells are introduced into NHP embryos, pending further research that will clarify the potential of such introduced cells to contribute to neural tissue or to the germ line.
- **Prohibition on Breeding:**
  No animal into which human pluripotent stem cells have been introduced such that they could contribute to the germ line should be allowed to breed.[459]

Multipotent neural stem cells

'It is also relevant to note that neural stem cells, although not pluripotent, are multipotent and may have the potential to contribute to neural tissue in chimeric animals. ESCRO committees should decide whether they wish to review and monitor such experiments with neural stem cells in a similar fashion.' [460]

459 *Ibid* Section 7.5.
460 *Ibid* Section 7.4.

Box 7.4 Other initiatives

- **Human–animal combinations in stem cell research.** In 2010 a working group of the Singapore Bioethics Advisory Committee reviewed national ethical, legal and social issues related to research involving cytoplasmic hybrids and human–animal chimæras involving human stem cells. Ethical issues and regulatory policies in other major scientific jurisdictions were also examined. The group recommended a prohibition on breeding animals into which human pluripotent stem cells had been introduced, and emphasised the need, where research involves the introduction of pluripotent human stem cells into animals, to avoid the creation of entities in which human sentience or consciousness might occur.[461]
- **German Ethics Council Opinion on human–animal mixed-species entities.** The German Ethics Council's opinion is under consideration following a public survey and meeting of international experts on 'human–animal mixed-species entities' in 2010.[462]
- **Chimbrids.** The 'Chimæras and hybrids in comparative European and International research' study involved researchers from 15 European states and six further nations in 2005–2007. Scientific, ethical, philosophical and legal aspects of research involving inter-species mixtures were addressed. It was recommended that 'chimbrid' research proposals should be independently examined by an interdisciplinary body; and particular experiments to be subject to prohibition or special consideration were identified.[463]
- **ESTOOLS Ethics Workshop 2.** This multi-national group of European stem cell researchers held a workshop in Lund, Sweden, in October 2008 which considered 'ethical aspects of research on inter-species embryos and iPS cells', including ethical and regulatory aspects of inter-species embryo research.[464]
- **Man or mouse? Ethical aspects of chimæra research.** In 2006–7 the Danish Ethical Council for Animals and Danish Council of Ethics conducted a joint study which included ethical discussion of research involving human–animal chimæras. Modification of Danish regulation was recommended to ensure that chimæras 'difficult to place biologically, ethically and legally' would not be created; however, these recommendations have not yet been enacted.[465]
- **The Cultural, Ethical and Spiritual Dimensions of the Use of Human Genes in Other Organisms.** The New Zealand Bioethics Council's 2003–4 study included a programme of public consultation across broad demographics. Its recommendations included that genetic manipulations, intended to produce social or mental capacities in animals that are recognisably human-like, or produce significant morphological changes in life forms to make them more similar to human life forms, should not be pursued.[466,467]

461 The Bioethics Advisory Committee Singapore (2010). *Human–animal combinations in stem cell research.* http://www.bioethics-singapore.org/uploadfile/62913%20PMFull%20HAC%20Report.pdf

462 The German Ethics Council (2010). http://www.ethikrat.org/press/press-releases/2010/press-release-02-2010

463 The Coordination Action Chimbrids (Chimæras and Hybrids in Comparative European and International Research: scientific, ethical, philosophical and legal aspects) (2009). Taupitz J., & Weschka M, Springer. See http://www.jura.uni-mannheim.de/imgbchimbrids/index.php?option=com_content&task=view&id=12&Itemid=31

464 ESTOOLS is the largest grouping of human embryonic and induced pluripotent stem cell researchers in Europe. Spanning 10 countries, the project brings together the combined expertise of 21 academic and commercial research teams. For the report of the Ethics Workshop 2 see http://www.estools.eu/assets/files/Uploaded_Files_1/Lund%20workshop/ESTOOLS%202nd%20Ethics%20workshop%20October%202008%20Lund%20Report.pdf

465 The Danish Council of Ethics (2008) *Man or mouse? Ethical aspects of chimæra research* http://etiskraad.dk/upload/publications-en/stem-cell-research/man-or-mouse/index.htm.

466 The Bioethics Council of New Zealand (2004). *The Cultural, Ethical and Spiritual Dimensions of the Use of Human Genes in Other Organisms* http://ndhadeliver.natlib.govt.nz/ArcAggregator/frameView/IE1074184/http://www.bioethics.org.nz/. The Council disbanded in 2009.

467 For completion we note that a draft US Senate Human Chimæra Prohibition Act was introduced in 2005 following a recommendation in a report by the President's Council on Bioethics (President's Council on Bioethics (2004). Reproduction and Responsibility: The Regulation of New Biotechnologies; http://bioethics.gov/reports/reproductionandresponsibility/chapter10.html). The Bill sought to prohibit the creation human chimæras, including attempts to create, transfer or receive them. The Bill was endorsed in the 2006 State of the Union Address but did not become law. A draft Human–animal Hybrid Prohibition Act, introduced in 2009, did not become law.

As in many fields of science, much research involving ACHM depends on collaborative working between groups of scientists working in different legal jurisdictions. Whilst intra- and international scientific collaboration is vital to the success of such research, this can create regulatory challenges where work is conducted under different legislative frameworks.

We endorse the views of bodies such as the Hinxton group in encouraging international coordination, which stated in relation to human ES cell research: '*Steps should be taken to develop consensus in ethical standards and practices in hESC research for international collaboration to proceed with confidence and for research from anywhere in the world that adheres to these standards and practices to be accepted as valid and valuable by the scientific community and academic journals. To achieve this goal, it will be necessary to specify what these standards and practices should be through the international efforts of scientists, philosophers, bioethicists, lawyers, clinicians, journal editors and regulators involved in this field, in collaboration and consultation with the public. This process of identification of international ethical standards and practices should include concerted efforts to engage people throughout the world in honest and realistic conversations about the science and ethics of stem cell research and its emerging applications.*'[468]

We believe this statement has equal validity in relation to ACHM research, particularly given the diversity across national regulation and practice outlined in this Chapter. **We therefore strongly encourage initiatives to raise awareness and promote consistency in research practice at an international level, which could be led by regulators, policy-makers, national and international bioethics bodies, medical research councils or the research community itself. The UK is well placed to take a lead in encouraging such dialogue** (see 8.7).

468 Hinxton Group Consensus statement 2006: http://www.hinxtongroup.org/au_trans_cs.html

8 Conclusions and recommendations

8.1 Overview

We have reviewed the types of research conducted using animals incorporating human gene sequences or human cells. The overall purposes of such work are to study the function of human genes and cells, to create improved animal models of human disease, and to develop, produce and test novel therapeutic products. Not all such experiments are successful, as in all types of science, but this research has yielded important new knowledge and significant insights with promise for the future, as well as methods and products that have considerable clinical value.

8.1.1 ACHM and animal research

Consideration of the research use of ACHM must always be set in the general context of animal research, which is tightly regulated in the UK under the Animal (Scientific Procedures) Act (ASPA), such that any suffering inflicted on a protected animal must be justified by the potential value of the research, and animal welfare principles, as commonly embodied in 3Rs, must be applied.[469] Comparable national regulation exists in many scientifically advanced countries, and is incorporated in the European Directive (2010/63/EU). We see no reason to either relax or tighten UK standards in the case of ACHM. However, we have considered whether any additional scrutiny might be required for ACHM research.

8.1.2 ACHM history and prospects

Research involving ACHM has a long history. No specific safety or regulatory concerns have emerged from such research to date, although a few issues have prompted ethical debate (see 8.5 for discussion of safety issues). Developments in transgenesis and particularly in stem cell research lead us to anticipate a major increase in the use of these techniques to investigate the biological effects of normal and abnormal human genes and cells in animals: to study their roles in development, normal function and human disease processes; to test the safety and efficacy of novel therapeutics (particularly biological therapeutics); and to produce clinically useful proteins, cells and tissues.

These approaches hold promise for advancing biomedical and biological research but, as with virtually all scientific developments, we repeat our caution that not all avenues explored will prove fruitful; and that the timescales between initial research and applicable health interventions are long (up to decades), variable and impossible to predict with confidence. The use of ACHM can also offer approaches which may advance the 3Rs principles, improving the effectiveness of animal use by making individual experiments more informative about human biology.[470]

8.1.3 ACHM ethical and societal aspects

The great majority of experiments that we can currently anticipate do not present novel ethical issues and should continue to be satisfactorily regulated under the existing framework governing all animal research. They include familiar experiments such as the creation of transgenic rodents containing relatively small numbers of human genes, tissue grafting, and the transfer of tissue-specific stem cells to humanise individual organs.

Evidence we received, the public dialogue, the published literature and our own deliberations, identify a limited number of research areas which may require greater scrutiny. These include research that may raise issues of ethical and social acceptability or have unusual implications for the animals involved. Experiments that approach these sensitive areas may, however, be of substantial medical and scientific importance. We therefore propose that such research projects should remain eligible for consideration for licensing by the appropriate regulatory authorities (see sections 8.3 and 8.6), but subject to additional expert scrutiny.

469 The 3Rs principles are that experiments involving animals can be licensed only if there are no scientifically suitable alternatives that *replace* animal use, *reduce* the number of animals needed or *refine* the procedures used to cause less suffering (see 4.1 and 6.2.1).

470 This is not to imply that we expect *overall use* of animals in medical research to diminish in the short term as a result of research involving ACHM, in part because their development will open up new avenues of research involving animal experimentation.

8.2 Categorisation of ACHM

We propose that experiments involving ACHM could be usefully classified into three categories:[471]

8.2.1 Category 1

The great majority of ACHM experiments, as outlined in section 8.1.3 above, which do not present issues beyond those of the general use of animals in research, should be subject to the same oversight and regulation under ASPA as other animal research.

8.2.2 Category 2

A limited number of types of ACHM research, outlined below in this section (8.2.2), should be permissible subject to additional specialist scrutiny by the national expert body we propose in section 8.3. Such experiments should be approached with caution. Strong scientific justification should be provided to the national expert body, who should closely consider the ethical and any safety issues in addition to the potential value of the research. Authorisation may require studies to adopt an incremental (graduated) approach as described in section 8.2.4 and Box 3.8. Proposed studies should be assessed on a case-by-case basis, at least until experience allows the formulation of guidelines. Although we would expect this list to evolve over time as knowledge advances, the major types of research that we would currently include in this category are:

- Substantial modification of an animal's brain that may make the brain function potentially more 'human-like', particularly in large animals.
- Experiments that may lead to the generation or propagation of functional human germ cells in animals.
- Experiments that could be expected to significantly alter the appearance or behaviour of animals, affecting those characteristics that are perceived to contribute most to distinguishing our species from our close evolutionary relatives.
- Experiments involving the addition of human genes or cells to NHPs. We recognise that research on NHPs is appropriate, and in some types of research probably essential if it is to lead to clinical benefit, but such research should remain under a high degree of regulatory scrutiny.[472]

8.2.3 Category 3

A very narrow range of experiments should not, for now, be licensed because they either lack compelling scientific justification or raise very strong ethical concerns. The list of such experiments should be kept under regular review by the proposed national expert body, but should at present include:

- Allowing the development of an embryo, formed by pre-implantation mixing of NHP and human embryonic or pluripotent stem cells, beyond 14 days of development or the first signs of primitive streak development, (whichever occurs first), unless there is persuasive evidence that the fate of the implanted (human) cells will not lead to 'sensitive' phenotypic changes in the developing fetus.[473,474] This supplements the 14 day provision applied to human admixed embryos under the HFE Act, so that mixed embryos that are judged to not quite meet the criteria for being 'predominantly human', should nevertheless be regulated on the basis of the likely phenotypic effect on the embryos created. Currently, any mixed origin embryo judged to be 'predominantly human' is regulated by HFEA and cannot be kept beyond the 14 day stage, whereas an embryo judged to be predominantly animal is unregulated until the mid-point of gestation (likely to be increased to two-thirds on implementation of the European Directive 2010/63/EU) and can in principle be kept indefinitely. As to whether or not an admixed embryo is predominantly 'human' is an expert judgement, including an assessment of likely phenotype, but neither

471 A graded approach already operates to some degree under ASPA. Project licenses including certain types of experiment, including those that raise 'novel or contentious' issues, must be referred to the Animal Procedures Committee for review (see Box 6.2). The principle of a graded approach has also been enunciated by the International Society for Stem Cell Research (see 7.4.2), the US National Academy of Sciences (Box 7.2-3), and in reference to the 'human neuron mouse' by Greely *et al.* (see 3.4).

472 For example, stem cell therapeutic approaches may need to be tested on NHPs because their greater similarity (cell cycle time, brain structure, molecular homology) to humans will provide better assessment of colonisation and neural contact development.

473 This applies whether the embryo is implanted within an animal uterus or maintained as an intact embryo *in vitro.*

474 Equivalent statutory restrictions are applicable to human and human admixed embryos under the HFE Act (see 6.2.2).

the precise eventual composition of an individual embryo nor the phenotypic effect of the admixture will be easily predictable in the current state of knowledge.
- Transplantation of sufficient human-derived neural cells into an NHP as to make it possible, in the judgement of the national expert body, that there could be substantial functional modification of the NHP brain, such as to engender 'human-like' behaviour. Assessing the likely phenotypic effect of such experiments will be informed by prior work on other species (possibly including stem cell transfer between NHPs) or by data on the effects of 'graded' transplantation of human cells into NHPs.
- Breeding of animals that have, or may develop, human-derived germ cells in their gonads where this could lead to the production of human embryos or true hybrid embryos within an animal.[475]

8.2.4 Graduated licensing

Since the outcome of many of the experiments outlined in category 2 (8.2.2) will be somewhat unpredictable until initial studies have been conducted, we recommend consideration of graduated licensing. By this we mean licensing limited initial experiments, involving small numbers of animals, starting with those species considered least likely to experience pain, suffering, or long-lasting harm, and with careful monitoring of the outcomes according to agreed measurable criteria, before further work is permitted.[476] Given the exploratory nature of the work, there should be active dialogue between investigator and the national expert body, and the results of such experiments should in turn inform the future regulatory process for similar experiments. In Chapter 3 (Box 3.8) we outline an example of this approach in neuroscience, but the principles are generic.

8.2.5 Flexibility of regulation

The types of experiment in these categories, and the boundaries which are set, are virtually certain to evolve with time, new knowledge and changing social norms. Regulators should monitor and respond to changes in societal views and scientific knowledge, and regulatory mechanisms should be sufficiently flexible to accommodate such change.

8.3 National expert body

The limited number of such experiments, the specialist knowledge required to evaluate their likely outcomes and the socially sensitive nature of the judgements to be made, dictate that oversight of research involving ACHM should be carried out by a single, national, expert, review body. **We recommend that the Home Office ensures that a national expert body with a duty to advise on the use of ACHM in research is put in place.**

We recommend that this national expert body should:

- **Be multidisciplinary, involving people with knowledge of ethics, the humanities, social sciences, law and the biological sciences as well as people without specific expertise in these fields, and be able to co-opt additional expertise when relevant.[477]**
- **Be transparent, making its proceedings, deliberations, reasoning, conclusions and recommendations available for public scrutiny.**
- **Be outward facing so that interested persons are aware of its function and feel able to input into its work programme.**
- **Be actively involved in public engagement and consultation; and maintain regular forward-looking dialogue with the scientific community.** This will enable it to anticipate future scientific directions. A major strength of this approach would be the ability to ensure that scientific work in this area proceeds with reasonable

475 Placement of human embryos into animals is prohibited by the HFE Act, and this seems likely to be interpreted to include placement of human embryos into animals modified to contain human uterine tissue.
476 We do not intend this to lead to the duplication of animal experiments. Where there is satisfactory evidence from previous experiments this should be taken into account and not repeated.
477 Given the special issues associated with experiments on NHPs, we recommend that the national expert body should include, either in its membership or as an advisor, an independent scientist with experience in NHP research who should be present to advise the group when such issues are discussed.

public understanding and support, and is not unduly influenced by extreme views. Responses from public participants in our dialogue indicated that the UK public would be receptive to such an approach.

- **Have the power to develop guidelines to promote consistency and transparency in the regulatory process.**

To ensure a consistent approach in ethical and animal welfare matters (see Chapters 4 and 5), we consider it desirable that research involving ACHM is considered by the same body that advises Government on other aspects of animal research. We are aware that, in implementing the EU Directive 2010/63/EU, the UK is required to establish a 'national committee for the protection of animals used for scientific purposes'.[478] We anticipate this body will succeed the currently constituted Animal Procedures Committee. **We recommend that the Home Office ensures that the body which meets the requirement of the 'national committee for the protection of animals used for scientific purposes' in the UK has within its remit and competence the function of the national expert body for ACHM.**

8.4 Welfare

We have commented that research involving ACHM does not have a generally increased potential for causing animal suffering compared with other experiments permitted under existing regulation, and that the development and use of ACHM could contribute to 3Rs principles. There may, however, be a few specific situations in which modification of the appearance or behaviour of a normally social animal may cause it to experience distress, including as a result of the actions of others of its own species, or of its human carers. Such effects can also occur in other experimental situations. This type of harm should be taken into account in the overall assessment of potential animal suffering in ACHM experiments, as it would with similar changes induced by other experimental procedures. We emphasise that research involving ACHM should be subject to scrutiny, and advancement from the perspective of animal welfare, in a manner no different from other animal research.

8.5 Safety

We have considered a variety of safety issues that could arise from experiments involving ACHM. There are some hazards that are specific to the purpose and nature of individual research protocols, such as those altering an animal's susceptibility to human infections, which must be appropriately regulated and managed according to established procedures. We have also considered more generic issues, predominantly relating to the risk of activating endogenous viruses or altering the host range of infectious agents. The risk levels are thought to be very low, but not zero.[479] Any manipulation which is known to, or could, alter viral or other pathogen recognition sites, or in any other way affect susceptibility to pathogens, or which deliberately involves the activation of human and animal proviruses within the same ACHM (such that they could recombine) should be carefully risk-assessed and appropriate control mechanisms put in place. It is critical that the provenance of human material to be used clinically is known and considered during the risk assessment.

The nature of the risks, and ways of mitigating them, are similar to those regularly used for other research involving potentially infectious materials. **We recommend that, for those classes of ACHM where it is relevant, a risk assessment should be undertaken and appropriate containment levels specified. The risk assessment is the responsibility of investigators, research institutions, and regulators; and should where relevant take the advice of an independent virologist.**

478 Article 49, Directive 2010/63/EU on the protection of animals used for scientific purposes. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31986L0609:en:HTML

479 Notably when human cells are isolated from ACHM and then maintained in culture or introduced into humans.

8.6 Interfaces between regulatory authorities

Research involving human embryos is regulated by the HFEA under the HFE Act (see 6.2.2). As was recognised during the passage of this Act, there are situations in which this regulation of human embryo research and the matters discussed in the current report interface very closely, and may partly overlap. Chimæric embryos containing both human and animal stem cells are examples, because whether they are considered 'human' for the purposes of regulation depends on the proportion of human cells, their distribution and, most importantly, their expected effect on the phenotype of the resultant embryo. The proportions and distribution of cells of different species in a single structure may evolve over time; such change may be unanticipated or result from experimental design; and the state of current knowledge is such that predicting phenotypic effects may be difficult. In each case, an expert judgement will have to be made, as to whether and how to proceed. The technical potential to create transgenic animals containing ever larger amounts of human DNA sequence raises similar issues.

The existing UK legislative structure is such that some awkward cases may fall at the boundary of jurisdiction. **We recommend that the Home Office and the Department of Health work closely together to ensure that there are no regulatory gaps, overlaps, or inconsistencies, between the two regulatory systems.** They should bear in mind that animal embryos are not regulated until the middle of gestation (likely to be increased to two-thirds of gestation under the new European Directive), although we recognise that maternal animals carrying these embryos may be regulated under ASPA.

We consider it essential that the Home Office and the HFEA (or, as appropriate, the Department of Health) work together to develop and maintain a smooth, functionally integrated operational interface at the boundaries of their areas of responsibility. This should be supported by clear guidance to the research community, to ensure the timely and appropriate adjudication of innovative scientific projects without undue bureaucracy. Such an interface may well involve the expert advisory bodies in the two systems, as well as officials acting for the agencies concerned.

The Home Office (and, where relevant, the Department of Health) should consult, as appropriate, with other bodies who may sometimes have a role in the regulation of ACHM, namely, the Human Tissue Authority, the Health and Safety Executive, the Department for the Environment, Food and Rural Affairs and the Steering Committee of the National Stem Cell Bank.

8.7 International regulation

We have considered other recent (non-UK) national and international studies which have examined aspects of the use of ACHM in research (Chapter 7). To date, consideration of ACHM research from policy, societal, ethical and regulatory perspectives is limited. We have also noted that this field of science, like so many, could take place across several jurisdictions with differing regulatory requirements, allowing funders and researchers to exercise choice about the location of their research. **We recommend raising international awareness of ACHM, promoting international consistency in research practice involving their use, and exploring the development of international standards or guidance. This might be achieved through international collaboration amongst regulators, policy-makers, national and international bioethics bodies and medical research councils, or initiatives within the research community. This is an area in which the UK should provide leadership.**

8.8 Summary

In short, we advocate a tiered approach to regulation such that the great majority of uncontentious experiments proceed as under current ASPA regulation, while a small number of categories of experiment are referred for more expert scrutiny, with graduated licensing allowing progress to be made under regular review. A very limited number of experiments should not be licensed at the current time. The graduated licensing process should be interfaced with the corresponding processes that regulate human embryos so that the regulators are aware of each other's activities and so that there is no gap or unnecessary overlap between their jurisdictions.

Annex I Report preparation

Working group membership

This report was prepared by a working group of the Academy of Medical Sciences. Members participated in a personal capacity, not as representatives of the organisations listed. A summary of working group members' interests is given below.

Chair

Professor Martin Bobrow CBE FRS FMedSci is Professor Emeritus of Medical Genetics at the University of Cambridge. His research interests are primarily in genetic disease and molecular diagnostics, with clinical specialism in genetics, including genetic diagnosis and genetic counselling. Currently a non-executive Director of the Cambridge University Hospitals NHS Foundation Trust, and Chair of the Muscular Dystrophy Campaign, Professor Bobrow has been Chairman of Unrelated Living Donor Regulating Authority (ULTRA) and the Advisory Committee on Radiation in the Environment (COMARE), Deputy Chairman of Wellcome Trust and the Nuffield Council on Bioethics, and a member of the MRC Council, Human Genetics Advisory Commission and NHS Central R&D Committee. In continuity with the current study, Professor Bobrow also chaired the Academy of Medical Sciences' *'Inter-species embryos'* study, which informed revision of medical research aspects of the UK Human Fertilisation and Embryology Act (2008).

Members

Professor Thomas Baldwin is a Professor of Philosophy at the University of York; he is also the Editor of Mind, the leading UK philosophy journal. His research focuses on 20th century philosophy, both analytical and continental (he has just completed a critical edition of some unpublished writings by G E Moore and is now writing a book about J-P Sartre). He currently teaches metaphysics, political philosophy and ethics. He is a member of the Human Genetics Commission and of the Government's Expert Advisory Committee on obesity. He has been Deputy Chairman of the Human Fertilisation and Embryology Authority, and a member of the UK Stem Cell Bank Steering Committee and of the Nuffield Council on Bioethics.

Reverend Dr Michael Banner is Dean and Fellow of Trinity College, Cambridge. He was previously Professor of Public Policy and Ethics in the Life Sciences at the University of Edinburgh, and of Moral and Social Theology at King's College London. He currently Chairs the Cambridge University (Animal Procedures) Licence Review Committee, and is a member of the Human Tissue Authority. He has been Chairman of the Home Office Animal Procedures Committee, the Shell Panel on Animal Testing, the Government Committee of Enquiry on the Ethics of Emerging Technologies in Breeding Farm Animals, and the Department of Health CJD Incidents Panel, Director of the UK Economic and Social Research Council's Genomics Research Forum, and a member of the Royal Commission on Environmental Pollution, and the Agriculture and Environment Biotechnology Commission. He is currently writing a book on animals and ethics for Oxford University Press.

Professor Peter Brophy FRSE FMedSci is Director of the Centre for Neuroregeneration and Professor of Anatomy at the University of Edinburgh. His research specialties are in the molecular and cell biology of myelination and demyelination, particularly axon–glia interaction and the genetics of inherited peripheral neuropathy using transgenesis and gene targeting in mice. Professor Brophy chaired the 2008 International Gordon Conference on Myelin and the Committee on Stokes Professorship Awards, Science Foundation Ireland, and currently chairs

the Scientific Advisory Board for the INSERM 'Institut du Fer à Moulin', Paris. He has also been a member of Wellcome Trust's Neurosciences and Mental Health Panel, the French 'Agence Nationale de Recherche', the Canadian Government's Foundation for Innovation Neuroscience Panel and a research panel member for bodies including Action Research and the Multiple Sclerosis Society.

Ms Tara Camm is a UK qualified solicitor, who has spent over 15 years in the not-for-profit sector, bringing her legal training and expertise to bear on a diverse range of strategic, policy and operational issues affecting not-for-profit organisations in the UK and internationally. She is currently General Counsel and Company Secretary for Plan International, one of the largest non-governmental organisations in the world promoting child rights to relieve child poverty. Previously Principal Solicitor for Wellcome Trust, Ms Camm has significant interest and experience in biomedical science law. She led the legal work for the creation of the UK Biobank, including its Ethics and Governance Framework and Council, and has had extensive involvement in the development of UK and international guidance and legislation affecting biomedical science, including in the UK, the Human Tissue Act (2004), the Mental Capacity Act (2005) and the Human Fertilisation and Embryology Act (2008).

Professor Dame Kay Davies DBE CBE FRS FMedSci is Head of Department of Physiology, Anatomy and Genetics, and Director of the MRC Functional Genomics Unit at the University of Oxford. Her research interests centre on the molecular genetic analysis of human muscular and neurological diseases, particularly muscular dystrophy, motor neuron disease and ataxia. She also has an active interest in the ethical implications of genetics research and the public understanding of science, and considerable experience of the use of biotechnology companies as a conduit for translating the results of experimental science into new therapeutics and diagnostics. Professor Davies is Executive Editor of the journal Human Molecular Genetics, and a member of Wellcome Trust Board of Governors.

Professor John Harris FMedSci is Lord Alliance Professor of Bioethics, and Director of the Institute for Science, Ethics and Innovation, at The University of Manchester. His specialities are in the ethics of scientific and technological innovation, including areas of genetics, transplantation, human enhancement and reproduction; he leads the Wellcome Strategic Programme in 'The Human Body, its Scope Limits and Future'. Currently joint Editor-in-Chief of The Journal of Medical Ethics, Professor Harris is a member of several editorial boards including that of the Cambridge Quarterly of Healthcare Ethics. A member of the Human Genetics Commission, he was formerly a member of the Medical Ethics Committee of the British Medical Association, and the Government Advisory Committee on Genetic Testing. Professor Harris was a Founder Director of the International Association of Bioethics, and has been consultant to bodies including the European Parliament and the World Health Organization.

Professor Roger Lemon FMedSci is Sobell Chair of Neurophysiology and Head of the Sobell Department of Motor Neuroscience and Movement Disorders at the Institute of Neurology, University College London. His main research interest is in the control of skilled hand movements by the brain, including the impacts on these movements of damage to the cortex, for example as a result of stroke or in cerebral palsy. He sits on the Ethical Review Panel of the UK Centre for Macaques, is a member of the Council of Understanding Animal Research, Chairs the Expert Group of the EU Animals Directive of the European Science Foundation, and is Associate Editor at the Journal of Neuroscience, Guarantor and Associate Editor at Brain and a Receiving Editor of Neuroscience Research.

Dr Robin Lovell-Badge FRS FMedSci is Head of Division of Stem Cell Biology and Developmental Genetics, at the MRC's National Institute for Medical Research. His research specialties are in genetics, early embryonic development, sex determination and the development of the mammalian nervous system, as well as the biology and use of stem cells. Dr Lovell-Badge's work in the wider communication of science has included school lectures, National Institute for Medical Research programmes, media interviews, and parliamentary and public debates on embryo and stem cell research and genetics. Dr Lovell-Badge is President of the Institute of Animal Technologists, a Visiting Professor at the University of Hong Kong and an honorary professor at University College London. He is a member of the Academy of Medical Sciences' Communications Group and has advisory board membership including the Scientific and Clinical Advances Advisory Committee of the Human Fertilisation and Embryology Authority, and the Science Media Centre. He is also a member of the organising committee of the Hinxton Group.

Professor Jack Price is Professor of Developmental Neurobiology at King's College London. He got his first degree with the Open University, then a PhD in Neurobiology from University College London. Following post-doctoral training at Massachusetts Institute of Technology, he ran a research group at the National Institute for Medical Research for eight years. He was then Director of Molecular Neuroscience at SmithKline Beecham Pharmaceuticals, until taking up his present position of Professor of Developmental Neurobiology in 1998. He became Head of the newly formed Centre for the Cellular Basis of Behaviour in 2006. He has worked on neural stem cells in various guises for about twenty years and has more recently been pursuing an interest in the development of psychiatric disorders. He is also currently Consultant and Director of Cell Biology for ReNeuron Ltd., a UK biotechnology company developing stem cells for therapeutic and drug-discovery applications.

Professor Terence Rabbitts FRS FMedSci works at the Leeds Institute of Molecular Medicine where he was Scientific Director until 2010. His research interests centre on the molecular analysis and modelling of chromosome abnormalities in human cancer, immunogenetics and the development of cancer biotherapies. Professor Rabbitts was formerly the joint Head of the Division of Protein and Nucleic Acid Chemistry at the Medical Research Council Laboratory of Molecular Biology, Cambridge. He chaired the Scientific Advisory Boards of Cambridge Antibody Technology and Quadrant HealthCare, and was a Domantis scientific advisory board member. He is currently a member of the scientific advisory boards of Oryzon, DiThera and the Institute of Genetics and Molecular Medicine, Edinburgh. He is a member of the Academy of Medical Sciences' Council, the European Molecular Biology Organization and has been awarded the Colworth Medal of the Biochemical Society and the CIBA Prize.

Professor Martin Raff CBE FRS FMedSci is Emeritus Professor of Biology at the Medical Research Council Laboratory for Molecular Cell Biology, University College London. His research interests were in cell biology, with focus on developmental neurobiology and mammalian cell proliferation and differentiation. A Fellow of the Academia Europaea, foreign member of the American Academy of Arts and Sciences and the National Academy of Sciences, and a member of the Lasker Awards jury, Professor Raff is also co-author of 'Molecular biology of the cell'. He is a Director of the Company of Biologists, and is a member of scientific advisory boards in America and Europe, including Wellcome Trust Centre for Human Genetics, the Weatherall Institute of Molecular Medicine, and the Medical Research Council Clinical Sciences Centre within the UK. Professor Raff has been President of the British Society of Cell Biology and Chairman of the UK Life Sciences Committee.

Professor Trevor Robbins FRS FMedSci was elected to the Chair of Experimental Psychology (and Head of Department) at the University of Cambridge in October 2002. He is a Fellow of the British Psychological Society, the Academy of Medical Sciences, and the Royal Society. He has been President of the British Association for Psychopharmacology (1994–1996) and the European Behavioural Pharmacology Society (1992–1994), winning the latter Society's inaugural Distinguished Scientist Award in 2001. He was the F. Kavli Distinguished International Lecturer at the Society for Neuroscience meeting in 2005 and he gave the Staglin Mental Health Music Festival Keynote address in 2008. He was recently jointly given the prestigious Distinguished Scientific Achievement Award for 2011 by the American Psychological Association. He has been a member of the MRC Council and chaired the Neuroscience and Mental Health Board from 1996 until 1999. Currently, he directs the MRC/Wellcome Trust-funded 'Behavioural and Clinical Neuroscience Institute', the mission of which is to enhance translation from basic to clinical neuroscience.

Professor Nikolas Rose is the Martin White Professor of Sociology and Director of the BIOS Centre for the study of Bioscience, Biomedicine, Biotechnology and Society at the London School of Economics and Political Science. His current research concerns the social, ethical, cultural and legal implications of biological and genetic psychiatry and behavioural neuroscience, examining in particular the emergence of novel ways of governing human mental life and conduct, and their consequences. He is also working with colleagues at Imperial College London in the joint Imperial–LSE Centre for Synthetic Biology and Innovation. He has published on areas including the social and political history of the human sciences, the history of empirical thought in sociology, and changing rationalities and techniques of political power. He is a member of the Nuffield Council on Bioethics, Chair of the European Neuroscience and Society Network, Editor of BioSocieties and a Visiting Professor at the Institute of Psychiatry, King's College, London.

Professor Christopher Shaw FMedSci is Professor of Neurology and Neurogenetics at the Institute of Psychiatry, King's College London. He is also Head of the Department of Clinical Neurosciences and Director of the MRC Centre for Neurodegeneration Research and Director of the Maurice Wohl Clinical Neurosciences Institute. He is also an Honorary Consultant Neurologist at King's College and Guy's Hospitals. His early training in General Medicine and Clinical Neurology was conducted in New Zealand. He was awarded a Wellcome Trust New Zealand Health Research Council Fellowship to come to the UK and study Neurobiology in the Neurology Unit of Cambridge University from 1992 to 1995. From that time he was a Neurologist at King's College Hospital and running a research laboratory in the Institute of Psychiatry. His major area of clinical and research interest is in the genetic and molecular basis of motor neuron disease. He runs a clinic at King's College Hospital for people with motor neuron disease.

Professor Veronica van Heyningen CBE FRS FRSE FMedSci is a Group Leader and joint Section Head of the Medical and Developmental Genetics Section at the MRC Human Genetics Unit in Edinburgh. Her research focuses on human eye anomalies such as aniridia and anophthalmia/microphthalmia to define gene networks implicated in disease and normal development. Her broader interests include exploration of the mechanisms of mutation, long-range regulation of gene expression and phenotype modulation. Professor van Heyningen has been a member of UK Human Genetics Commission, and is the current President of the UK Genetics Society.

Observers

Representatives from the study sponsors and Government stakeholders were invited to join working group meetings as observers to clarify factual points. They were not present for the discussions of the study's conclusions and recommendations. The observers were:
Dr Joseph Chan, Animals (Scientific Procedures) Inspectorate, Home Office
Dr John Connolly, Head of Advanced Therapies, Department of Health
Mr Andrew Earnshaw, Senior Policy Manager, Advanced Therapies, Department of Health
Ms Eve Jacques, Corporate Affairs Group, Medical Research Council
Ms Nancy Lee, Senior Policy Adviser, Wellcome Trust
Dr Frances Rawle, Head of Corporate Governance and Policy, Medical Research Council
Mr Carl Reynolds, Dialogue and Engagement Specialist, Department of for Business, Innovation and Skills Sciencewise Expert Resource Centre
Dr Neil Watt, Animals (Scientific Procedures) Inspectorate, Home Office

Secretariat

Dr Laura Boothman (Lead Secretariat), Policy Officer, Academy of Medical Sciences
Ms Catherine Luckin, Policy Officer, Academy of Medical Sciences
Dr Rachel Quinn, Director, Medical Science Policy, Academy of Medical Sciences

Review group membership

The report was reviewed by a group on behalf of the Academy's Council. Reviewers were asked to consider whether the report met the terms of reference and whether the evidence and arguments presented in the report were sound and supported the conclusions. Reviewers were not asked to endorse the report or its findings. Review group members were:
Professor Ronald Laskey CBE FRS FMedSci (Chair)
Emeritus Professor of Embryology, University of Cambridge
Professor Sir Richard Gardner FRS
Emeritus Professor, Department of Biology, University of York
Lord Richard Harries of Pentregarth FMedSci
Former Bishop of Oxford
Dr Stephen Inglis
Director of the National Institute for Biological Standards and Control (NIBSC)
Professor Ian Kimber
Chair, Board of National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)
Professor Ian McConnell FRSE FMedSci
Emeritus Professor of Veterinary Science, Department of Veterinary Medicine, University of Cambridge
Dr Paul Whiting
Head of Molecular and Cellular Biology and Site Head, Regenerative Medicine, Pfizer

Annex II Consultation and evidence gathering

Call for evidence

The Academy issued an open call for evidence to inform the study. Those who submitted written evidence are listed below.

Organisations

Animal Procedures Committee (APC)
The Anscombe Bioethics Centre
AstraZeneca
Biotechnology and Biological Sciences Research Council
British Pharmacological Society Animal Welfare and Integrative Pharmacology Committee
British Union for the Abolition of Vivisection
Church of England Mission and Public Affairs Council
Department of Health
Fund for the Replacement of Animals in Medical Experiments
Genetic Interest Group
Human Fertilisation and Embryology Authority
Human Tissue Authority
Institute of Animal Technology
Medical Research Council (MRC)
National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)
Northeast England Stem Cell Institute
Nuffield Council on Bioethics
Royal Society for the Prevention of Cruelty to Animals (RSPCA)
Safer Medicines Trust
Scottish Council on Human Bioethics
Wellcome Trust Sanger Institute

Individuals

Professor Richard Anderson, University of Edinburgh
Dr Sian Beynon-Jones, University of York
Elio Caccavale, University of Dundee; Professor Richard Ashcroft, Queen Mary University of London; and Professor Michael Reiss, Institute of Education (joint submission)
Ms J Deeks
Professor Robert Dingwall, Ms Michelle Hudson and Ms Kathleen Job, University of Nottingham (joint submission)
Professor Hank Greely, Stanford University
Dr Mark Greene, University of Delaware
Dr Gill Haddow, INNOGEN, University of Edinburgh
Dr Alison Harvey, King’s College London
Dr D Jones
Dr Jonathan Kelley, University of Nevada

Dr Edward Moore OStJ
Dr Barbara Nicholas, formerly secretariat to New Zealand Bioethics Council
Miss J M Pick
Sir Robert Worcester KBE DL, Ipsos MORI

Additional evidence gathering

The following individuals provided oral evidence to the working group:
Sir Patrick Bateson FRS, Emeritus Professor of Ethnology, Department of Zoology, University of Cambridge
Professor Allan Bradley FRS FMedSci, Director Emeritus, Wellcome Trust Sanger Institute
Mr Phil Banks, APC Secretariat
Dr John Connolly, Head of Advanced Therapies, Department of Health
Professor Elizabeth Fisher FMedSci, Professor of Molecular Genetics, Department of Neurodegenerative Disease, University College London
Dr Simon Glendinning, APC member
Dr Maggie Jennings, Head of Research Animals Department, RSPCA
Professor Keith Kendrick, APC member
Dr Sophie Petit-Zeman, Head of External Relations, Association of Medical Research Charities
Dr Vicky Robinson, Chief Executive, NC3Rs
Dr Victor Tybulewicz FMedSci, Head of Division of Immune Cell Biology, MRC National Institute for Medical Research
Mr Martin Walsh, Head of Policy, Animals (Scientific Procedures) Division, Home Office

We are particularly grateful for the advice and assistance of:
Professor Robin Weiss FRS FMedSci, University College London
Dr Jonathan Stoye, MRC National Institute for Medical Research

The following individuals submitted evidence, information or relevant publications through correspondence with the working group and the secretariat:
Professor Robin Ali FMedSci, University College London
Professor Jeffrey Almond FMedSci, Sanofi Pasteur
Professor Peter Andrews, University of Sheffield
Dr Roger Barker, University of Cambridge
Antony Blackburn-Starza
Dr Gary Burns MBE, AstraZeneca
Professor Hilary Critchley FMedSci, University of Edinburgh
Anne Lykkeskov, The Danish Council of Ethics
Dr John Dick, University of Toronto
Professor Stephen Dunnett FMedSci, Cardiff University
Dr Kristina Elvidge, Muscular Dystrophy Campaign
Dr Maurizio Salvi, European Group on Ethics
Professor Sir Martin Evans FRS, Cardiff University
Dr Simon Fisher, University of Oxford
Professor Richard Flavell FRS, Yale School of Medicine
Professor Robin Franklin, University of Cambridge
Dr Carrie Friese, London School of Economics

Dr Jonathan Gawn, Health and Safety Laboratory
Professor Daniel Geschwind, University of California, Los Angeles
Professor Roger Gosden
Professor Melvyn Greaves FRS FMedSci, Institute of Cancer Research
Dr Christine Hauskeller, University of Exeter
Professor Douglas Higgs FRS FMedSci, University of Oxford
Julian Hitchcock, Field Fisher Waterhouse
Calvin WL Ho, Bioethics Advisory Committee Singapore
David Jones, Medicines and Healthcare products Regulatory Agency
Mary Kirwan, Chase Paymentech
Professor Andrew Lever FMedSci, University of Cambridge
Professor Alison Murdoch, Newcastle University
Professor Trevor Owens, University of Southern Denmark
Professor Andrew Parker, University of Oxford
Professor David Rubinsztein FMedSci, University of Cambridge
Dr Sebastian Sethe, Lawford Davies Denoon
Professor Richard Sharpe, MRC Centre for Reproductive Health
Professor Pamela Shaw FMedSci, University of Sheffield
Dr William C Skarnes, Wellcome Trust Sanger Institute
Professor Peter St George-Hyslop FRS FMedSci, University of Cambridge
Dr Glyn Stacey, UK Stem Cell Bank
Professor Francis Stewart, Biotechnology Center, Technische Universität, Dresden
Professor Jerome Strauss, Virginia Commonwealth University
Professor Swee Lay Thein FMedSci, King's College London
Professor Adrian Thrasher FMedSci, University College London
Professor John Todd FRS FMedSci, University of Cambridge
Professor Arthur Toga, University of California, Los Angeles
Dr Irving Weissmann Institute for Stem Cell Biology and Regenerative Medicine, Stanford Cancer Center and Ludwig Center, Stamford
Professor Charles Weissmann ForMemRS FMedSci, The Scripps Research Institute
Professor Bruce Whitelaw, University of Edinburgh

We are very grateful to all those who have contributed information to the study, including those who submitted evidence anonymously and anyone that we have inadvertently omitted from this list.

Annex III Overview of dialogue methodology and evaluation

Background and objectives

To ensure that the working group's discussions and recommendations were informed by public concerns and aspirations alongside the scientific evidence and the social, ethical and legal perspectives, the Academy commissioned a programme of public dialogue. 'Exploring the boundaries' was designed and managed by a consortium led by Ipsos MORI and including Dialogue by Design and the British Science Association. It was supported by the Sciencewise Expert Resource Centre programme, funded by the Department for Business, Innovation and Skills. Oversight was provided by a group consisting of members of the working group, the Department of Health and Sciencewise Expert Resource Centre. A comprehensive report of the dialogue methodology and findings has been published separately.[480]

The dialogue focused specifically on public awareness of, and attitudes towards, research using ACHM, distinguishing this from more general use of animals in research. The purpose of the 'Exploring the boundaries' dialogue was to provide a forum in which individuals could explore their concerns and aspirations around this unfamiliar topic. In introducing the subject, the dialogue set out to identify areas of consensus, disagreement or uncertainty on a broad range of issues raised by current and possible future uses of ACHM. It was designed to actively seek the views of a range of different audiences, including patients and carers, those with strong views on animal welfare and individuals for whom religious faith is important. The dialogue sought to provide an in-depth assessment of the attitudes towards research using ACHM of these varied audiences, but also to indicate the views of the public overall towards such work.

The dialogue programme was a core aspect of the study's evidence-gathering process. The working group considered its findings alongside the other evidence throughout their discussions and in considering their recommendations. The working group members were involved in providing oversight, including contributing towards the development of the dialogue materials, and attending the events.

Aims and objectives

The overall aim of the dialogue was to engage members of the public on the issues raised by the current and future uses of research involving ACHM. The objectives of the dialogue were to:

- Provide opportunities for members of the public to discuss and explore their aspirations and concerns relating to the scientific, social, ethical, safety or regulatory aspects of research involving ACHM.
- Identify areas of consensus, disagreement or uncertainty on a broad range of issues raised by current and possible future scientific developments, and explore both initial views and changes in opinion.
- Inform the final recommendations made by the Academy for public policy and research needs.

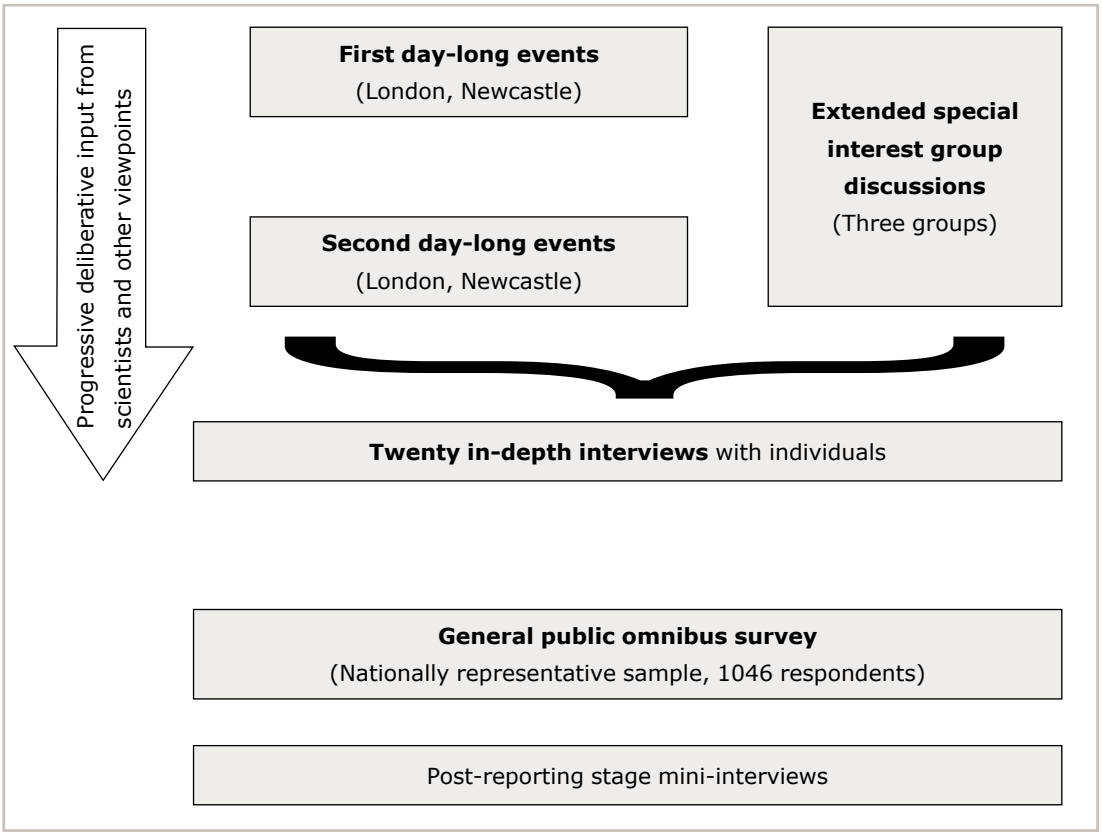
A secondary objective was to enable the Academy and the wider science community to build on previous experience in public dialogue, to pioneer innovative approaches in public engagement where appropriate, and to develop knowledge and understanding of public dialogue and its potential for future applications.

480 Ipsos MORI (2010). *Exploring the Boundaries: report on a public dialogue into animals containing human material* http://www.acmedsci.ac.uk/download.php?file=/images/page/128619890736.pdf

Methodology

The dialogue consisted of qualitative and quantitative elements, as outlined in the summary diagram below. In total, over 1100 individuals were involved. Following an initial literature review, a process of stakeholder engagement, including a workshop, was undertaken to agree the detailed aims of the dialogue and to inform the development of its themes and the stimulus materials. The stakeholders involved included members of the dialogue oversight group and representatives from non-governmental organisations, industry, religious organisations and animal welfare organisations.[481] The qualitative work then took place and its emerging findings were used to develop the questions for the quantitative study.

Summary of the dialogue methodology



Qualitative work

Seventy participants took part in the qualitative dialogue, the most substantial element of which was the general public dialogues that consisted of two groups in Newcastle and London. These groups met twice each in May and June 2010, for one day each time. Additional discussions were held with special interest groups. These were patients or carers of people with serious illnesses; people with religious faith and who stated that their beliefs were directly and practically important to them; and those who attached importance to animal welfare. Structuring the dialogue in this way enabled the views of a range of different audiences to be sought and explored thoroughly, provided an environment in which participants felt able to express their views and ensured that those with particularly powerful or emotive experiences or views did not unduly influence the dialogue as a whole.

Participants were recruited face-to-face by experienced recruitment professionals to

481 Ipsos MORI (2010). *Exploring the Boundaries: report on a public dialogue into animals containing human material.* http://www.acmedsci.ac.uk/download.php?file=/images/page/128619890736.pdf

ensure that a mixed and broadly representative group of people took part. A modest cash incentive was paid to encourage a diverse range of participants.

The dialogue process was designed to reveal how participants responded to information about research involving ACHM. The emphasis was on encouraging participants to reflect on information that was provided and on their interactions with each other and with the scientists and facilitators present. 'Focus group'-style sessions were used, in which participants could share their views, first spontaneously, then after reflection. Their eventual conclusions were the focus of analysis.

A sub-sample of 20 participants from both the general public dialogue and special interest groups were interviewed individually by telephone during July and August 2010. Interviewees for this stage were selected in part because the views they expressed during the day were distinct, which enabled issues raised by preliminary analysis of the qualitative data to be more thoroughly investigated and to confirm the validity of these views, for example that individuals were not unduly influenced by others in the group.

Data analysis

Notes were taken throughout each dialogue session and insights that each facilitator gained were then shared during analysis meetings at the end of each day and at the end of the dialogue process as a whole. During the general public dialogue events, an observational researcher also made notes on body language, facial expressions and evidence of behaviours, without taking part in the facilitation, as well as carrying out *ad hoc* interviews to explore participants' thoughts and feelings. The purpose of this was to understand more subtle and unspoken reactions alongside the main discussions and to help build hypotheses as to participants' thoughts and feelings throughout the process as their views developed.

Quantitative work

The quantitative findings in the report provide an indication of the views of the wider British public. The findings of the qualitative dialogue informed the survey questions. The findings were collected through the Ipsos MORI weekly computer-assisted personal interviewing (CAPI) survey, which is a cross-sectional representative survey of individuals aged over 15 years across Great Britain, performed face-to-face by trained interviewers. 1046 participants completed the 'Exploring the boundaries' survey in July 2010.

Findings

The findings of the public dialogue are highlighted throughout this report and are outlined and discussed in detail in the full report produced by Ipsos MORI.[482]

Evaluation

The Academy commissioned Laura Grant Associates to conduct an independent evaluation of the dialogue. The evaluation aimed to provide an independent assessment of the dialogue programme's credibility, effectiveness and success against its deliverables and objectives, throughout the programme and at its conclusion; and to assess its contribution to the overall Sciencewise Expert Resource Centre aim of creating excellence in public dialogue to inspire and inform better policy in science and technology. A comprehensive report of the full evaluation methodology and findings produced by Laura Grant Associates is available online.[483]

482 Ipsos MORI (2010). *Exploring the Boundaries: report on a public dialogue into animals containing human material.* http://www.acmedsci.ac.uk/download.php?file=/images/page/128619890736.pdf

483 Laura Grant Associates (2010). *Exploring the Boundaries: A dialogue on Animals Containing Human Material Evaluation Report.* http://www.acmedsci.ac.uk/download.php?file=/images/page/129111803577.pdf

Annex IV Glossary of terms and abbreviations

This glossary is intended to assist readers with the terminology and abbreviations used in this report; it is not presented as a definitive list of terms. Cross-references (e.g. see 2.2) refer to the sections of this report.[484]

ACHM: Animals containing human material. See 2.2, page 18.

Admixed: 'Admixture' is the process of mingling one substance with another. The Human Fertilisation and Embryology Act (as amended in 2008) defines five classes of 'human admixed embryos' containing both human and animal material, with the human contribution predominating. See Box 6.4, page 90.

Adult stem cell: Another term for 'tissue-specific' stem cell. See Box 3.3, page 38.

Amino acid: One of a group of chemical compounds that are the basic units of proteins.

Amniocentesis: A prenatal diagnostic technique in which a sample of amniotic fluid is withdrawn and examined for information about the fetus. (The 'amnion' is the innermost membrane enclosing the fetus.)

Amniotic stem cell: See Box 3.3, page 38.

Animal: In this text 'animal' (rather than 'non-human animal') is used to refer to animals of all species in the animal kingdom except humans. (In correct scientific taxonomy, humans are both primates and animals.)

Animal model: A living animal in which normal and abnormal biological processes can be studied, to gain insight into human health and disease. The more closely the process being modelled resembles the process in humans, the more scientifically valuable the model is likely to be.

Antibody: An antibody is a large protein found in blood and tissues, used by the immune system to identify and neutralise foreign material such as cells, bacteria and viruses. The antibody recognises a unique part of the foreign target, termed an antigen. Antibodies are produced as part of the immune response.

Antigen: A foreign substance that, when introduced into a living organism, stimulates the production of an antibody.

Aneuploid: Used to describe cells, tissues or organisms in which the number of chromosomes is abnormal in that it differs from the euploid. 'Euploid' entities are those in which each of the chromosomes of the set is represented in equal number (e.g. two copies of each chromosome is termed 'diploid'; one of each is 'haploid'). See also haploid and diploid.

ASPA: Animals (Scientific Procedures) Act (1986). See 6.2.1, page 83.

APC: Animal Procedures Committee. See 6.2.1, page 83.

484 Terms are drawn from sources including Department of Health (2010). *Code of Practice for the use of Human Stem Cell Lines*. http://workspace.imperial.ac.uk/clinicalresearchgovernanceoffice/Public/Code%20of%20practice%20for%20stem%20cell%20lines.pdf; Nuffield Council on Bioethics (2005). *The ethics of research involving animals*. Nuffield Council on Bioethics, London; The National Institutes of Health resource for stem cell research glossary, http://stemcells.nih.gov/info/glossary.asp; The Standard Oxford English Dictionary; Understanding Animal Research, http://www.understandinganimalresearch.org.uk/

ASPD, ASPI: Animals Scientific Procedures Division (ASPD) and Inspectorate (ASPI) of the Home Office.

Autologous: Derived from the same individual.

Autophagy: Literally, 'feeding upon oneself'. A biological process by which a cell digests internal components, for example to break down and recycle cellular components or to rid itself of toxins.

BIS: Department for Business, Innovation and Skills.

B-lymphocyte, B cell: A type of lymphocyte (a form of white blood cell), which forms part of the immune system and produces antibodies in response to antigens.

Bioluminesence: The emission of light by living organisms such as fireflies and deep-sea creatures. In biomedicine, cells or tissues can be made to emit light in this way as a form of marker (see GFP).

Blastocyst: An early embryo consisting of a hollow ball of 50–100 cells reached after about 4 or 5 days of embryonic development (depending on species), just prior to implantation in the uterus. The outer 'trophectoderm' cells give rise to part of the placenta, while a distinct group of about 15–20 'inner cell mass' cells give rise to the embryo proper and other extraembryonic tissues (e.g. placenta, yolk sac).

cDNA: Complementary deoxyribonucleic acid. A DNA strand that has been produced by 'reverse copying' a messenger ribonucleic acid (mRNA) (either by a laboratory technique or by a retrovirus). cDNA has the same sequence as DNA, except that it lacks introns.

Cell: The fundamental, usually microscopic, structural and functional unit of all living organisms, which consists of a small quantity of cytoplasm enclosed within a membrane, typically contains a nucleus and other organelles and internal compartments, and is capable of utilising energy, synthesising proteins and other biomolecules, and (usually) of self-replicating.

CNS: Central nervous system. The largest part of the nervous system, including the brain and spinal cord.

Cephalopod: An animal within the most highly organised class of molluscs. Cephalopods are characterised by a distinct head with 'arms' or tentacles attached. Examples are Cuttlefish and Octopuses.

Chimæra, chimæric animal: An animal comprised of whole cells from two different organisms. See 2.2.2, page 18.

Chloride channel: A protein 'pore' that enables the transit of chloride ions into and out of cells, across the cell membrane.

Chromosome: One of the threadlike structures containing identical sister strands of DNA, protected by proteins and carrying genetic information, that can be microscopically visible within a cell.

Cleavage stage embryo: An embryo prior to formation of a blastocyst, undergoing 'cleavage' divisions where there is little or no cell growth; thus during cleavage each cell of the one, two, four, and then eight cell embryo becomes progressively smaller.

Cognition, cognitive capacity: In its broadest sense, human 'cognition' can be defined as the 'faculty of knowing', to include aspects such as knowledge, reason, intelligence, understanding, sensation and perception (as distinguished from feeling and volition). See 3.4, page 46.

Complement: A protein complex found in blood and other body fluids, which forms part of the adaptive immune system. When combined with an antigen–antibody complex, complement produces a series of reactions (the 'complement cascade') to bring about cell lysis.

Congenital: Present from the time of (and often before) birth.

Cord blood stem cell: See Box 3.3, page 38.

Cytoplasm: The gel-like substance enclosed by the cell membrane. It contains many important molecules and organelles concerned with cell metabolism and movement.

Cytoplasmic hybrid: Cytoplasmic hybrid cells (cybrids) are those created by combining the nucleus (with a minimal amount of cytoplasm and mitochondria) of a cell of one species with the cytoplasm (including the mitochondria) of another species. Cytoplasmic hybrid embryos are those created by transferring a somatic cell nucleus from one species into the enucleated oocyte of another species.

Deontological: Relating to an ethical approach based on rules and duties. (Deontology is the study of duty, a branch of knowledge that deals with moral obligations.)

Defra: Department for Environment, Food and Rural Affairs.

DH: Department of Health.

Differentiation: The process by which cells become progressively more specialised towards their final function, both during development and adult maintenance.

Diploid: The state in which each type of chromosome (except the sex chromosomes) is represented twice. This is the normal state of all cells of the body, except the germ cells (sperm and eggs), which have only a single (haploid) set of chromosomes.

DNA: Deoxyribonucleic acid. A type of double-stranded nucleic acid molecule that encodes the genetic instructions used by almost all living organisms.

DNA base pair: Two nucleotides (the structural units of which DNA is composed), on opposite complementary DNA strands, which are connected by a hydrogen bond.

DNA regulatory region: A section of DNA that functions as a 'switch' to control gene expression. They may lie either side of the gene or even within its introns, and they are often highly conserved in evolution. Regulatory proteins (such as transcription factors) bind to regulatory regions. See Box 2.2, page 21.

DPA: Data Protection Act (1998).

Double-blind: A clinical trial or experiment, conducted by one person on another, in which information (such as whether a substance being administered is active or placebo) that may lead to bias in the results is concealed from both the tester and the subject. This method is used to eliminate subjective bias.

Ectoderm: The outermost of the three primary germ layers. It gives rise to the epidermis (outer part of the skin, including hair, nails, and sebaceous glands) the central nervous system (brain and spinal cord) and to sensory systems, such as the eye, olfactory system and inner ear. See also endoderm and mesoderm.

Ectopic: The location of cells or tissues at an abnormal site in the body. For example, ectopic pregnancy involves the implantation of a fertilised egg outside the uterus.
EG cell: Embryonic germ cell. See Box 3.3, page 38.

EMEA: European Medicines Agency.

Embryo: the first stages in the development of an animal, usually the result of fertilising an egg with a sperm. In humans, the embryo is usually referred to as a fetus from about the eighth week of fertilisation. In other mammals, 'fetus' may be used to refer to older embryos, but there is no strict definition of when fetal stages begin.

Embryonic stem cell: See Box 3.3, page 38.

Emphysema: A long-term, progressive disease of the lungs that primarily causes shortness of breath.

Endoderm: There are two types of endoderm; 'primitive' or 'extra-embryonic' endoderm (also sometimes called the 'hypoblast') forms the lower or outer layer of the early embryo (around the time of implantation in mammals) and contributes to the yolk sac, but contributes little to the embryo proper. 'Definitive' or 'embryonic' endoderm develops during gastrulation where it displaces the extra-embryonic endoderm and gives rise to the larynx, lungs, gut and associated organs, such as the thyroid and liver. See also ectoderm and mesoderm.

Endogenous: Having a cause (or origin) inside the body or self, not attributable to any external or environmental factor.

Endometriosis: a condition resulting from the development of endometrial (womb lining) tissue in an abnormal location outside the uterus.

Engraft: To insert a piece of material (e.g. cells, tissues or an organ) into an organism as a graft. (Autologous grafts involve movement of material from one location to other within the same body. Secondary chimæras are created by grafting cells tissue or organs from one animal into another.)

Enucleate: A cell lacking a nucleus; or the process of removing the nucleus of a cell.

Enteric nervous system: The part of the nervous system that directly controls the gastrointestinal system (gut).

Enzyme: A protein that catalyses (increases the speed of) a specific biochemical reaction.

Epiblast: The upper layer of cells present in the embryo just prior to gastrulation. These cells are pluripotent, and give rise to all three primary germ layers (ectoderm, mesoderm and endoderm) as well as to germ cells and to extra-embryonic mesoderm.

Epigenetic: ('Over' or 'above' genetics). Epigenetic factors are heritable changes in phenotype or gene expression, which result from mechanisms other than changes to the underlying DNA sequence. For example, characteristics resulting from alterations in DNA methylation or changes in chromosomal proteins.

Epitope: Part of an antigen, to which a particular antibody binds with a high degree of specificity.

EPO: European Patent Office.

ERP: Ethical review process. See 6.2, page 83.

ESC, ES cell: Embryonic stem cell. See Box 3.3, page 38.

ESCRO: Embryonic Stem Cell Research Oversight Committee. See Box 7.2, page 104.

Exogenous: Originating outside the body.

Exon: See intron.

Express, expression: Gene expression is the process by which information from a gene is used to synthesise a functional gene product. This involves transcription to produce an RNA molecule called messenger RNA (mRNA). mRNA is exported from the cell nucleus to the cytoplasm where its code is translated into proteins by assembling amino acids in the right order. The polypeptide chains produced are ultimately folded into proteins. Some genes only produce RNA products that fulfill different important functions in cells. See Box 2.2, page 21.

Extra-embryonic tissue: Tissue that contributes to the growth or development of an embryo without forming part of the embryo itself. Placental and yolk sac tissues are extra-embryonic.

Extra-embryonic stem cell: See Box 3.3, page 38.

Fetus: See embryo.

Fibroblast: A type of cell ubiquitously found in connective (supporting) tissues in most organs. Fibroblasts play a role during wound healing or tissue repair. They are the type of cell that most commonly grows in tissue culture, emerging from explants of pieces of most body tissues.

Fetal stem cell: See Box 3.3, page 38.

Fluorescence: Coloured light emitted by some chemicals, including some proteins, in response to the action of light (especially violet and ultraviolet rays) upon them.

Gamete: A mature haploid sexual reproductive cell, for example a sperm or egg, which can unite with another gamete to form a new organism. See germ cell.

Gastrulation: A phase early in the embryonic development of most animals, during which the single layer of cells called the blastula (or in higher vertebrates the epiblast), is reorganised into a three-layered patterned structure that will go on to form the three primary tissues of the embryo proper (ectoderm, mesoderm, endoderm). In human embryonic development it begins at around 14 days after fertilisation, in the mouse at about 7 days.

Gene: The basic unit of heredity in living organisms, now known to consist of a sequence of DNA (or RNA in certain viruses) containing a code for an RNA molecule that in many cases encodes a protein. The gene also includes any associated regulatory sequences. See Box 2.3, page 23.

Gene product: A substance produced by the expression of a gene, for example a protein molecule.

Gene replacement therapy: The insertion of gene copies within some of an individual's cells for the purpose of treating disease. See Box 2.3, page 23.

Genotype: The genetic constitution of an individual.

Genetic sequence: The order of nucleotide bases (the individual units of which DNA is composed) in a section of a DNA molecule.

Genetically altered: A cell, or organism, in which the DNA sequence has been modified. See 2.2.1, page 18.

Genome: The complete DNA sequence of an individual, or a representative sequence for a species.

Germ cell: A sex cell or gamete (e.g. an egg or sperm); a reproductive cell that fuses with one from the opposite sex in fertilisation to form a single-celled zygote. The term is also used to refer to the progenitors of eggs and sperm during development.

Germ-line: The lineage of special cells set aside early in development that eventually differentiate into mature germ cells. (Cells that are not part of the germ-line are referred to as somatic cells.)

Gestation: The process of carrying young in the womb.

GFP: Green fluorescent protein. A protein that occurs naturally in some marine organisms. GFP fluoresces bright green under blue light, and is widely use as a marker in biomedical research. Its gene can be readily transfected into cells of many species, and confers the fluorescent property on the cells, which make the fluorescent protein and can then be easily visualised under appropriate illumination.

Glia: The supportive non-neuronal tissue of the nervous system, composed of different types of glial cell.

GM, GMO: Genetically modified, genetically modified organism.

Gonad: Any organ in an animal that produces gametes (e.g. a testis or an ovary).

Haematopoietic: A haematopoietic cell is one that is able to produce blood cells.

Haematopoietic stem cell: A stem cell that can give rise to all types of blood cell.

Haploid: A cell in which each type of chromosome is represented once (half the diploid number).

Hepatocyte: A type of cell that makes up the majority of liver tissue.

HFE Act: UK Human Fertilisation and Embryology Act. Unless specified, this term is used to refer to the 1990 Act as amended in 2008.

HFEA: Human Fertilisation and Embryology Authority.

HLA: Human leukocyte antigen. The HLA genes are the human versions of the major histocompatibility complex genes (MHC) found in most vertebrates. These genes encode cell-surface antigen-presenting proteins, antigens and other proteins. The major HLA antigens are the most important determinants of 'tissue type', which must be matched for optimal organ transplantation.

Homologous: Sharing a common ancestral origin; entities that are homologous have a similar structure. This term is used to describe genes that have a similar DNA sequence, or proteins that have the same (or very similar) structure. It is often used in describing genes or proteins of common evolutionary origin, found in different species.

Human (man): Individuals of the species *Homo sapiens*; human beings. Although in correct taxonomy, humans are both primates and animals, in this text 'animal' (rather than 'non-human animal') is used to refer to animals of all species *except* humans; and non-human primate (NHP) is used to refer to primates *except* humans.

HSC: Haematopoietic stem cell. See Box 3.3, page 38.

hESC, hES cell: Human embryonic stem cell. See stem cell.

hiPSC, hIPS cell: Human induced pluripotent stem cell. See stem cell.

HSE: Health and Safety Executive.

HTA: Human Tissue Authority.

HT Act: Human Tissue Act (2004).

Human lineage-specific sequence: A section of the genome that is unique to humans, or to humans and their near ancestors. See 3.2, page 32.

Humanised: An aspect of the biology of an animal (including for example a gene, protein, organ, element of external appearance or behavioural characteristic) that has been modified so that it more closely resembles that of the human.

Humanised antibody: An antibody produced by an animal (typically a mouse) whose antibody-producing genes have been replaced by human DNA sequence, causing it to produce antibody molecules that resemble those of the human. See 2.3.2, page 25.

Hybrid: An animal or plant that is the offspring of individuals of different kinds (usually, different species) (see 2.2.3); hybrid embryos (see Box 6.4); inter-species cell hybrids are cells created by the *in vitro* fusion of (usually somatic) cells from two different species (see 2.2.3), page 20.

Hyperacute response: A type of immune response that can occur rapidly after the transplantation of cells or tissues from one species into another (e.g. xenotransplantation of organs from pigs into humans). It is mediated by the binding of host antibodies to the donor graft causing damage to, and rejection of, the transplanted tissue.

ICSI: Intra-cytoplasmic sperm injection. The injection of a sperm directly into an egg.

Immature germ-line cell: A cell of the germ-line that has not fully differentiated into a mature gamete.

Immortalised cell line: A cell line that has the ability to grow through an indefinite number of divisions in cell culture. Some stem cell types and many cancer cell lines are immortal; such cell lines can also be produced deliberately in the laboratory, usually as the result of genetic manipulation. See 3.3.1, page 34.

Immune system (adaptive): The adaptive (or specific) immune system, found in vertebrates, is composed of highly specialised cells and processes that recognise and destroy foreign proteins, cells or micro-organisms entering the body. This is the body's first defence against infections. The adaptive immune response provides the ability to recognise and remember specific antigens and so to generate immunity.

Immunodeficiency: A lack, or deficiency, of a functional immune system. The term 'immuno-compromised' is used similarly. See 2.3.3, page 27.

Immuno-suppressive drug: A drug that prevents or inhibits immune system function.

In vitro: Literally, 'in glass'; an experiment performed in a test tube, culture dish, or other non-living environment.

***In vivo*:** An experiment conducted within a living organism.

iPSC, iPS cell: Induced pluripotent stem cell. See Box 3.3, page 38.

Intron: A segment of a DNA molecule, which separates the exons (protein coding sections) of a gene. An intron does not code for protein. See Box 2.3, page 23.

Ion channel: A protein 'pore' that enables the transit of ions into and out of cells across the cell membrane.

IVF: *In vitro* fertilisation. The fertilisation of an egg by a sperm outside the body.

ISSCR: International Society for Stem Cell Research.

kb: Kilobase. A unit of length for measuring DNA or RNA sequences, equivalent to the length of 1000 bases.

Latent: Latent diseases are those in which the usual symptoms are not yet manifest. For infectious disease, this may be because the causative microorganisms are lying dormant within the body until circumstances are suitable for the development of overt disease.

Limbal stem cell: A stem cell found towards the edge of the cornea of the eye.

Longitudinal: Refers to a study that involves repeated observations on the same subject over a long period of time.

Lymphatic system: A network of vessels through which lymph (a colourless fluid containing white blood cells, vital in immune system function) drains from the tissues into the blood.

Lysis: The disintegration, for example of a cell, brought about by the breakdown of the containing wall or membrane.

Lytic: Relating to, or causing, lysis. For example, in lytic viral infection a virus replicates within a cell and, in the process of its release, destroys the cell.

Macular degeneration: A condition that results in a loss of vision in the centre of the visual field, owing to the deterioration of the macula (an area near the centre of the retina in the eye).

Matrigel™: A gelatinous protein mixture that resembles the extracellular environment found in many tissues and is used by cell biologists as a substrate for cell culture.

Meiosis: Part of the process of gamete formation, involving two cell divisions, in the course of which the diploid chromosome number becomes reduced to the haploid.

Mesenchyme: Undifferentiated loose connective tissue that is derived mostly from embryonic mesoderm. It contains cells capable of developing into various tissues such as bone, cartilage and blood vessels.

Mesenchymal stem cell: see Box 3.3, page 38.

Mesoderm: The middle of the primary germ layers, which gives rise to many of the internal tissues, such as bone, cartilage, muscle, blood, dermis and connective tissues. See also ectoderm and endoderm.

Mesodermal stem cell: A stem cell of a type found in or derived from the mesoderm (the middle layer of cells or tissues of the embryo).

Metabolism: The chemical processes that occur within a living organism to maintain life, including both the synthesis of substances and their breakdown to produce energy.

Mitosis: The normal form of cell division in body tissues, resulting in two diploid daughter cells. See also meiosis.

Molecular phylogenetics: The study of similarities of DNA sequence, to gain information on the evolutionary relationships between organisms and species. See Box 2.1, page 17.

Monoclonal antibody: An antibody produced in the laboratory from a single clone (a genetically identical population) of cells. Monoclonal antibodies from the same clone are identical, so they recognise the same epitope on the antigen.

Monoclonal antibody therapy: A medical treatment that makes use of the highly specific binding of a monoclonal antibody to a specific biological target. The antibody itself can act as a therapeutic agent (e.g. by blocking receptors) or can carry with it an active drug molecule.

Mosaic animal: An animal containing two or more genetically distinct cell types that have arisen from the same zygote. Mosaic animals can occur as a result of naturally occurring mutations, or manipulations such as retroviral transfer. They are distinct from chimæras. See Box 2.3, page 23.

MHRA: Medicines and Healthcare products Regulatory Agency.

mRNA: Messenger ribonucleic acid. The molecule that carries the information from DNA to act as a template for protein synthesis (some mRNAs are non-protein-coding and have other functions). See Box 2.2, page 21.

MRI: Magnetic resonance imaging. A technique for producing images of bodily organs by measuring the response to high-frequency radiowaves in a strong magnetic field.

MSC: Mesenchymal stem cell. See Box 3.3, page 38.

Multipotent: See potency and Box 3.3, page 38.

Mutation: The modification of a DNA sequence that has the potential to lead to a change in the function of a gene. Mutations may be caused by mistakes in copying of DNA during cell division, or by exposure to DNA-damaging agents in the environment. Mutations can be harmful, beneficial or, most commonly, of no consequence. They can be caused by the alteration of single base units in DNA, or the deletion, insertion or rearrangement of larger sections of genes or chromosomes. Mutations can only be passed on to offspring if they occur in cells that make eggs or sperm.

NAS: National Academy of Sciences. One of the four United States National Academies.

Neuron: A specialised cell that transmits nerve impulses; a nerve cell.

Neural stem cell: A stem cell of a type found in the brain, from early development to adulthood.

Niche: The cellular microenvironment providing support and stimuli necessary to sustain stem cell self-renewal and to control stem cell differentiation. See 3.3, page 38.

NHP: Non-human primate. In this text 'NHP' is used to refer to species of primates *except* humans. 'Great Apes' is used to refer to chimpanzee, pygmy chimpanzee, gorilla and orang-utan (see Box 6.1, page 83), and 'monkey' to refer to NHPs *other than* humans and Great Apes (e.g. 5.6.2), page 78.

NRES: UK National Research Ethics Service, which provides ethical assessment of proposed medical research involving human subjects.

Nucleotide: The structural unit of nucleic acids, DNA and RNA.

Nucleus (cell nucleus): A membrane-bound structure, often spherical, present in most living cells, which contains the DNA in the form of chromosomes.

Oocyte: A cell of the female germ-line that may undergo division to form an ovum (a mature female reproductive cell, egg). However, the term oocyte is often loosely used in place of ovum or egg.

Olfactory: Relating to the sense of smell.

Oligodendrocyte precursor: A cell that can develop into an oligodendrocyte, a kind of glial cell that produces myelin (a substance that provides an insulating sheath around many nerve fibres) in the central nervous system.

Oncogene: A gene that in certain circumstances can transform a cell into a tumour cell.

Oncology: The study and treatment of cancer.

Open-label: Describes a clinical trial in which both the researchers and participants know the treatment that is being administered. This contrasts with the single blind method where participants are not aware of what treatment they are receiving, and the double-blind trial where neither experimenter nor the subject know whether active treatment or placebo is being administered.

Organism: An individual animal, plant or single-celled life form.

Ovariectomy: Surgical removal of one or both ovaries.

Perinatal: Relating to the time immediately before and after birth. In humans this is usually a period of several weeks; in rodents, a few days.

PET: Positron emission tomography. A medical imaging technique that produces a three-dimensional image of internal body structures. During a PET scan, the recording system detects rays emitted by a radioactive substance that is introduced into the body on a biologically active molecule.

Phenotype: The physical manifestation of an organism, which results from the expression of the genotype together with non-genetic influences.

Plasticity: The ability of a cell or organism to adapt to changes in its environment.

Pluripotent: See potency and Box 3.3, page 38.

Polypeptide: A molecule made up of several amino acids joined together in a chain. Proteins consist of one or more polypeptides.

Potency (or potential): Generic terms to denote the range of specialised cells that a stem cell may/can give rise to. Stem cell potency can be more specifically described as unipotent, multipotent or pluripotent. See Box 3.3, page 38. (A totipotent cell is one that can give rise to all cell types in an animal or human, including extra-embryonic tissues, and (according to a commonly accepted definition), carries the information required to organise development of the embryo correctly. The only cells that are totipotent are therefore the fertilised egg and those of early cleavage stage embryos. These do not self-renew, therefore they are not stem cells.)

Pre-clinical: Denotes research, or the stages of the drug development process, conducted before that in the clinic. It may include approaches such as *in vitro* research, computer simulation and research using animals.

Primitive streak: A structure that forms during the early stages of mammalian embryogenesis; its appearance is one of the first signs of gastrulation.

Prion: A misfolded form of protein believed to act as an infectious agent. Diseases including bovine spongiform encephalopathy (BSE, in cattle), scrapie (in sheep) and Creutzfeldt–Jakob disease (CJD, in humans) are thought to be prion-mediated.

Progenitor cell type: A generic term for any dividing cell with the capacity to differentiate. It includes putative stem cells in which self-renewal has not yet been demonstrated.

Pronucleus: Either of a pair of nuclei from gametes (in the haploid state following meiosis) in the egg after fertilisation (or activation) but before they come together to form the (diploid) chromosome complement of the zygote.

Protein: Large molecules composed of one or more long chains of amino acids (polypeptides). Proteins are an essential part of living organisms, as both structural and functional components of all body tissues.

Provirus: The genetic material of a virus as incorporated into the genome of a host cell.

Quiescent: In a state or period of inactivity or dormancy.

Receptor: A molecule within a cell (frequently in a cell membrane), which binds and responds specifically to a particular transmitter (cell signalling molecule), hormone, antigen or other biologically active molecule.

Recombinant DNA: DNA formed artificially by combining sections of DNA, often from different organisms.

Regenerative medicine: Approaches aimed at creating living, functional tissues to repair or replace the function of cells, tissues or organs lost because of damage or congenital defects. Many such approaches involve the use of stem cells.

Restricted: Limitation of the potency of a stem cell, meaning that it cannot give rise to some types of specialised cells in the body.

Retrovirus: An RNA virus that inserts a DNA copy of its genome into the host cell to replicate, for example human immuodeficiency virus (HIV).

RGF: Research Governance Framework.

SCRO: Stem Cell Research Oversight Committee. See Box 7.2, page 104.

Self-renewal: Cycles of division that generate at least one daughter cell equivalent to the mother cell, with latent capacity for differentiation. The defining property of stem cells. See 3.3, page 34.

Somatic cell: Any cell that forms part of the body of an organism, not including germ cells.

SCNT: Somatic cell nuclear transfer. The transfer of the nucleus from a somatic (e.g. fetal or adult body) cell into an oocyte from which the nucleus (or the nuclear DNA) has been removed. The basis of the technique used to clone mammals, famously including Dolly the sheep.

Species: A species is one of the basic units of biological classification and a taxonomic rank. A species is often defined as a group of organisms capable of interbreeding and producing fertile offspring. Although in many cases this definition is adequate, more precise or differing measures are often used, such as similarity of DNA or morphology. See Box 2.1, page 17.

Sperm: An abbreviated term used to denote a male sex cell of an animal. In scientific terminology, the developing male sex cells are named at different stages (e.g. 'spermatogonium', 'spermatocyte', 'spermatid'), and a mature, motile male sex cell is referred to as a 'spermatozoon'.

Spermatagonial stem cell: See Box 3.3, page 38.

Splicing: The modification of a primary messenger RNA (mRNA) transcript to remove the non-coding 'introns' and join up the coding 'exons' to make a functional mRNA, usually one able to be translated to form proteins.

Stem cell: A stem cell is a cell that can continuously produce unaltered daughters and has the ability to produce daughter cells that have different, more restricted properties. In this text, the term 'stem cell' is sometimes used to encompass other progenitor cells types. Human stem cells are abbreviated 'h', e.g; human ES cell (human embryonic stem cell); human iPS cell (human induced pluripotent stem cell).

Telomere: Repetitive nucleotide sequences at the ends of chromosomes that serve as a 'capping' structure. Telomeres are shortened with each cell division. Short telomeres are consequently considered a sign of ageing.

Teratocarcinomas: A form of malignant teratoma occurring especially in the testis.

Teratoma: A type of tumour that contains several different tissue types. See 3.6.1, page 56.

Tetraploid: A cell or nucleus in which four homologous sets of chromosomes are represented, in contrast to the two sets (diploid) normally found in somatic cells.

Tissue: A distinct type of material of which the body is composed, consisting of specialised cells and their products, for example connective tissue, muscle.

Tissue-specific (or adult) stem cell: See Box 3.3, page 38.

Toxicity testing: A stage in the development of therapeutic products, in which they are tested for their potential to cause unanticipated or harmful effects to the body. Toxicity tests are often conducted on animals to establish dose–toxicity relationships and maximum safe dosage levels before clinical trials are conducted in man.

Transfection: A process in which DNA is introduced into a cell containing a nucleus, and integrates into the recipient cell's nuclear DNA.

Transgenic: A cell, embryo or animal created by the insertion of some additional genetic material from another genome. See Box 2.3, page 23.

Transgene: A sequence of genetic material taken one organism (or artifically synthesised) and inserted into the genome of another cell, embryo, or animal. See Box 2.3, page 23.

Translation: The process by which a sequence of nucleotides in a mRNA molecule is read and 'translated' by cellular machinery to a specific sequence of amino acids, during synthesis of a protein.

Trophectoderm, trophoblast: A layer of tissue on the outside of a mammalian blastocyst, which supplies the embryo with nourishment and later forms the major part of the placenta.

Tumour: A swelling of a part of the body, generally without inflammation, caused by an abnormal growth of tissue.

Tumourigenic: Tumour-causing.

UNESCO: United Nations Educational, Scientific and Cultural Organization.

Unipotent: See potency and Box 3.3, page 38.

Vaccine: An antigenic substance prepared from the causative agent of a disease or a synthetic substitute, used to produce immunity against disease.

Vascularisation: The process of developing blood vessels.

Vector: A biological construct (e.g. a plasmid) used as a vehicle for transferring genetic material into a cell. See Box 2.2, page 21.

Vertebrate: An animal possessing a backbone or spinal column, such as a mammal, bird, reptile, amphibian or fish.

Xenograft: A tissue graft or organ transplant from a donor of one species into a recipient of another. ('Xeno-' from Greek meaning 'stranger'.)

X-ray: An electromagnetic wave of high energy, which is able to pass through many materials opaque to light; a photographic or digital image of the internal composition of something, especially a part of the body, produced by X-rays being passed through it.

Zygote: The diploid cell resulting from the union of haploid male and female gametes.

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