



Strasbourg, 21 February 2003
[cdbi/cdbi-cdsp-xeno/doc/divers/
XENO(2003)1E state of art final]

CDBI/CDSP-XENO (2003) 1

REPORT ON
THE STATE OF THE ART
IN THE FIELD OF
XENOTRANSPLANTATION

In 1999, the Committee of Ministers of the Council of Europe set up a Working Party on Xenotransplantation under the joint responsibility of the Steering Committee on Bioethics (CDBI) and the European Health Committee (CDSP) which decided to prepare a Report on the State of the Art in the field of Xenotransplantation.

A previous version of this Report was used as a scientific basis for the preparation by the Working Party of a draft Recommendation on Xenotransplantation.

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1. INTRODUCTION

Xenotransplantation (the transplantation of cells, tissues and organs from one species to another) was first considered almost a hundred years ago. Since then, there have been sporadic instances of clinical applications in the history of medicine but interest was only rekindled in the early 1990s as a result of new progress in the biomedical sciences. Indeed, because of the great success of allotransplantation (human to human) an ever increasing number of operations are being performed and the need for human transplants now exceeds many times the supply. It is because of this shortage and the possibility for scientists to create a virtually unlimited supply of transplants through the use of animal material, that xenotransplantation is currently being studied as a therapeutic solution to several previously incurable diseases relating to heart, liver, lung and kidney disorders. Additionally, there are other unmet medical needs which could potentially be treated by xenotransplantation such as incurable neurological diseases (Parkinson's and Alzheimer's disease), paraplegia due to spinal cord lesions and pancreatic islet or beta cell transplants for treatment of diabetes.

Xenotransplantation, however, raises medical, legal, cultural, religious and ethical issues. For example, there are questions relating to safety since certain dangers in xenotransplantation exist, such as immunological and infectious risks, which are not present or are less significant in allotransplantation. Issues related to the quality of xenotransplants, their size and their origin are also important. Furthermore, there are concerns relating, for example, to the adequacy of the human recipient's informed consent. Indeed many patients are in such acute medical conditions that they might be tempted to ignore some xenotransplantation risks. What is more, these risks do not only concern the patient since the transmission of serious viral or microbiological diseases could affect the patient's close contacts or even the general public.

Finally, ethical issues relating to the animals used should be considered. For example, the source animals will have specific needs related to their species and the potential biological requirements of the xenotransplant.

Doubts also exist with respect to the economic prospects and the usefulness of xenotransplantation. For example, competing biotechnologies, such as stem cell technology, have recently been emerging which could potentially address the needs for cell and tissue (but not for complete organ) transplantations. At the moment, it is uncertain whether these new discoveries will have similar or even better prospects for clinical applications than xenotransplantation and one would need to take into account the presence of other possible options and their potential developments.

To address these issues, the Parliamentary Assembly of the Council of Europe, having considered the risks to public health which xenotransplantation could involve asked the Committee of Ministers, on the 29th of January 1999 (Recommendation 1399 (1999) on Xenotransplantation), to initiate a study concerning the different aspects of the relevant issues relating to xenotransplantation taking into account the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (European Treaty Series No.164).

The same year, the Committee of Ministers established a Working Party (CDBI/CDSP-XENO) under the joint authority of the Steering Committee on Bioethics (CDBI) and the European Health Committee (CDSP) to evaluate the risks in xenotransplantation and establish appropriate safeguards. Indeed it was recognised that the legal and regulatory framework balancing the risks versus benefits for xenotransplantation was often incomplete in many Council of Europe member States and that a need to harmonise guidelines existed.

The Working Party finalised a draft Recommendation on xenotransplantation in September 2001. In this Recommendation, the Working Party drafted stringent and careful provisions in order to

address the concerns expressed by the Parliamentary Assembly. This Recommendation is currently being considered by the other bodies of the Council of Europe and is expected to be adopted by the Committee of Ministers during the course of 2003.

Finally, it should be noted that during the course of this preparatory regulatory work, the xenotransplantation of organs in clinical practice was increasingly being accepted as less likely, in the short term, by experts and that more time was needed to address all the different issues. These include the continuing concerns with respect to possible adverse infections and uncertainties relating to the immunological rejection of xenotransplants.

2. INTENTION OF DOCUMENT

On 30th of September 1997, the Committee of Ministers of the Council of Europe adopted Recommendation R (97) 15 on xenotransplantation proposing that Member States establish a mechanism for the registration and regulation of certain aspects of xenotransplantation including:

- basic research and clinical trials;
- the source and care of animals for use in xenotransplantation;
- xenotransplantation programmes;
- long term follow-up and review of xenotransplant recipients and
- the xenotransplant source animals.

On 29 January 1999, the Parliamentary Assembly of the Council of Europe unanimously adopted, in the name of the precautionary principle, Recommendation 1399 (1999) on xenotransplantation, which among other things called for a legally binding moratorium on all xenotransplantation relating to humans, including clinical trials.

Without taking a stance on the proposition of a moratorium, the Committee of Ministers established a Working Party under the joint direction of the Steering Committee on Bioethics and the European Health Committee of the Council of Europe to study the problems arising from xenotransplantation. This multidisciplinary Working Party (which met from 1999 to 2001) had 12 members who specialised in ethics, law, medical research, clinical practice, epidemiology, immunology and animal protection.

As a result of its work (and after much careful reflection), the Working Party prepared a non legally binding draft Recommendation on Xenotransplantation.

In this draft Recommendation, the Working Party sought to address the concerns raised by the Parliamentary Assembly while recognising that public health problems could take on an international character requiring common provisions applicable in all member States of the Council of Europe. The document also emphasises that stringent regulations relating to efficacy, safety and animal welfare issues are urgently required since xenotransplantation is already being carried out in a number of countries.

The terms of reference for the Working Party also included the preparation of a report on the state of the art in the field of xenotransplantation for the attention of the Council of Europe member States.

Therefore, this report was written with the aim of putting xenotransplantation into context while providing interested parties and those responsible for drafting the Recommendation on Xenotransplantation with the most recent information concerning advances in this new therapeutic field.

The Working Party was Chaired by Mr. Bart Wijnberg (The Netherlands) and was composed of Prof. Didier Houssin (Vice-Chair, France), Prof. Annika Tibell (Vice-Chair, Sweden), Prof. Pekka Häyry (Finland), Prof. Karin Ulrichs (Germany), Dr. Marialuisa Lavitrano (Italy), Dr. Dag Sorensen (Norway), Prof. Alexander Tonevitsky (Russian Federation), Dr. Rafael Manez (Spain), Dr. Theodor Weber (Switzerland), Dr. David Cook (United Kingdom), Dr. Maggy Jennings (United Kingdom) and Dr. Line Matthiessen (European Community).

It should be noted that representatives from several non-member States (Prof. Eda Bloom (United States) and Dr. Larry Whitehouse (Canada)) in addition to several organisations ((International Xenotransplantation Association (IXA), OECD, Office International des Epizooties (OIE) and WHO)) were active participants, as observers, in the work. Indeed, it was considered that world-

wide cooperation between States was necessary in this field and that the participation of representatives of these non-member States and international organisations would enable the drafting of common standards, especially with respect to protecting public health.

Structure of this report:

This report begins with the state of the art concerning xenotransplantation research in order to enable the reader to understand the general context in which xenotransplantation is being examined. The important scientific and medical issues and their consequences are then discussed in the light of cultural, ethical and religious considerations.

3. OVERVIEW OF CONTENTS

DEFINITION (SEE CHAPTER 4)

For the purpose of this document, xenotransplantation is defined as any procedure that involves the transplantation or infusion into a human recipient of:

- live cells, tissues or organs from an animal source or
- human body fluids, cells, tissues or organs that have had *ex vivo* contact with live animal cells, tissues or organs.

This definition of xenotransplantation does not include non-living animal products, many of which are regulated as devices (e.g. porcine heart valves), drugs (e.g. porcine insulin) and other biological products (e.g. vaccines prepared from animal sources or animal sera used for the culture of human cells).

RESEARCH IN THE FIELD OF XENOTRANSPLANTATION (SEE CHAPTER 5)

Recent activities in organ xenotransplantation (see paragraph 5.1.)

Because of the considerable and growing success of transplantation between human beings since the 1960s, xenotransplantation has increasingly been studied as a means of providing a clinical solution for the requirement of cells, tissues and organs in medical practice. For example, skin cells grown on mouse feeder cells used to treat burns patients have been in use for over 10 years in some countries. Moreover, the acute general shortage of human transplants for potential patients could eventually be addressed by xenotransplantation. Indeed, one of the main advantages of this technique is the possibility to consider a variety of whole organs as possible transplant materials addressing different kinds of deficiencies and diseases in patients.

For example, it was because of this possibility that a porcine heart was tested in 1990 by a Polish surgeon with the intention, initially, to use it to bridge the patient until an allotransplant could become available. Unfortunately, the patient died 24 hours after the operation.

Following the remarkable survival of a patient living for nine months with a chimpanzee's liver in the 1960s, liver xenotransplantations have also been continuing. Between 1992 and 1993 two patients in the USA with hepatitis B who were at the time not suitable for allotransplantation were transplanted with baboon livers. In these clinical trials one patient survived for 10 weeks and the other for 3 weeks after the operation. Another trial in 1993 was performed in the USA using a pig liver on a young woman with terminal liver failure. In this experiment, scientists were again trying to use the pig liver as a bridge until a human liver became available. Sadly, the xenotransplant was rejected after 34 hours.

Kidneys from non-human primates have also been xenotransplanted into human beings but no operations have been recorded since 1966.

Recent activities in cellular xenotransplantation (see paragraph 5.2.)

With respect to cellular xenotransplants research in the last 10 years has been ongoing with respect to the transplantation of:

- cells from the adrenal medulla,
- cells producing neurotrophic growth factor,
- porcine neurons,
- pancreatic xenoislets,
- baboon bone marrow and
- encapsulated transgenic hamster cells.

Though cellular xenotransplants may, from a physiological perspective, be somewhat less complicated than whole organs and are easier to study, difficulties with these xenotransplants remain considerable. For example, the substances produced by the cells may be species-specific. Chemical structural differences in the produced substances may also make them physiologically non-functional and may induce immunological reactions.

Cell transplantation often only requires a minor intervention. This may partly explain why several clinical trials have been initiated without sufficient pre-clinical data first being obtained in primate models. From a physiological viewpoint, present data indicate that some xenotransplants including dopaminergic neurons, pancreatic islets and cells from the adrenal medulla can have a therapeutic effect in humans but more studies on the physiological aspects of this kind of xenotransplantation are required.

Extracorporeal exposure to xenoorgans and xenocells (see paragraph 5.3.)

During the 1990s, many patients have participated in clinical xenotransplantation trials using extracorporeal treatments with, for example, encapsulated porcine hepatocyte cells (HepAssisst). Placebo-controlled phase II or III trials are now being performed for two applications, i.e. fetal porcine neuron transplantation in Parkinsons disease and the HepAssisst device in patients with liver insufficiency.

However, by far the best known application of human cells having been exposed to xenocells is in the culture of epithelial (skin) cells grown on murine feeder cells to replace the skin of severely burned patients (Epicells). The process was developed by Dr Howard Green in the mid 1970s. This technique has been in use for some 20 years so far without problems. However there are mouse viruses (MuERVS) that could be transmitted and it is also possible that a few remaining mouse cells will be present with the skin cells. The process can be very effective, even life saving if the burns are very extensive.

With respect to extracorporeal organ xenotransplantations, only a few attempts have been reported during the 1990s. However, in a few cases, livers from transgenic pigs have been used for extracorporeal perfusion in patients with liver insufficiency.

In most cases, extracorporeal exposure to xenoorgans and xenocells seems to have been well tolerated and in many studies no side effects to the xenotransplantation, as such, have been reported. An increasing numbers of patients have been evaluated for porcine and bovine endogenous retrovirus (ERV) but no transfer of these retroviruses have yet been detected. In one recipient connected to a baboon liver, a retrospective study revealed baboon Cytomegalovirus in a blood sample. In other patients who were connected to porcine islets and treated with rabbit antithymocyte globulin (ATG-F) mild signs of serum sickness occurred but it was unclear whether this was related

to the ATG-F or the porcine cells themselves. One patient who was temporarily connected to a pig kidney also developed an anaphylactic (widespread and very serious allergic) reaction.

When it comes to evaluating the efficacy of these treatments, data are still scarce. Indeed, most studies are at a preliminary stage and even if occasional patients have reported improved conditions, the studies do not provide any conclusive data on the efficacy of the treatments.

Immunological and physiological challenges relating to Xenotransplantation (see paragraph 5.4.)

The Immunobiology of Xenotransplantation (see paragraph 5.4.1.)

One of the main problems posed by allo- and xeno-transplantation is one of transplant rejection whereby the body recognizes the foreign invader and activates an immune response. Moreover, in the case of xenotransplantation, the response to animal materials is much stronger when compared to the use of human materials. The rejection consists of the following:

Hyperacute rejection (see paragraph 5.4.1.1.)

This immune reaction occurs within minutes to hours of the transplantation and is mediated by natural or preformed antibodies and complement (or complement alone) in the human serum which bind to distinct target antigens on the endothelial cells that line the inner wall of the porcine organ's blood vessels. None of the modern immunosuppressive drugs – alone or in combination – that are used with good effect in human allotransplantation can prevent hyperacute rejection. This failure led to the manipulation of porcine endothelial cells using molecular biology methods. After grafting such transgenic or “humanised” porcine organs, natural antibodies still bind to the xenotransplant, however, activation of human complement (necessary to start the pathophysiological events that lead to hyperacute rejection) is effectively inhibited by the cell surface expression of the human complement inhibitory protein. Transgenic porcine kidneys, for example, survived up to 60 days in primates, which – from the immunological perspective – must be seen as promising.

Acute vascular rejection (see paragraph 5.4.1.2.)

In contrast to hyperacute rejection, acute vascular rejection needs time to develop and is thus sometimes termed “delayed vascular rejection” – vascular, because the antibodies' prime target structures are the porcine endothelial cells in the blood vessels. Acute vascular rejection occurs within days after xenotransplantation. Though the immune mechanisms of this type of rejection are not yet fully understood, it appears to be predominantly caused by xenogeneic antibodies that are newly formed when the human recipient's immune system makes a first contact with the xenotransplanted cells. Prevention strategies are not yet effective enough to enable the clinical xenotransplantation of animal materials within the near future.

Acute cellular rejection (see paragraph 5.4.1.3.)

This type of rejection reaction is mediated by various immunological effector cells and occurs within days after xenotransplantation. Experimental data clearly indicate that acute cellular rejection of a xenotransplant is as powerful as acute cellular rejection of an allotransplant. Thus, it is suggested that the immunosuppressive drugs currently used in humans, such as cyclosporin A, should also be capable to suppress acute cellular rejection arising from xenotransplantation.

Chronic rejection (see paragraph 5.4.1.4.)

Chronic rejection remains the most common cause for long-term failure of solid organ allotransplants. It occurs within weeks, months or even years after clinical transplantation. The immune mechanisms of this phenomenon are not well understood at present. Chronic rejection in allotransplantation has now become a major focus of research in modern transplantation medicine.

The understanding of this phenomenon during the past 20 years clearly indicates that this type of rejection cannot be sufficiently addressed with immunosuppressive drugs. The exact role of chronic rejection in xenotransplantation is even less clear, since xenotransplants have not yet survived long enough to study this rejection mechanism in detail.

Strategies to prevent immunological rejection reactions (see paragraph 5.4.4.5.)

Clinical xenotransplantation can be taken into consideration – from an immunological perspective – when the above immunological rejection reactions can be handled safely. If hyperacute rejection can be avoided by using genetically modified (“humanised”) source animals, acute vascular, acute cellular and chronic rejection still remain three major hurdles to be overcome. Life-long immunosuppressive drugs to down-regulate immune reactions are used by physicians to treat an allotransplant recipient. However, there is no immunosuppressive treatment protocol known to date, which allows the safe xenotransplantation of solid porcine organs to primates, or to human patients.

Microencapsulation of small tissues and single cells with biocompatible membranes may be a concept that has a realistic chance to soon enter the clinical stages. Microcapsules protect the xenotransplant from being attacked by the recipient’s immune system, yet enable exchange of nutrients and hormones. They may even prevent viruses crossing over from the porcine xenotransplant to the human recipient.

The Physiology of organ xenotransplantation (see paragraph 5.4.2.)

Apart from rejection problems, physiological changes after solid organ xenotransplantation, particularly when pigs are used as a source of organs, are not well understood. In this respect, there are two main physiological concerns about the use of animal organs. The first relates to the duration of function and whether, for example, a pig’s heart can continue to pump the volumes needed by a human which walks upright, and whether or not the organ will age at the rate of a pig or of a human organ. The second concern relates to whether the xenotransplant will produce the appropriate biochemicals that humans beings require and whether they will respond to the regulatory hormones and other biomolecules produced in the human body.

In this regard, it is necessary to obtain longer xenotransplant survival times through an improvement of immunosuppression strategies before a better understanding of the new physiological situation resulting from organ xenotransplantation can be achieved.

PRECLINICAL ACTIVITIES (SEE CHAPTER 6)

The use of non-human primates in xenotransplantation (see paragraph 6.1.)

Though xenotransplantation, including *ex vivo* perfusion using cells, tissues or organs, from non-human primates, would not induce an hyperacute rejection as strong as if other animals were used (because of their relatedness to humans), other problems, however, exist. Indeed xenotransplantation using materials from non-human primates would only be feasible if it were possible to eliminate known human pathogens and to identify and eliminate as yet unknown microorganisms. A thorough evaluation of risks and the development of comprehensive monitoring strategies for transplant recipients would also be essential as would a conclusive scientific and public discussion of all the issues involved including the ethical and animal welfare concerns.

The use of pigs in xenotransplantation (see paragraph 6.2.)

Pigs have always been the preferred source of xenotransplants since they are already bred in large quantities for human consumption, the size of their organs are compatible with those of humans, they reproduce quickly and a lot of experience exists with respect to their social and medical requirements. However, risks still exist when they are used for xenotransplantation and risk management schemes have been developed that give some assurance that pigs are free from microbiological agents. But other problems remain such as the potential transfer to humans through xenotransplantation of retroviral diseases. In other words, the rearing of pigs in pathogen free environments can reduce the risk of transfer of many potential pathogens but cannot eliminate the risk of transfer of a retrovirus. Furthermore it is important to consider all the ethical and welfare implications of using pigs in such procedures.

CULTURAL, ETHICAL AND RELIGIOUS ASPECTS OF XENOTRANSPLANTATION (SEE CHAPTER 7)

Xenotransplantation raises a host of cultural, ethical, animal welfare and religious issues. These are outlined to offer an overall framework for the evaluation of xenotransplantation and the public's response to it.

Culture (see paragraph 7.1.)

In relation to xenotransplantation, a positive public opinion will be crucial if the technology is to become an accepted part of medical practice. This necessarily involves reflection on moral and cultural concerns raised by such a procedure.

With respect to some modern biotechnological techniques, there is often a negative emotional reaction, frequently referred to as the 'yuck' factor which needs to be adequately assessed. Moreover, the potential benefits of xenotransplantation to patients, the continuing shortage of organs in spite of various attempts to increase the number of human organs available and the sensitivities of people in life and death situations, are all part of the wider cultural perspectives that need to be understood when discussing the implications of xenotransplantation.

The level of public understanding and the role of the various media in creating a genuine dialogue is critical. Thought needs to be given to the fora and conduct of the public debate as well as by whom and on what basis it will be delivered. For all involved in the xenotransplantation debate, it is vital to examine the grounds in favour and against xenotransplantation.

Attitudes (see paragraph 7.2.)

Surveys of attitudes to xenotransplantation in different countries including Australia, Canada, United States, France, Germany, Sweden, and the United Kingdom are studied. The recognition and understanding of these attitudes is an important factor in assessing the public's attitude towards xenotransplantation.

National Policies (see paragraph 7.3.)

National policies towards xenotransplantation in 8 different countries are examined. The development of such policies has usually included extensive consultation with the community, ranging from public meetings to various pressure groups and advisory bodies. These policy statements offer insight into the wider cultural attitudes and views regarding xenotransplantation as a technology.

International Organisations and Policies (see paragraph 7.4.)

As a response to concerns relating to xenotransplantation, the Council of Europe but also a number of other international organisations including the Organisation for Economic Co-operation and Development (OECD), the World Health Organisation (WHO) and the European Union (EU) have been developing a coordinated approach to evaluate, regulate and supervise the new advances in this field. In this respect, the Council of Europe is preparing, with the help of the aforementioned international organizations, a Recommendation on Xenotransplantation. These organizations have also been drafting reports concerning general and also specific issues relating to xenotransplantation. And though this important work is still ongoing, it has already been recognized that only an international approach will be able to sufficiently address issues such as infectious diseases which do not respect national borders.

An Ethical Overview of Clinical Xenotransplantation (see paragraph 7.5.)

One of the most important issues relating to xenotransplantation concerns the ethical evaluation of the procedure. In this respect, questions relating to the public's participation and understanding of xenotransplantation are studied including the view that the technique will be seen as interfering with nature. Issues of consent and the effect of any intervention on others were also examined since xenotransplantation does not only affect the patient himself or herself. These are important elements since the balance of risk against benefit (principle of proportionality) is crucial to any ethical evaluation of a new procedure. Finally, the effects of commercial interests, both in terms of setting the agenda and controlling the outcomes were studied.

The Ethical and Welfare Issues relating to the Use of Animals for Xenotransplantation (see paragraph 7.6.)

Most of the discussion regarding the acceptability of using animals for xenotransplantation and the related welfare concerns have focussed on the use of animals as a source of organs or tissues rather than their use in research. This reflects the fact that until recently it has been assumed that clinical xenotransplantation of organs was fairly imminent. However, this has now proved to be somewhat optimistic.

It is agreed that the acceptability of xenotransplantation depends on the full evaluation of the 'costs' and benefits of the technology. The 'costs' includes the full impact on the animals of the xenotransplant programme. These costs are not generally recognised and understood within the public arena, particularly with respect to the use of animals in the international research effort. The assessment of the ethical acceptability of any new technology should be an ongoing, rather than a single event, and thus should be kept under review as the situation develops. From an animal welfare perspective, it is important that the ethical acceptability of this use of animals be revisited weighing the real, practical costs for animals, against a critical reassessment of the actual achievements to date. The ethical and welfare issues with regard to both the use of animals as sources of cells, tissues and organs and in experimental work to develop the technology require serious consideration.

Religious faiths and xenotransplantation (see paragraph 7.7)

The views of different religions concerning xenotransplantation often depend on the manner in which these religions consider animals and how they should be treated. Moreover, because xenotransplantation results in living animal parts being incorporated into the physical body of a human person this may cause some concerns to followers of different religions, especially if the

animals used are considered by them as impure or as having a special status. For some, there may even be problems with respect to the manner in which they consider their new identity after the xenotransplantation of an animal part into their body. For this reason, a study was undertaken to examine the respective views of the major religions concerning xenotransplantation.

LEGISLATIVE AND REGULATORY FRAMEWORKS (SEE CHAPTER 8)

A survey collected legal, regulatory and scientific data in the field of xenotransplantation from 27 States. The replies showed that clinical xenotransplantation trials are planned or are presently underway in some 8 countries and close to 12 States mentioned that they perform xenotransplantation research projects on animal models.

The survey also revealed that only a minority of States had legislation currently in place covering clinical or pre-clinical xenotransplantation. However, even if some States did not have legislation in place many did, on the other hand, have specific regulatory and/or administrative arrangements concerning xenotransplantation. These arrangements usually prohibit human clinical xenotransplant protocols being carried out without specific authorisation from a regulatory board or government body. Thus, 67% of the States require that a specific authorisation be obtained before any animal xenotransplantation research protocol is carried out, and this percentage reaches 80% with respect to human clinical xenotransplantation research protocols.

Furthermore, the results showed that these legal and/or regulatory frameworks concerning xenotransplantation are significantly more developed in States where research projects and/or xenotransplantation clinical trials are performed in comparison to States where no trials are taking place.

In the field of registries, 8 States perform – or plan to perform – the registration of all xenotransplantation research protocols. France, the United-Kingdom and the United-States have already established a national procedure for registering and monitoring xenotransplantation recipients and their close contacts at the national level.

It also appears that the archiving of biological samples kept during (a) animal research or (b) clinical xenotransplantation trials is organised in 8% and 13% of States respectively.

Finally, the survey showed that initiatives for public debate on xenotransplantation have only taken place in 36% of States.

4. DEFINITION

Xenotransplantation is defined as any procedure that involves the transplantation or infusion into a human recipient of:

- live cells, tissues or organs from an animal source. This covers the transplantation of parenchymal organs (e.g., kidney, heart, liver, pancreas, lung) and the implantation or infusion of tissues and cells (e.g., skin, bone marrow, blood, pancreatic islets or beta-cells) that have been derived from animals into a human recipient, or

- human body fluids, cells, tissues or organs that have had *ex vivo* contact with live animal cells, tissues or organs. This covers the exposure by a person to:

- a. human blood or blood constituents that have been in contact with live animal tissues (for example via perfusion), or
- b. human organs, cells and tissues cultured on, or in contact with, live animal cells (regardless of whether they are alive or lethally irradiated but metabolically active), or implanted (stored) in animals.

This definition of xenotransplantation does not include non-living animal products, many of which are regulated as devices (e.g. porcine heart valves), drugs (e.g. porcine insulin) and other biological products (e.g. anti thymocyte globulin, vaccines prepared from animal sources or animal sera used for the culture of human cells).

5. RESEARCH IN THE FIELD OF XENOTRANSPLANTATION

At the beginning of the 20th century, the transplantation of animal body parts to human beings was being performed on a trial and error basis without any real understanding of the medical effects which were taking place. For example, several hundred chimpanzee testicle transplantations were performed in Europe during the years 1920 to 1930.

It was only after the 1950s that xenotransplantation was studied in a more rational manner. The cases of clinical xenotransplantation during the 1960s, 1970s and 1980s are summarised in Table:1¹. In most cases, chimpanzees or baboons were used as source animals. But with the exception of a remarkable case reported by Reemtsma et al - a chimpanzee kidney functioning for nine months after transplantation to a young woman - the xenotransplants usually failed within the first few weeks.

Another well-known attempt was performed in 1984 when a new-born baby (the so-called baby Fae case) was transplanted with a baboon heart which functioned for 20 days. The transplantation attempt created a lot of discussion and the ethical and scientific basis was questioned by critical voices both in the scientific community and amongst the general public.

For an overview of the research during the 20th century see tables: 1 and 13.

Since 1990 a new dynamic in xenotransplantation has been observed especially with trials relating to cellular xenotransplants (see table: 2). Extracorporeal perfusions have also been studied in addition to gene therapy trials where animal cells have been used to introduce new genes into human individuals.

5.1. Recent activities in organ xenotransplantation

5.1.1. Heart xenotransplantation

In 1990 a Polish surgeon, Dr Religa, performed one case of porcine heart transplantation². The intention was to bridge the patient until an allotransplant could become available. In preparation for the transplant, the patient's blood was passed extracorporeally through a pig heart to reduce the levels of anti-pig antibodies. The patient died 24 hours post-transplant in graft failure. Histology was reported not to show any signs of rejection. However, the histological evaluation of this case was limited and did not include immunohistological studies to detect the presence of antibodies and complement.

5.1.2. Liver xenotransplantation

In 1992 and 1993, Thomas Starzl, Pittsburg, USA performed two cases of baboon-to-man liver transplantation^{3,4}. Both patients had hepatitis B and the first patient also had HIV. They were at the time considered not suitable for allotransplantation. The rationale for offering these patients a xenotransplant was that the baboon liver is resistant to hepatitis B.

In the first case, early transplant function was good and the patient was off the ventilator within 24 hours and fully mobile after 5 days. As expected, several biochemical parameters e.g. albumin, coagulation factors and urate changed to a baboon profile. The liver was small in relation to the patients and significant regeneration of the liver occurred during the first weeks after transplantation which is similar to what would be expected with a human transplant. The patient gradually developed complications to high-dose immunosuppression with renal insufficiency and infection. There was also suspicion of xenotransplant rejection. The patient died 70 days after transplantation because of an intracerebral bleeding caused by an *Aspergillus* infection.

The second recipient did not recover as well during the first days after xenotransplantation and died 3 weeks after the operation because of a liver insufficiency.

Table 1: Summary of clinical organ xenotransplantation during the 1960's, 1970's and 1980's

	Year	Source Animal	N	Investigator
Kidney	1964	Chimpanzee	12	Reemtsma
	1964	Monkey	1	Reemtsma
	1964	Baboon	1	Hitchcock
	1964	Baboon	6	Starzl
	1964	Chimpanzee	1	Hume
	1964	Chimpanzee	3	Traeger
	1965	Chimpanzee	2	Goldsmith
	1966	Chimpanzee	1	Cortesini
Heart	1964	Chimpanzee	1	Hardy
	1968	Sheep	1	Cooley
	1968	Pig	1	Ross
	1968	Pig	1	Ross
	1969	Chimpanzee	1	Marion
	1977	Baboon	1	Barnard
	1977	Chimpanzee	1	Barnard
	1984	Baboon	1	Bailey
Liver	1966	Chimpanzee	1	Starzl
	1969	Chimpanzee	2	Starzl
	1969	Baboon	1	Bertoye
	1970	Baboon	1	Leger
	1970	Baboon	1	Marion
	1971	Baboon	1	Poyet
	1971	Baboon	1	Motin
	1974	Chimpanzee	1	Starzl

Table 2: Summary of clinical trials on organ and cell xenotransplantation during the 1990's.

	Graft	Indication	n	Country	Presently including patients
Organ transplantation	Pig heart	Heart failure, bridging procedure	1	Poland	No
	Baboon liver	Hepatitis B with liver failure	2	USA	No
	Pig liver	Liver failure, bridging procedure	1	USA	No
Cellular grafts	Neonatal bovine cromaffine cells	Pain	more than 100	Poland, Czech Republic, Switzerland & USA	No?
	Encapsulated transgenic hamster cells	ALS	6	Switzerland	No?
	Fetal porcine neurons	Parkinson	21	USA	Yes
		Huntington	12	USA	Yes
		Epilepsy	3	USA	Yes
		Stroke	3	USA	Yes
	Fetal porcine islets	Diabetes	10	Sweden	No
	Neonatal porcine islets	Diabetes	6	New Zealand	No
Fetal rabbit islets	Diabetes	Several 100	Russia	Yes	
Baboon bone marrow	HIV	1	USA	No	

In 1993, Makowka at the Cedars Sinai Hospital, Los Angeles, USA performed an auxiliary pig liver transplantation on a young woman with terminal liver failure⁵. The intention was to use the pig liver as a bridge until a human liver became available. Before transplantation, plasmapheresis and extracorporeal perfusion of pig kidneys were performed to reduce the levels of anti-pig antibodies. Initially the xenotransplant seemed to function as indicated by bile production and improvement in some of the biochemical parameters. After transplantation, antibody levels increased rapidly and the xenotransplant was totally rejected when the patient died 34 hours after xenotransplantation.

To our knowledge, these are the only cases of organ xenotransplantation performed during the 1990s. So far, no case of xenotransplantation using organs from transgenic pigs has been reported.

5.2. Recent activities in cellular xenotransplantation

Cell transplantation is leading clinical developments in xenotransplantation. Several 100 patients have been exposed to cellular xenotransplants using pigs as well as other non-primate species as source animals. This overview will focus on the different kinds of cellular xenotransplantations that have been clinically tested. The use of animal cells to introduce vectors in gene therapy could also be considered as a kind of xenotransplantation but will not be discussed in this document.

5.2.1. Transplantation of cells from the adrenal medulla

The cells in the adrenal medulla produce adrenaline, noradrenaline and an opioid-like substance, met-enkephalin. These substances are also produced in the central nervous system where they have analgesic effects by inhibiting pain signals from the periphery. Cells from the adrenal medulla can easily be obtained from newborn calves while it is much more difficult to isolate them from pig adrenals. The structure of these three substances is similar between cattle (and several other mammals) and man.

Several reports indicate that transplantation of allogeneic adrenal medulla cells can improve patients with severe chronic pain. Thus, encapsulated bovine chromaffine cells from new-born calves have been transplanted to the spinal channel of terminal cancer patients with severe morphine resistant pain⁶. It was then hoped that these cells would produce the substances that have pain-relieving effects when found in the central nervous system.

The cells were encapsulated and placed in an immunoprotected site, the spinal canal. No pharmacological immunosuppression was given to the patients. The trials were initiated by the American Biotech Company, Cytotherapeutics Inc. (CTI). The majority of the patients had advanced cancer but the treatment was also tested in a small number of patients with neurologic pain. Phase I trials have been performed both in Europe and in the USA and have indicated significant reduction in morphine intake in some of the patients.

During 1998 and 1999 a Phase II trial involving some 85 patients was performed in three European countries, Poland, the Czech Republic and Switzerland. This trial was performed by AstraZeneca and was a blinded placebo-controlled study. During the first 10 weeks, patients received either an empty implant or a device filled with bovine cells. The implants were then removed and all patients were offered a filled device and monitored for another 6 months. Most patients wanted a second device and the last patients have now finished their 6-month follow-up. Evaluation of the 10-week placebo-controlled implantation period indicated insufficient efficacy of the treatment but no serious adverse events (personal communication). The best effects were seen in patients with pain in the lumbar or sacral regions which are close to the implantation site. Similar observations were made in the phase I trial. The cells survived well during the implantation period. The future of this application of xenotransplantation is, at present, uncertain. Due to technical problems with the device some of the American patients are still carrying their implants and will be followed life-long. AstraZeneca will not perform further studies and CTI has sold the concept to a Swiss company.

5.2.2. Transplantation of cells producing neurotrophic growth factor

Encapsulated transgenic fetal hamster cells producing a neurotrophic growth factor have been placed in the spinal canal of at least six patients with ALS (amyotrophic lateral sclerosis, a neurological disease leading to paralysis) in Switzerland⁷. The principal investigator was Dr Patrick Aebischer, Lausanne, Switzerland. Significant levels of the growth factor were detected in the spinal fluid but so far there are no reports indicating a clinical improvement of the patients' symptoms.

5.2.3. Transplantation of porcine neurons

Porcine dopaminergic neurons are the most studied neural cells in xenotransplantation. The structure of dopamine is similar in man and pigs but its production seems higher in pigs. The systems for re-transport of dopamine from the nerve-endings has similar activity in pig and man while man has two pathways for dopamine metabolism and the pig only has one. After allotransplantation of dopaminergic neurons, patients usually have to be maintained on anti-Parkinson drugs. These drugs seem to have the same therapeutic effects on porcine neurons.

Fetal dopaminergic cells have been transplanted in a number of species combinations. Transplantation of fetal porcine neurons improves symptoms in rats and monkeys with chemically induced Parkinson's disease. Histological evaluation shows that the cells grow and differentiate into mature dopaminergic neurons and establishes contact with the relevant parts of the recipient's brain.

Fetal porcine dopaminergic neurones have been transplanted to 12 patients with Parkinson's disease in a phase I trial starting 1995 and performed by Diacrine, USA. A total of 12 million cells were injected unilaterally into the putamen and caudate. For one patient who died 7.5 months post transplant in a non-transplant related disease, small numbers of surviving neurones were identified⁸. Some patients have reported clinical improvement, though placebo effects cannot be excluded. A placebo-controlled double-blind phase II trial is now ongoing in the USA (Boston, New England, Atlanta, Georgia and Tampa, Florida). The trial includes 18 patients, half of them have received bilateral porcine xenotransplant implants with 25 million cells while the others underwent sham operations.

The same company has also provided porcine fetal GABA neurones (neurones having inhibitory effects on the transmission between nerve cells) for transplantation to 12 patients with Huntington's chorea. The structure of GABA is similar between pigs and man. But in these cases no clinical improvements have been reported among the Huntington patients. However, 3-4 patients who were transplanted reasonably early in their disease seemed to have stabilised after the transplantations. The significance of this observation is uncertain as this was a phase I trial. A phase II trial is being discussed. Both the Parkinson and the Huntington programs are performed in collaboration with the American company Genezyme.

Porcine GABA neurones have also been transplanted to 3 patients with focal epilepsy planned to undergo neurosurgical resection of the epileptic focus. The trial started in late 1998. In one case, the patient had seizures every 2-3 weeks before transplantation but has been free from seizures after the xenotransplantation. This patient has cancelled his operation. The other two patients did not have the same effect and have later undergone surgery. Another 3 patients are planned in this trial and FDA has also approved a trial in multifocal epilepsy.

Three patients who had suffered a stroke have also been transplanted with porcine fetal neurons in a trial starting September 1999. The trial is open for patients 6 months to 10 years after a stroke and with stable deficits. Two patients are reported to have experienced significant improvements, especially regarding their facial palsy. Again the significance is impossible to evaluate due to the lack of controls.

In all these trials, the neurons are placed in an immunoprivileged site, the central nervous system that reduces the need for treatment to prevent rejection. In the phase I Parkinson study, half the patients received immunosuppression with cyclosporin A (CsA). Treatment is planned to be life-long. In the other cases, the porcine cells were treated in culture with antibodies to the Major Histocompatibility Complex class I (MHC includes the most important genes deciding the fate of a transplanted cell, tissue, or organ) the intention being to reduce the antigenic exposure. In the phase II Parkinson trial, CsA is used in all patients while in the epilepsy and stroke patients the anti-MHC antibodies are used to protect the transplants.

Diacrine has several other preclinical programs in xenotransplantation. For example, they have FDA approval for transplantation of GABA neurons to treat pain secondary to spinal injury. The cells will be injected in the dorsal horn and the intention is to balance the uncontrolled firing from the traumatised cord and restore the pain-suppressing circuit.

5.2.4. Transplantation of pancreatic xenoislets

Pancreatic islets contain the insulin-producing beta cells which are important in the regulation of the blood sugar level. The structural difference between porcine and human insulin is limited to one amino acid. From clinical experience we know that porcine insulin works well in humans as it was used to treat diabetic patients for decades until human recombinant insulin became available in the 1980s. The tendency to produce anti-insulin antibodies was also low in non-immunosuppressed humans. It is expected that after transplantation, the islet graft will regulate blood sugar to a level that is normal in the source species. This should not constitute a problem since both pigs and human beings have the same normal range for blood sugar and insulin secretion is regulated by similar mechanisms.

C-peptide is a polypeptide chain, which is split off from the pro-insulin molecule and secreted from the betacells together with insulin. C-peptide was long considered a waste product without biological activity but recently it has been shown that a lack of C-peptide in the diabetic patients may contribute to the secondary complications of diabetes. There are significant structural differences between human and porcine C-peptide. The active site of the molecule has been identified but it is still not known whether porcine C-peptide will be active in man.

The islets also secrete glucagon, a molecule that raises blood sugar levels and which together with growth hormone and noradrenaline, balance the effects of insulin. The structure of glucagon is similar in pig and man.

Somatostatin is produced in the islets, but also in the brain and the bowel. The production from the islet graft is not considered necessary for the somatostatin effects.

Experiences from experimental studies indicate that fetal and adult porcine islets can cure diabetes in rodents. Function has been maintained for up to two years. There are also examples of successful reversal of diabetes in dogs by transplantation of encapsulated porcine islets, though in most cases survival time was limited to a few weeks or months. One investigator reported the spontaneous cure of diabetic cynomolgus monkeys after transplantation of microencapsulated adult porcine islets. Without immunosuppression 7 of 10 monkeys became insulin-independent for periods ranging between 120-804 days.

To date, more than 500 patients have undergone xenotransplantation with fetal xenoislets. In Moscow, Russia, Professor Shumakov has transplanted diabetic patients with islet of Langerhans obtained from rabbit pancreas. However, the reports on these studies is very incomplete and no further information on the outcome of this trial is available at present. Transplantations of fetal pancreatic tissue from pigs and calves have also been performed to significant numbers of patients in China but the results have not been reported internationally.

Non-encapsulated fetal porcine islets were transplanted in 10 patients in Stockholm, Sweden, between 1990 and 1993 by the group of Carl Groth and Claes Hellerström⁹. Eight patients were already carrying renal allotransplants and in these patients the porcine islets were injected into the portal vein. In two additional cases, the islet xenotransplantations were performed in conjunction with living donor renal transplantation whereby the porcine islets were placed under the capsule of the renal allotransplant. In four patients there were signs of xenotransplant survival as indicated by the presence of porcine C-peptide in urine up to 450 days after transplantation. In another patient, viable porcine islets were identified in a renal transplant biopsy performed three weeks after the xenotransplantation. However, no patient could terminate their exogenous insulin injections.

Encapsulated porcine islets have been xenotransplanted to six diabetic patients in New Zealand. The trial was performed by Dr Elliott in collaboration with an American Biotech Company, Vivorex. The results of this trial have not been reported. At least one of the patients was a renal transplant recipient maintained on conventional immunosuppressive therapy. In two cases, C-peptide production and a reductions in the insulin requirement for up to two years were reported briefly in an abstract dealing with surveillance of PERV in these patients.

5.2.5. Transplantation of baboon bone marrow

There are no reports on the physiological compatibility between human and baboon bone marrow cells. However, when pig bone marrow was transplanted to cynomolgus monkeys it became apparent that many of the growth factors were species-specific and engraftment of pig bone marrow required that porcine growth factors were given to the monkey.

In 1996, Suzanne Ilstaad, Pittsburgh, USA, performed one case of baboon-to-man bone marrow transplantation in a patient with advanced HIV. The intention was to provide the patient with white blood cells that were resistant to HIV infection¹⁰. The post-transplant course was reported to have been uneventful. Microchimerism (presence of baboon cell) was reported for the first 13 days after the transplantation. The xenotransplant was then presumably rejected.

5.2.6. Summary remarks relating to cellular xenotransplantation

Cellular xenotransplants may, from a physiological perspective, be somewhat less complicated than whole organs. Yet, the substances produced by the cells may be species-specific. Structural differences may have two consequences, the substance may not exert its physiological function and secondly the substance may be immunogenic. Thus, a structural difference not affecting the active site of a hormone may still lead to the development of neutralizing antibodies affecting function. In addition, species differences in the hormones and other factors produced by the recipient regulating transplant function must be taken into account.

Cellular xenotransplants are often implanted in ectopic sites. Thus normal paracrine interaction with the surrounding tissues will not be possible. This may also influence the prospects for long-term function. Recent studies indicate that in adult pancreatic glands, new islets are continuously developed from ductal structures. This will obviously not occur in the islet graft, which may then have a more limited life span.

Cell transplantation often only requires a minor intervention. This may partly explain why several clinical trials have been initiated without significant experience first being obtained in primate models. From a physiological perspective, present data indicate that some xenotransplants including dopaminergic neurons, pancreatic islets and cells from the adrenal medulla can have a therapeutic effect in humans but more studies on the physiological aspects of xenotransplantation are obviously needed.

5.3. Extracorporeal exposure to xenoorgans and xenocells

5.3.1. Developments in the use of extracorporeal cellular xenotransplants

The American Company Circe Biomedical has developed a bioartificial liver dialyses machine in which the patient's plasma is passed along encapsulated porcine hepatocytes¹¹. In phase I-II studies, improvements in hepatic encephalopathy and in some biochemical parameters were observed, again the significance is unclear due to the lack of controls. So far, no signs of synthetic activity such as

production of coagulation factors from these cells have been reported. The phase I-II trial involved 52 patients of which 39 were treated within the protocol and 13 on compassionate basis.

A placebo-controlled phase III trial in which patients are treated either with empty columns or columns filled with porcine hepatocytes is now ongoing. So far, some 60 of the 100 patients have been enrolled. The study is a multicenter trial and includes American and European centres.

A similar concept is also being evaluated at the University of Pittsburgh Medical Centre. This device was developed by Excorp. Medical, Oakdale, MN, USA. The clinical trials began in November 1998. In October 1999, six patients had been treated with their device. No unanticipated adverse effects have been observed. The phase I trial is planned to include a total of 15 study and 30 reference patients.

The U.S. Food and Drug administration indicated at the beginning of 2002¹² that the co-culture of human embryos with non-human animal cells would be considered as xenotransplantation if the embryos were implanted into a woman. This is because during co-culturing, human embryos and non-human animal cells are maintained together outside the body, in *ex vivo* contact. This statement came as a response to procedures such as the one undertaken in France in 1999 whereby an *in vitro* fertilization technique was used in which a Vero cell (long ago obtained from African Green Monkey kidney cells) feeder layer was used^{13,14,15}. In this technique, co-cultures of human embryos, particularly with Vero cells, were primarily developed for cases of successive implantation failures.

However, by far the best known application of human cells having been exposed to xenocells, is in the culture of epithelial (skin) cells on murine feeder cells to replace the skin of severely burned patients (Epicells). The process was developed by Dr Howard Green in the mid 1970s. The process consists in a small sample of skin cells (3cm square) from the burned patients being taken to the lab and digested with enzymes to break down the skin and enable the keratinocytes (skin cells) to be extracted. These are then cultured in flasks with a special cell line of murine (mouse) 3T3 fibroblasts (type of connective tissue cell). The mouse cells are necessary to promote the growth of the human cells. The mouse cells have been irradiated and normally die after a week by which time the skin cells have grown and started to differentiate. The new sheet of human cells is removed from the flask and laid on the patient. The bigger the burn the more new skin cells are required.

This technique has been in use for some 20 years so far without problems. However there are mouse viruses (MuERVS) that could be transmitted and it is also possible that a few remaining mouse cells will be present with the skin cells. The process can be very effective, even life saving if the burns are very extensive.

5.3.2. Developments in the use of extracorporeal organ xenotransplants

Livers from pigs and other species have been used for extra corporeal perfusions in patients with liver failure either while waiting for an allotransplant or with the intention to treat the patient while the native liver is recovering. Occasional cases and small series of patients have been reported since the 1960s. Some patients are reported to have improved during treatment or having survived long-term because of the treatment but again there is a lack of controls. The early experiences have been summarised in an overview by Abouna in the book *Xenotransplantation*¹⁶.

Five patients have also been treated at Dukes University, North Carolina, USA and two cases at the Medical University of South Carolina, Charleston, USA. These patients were exposed to livers from normal (non-transgenic) pigs. Transient improvements were reported in several of the patients. Recently a case of extracorporeal pig liver perfusion was also performed by the group of Prof Neuhaus, Berlin, Germany. The patient initially stabilised and his own liver showed signs of

regeneration but the course was complicated by a fungal septicemia and the patient died 10 days after the perfusions.

Recently, livers from transgenic pigs expressing human complement regulatory proteins (hCD55 and hCD59) have been used for extracorporeal perfusions in at least two patients. The perfusions were performed by the group of Goran Klintmalm in Dallas Texas, USA and the transgenic pigs were produced by Nextran. The technique for the extra-corporeal perfusions was not optimised and therefore it is hard to evaluate whether the transgenicity provided a superior xenotransplant function.

In 1994, two dialyses patients in Gothenburg, Sweden, had pig kidneys connected to their dialyses fistulas^{17,18}. The intention was to study the early immune response. Both patients underwent plasmapheresis prior to perfusions to reduce the levels of anti-pig antibodies. In one patient the kidney was rejected after approximately one hour. In the other case, the patient developed an anaphylactic reaction some 20 minutes after the perfusion and the experiment had to be terminated. The patient recovered rapidly. These studies are an expansion of a similar study performed in one patient by Welsh, Taube et al in the UK¹⁹. The Gothenburg group sought to compare kidneys from normal pigs with kidneys from transgenic pigs, however the latter studies have not been performed so far.

5.3.3. Conclusion

During the 1990s several hundred patients have participated in clinical xenotransplantation trials mainly in the field of cell transplantation and extracorporeal treatment with encapsulated porcine hepatocytes (HepAssist). Placebo-controlled phase II or III trials are now being performed for two applications, fetal porcine neuron transplantation in Parkinsons disease and the HepAssist device in patients with liver insufficiency. However, with the exception of skin cells grown on mouse feeder cells (such as Epicells) there is little evidence concerning clinical effectiveness of any of the xenotransplantation techniques reported.

Only a few attempts of extracorporeal organ xenotransplantation have been reported during the 1990s and so far organs from transgenic pigs expressing human complement regulatory factors have not been transplanted to humans. In a few cases, livers from such pigs have, however, been used for extracorporeal perfusion in patients for liver insufficiency.

In most cases, the procedures seem to have been well tolerated and in many studies no side effects to the xenotransplantation as such have been reported. An increasing numbers of patients have been evaluated for porcine and bovine endogenous retrovirus (ERV) but, so far, no transferral of ERV has been detected. In one recipient of a baboon liver, a retrospective study revealed baboon Cytomegalovirus (CMV) in a blood sample which suggests that a transferral of baboon CMV may have occurred. Alternatively, reactivation of baboon CMV may have occurred in the transplanted baboon cells without transferral to human cells. In another patient undergoing extracorporeal liver perfusion, development of antibodies to coagulation factor V that cross-reacted with the corresponding human coagulation factor was suspected. The patient later died because of bleeding complications. In two of the Stockholm patients given porcine islets and treated with rabbit antithymocyte globulin (ATG-F) mild signs of serum sickness occurred. It is unclear whether this was related to the ATG-F or the porcine cells. As described above, one of the Gothenburg patients who was temporarily connected to a pig kidney developed an anaphylactic reaction.

When it comes to evaluating the efficacy of the treatments, data are still scarce. Nevertheless, most studies are at a preliminary stage and even if occasional patients have reported improved conditions, the studies do not provide any conclusive data on the efficacy of the treatments.

5.4. Immunological and physiological challenges relating to xenotransplantation

5.4.1. The Immunobiology of Xenotransplantation

The pig is currently considered as the most suitable source species mainly for physiological and ethical reasons but also because of the low breeding and keeping costs. Organs of suitable age and size can be provided in large numbers. Non-human primates (e.g. baboons), on the other hand, are generally excluded as a source because of the high breeding and keeping costs, ethical problems and the greater risks of infections (xenoses).

The enormous immunogenetic difference between pig and human beings leads to much more vigorous immunological rejection reactions than in human-to-human allotransplantations. Besides current controversies on physiological and biochemical incompatibilities and virological problems (e.g. the debate on porcine endogenous retroviruses) that must also be solved prior to clinical xenotransplantation, the relevant immune reactions must be fully understood in order to successfully limit them and achieve long-term survival of porcine organs in human patients.

The immunological rejection reactions following transplantation of a porcine organ to man are summarised in Table 3.

Table 3: Immune reactions after xenogeneic transplantation of organs, tissues and cells and strategies to prevent them

Type of Immune Reaction	Time point when reaction occurs	Affected xenogeneic organs, tissues or cells		Prevention Strategies
		Vascularized:e.g. heart, kidney, liver, pancreas, lung, small bowel	Non-vascularized: e.g. pancreatic islets, neurons, hepatocytes, parathyroid glands	
Hyperacute rejection (mediated by antibodies and complement or complement alone)	Minutes to hours	Yes	No	(1) Transgenic donors (2) Antibody absorption techniques (3) New immunosuppressive drugs (4) Concepts for tolerance induction
Acute vascular rejection (mediated by antibodies and complement)	Days	Yes	Yes	See (1)-(4) (5) Microencapsulation of small tissues and cells
Acute cellular rejection (mediated by various types of cells)	Days	Yes	Yes	See (1)-(5)
Chronic rejection (mediated by unspecific and xenoantigen-specific factors)	Weeks, months, years	Yes	???	Not yet available

5.4.1.1. Hyperacute rejection

This immune reaction occurs within minutes to hours of the transplantation and is mediated by natural or preformed antibodies of immunoglobulin M type (IgM; immunoglobulins are proteins which attach to foreign substances such as bacteria, and assist in destroying them) and complement (or complement alone) in the human serum which bind to distinct target antigens on the endothelial cells that line the inner wall of the porcine organ's blood vessels. A series of subsequent pathobiochemical and pathophysiological processes lead to dysfunction and finally destruction of the porcine organ. The major target antigen is galactose α 1,3 galactose (gal α 1,3 gal) which is part of a glycoprotein expressed on the porcine endothelial cells. This type of rejection (see Tab: 3) does not affect non-vascularized small porcine tissues and single cells because they are transplanted without functional blood supply via re-anastomosed blood vessels. Elimination of natural IgM antibodies from the human serum prior to transplantation by antibody-absorption techniques, e.g., plasmapheresis, is possible, yet antibodies will reoccur after a short time.

None of the modern immunosuppressive drugs – alone or in combination – that are used with good effect in human allotransplantation can prevent hyperacute rejection. This failure led to the idea of manipulating the porcine endothelial cells using molecular biological methods: human complement inhibitory genes are inserted into the porcine genome. Pigs that express the human decay accelerating factor, hDAF (a complement inhibitory protein) on their endothelial cells are now commercially available in the UK and USA, and are presently used for multiple preclinical studies all over the world. After grafting such transgenic or “humanised” porcine organs, natural antibodies still bind to the gal- α 1,3-gal epitope, however, activation of human complement, necessary to start the pathophysiological events that lead to hyperacute rejection, is effectively inhibited by the cell surface expression of the human complement inhibitory protein. Transgenic porcine kidneys, for example, survive up to 60 days in primates, which – from the immunological viewpoint – must be rated a tremendous success. Other approaches to circumvent hyperacute rejection concentrate on the elimination of the target epitope gal- α 1,3-gal by gene “knock-out” technologies.

5.4.1.2. Acute vascular rejection

Acute vascular rejection occurs within days after xenogeneic transplantation. Though the immune mechanisms of this type of rejection are not yet fully understood, it appears to be predominantly caused by xenogeneic antibodies (Immunoglobulin G, IgG, type) that are newly formed when the human recipient's immune system makes a first contact with the xenogeneic donor cells. These antibodies also recognise the gal- α 1,3-gal antigen and may then activate complement with all subsequent dysfunction and destruction processes.

In contrast to hyperacute rejection, acute vascular rejection needs time to develop and is thus sometimes termed “delayed vascular rejection” – vascular, because the antibodies' prime target structures are the porcine endothelial cells. Yet, any other grafted porcine cell, carrying a variety of xenogeneic antigens, may also induce an anti-porcine (anti-donor)-directed antibody response within the recipient. Thus, non-vascularized “organs”, such as porcine islets of Langerhans, parathyroid glands or liver cells may be directly affected by this type of immune rejection, and not only the blood vessels of porcine hearts or kidneys. Here too, antibody-absorption techniques or common immunosuppressive drugs are unable to fully prevent this rejection process. When these manipulative steps were used in combination with hDAF transgenic donor organs, limited success was achieved in some pig-to-primate models. However, these prevention strategies are not yet effective enough to enter clinical xenotransplantation of solid porcine organs, tissues or cells within the near future.

5.4.1.3. Acute cellular rejection

This type of rejection reaction is mediated by various immunological effector cells, namely T-lymphocytes, macrophages and/or natural killer (NK) cells, and occurs within days after xenotransplantation. Similar to allotransplantation CD8⁺ and CD4⁺ T-lymphocytes were shown to be the major effector cell populations also in xenotransplantation and responsible for cellular xenotransplant rejection. Still unclear is the exact role of macrophages and NK cells in this context. Experimental data clearly indicate that acute cellular rejection of a xenotransplant is as powerful as acute cellular rejection of an allotransplant. One must assume therefore sufficient homologies between porcine and human immunological regulator molecules. Thus, the immunosuppressive drugs cyclosporin A, tacrolimus (FK506), azathioprin and/or prednisolone should be capable to suppress acute cellular rejection in xenotransplantation quite effectively.

5.4.1.4. Chronic rejection

Chronic rejection remains the most common cause for long-term failure of solid organ allotransplants. It occurs within weeks, months or even years after clinical transplantation. The immune mechanisms of this phenomenon are not well understood at present. Major causes for concern are the pathophysiological effects of repeated acute rejection crises or perfusion and re-perfusion injuries of the xenotransplanted tissues. Ongoing inflammatory reactions and diffuse concentric intimal proliferation in the arterial vessels, i.e. interactions between non-specific and antigen-specific factors, finally lead to xenotransplant arteriosclerosis. Chronic rejection in allotransplantation has now become a major focus of research in modern transplantation medicine. Our knowledge gained during the past 20 years clearly indicates that this type of rejection cannot be handled sufficiently with immunosuppressive drugs. The exact role of chronic rejection in xenotransplantation is even less clear, since xenotransplants have not yet survived long enough to study this rejection mechanism in detail.

5.4.1.5. Strategies to prevent immunological rejection reactions

Clinical xenotransplantation can be taken into consideration – from an immunological perspective – when the above immunological rejection reactions can be handled safely. If hyperacute rejection can be avoided by using genetically modified (“humanised”) source animals, acute vascular, acute cellular and chronic rejection still remain three major hurdles to be overcome. Life-long immunosuppressive drugs to down-regulate immune reactions were, and still are used by physicians to treat an allotransplant recipient. However, there is no immunosuppressive treatment protocol known to date, which allows the safe xenotransplantation of solid porcine organs to primates, respectively the human patient. Thus, research for more selectively and more specifically acting drugs, with less side effects, is in constant progress. But life-long treatment with immunosuppressive drugs is accompanied by an increased risk of developing infections and tumours.

Thus, from an immunological perspective, solutions should be found to induce immunological tolerance against the xenotransplants: a tolerant recipient’s immune system is unable to react against the transplanted antigens, but remains fully reactive towards pathogens, e.g., bacteria, fungi and viruses and tumour cells. The goal of achieving tolerance applies to allo- as well as to xenotransplantation. Various approaches to induce immunological tolerance are being considered, e.g. a time-limited phase of immunosuppression or combining an allotransplant with a haematopoietic stem cell transplant from the same donor. As to the latter, co-existing human and porcine stem cells in a human recipient, which is termed “mixed haematopoietic cell chimerism”, may create new ethical problems. Though the majority of such concepts are still in an experimental phase and far from being clinically applicable, a first clinical case was recently reported: a

combined kidney and bone marrow (carrying haematopoietic stem cells) allotransplantation had been successfully carried out without further need of immunosuppression.

Particularly, microencapsulation of small tissues and single cells with biocompatible membranes (see Tab: 3) may be a concept that has a realistic chance to be considered at the clinical stage. Microcapsules protect the xenotransplant from being attacked by the recipient's immune system, yet enable exchange of nutrients and hormones, e.g. release of insulin from porcine pancreatic islets or parathyroid hormone from parathyroid glands. They may even prevent viruses crossing over from the porcine transplant to the human recipient.

Undoubtedly, progress in immunology, molecular biology and biotechnology has achieved prolonged survival of xenogeneic cells, tissues and organs in preclinical animal models. However, further success is needed before xenotransplantation can be safely performed in human patients.

5.4.2. Physiological changes resulting from organ xenotransplantation

Experience relating to the function of solid organs transplanted between different species is limited. This is the case with respect to the duration of function of the organ. For example, questions remain as to whether a pig's heart can continue to pump the volumes needed by a human which walks upright, and whether or not the organ will age at the rate of a pig or of a human organ. Concerns also exist as to whether the organ will produce the appropriate biochemicals that humans require, or respond to the hormones that other human organs produce.

Most of the information available for the moment concerns pig organs transplanted into baboons which showed a maximal survival time of 39 days for orthotopic heart and 99 days for kidney xenotransplantation. Thus, nobody knows what may happen with issues such as xenotransplant growth or longevity that will require longer survival times for its evaluation.

5.4.2.1. Heart xenotransplantation

In the case of heart xenotransplantation the pig organ maintained normal haemodynamics (blood pressure, cardiac output) in the recipient during the survival time which may mean that the xenotransplant size matched that of the primate weight during this period. In contrast, pig kidneys apparently grew faster than *cynomolgus monkey* recipients during 4-6 weeks after transplantation, being more equivalent thereafter. The cause for this early mismatch between pig organ and body primate growths is unclear and studies comparing the progression of organs in pigs and xenotransplants are warranted.

5.4.2.2. Kidney xenotransplantation

Several extrinsic hormones regulate kidney function which itself produces other intrinsic hormones. The former include:

- antidiuretic hormone (ADH) that regulates water homeostasis and produces "diabetes insipidus" if defective;
- aldosterone that adjusts sodium and potassium levels leading to hypotension, hyperkalemia and hyponatremia in case of dysfunction; or
- parathormone (PTH) that controls calcium and phosphorus metabolism causing hypocalcemia and hyperphosphatemia if it does not work properly.

The intrinsic hormones produced by the kidney include renin that participates in blood pressure control and erythropoietin responsible for the synthesis of blood red cells that in case of inadequate function will lead to anaemia.

Porcine kidneys xenotransplanted into non-human primates have shown that they are competent to maintain normal blood pressure, sodium, potassium and calcium, although the latter may be low just after transplantation but normalises over time. However, a persistent hypophosphatemia and anaemia have been observed in all recipients. The cause of the former is unclear, whereas the anaemia is secondary to inadequate erythropoietin function. This may be due to a failure of production, failure of recognition by recipient cells or destruction by antibodies against this pig hormone. The specific factor responsible for anaemia is unknown. Nevertheless, treatment with human erythropoietin allows the recovery of normal blood red cell counts.

The hypophosphatemia and anaemia observed in non-human primates after porcine kidney transplantation are the only evidence for the moment of physiological changes after solid organ xenotransplantation. These variations can be considered minor since the normal levels are restored with the appropriate treatment. However, they raise questions about what may occur with the transplantation of organs with more metabolic complexity such as liver. In the last two baboon to human liver xenotransplantations performed in 1992 and 1993, recipients showed a disappearance of uric acid one week after transplantation and persistent low albumin level during the postoperative course. The first resulted from a particular metabolism of uric acid by baboons since they have an enzyme that practically eliminates this element, but the cause of hypoalbuminemia is unknown because baboons have a level of albumin similar to humans.

5.4.2.3. Liver xenotransplantation

Recently (hDAF) pig livers have been transplanted into baboons. The longest survival time achieved was 8 days. During this short period of time the coagulation and acid-base equilibrium were maintained in the recipient, although a low albumin concentration was present. Also, in several occasions an extracorporeal pig liver has been used to support patients undergoing an acute hepatic failure until a human liver was available for transplantation. The conclusion of all these clinical and pre-clinical studies is that pig livers can support baboon or human life for a period of time that does not exceed one week. Therefore, they can be used in acute liver failures before a human liver is available. However, it is not known what may happen in the long term.

5.4.2.4. Conclusion

In summary, the impact of physiological changes after solid organ xenotransplantation, particularly when pigs are used as source of organs is not well understood. The information is limited to heart and kidney transplantation where an average survival time of around 20 and 40 days, respectively, has been achieved. In the specific case of kidney xenotransplantation a persistent anaemia and hypophosphatemia, which can be reversed with suitable treatments, has already been observed. However, it is necessary to obtain longer xenotransplant survival times through an improvement of the immunosuppression strategies before any conclusion can be made in this particular subject.

6. PRECLINICAL ACTIVITIES

At present, two main sources for xenotransplantation are under discussion: transgenic pigs and non-human primates. The main advantages and disadvantages of each source are described in Tab: 4²⁰. In both cases microorganisms may be transmitted from the source animal to the transplant recipient or to the perfusion patient (xenozoonosis, xenosis).

Table 4: Advantages and disadvantages of different animal sources for xenotransplantation in humans

	Pigs	Non-human primates	
		Chimpanzees	Old World Primates
Physiology	similar	nearly identical	Similar
Transplant rejection	very strong	not very strong	Strong
gal a 1,3 gal^A	Yes	no	No
Animal protection	yes	yes, very strong	Yes
Size of organs	similar	similar	too small
Posture	horizontal	upright	Upright
Time of gestation	100 days	251-289 days	170-193 days
Number of progeny	10 – 18	1, rarely 2	1, rarely 2
Availability	unlimited	none	Low
Costs	low	very high	High
Specific Pathogen Free containment	possible	possible in future at very high costs	possible in future at high costs
Cloning	possible	possible - if at all - in far future	possible in far future

6.1. The use of non-human primates in Xenotransplantation

The advantage of non-human primates as source animals is their genetic relatedness and the close similarity to humans with regard to their anatomy, physiology, immune system, hormones and enzymes (Tab: 5²¹). However, this relatedness also implies that microorganisms infecting non-human primates may more easily adapt to humans, for example due to the presence of related receptor molecules. Like humans, non-human primates do not carry galactosyl α 1,3 galactosyl (gal α 1,3 gal) epitopes on the surface of their cells, whereas pigs do. Gal- α 1,3-gal epitopes are the main reason for the hyperacute rejection (HAR) of pig organs. Preformed natural antibodies, originally produced against gal- α 1,3-gal epitopes on bacteria, bind to these epitopes on the cell surface of the pig transplant, interact with complement factors and destroy the organ in a very short time. HAR can be overcome using transgenic pigs expressing human regulators of complement activation such as the decay accelerating factor (DAF) or the membrane cofactor protein (MCP)²². Since non-human primates, like humans, do not express gal- α 1,3-gal epitopes, HAR does not take place when transplanting their organs into humans.

Table 5: Index of dissimilarity (ID) established on the basis of albumin evolution²³

Species	ID
Primates	
Man	1.00
Gorilla	1.09
Chimpanzee	1.14
Orangutan	1.22
Baboon	2.23
Pig	> 35.00

However, there are a number of negative aspects concerning the use of non-human primates as source animals for xenotransplantation including the ethical and welfare implications of such use, their limited availability and the infectious risks. (Ethical and welfare issues are dealt with in the following sections and only the latter two points are considered here.)

The numbers and species of suitable non-human primates available is limited. Indeed, some species are endangered, with great apes in particular having additional protection in law. Availability is further affected by the long gestation time and low numbers of progeny and these factors have to be considered from both a practical and animal welfare perspective when planning specific pathogen free (SPF) containment of non-human primates for medical research and its application. The infectious risks, combined with the problems of maintaining these animals in SPF conditions in order to reduce such risks, is probably the main reason why non-human primates are currently not considered as an acceptable source of xenotransplants.

6.1.1. Microorganisms of non-human primates (with exception of retroviruses) and their transmission to humans

Numerous pathogenic bacteria, viruses, fungi and parasites have been described in non-human primates. Some of these have been shown to be transmissible to humans and to even induce diseases (Tab: 6).

Table 6: Selected microorganisms from non-human primates (with exception of retroviruses) shown to infect humans

Microorganism	Non-human primate	Human disease	Disease in the non-human primate
Viruses			
Herpesvirus simae (B virus)	Macaca spp.	paralysis, fatal	usually inapparent
Monkeypoxvirus	Macaca mulatta, M. fascicularis, Cercopithecus hamlyni, Hylobates lar, Pan troglodytes	benign papules	fever, pox-like exanthema, frequently fatal
Yaba-Virus (Poxgrup)	Macaca spp.	benign, pseudotumors, fever	pseudotumors (histiocyomas)
Yaba-like virus (Poxgrup)	Macaca mulatta, M. fascicularis	benign, pseudotumors, high fever	histiocytic proliferation
Or-Te-Ca-Virus	Macaca sp.	benign cutaneous lesions	Pock-like lesions
SV 5 (Myxovirusgrup)	Macaca mulatta	antibody formation	respiratory symptoms
Yellow fever virus	Colobus sp., Cercopithecus spp., Saimiri sp., Cebus spp.	mild to severe disease, haemorrhages	fatal disease in New World monkeys, haemorrhages
SV 40 (Papovagrup)	Macaca mulatta, M. fascicularis, Chlorocebus aethiops	antibody formation (oncogenic in hamsters)	none
Poliomyelitis virus	Pan troglodytes, Gorilla gorilla	poliomyelitis	anorexia, occasionally fatal, encephalomyelitis
Marburg disease virus (Filogrup) Ebola virus (Filogrup)	Chlorocebus aethiops, Pan troglodytes	haemorrhagic fever, fatal	febrile illness, occasionally fatal, splenomegaly, haemorrhages
Hepatitis virus	Pan troglodytes, Hylobates sp., Gorilla gorilla	hepatitis, occasionally fatal	usually inapparent, antibody formation, in P. troglodytes occasionally mild disease, diarrhoea

Microorganism	Non-human primate	Human disease	Disease in the non-human primate
Bacteria			
Shigella spp.	all non-human primates susceptible	diarrhoea, haemorrhagic enteritis, sometimes fatal	from inapparent infection to fatal haemorrhagic diarrhoea
Salmonella spp.	all species susceptible	fever, diarrhoea	from inapparent infection to fatal haemorrhagic diarrhoea
Leptospira haemorrhagae	Pan troglodytes, Papio sp., Macaca spp.	Weils's disease, occasionally death from renal failure	usually inapparent, antibody formation, occasionally jaundice
Mycobacterium tuberculosis, Mycobacterium bovis	Macaca mulatta, all Old World, rare New World monkeys	respiratory disease, subacute or chronic disease	fatal disease
Dermatophilus congolensis	Aotus trivirgatus, Lagotrix lagotricha	dermatitis, furunculosis	dermatitis
Fungi			
Microsporium canis	Macaca mulatta, Pan troglodytes, Hylobates lart, Gorilla gorilla	eczema	eczema
Parasites			
Trypanosoma brucei	Cercopithecus sp.	sleeping sickness	unknown
Plasmodium schwetzi	Pan troglodytes Gorilla gorilla	tertian malaria	asymptomatic, occasionally fever
Plasmodium brasilium	Aotus spp., Callitrix spp.	anorexia, headache	asymptomatic, occasionally anaemia, fever
Plasmodium cynomolgi	Macaca fascicularis	tertian malaria	asymptomatic tertian malaria
Plasmodium knowlesi	Macaca fascicularis	quotidian malaria	experimentally jaundice
Plasmodium Inui inui	Macaca fascicularis	quartan malaria	asymptomatic, occasionally fever
Plasmodium simium	Callitrix sp.,	tertian malaria	asymptomatic
Plasmodium catneyi	Macaca spp.	quartan malaria	asymptomatic, occasionally fever

According to the OIE (Office International des Epizooties) the risk of carrying zoonotic pathogens is related to the taxonomic position and increases from prosimians through New World primates to Old World primates and finally to chimpanzees²⁴. The OIE recommends that animals imported from uncontrolled environments should be held under quarantine for 12 weeks and that several tests and treatments should be carried out (Tab: 7). In addition, the OIE underlines the public health importance of other zoonoses such as measles, hepatitis A, monkey pox, Marburg and Ebola viruses, as well as herpes viruses. For the use of non-human primates for xenotransplantation, these tests should be obligatory (see below).

Table 7: Tests and treatments required by the OIE for non-human primates from an uncontrolled environment²⁵

Disease/Agent	Animal groups	Schedule	Methods
Hepatitis B	Gibbons and apes	First testing during first week, second test after 3 or 4 weeks	Serological tests
Tuberculosis (Mycobacterium hominis and M. bovis)	Marmosets and tamarins	Two tests at an interval of 2 to 4 weeks	Skin tests or serology
	Prosimians, New World monkeys, Old World monkeys, gibbons, apes	At least three tests at intervals of 2 to 4 weeks	
Other bacterial pathogens (Salmonella, Shigella, Yersinia and others)	All species	Daily test for 3 days within the first 5 days after arrival, 1 or 2 more tests at interval of 2 to 4 weeks	Faecal culture
Endo- and ectoparasites	All species	At least 2 tests, 1 at the start, the other towards the end of the quarantine	Testing methods and antiparasite treatment as appropriate to species

Filoviruses such as the Ebola virus and the Marburg disease virus as well as herpesviruses such as the B virus have been repeatedly transmitted from non-human primates to humans where they often induced fatal diseases (Tab. 8).

Table 8: Human infections linked to contact with non-human primates²⁶

Virus	Number of human cases
B virus	40
Marburg	35
Ebola	42
Simian immunodeficiency virus	2
Simian foamy virus	3
Monkeypox	1

Several other herpesviruses and hepatitis viruses have been described in primates which may also be transmitted to humans (Tab. 9 and Tab. 10).

Table 9: Herpesviruses of humans and non-human primates^{27,28,29,30,31,32}.

Scientific name	Common name	Host	Disease
a-Herpesviruses			
Human herpesvirus 1, 2 (HHV-1, HHV-2)	Herpes simplex types 1 and 2	Humans	Self-limiting mucocutaneous vesicles
		Gibbons	Self-limiting vesicles or encephalitis
		Owl monkeys, marmosets	Fatal infection
Saimirine herpesvirus 1	Herpes T	Squirrel monkey	Self-limiting mucocutaneous vesicles
Cercopithecine herpesvirus 1	Herpes simiae, B virus	Macaques	Self-limiting mucocutaneous vesicles
		Humans	Fatal encephalomyelitis
Cercopithecine herpesvirus 2	SA 8	African green monkey (<i>Chlorocebus aethiops</i>) Baboon	Myelitis latent
Human herpesvirus 3 (HHV-3)	Herpes varicella, Herpes zoster	Humans Great apes	Chicken pox, shingles Chicken pox
Cercopithecine herpesvirus 6, 7, 9	Simian varicella	African green monkey (<i>Chlorocebus aethiops</i>), Macaque (<i>Macaca nemestrina</i> , <i>Macaca fascicularis</i>)	Chicken pox-like disease
Ateline herpesvirus 1	Spider monkey herpesvirus	Spider monkey	Usually latent, may cause fatal infection
b-Herpesviruses			
Human herpesvirus 5 (HHV-5)	Cytomegalovirus (CMV)	Humans	Cytomegalic inclusion body disease
Aotine herpesvirus 1, 3, 4	Herpes aotus types 1, 3, 4	Owl monkey	Cytomegalic inclusion body disease / usually latent
Cercopithecine herpesvirus 3	SA 6	Vervet monkey (<i>Chlorocebus aethiops pygerytrus</i>)	Cytomegalic inclusion body disease / usually latent
Human herpesvirus 7 (HHV-7)		Humans	associated with exanthem subitum, pityriasis rosea, neurological manifestations
Cercopithecine herpesvirus 4	SA 15	Vervet monkey (<i>Chlorocebus aethiops pygerytrus</i>)	Cytomegalic inclusion body disease / usually latent
Cercopithecine herpesvirus 4	African green monkey CMV	African green monkey (<i>Chlorocebus aethiops</i>)	Cytomegalic inclusion body disease / usually latent
Cercopithecine herpesvirus 5	Rhesus monkey CMV	Rhesus monkey	Cytomegalic inclusion body disease / usually latent

Scientific name	Common name	Host	Disease
Cercopithicine herpesvirus ?	Baboon CMV	Baboon	?
Cercopithicine herpesvirus ?	Baboon CMV	Baboon	?
Callitrichine herpesvirus 1, 2	Herpesvirus saguinus, marmoset CMV	Cotton-top tamarin	Cytomegalic inclusion body disease / usually latent
Cebine herpesvirus 1	Capuchin herpesvirus (AL 8)	Cebus monkey	Cytomegalic inclusion body disease / usually latent
Cebine herpesvirus 2	Capuchin herpesvirus	Cebus monkey	Cytomegalic inclusion body disease / usually latent
Human herpesvirus 6 (HHV-6)	Human B lymphotropic virus (HBLV)	Humans	Exanthem subitum
g-Herpesviruses			
Gamma 1-viruses			
Human herpesvirus 4 (HHV-4)	Epstein-Barr virus (EBV)	Humans	Infectious mononucleosis, Burkitt's lymphoma, Nasopharyngeal carcinoma
Cercopithicine herpesvirus 14	African green EBV-like	African green monkey (<i>Chlorocebus aethiops</i>)	?
other EBV-like viruses		Macaques (<i>Macaca mulatta</i> , <i>Macaca fascicularis</i>)	associated with lymphoma
	Herpesvirus gorilla	Gorilla	?
	Herpesvirus pan	Chimpanzee	?
	Herpesvirus pongo	Orangutan	?
	Herpesvirus papio	Baboon	?
2. rhadinoviruses			
Saimirine herpesvirus 2	Herpesvirus saimiri (HVS)	Squirrel monkey Owl monkey Marmosets Spider monkey	Latent infection Lymphoma, leukaemia
Ateline herpesvirus	Herpesvirus ateles (HVA)	Spider monkey Owl monkey	Latent infection Lymphoma, leukaemia
Aotine herpesvirus 2	Herpesvirus aoti type 2	Owl monkey	?
Scientific name			
Common name			
Host			
Disease			
Cercopithicine herpesvirus 10, 11	Rhesus rhadinovirus (RRV) Retroperitoneal fibromatosis herpesvirus	Rhesus monkey Macaque (<i>Macaca nemestrina</i> , <i>M. mulatta</i>)	?
Cercopithicine herpesvirus 12	Herpesvirus papio	Baboon	?
Cercopithicine herpesvirus	Chlorocebus rhadinovirus 1 (ChRV 1)	African green monkey ¹⁾	
	Chlorocebus rhadinovirus 2 (ChRV 2)	African green monkey ¹⁾	
Human herpesvirus 8 (HHV-8)	Kaposi's sarcoma-associated herpesvirus (KSHV)	Human	Kaposi's sarcoma, Castleman's disease, primary effusion lymphoma

Table 10: Infection of non-human primates with hepatitis viruses with potential significance for xenotransplantation^{33,34,35,36,37}.

Virus	Classification	Size	Genomic Structure	Natural Infection	Experimental Infection
Hepatitis A virus (enterically transmitted)	Picorna-viridae	27 nm	ssRNA	Cynomolgus macaque; Orangutan ?; Baboon; African green monkey	Old and New World monkeys: Chimpanzee; Marmoset; Owl Monkey; Tamarin
Hepatitis B virus (transmitted by the parenteral route)	Hepadna-viridae	42-47 nm (22-27 core)	dsDNA	Woolly monkey Orangutan	Old World monkeys: Chimpanzee; Gibbon; Gorilla; Orangutan; Woolly monkey
Hepatitis C virus (transmitted by the parenteral route)	Flaviviridae	30-60 nm	ssRNA		Old World monkeys: Chimpanzee; Marmoset ?
Hepatitis D virus (transmitted by the parenteral route)	Viroid, related to plant satellite virus	36 nm	ssRNA		Old World monkeys: Chimpanzee
Hepatitis E virus (enterically transmitted)	Caliciviridae	27-34 nm	ssRNA		Old and New World monkeys: Chimpanzee; Marmoset; Macaque; Owl Monkey
Hepatitis F virus	(not a hepatitis virus)	-	-	-	-
Hepatitis G virus	Flaviviridae	?	ssRNA	Marmoset; Tamarins; Owl monkey; Chimpanzee	Tamarin
TTV (associated with hepatitis)	Circoviridae (related to Circoviridae)	12-18 nm	ssDNA	Chimpanzee (Pan troglodytes verus; Pan paniscus)	Chimpanzee Bonobo

The prevalence of these viruses is very high among captive animals as well as animals in the wild (Tab: 11, Tab: 12).

Table 11: Distribution of herpesviruses in apes^{38,39,40,41,42}.

Host	Viruses or antibodies detected
Gorilla (captive)	92% anti-HSV-1 + 8% anti-HSV-2
Gorilla (captive)	Varicella zoster virus
Gorilla (mountain)	58% anti- HSV-1, HSV-2, SA 8
Chimpanzee (captive)	71% anti-HSV-2
Gibbon (captive)	100% anti-HSV-1
Orangutan (wild)	59.4% anti-B virus,
Orangutan (captive)	0% anti-HSV-1, HSV-2, SA 8, B virus

Table 12: The number of orangutans seropositive for specific viral infections⁴³

Type of virus	Number of individuals	
	Total number n=143	%
Hepatitis B virus (HBV)	85	59.4
Hepatitis A virus (HAV)	50	34.9
Herpes simplex virus 1 (HSV-1)	21	14.7
Simian retrovirus 1 (SRV-1)	16	11.2
Human T cell leukaemia virus (HTLV)	2	1.4
Human immunodeficiency virus 1 (HIV-1)	0	0
Simian immunodeficiency virus (SIV)	0	0

Clinical xenotransplantations using organs or cells from non-human primates have been performed in the past (Tab: 13). Since the cells or organs were rejected within a very short time and in most cases the patients died soon after transplantation, no reliable records of infections with microorganisms exist. However, in one case, transplantation of a baboon organ resulted in an infection of the recipient by a herpes virus⁴⁴. Generally, animals used for xenotransplantations in the past have been tested for several microorganisms, including viruses (Tab. 14).

6.1.2. Exogenous and endogenous retroviruses of non-human primates and their transmission to humans

The special interest in retroviruses is based on their ability to integrate into the genome of infected cells. Retroviruses are RNA-viruses which use a viral enzyme, the reverse transcriptase (RT) to transcribe the single-strand RNA into double-strand DNA, which will be integrated as provirus. So called exogenous retroviruses such as HIV-1 infect specific target cells and integrate into the genome of these cells. Proviruses will therefore not be found in other cells of the organism. If, however, a retrovirus infects an oocyte or a sperm cell, which gives rise to a new organism, every cell of the body will contain the integrated provirus. Such retroviruses are termed endogenous (formed within). Endogenous retroviruses may, on the one hand, be expressed as infectious virus particles, and on the other they will be transmitted like normal genes to the progeny. These viruses are present in the genomes of all mammals including those of human beings.

Primate endogenous retroviruses (PriERV) have, until now, received little attention despite the fact that retrovirus particles have been found, using electron microscopy, in the placentas of all mammals studied, including rhesus monkeys^{45,46,47}, baboons⁴⁸, chimpanzees⁴⁹ other non-human primates^{50,51} and humans^{52,53,54,55,56}. Some PriERVs are produced by normal primate cells and some, such as the baboon endogenous retrovirus (BaEV), have been shown to infect human cells^{57,58}. The situation is the same in pigs. Porcine endogenous retroviruses (PERVs) have also been shown to be produced by normal pig cells and to infect human cells^{59,60,61,62,63,64}. Whether PriERVs and PERVs are able to infect humans *in vivo* and whether they are pathogenic, is still unclear. Many retroviruses, including viruses, which are closely related to BaEV, induce tumours and immunodeficiencies^{65,66}.

Table 13: History of clinical xenotransplantation using cells or organs from non-human primates^{67,68}.

Year	Reziipients (Surgeon)	Xenotransplant	Survival
1910	1 (Unger)	monkey kidney	< 2 days
1913	1 (Schons tadt)		
1963/64	13 patients (Reemtsma)	chimpanzee (12), monkey (1) kidneys	1 case 9 months
1964	3 (Traeger)	chimpanzee kidney	< 49 days
1964	1 (Hume)	chimpanzee kidney	1 day
1964	6 (Starzl)	baboon kidney	< 60 days
1964	1 (Hitchcock)	baboon kidney	5 days
1964	1 (Hardy)	chimpanzee heart	2 hours
1965	2 (Goldsmith)	chimpanzee kidney	4 months
1966	1 (Starzl)	chimpanzee liver	< 1 day
1966	1 (Cortesini)	chimpanzee kidney	31 days
1969	2 (Starzl)	chimpanzee liver	< 9 days, < 2 days
1969	1 (Bertoye)	baboon liver	< 1 day
1969	1 (Marion)	chimpanzee heart	4 hrs
1970	1 (Leger)	baboon liver	3 days
1970	1 (Marion)	baboon liver	< 1 day
1971	1 (Poyet)	baboon liver	< 1 day
1971	1 Motin	baboon liver	3 days
1974	1 (Starzl)	chimpanzee liver	14 days
1977	1 (Barnard)	baboon heart	5 hrs
1977	1 (Barnard)	chimpanzee heart	4 days
1984	"baby Fae", born premature with malformed heart (Bailey)	baboon heart	20 days
1992	1 (Starzl)	baboon liver	70 days
1993	1 (Starzl)	baboon liver	26 days
1995	HIV-infected patient	baboon immune cells	cells died

Table 14: Evaluation of infectious diseases and infectious agents for xenotransplant baboon donors at the University of Pittsburgh, 1993^{69,70}

<p>Serology for:</p> <p>Herpesvirus: HSV-1, HSV-2, EBV, VZV, B virus, SAS, CMV</p> <p>Retrovirus: SIV, SRV-1, -2, -5, STLV-1, HIV-1, HIV-2</p> <p>Hepatitis A, B, C</p> <p>Encephalomyocarditis</p> <p>Lymphocyte choriomenengitis virus (LCM)</p> <p>Monkeypox virus</p> <p>Simian hemorrhagic fever virus</p> <p>Marburg virus</p> <p>Foamy virus</p> <p>Toxoplasma</p> <p>Periodic physical examination</p> <p>Tuberculin skin tests every 3 months</p> <p>Microbiologic cultures:</p> <p>Stool for ova, parasites and pathogenic bacteria</p> <p>Blood for pathogenic bacteria</p> <p>Buffy coat, throat swab, urine, stool for viral cultures</p>
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Numerous exogenous retroviruses have been described in non-human primates (Tab: 15).

Table 15: Exogenous retroviruses in non-human primates

	Virus	Infected animals	Disease
1	Type C retroviruses		
	STLV-1 (STLV, Simian T cell leukaemia virus)	Gorilla (Gorilla gorilla), Chimpanzee (Pan troglodytes, P. paniscus), Baboons (Papio cynocephalus, P. anubis, P. hamadryas, P. sphinx), African green monkey (Chlorocebus aethiops, C. sabaues), Patas monkeys (Erythrocebus patas), Macaques (Macaca arctoides, M. fascicularis, M. fuscata, M. mulatta, M. nigra, M. radiata)	Asymptomatic, some lymphomas, leukaemia

2	Type D retroviruses	Macaques (<i>M. fascicularis</i> from Indonesia, not from Philippines, <i>M. nemestrina</i> from Indonesia, <i>M. radiata</i> from India, <i>M. tonkeana</i> from Sulawesi, <i>M. mulatta</i> from China)	AIDS
	SRV-1 (SRV Simian retrovirus) (SAIDS/CA, /NE)		
	SRV-2 (SAIDS/WA, SAIDS/OR)	Macaques, Celebes	Retroperitoneal fibromatosis, subcutaneous fibrosarcoma
	SRV-3 (MPMV, Mason-Pfizer monkey virus)		
	SRV-4	<i>M. fascicularis</i>	
	SRV-5		
	SRV-Pc	Baboon (<i>Papio cynocephalus</i>)	Not known to be pathogenic
	Type D retrovirus	Talapoins (<i>Miopithecus</i> sp.)	
	Virus	Infected animals	Disease
3	Lentiviruses		
		Macaques (<i>Macaca</i>)	
	SIVmac (SIV, Simian immunodeficiency virus)	Rhesus monkey (<i>Macaca mulatta</i>)	AIDS (in captivity only)
	SIVmne	Pigtailed macaque (<i>M. nemestrina</i>)	AIDS (in captivity only)
	SIVstm	Stump-tailed macaque (<i>M. arctoides</i>)	? (in captivity only))
		Guenons (<i>Cercocebus</i>)	
	SIVsyk	Sykes' monkey (<i>Cercopithecus albogularis</i>)	Apathogenic*
	SIVblu	Blue monkey (<i>C. mitis</i>)	Apathogenic*
	SIVlhoest	L'Hoest monkey (<i>C. lhoesti</i>)	Apathogenic*
	SIVsun	Sun-tailed monkey (<i>C. solatus</i>)	Apathogenic*
	SIV?	Hamlyn's monkey (<i>C. hamlyni</i>)	Apathogenic*
	SIVdeb	DeBrazza monkey (<i>C. neglectus</i>)	Apathogenic*
	SIVmon	Campbell's mona (<i>C. campbelli</i>)	Apathogenic*
	SIV?	Wolf's mona (<i>C. wolffi</i>)	Apathogenic*
		African green monkeys (<i>Chlorocebus</i>)	
	SIVagmVer	Vervet monkey (<i>Chlorocebus pygerythrus</i>)	Apathogenic*
	SIVagmGri	Grivet monkey (<i>C. aethiops</i>)	Apathogenic*
	SIVagmSab	Green monkey (<i>C. sabaeus</i>)	Apathogenic*
	SIVagmTan	Tantalus monkey (<i>C. tantalus</i>)	Apathogenic*
		White-eyelid mangabeys (<i>Cercocebus</i>)	
	SIVsm	Sooty mangabey (<i>Cercocebus atys</i>)	Apathogenic*
	SIVrcm	Red-capped monkey (<i>C. torquatus</i>)	Apathogenic*
	SIVwcm	White-crowned mangabey (<i>C. torquatus lunulatus</i>)	Apathogenic*
		Talapoins (<i>Miopithecus</i>)	
	SIVtal	Angolan talapoin (<i>Miopithecus talapoin</i>)	Apathogenic*
		Black and white colobus (<i>Colobus</i>)	
	SIVcol	Mantled guereza (<i>Colobus guereza</i>)	Apathogenic*
		Mandrills (<i>Mandrillus</i>)	

	SIVmnd/SIVmnd2	Mandrill (<i>Mandrillus sphinx</i>)	Apathogenic*
	SIVdrl	Drill (<i>M. leucophaeus</i>)	Apathogenic*
		Chimpanzee (<i>Pan</i>)	
	SIVcpz(P.t.t.)	Western chimpanzee (<i>Pan troglodytes troglodytes</i>)	Apathogenic*
	SIVcpz(P.t.s.)	Eastern chimpanzee (<i>Pan troglodytes schweinfurthii</i>)	Apathogenic*
		Patas monkey (<i>Erythrocebus</i>)	
	SIVagmSab	Patas monkey (<i>Erythrocebus patas</i>)	Apathogenic*
		Baboons (<i>Papio</i>)	
	SIVagmVer	Yellow baboon (<i>Papio cynocephalus</i>)	Apathogenic*
	SIVagmVer	Chacma baboon (<i>P. ursinus</i>)	Apathogenic*
4	Spumaviruses		
	Foamyviruses	Macaques and others	Apathogenic°

* apathogenic in its natural host (see Tab.16, 17); °apathogenic in non-human primates and in humans

Meanwhile it has been suggested that the human immunodeficiency viruses HIV-1 and HIV-2^{71,72} and the that human T cell leukaemia viruses HTLV-1 and HTLV-2^{73,74,75,76} originated from non-human primate viruses (Tab: 16).

Table 16: Natural trans-species transmission of retroviruses from non-human primates^{77,78}.

Original virus	Species	Pathogenicity		Species	Virus	Pathogenicity
SIVcpz	chimpanzee (<i>Pan troglodytes</i>)	apathogenic ?	→	human (<i>Homo sapiens</i>)	HIV-1	AIDS
SIVsm	sooty mangabey (<i>Cercocebus torquatus atys</i>)	apathogenic	→ →	human rhesus monkey (<i>Macaca mulatta</i>)	HIV-2 SIVmac	AIDS AIDS
STLV-1	mandrill (<i>Mandrillus sphinx</i>), chimpanzee (<i>P. t. troglodytes</i>), baboon (<i>Papio sp.</i>), rhesus monkey (<i>M. mulatta</i>)	T-cell leukaemia	→	human	HTLV-1	T-cell-leukemia, immunodeficiency
STLV-1	chimpanzee (<i>P. t. verus</i>)	T-cell-leukaemia	→	African green monkey (<i>C. a. sabaeus</i>)	STLV-1	T-cell-leukemia
SFV	African green monkey (<i>Chlorocebus aethiop</i>), baboon (<i>Papio sp.</i>)	apathogenic	→	human	SFV	apathogenic
PriERV (endogenous)	non-human primates	apathogenic	→	cat	RD-114 (endogenous)	apathogenic
PO-I-Lu (endogenous)	langur (<i>Presbytis obscuris</i>)	apathogenic	→	rhesus monkey	SRV	AIDS

Experimental transmissions of retroviruses to different monkey species show that while not every transmission results in infection and disease, many do^{79,80,81,82} (Tab: 17).

Table 17: Experimental trans-species transmission of retroviruses from non-human primates^{83,84}

Original virus	Species	Pathogenicity		Species	Virus	Pathogenicity
SIVagm	African green monkey (<i>Chlorocebus aethiops</i>)	apathogenic	⇒	pig tailed macaques (<i>M. nemestrina</i>), rhesus monkey (<i>M. mulatta</i>), crab eating macques (<i>M. fascicularis</i>)	SIVagm	apathogenic
SIVagm9063			⇒	pig tailed macaques (<i>M. nemestrina</i>)	SIVagm9063	AIDS
SIVsyk	Syke's monkey (<i>Cercopithecus mitis</i>)	apathogenic	⇒	rhesus monkey		apathogenic
SIVsm	sooty mangabey (<i>Cercocebus atys</i>)	apathogenic	⇒	rhesus monkey (<i>M. mulatta</i>), pig tailed macques (<i>M. nemestrina</i>)	SIVmac, SIVmne	AIDS

6.1.3. Non-human primates: General considerations on microbiological safety

In order to obtain pathogen free animals for xenotransplantation, known bacteria, fungi, parasites and viruses including exogenous retroviruses may be eliminated by:

- (i) selection of uninfected animals,
- (ii) treatment with antibiotics and antiparasitical reagents or
- (iii) vaccination of the animals.

To achieve this, animals have to be tested for the presence of all microorganisms listed in Tab: 6, 9, 10, 14 and 15. At present an additional risk comes from unknown microorganisms which may not be detected, and from endogenous retroviruses, which cannot be eliminated.

In order to identify unknown microorganisms in a given species, probes may be used which are designed to detect known related agents in other species (e.g. PCR primers or nucleic acid probes for highly conserved regions of the microorganism's genome), assays may be used which detect related enzymes (e.g. reverse transcriptase in the case of retroviruses) and pathogenic effects may be measured directly in human cell cultures (Tab: 18). It is unclear whether agents inducing transmissible spongiform encephalopathies (TSE) represent a risk and the possibility that cases of Creutzfeldt-Jakob disease exist among monkeys and apes cannot be excluded⁸⁵. However, it is well known that the transplantation of organs such as kidneys, livers and hearts is not associated with a high risk of TSE transmission. In certain animals and humans, high risk was associated with transplantation of lymphoid tissues as well as of brain tissues.

Table: 18 Tests for known human-pathogenic microorganisms of non-human primates used as a source of cells, tissues and organs for xenotransplantation, and strategies for detection of unknown microorganisms

1. All tests and examinations described in Tab. 6, 7, 9, 10, and 14 (bacteria, viruses, parasites, fungi), selection of non-infected animals or adequate treatment or vaccination of the source animals.
2. In addition, tests for species-specific exogenous retroviruses (simian T cell leukaemia viruses, STLV, immunodeficiency viruses, SIVmac in the case of rhesus monkeys, SIVagm in the case of African green monkeys etc., and type D retroviruses) (Tab. 15) according to the scientific state of the art.
3. In addition, tests for new species-specific herpesviruses, e.g. HHV-8-like, and new species-specific hepatitis viruses, e.g. Hepatitis E virus, Hepatitis G virus etc. (Tab. 10) according to the scientific state of the art.
4. In addition, tests for the expression of endogenous retroviruses of the given species.
5. In order to identify unknown microorganisms, probes may be used in a given species which are designed to detect similar agents in other related species (e.g. PCR primers or nucleic acid probes for highly conserved regions of the microorganism's genome).
6. In order to identify unknown microorganisms, assays may be used which detect related enzymes (e.g. reverse transcriptase in the case of retroviruses).
7. In order to identify unknown microorganisms, unspecific pathogenic effects may be measured directly in human cell cultures.

Again, it is still unclear whether PriERVs are able to infect humans *in vivo* and whether they are pathogenic. However, if they replicate to high titers, they may induce tumours and immunodeficiency disease in the transplant recipient. Furthermore, they may adapt to humans and pose a risk for third parties (clinic personnel, relatives, close personal contacts) and for the whole of society^{86,87}.

6.1.4. Health control considerations when using non-human primates

On the basis of all the data presented here it should be concluded that xenotransplantation using cells or organs from non-human primates should not be performed at present.

The Food and Drug Administration (FDA) of the United States of America issued a “Guidance for industry: Public health issues posed by the use of non-human primate xenografts in humans” (April 1999) to address concerns regarding non-human primates as a source of xenotransplantation products. This approach was accepted by other Public Health Service (PHS) agencies including the Centres for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) and by the Department of Health and Human Services’ (DHHS) Working Group on Xenotransplantation.

The FDA came to the conclusion that current data indicate that recipients, their close contacts, and the public would be exposed to significant risk. The FDA recommends that “clinical protocols proposing the use of non-human primates as sources of xenotransplantation products should not be submitted to the FDA until sufficient scientific information exists addressing the risk posed by non-human primate xenotransplants. Consistent with FDA Investigational New Drug (IND) regulations, any protocol submission that does not adequately address these risks is subject to clinical hold (i.e., the clinical trial may not proceed).”⁸⁸

In addition, “an appropriate federal xenotransplantation advisory committee, such as the Secretary’s Advisory Committee on Xenotransplantation (SACX) of the DHHS should address novel protocols and issues raised by the use of non-human primate xenotransplants, conduct discussions, including

public discussions as appropriate, and make recommendations on the questions of whether and under what conditions the use of non-human primate xenotransplants would be appropriate in the U.S.” This was declared to be one of the possible SACX topics⁸⁹.

6.1.5. Future prospects in the use of non-human primates

Although it has largely been agreed that xenotransplantation using cells or organs from non-human primates should not be performed at present, most regulatory authorities have not gone so far as to exclude non-human primates as a source of xenotransplants totally and forever. Some consider that there are a few conditions for which the use of non-human primates may be possible in future if the various problems outlined in previous sections can be overcome (Tab: 19) and if ethical issues are properly addressed. Specific pathogen free (SPF) containment of animals, satisfactory testing for and elimination of all known human-tropic pathogens, plus carefully conducted research to enable the detection and elimination of unknown, potentially human-tropic pathogens would be required. In addition, adequate *in vitro* and *in vivo* risk evaluation has to be performed. It has to be considered that organs from monkeys are generally suitable only for children (who require smaller organs).

Table 19: Criteria to be fulfilled before xenotransplantation using non-human primate cells or organs may be performed

1. SPF (specific pathogen free) containment of the animals.
2. Tests for all known human-pathogen microorganisms (bacteria, viruses, parasites, fungi), selection of non-infected animals or adequate treatment or vaccination of the source animals.
3. Research to evaluate the potential risk by still unknown microorganisms: Development of strategies for detection of such microorganisms and design of specific tests.
4. Research to evaluate the potential risk by endogenous retroviruses using *in vitro* and *in vivo* strategies, attempts to select low virus producers, future attempts to create “knock out” animals or to develop antiretroviral substances or vaccines.
5. Improvement of transplant survival by novel drug-based (chemical) or antibody-based immunosuppression or strategies for tolerance induction in the recipient.
6. Broad scientific and public discussion whether and under what condition xenotransplantation using non-human primates may be performed, involving regulatory bodies.
7. In the case that agreement is achieved and permission is given, testing of the human recipients, their relatives and contacts (based on adequate laws) for microorganisms of non-human primates before and regularly after xenotransplantation, archiving of donor and recipient blood and tissue samples with national or international documentation.

The situation will be even more difficult should xenotransplantation using transgenic pig organs (Tab: 4) fail. The urgent need for human organs will steadily increase and the question of whether and under what conditions the use of non-human primate xenotransplants would be appropriate and acceptable will be raised again with increased urgency. The decision has to be made by scientists, regulators and the public, taking into consideration ethical and animal welfare aspects, the expensive investments into SPF containment, the results of basic research and the need to develop assays to test animals. Cloning of monkeys and apes may help reduce the risk of infection but there are additional ethical and welfare concerns associated with this technology which must be carefully addressed. Some authors argue that cloning of non-human primates may also provide concise answers to critical HIV vaccine issues and that despite the technical, welfare and ethical difficulties, the potential gains are worthy of careful consideration⁹⁰. However, due to the long gestation period

and the low number of progeny, production of non-human primates in sufficient quantities will be very time consuming and expensive. Finally, neither endogenous retroviruses nor unknown microorganisms will be eliminated by this approach.

6.1.6. Conclusion

To summarise, xenotransplantation, including *ex vivo* perfusion using cells, tissues or organs from non-human primates, should not be performed at present or in the near future. Xenotransplantation using cells, tissues or organs from non-human primates would only be feasible if it were possible to eliminate known human pathogens and to identify and eliminate as yet unknown microorganisms. A thorough evaluation of risks and the development of comprehensive monitoring strategies for transplant recipients would also be essential as would a conclusive scientific as well as public discussion of all the issues including the ethical and animal welfare concerns.

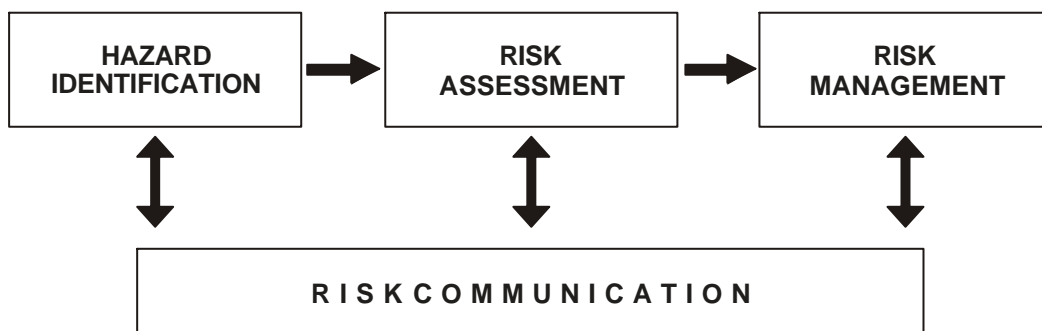
6.2. The use of Pigs in Xenotransplantation

Though pigs can be bred a lot more easily than non-human primates and their dissimilarity with human beings is greater, many of the problems relating to the use of pigs in xenotransplantation are comparable to the ones which exist for non-human primates. Thus the previous chapters concerning non-human primates can be of some assistance when considering pigs as a source of xenotransplants. However, some additional aspects on the risks arising from the use of pigs will be studied in the following sections.

6.2.1. Risk of zoonosis when using pigs

The risk analysis terminology used is as described in the OIE International Animal Health Code⁹¹ and outlined in figure 1:

Figure 1: The four components of risk analysis



- The hazard in this case is the transmission of an agent from pigs to humans;
- Risk assessment is the evaluation of the likelihood of transmission;
- Risk management is the identification and implementation of measures to reduce the level of risk;
- Risk communication is sharing information on the risk analysis with interested parties to ensure the process is transparent and defensible.

Concerns have been raised about the risk of disease transmission from animals to humans with the subsequent disease proliferation throughout the human population⁹². A response to these concerns has been to observe that when close contact with pigs existed, such as with workers in slaughterhouses and in farms where pigs were raised in close proximity to humans, there has been almost no evidence of transmission of disease from pigs to humans. As will be discussed later, there are relatively few agents that infect pigs which are a risk to humans. However, this argument is then countered with the concern that transplanting pig tissues into an immunosuppressed human is not the same as close physical contact with pigs or pig tissues⁹³. Some limited information has been published on the risk analysis of xenotransplantation^{94,95,96}. The statement has been made that the risk is currently unquantifiable⁹⁷. The risk of transmission of disease by a number of specific agents has been discussed and will be reviewed here^{98,99,100,101}.

Bacteria and parasites

Some of the zoonotic agents that have been found in pigs are:

Bordetella bronchiseptica,	Bacillus anthracis,
Erysipelothrix rhusiopathiae,	Leptospira spp.,
Mycobacterium spp.,	Chlamydia psittaci,
Pasteurella multocida,	Campylobacter spp.,
Streptococcus suis,	Listeria spp.,
Escherichia coli,	Brucella suis,
Salmonella spp.,	Toxoplasma gondi,
Trichinella spiralis.	

It has been stated that these agents, as well as other bacteria and parasites, can be controlled by the risk management methods that will be discussed later.

Viruses

The following are most of the viruses that cause disease in pigs and humans:

Rabies,	Influenza A (H1N1 and H3N2),
Nipah,	Vesicular stomatitis,
Eastern equine encephalitis,	Japanese encephalitis,
Encephalomyocarditis,	Rotavirus.

There are other viruses that have been reported to infect pigs, usually without clinical signs, which may cause disease in humans; these include:

Lymphocytic choriomeningitis,	Hanta,
Swine hepatitis E,	Human parainfluenza,
Murine parainfluenza virus (Sendai),	Other human influenza viruses,
Human adenoviruses,	Bornavirus,
Human rhinoviruses.	

The following are viruses that infect pigs but have not been shown to cause disease in humans:

Pseudorabies,	Classical swine fever,
African swine fever,	Transmissible gastroenteritis,
Foot and mouth disease,	Porcine enteroviruses,
Porcine respiratory coronavirus,	Haemagglutinating encephalomyelitis,

Porcine circoviruses,
Reproductive syndrome,
Bovine viral diarrhoea,
Swine pox.

Porcine respiratory
Porcine epidemic diarrhoea,
Border disease,

The viruses listed are believed to be the most significant, but there are additional viruses in each group which may represent a risk. It is very likely that more agents will be found as this work continues. The risk management methods that will be described are designed to control these agents.

The following agents are more difficult to address:

Porcine endogenous retrovirus (PERV),
Porcine cytomegalovirus.

Porcine parvovirus,

In a workshop on PERV which was held in August 1998 it was concluded that because the virus or provirus can be found in most tissues and in all pigs, it will be very difficult or impossible to breed pigs who do not have PERV¹⁰². Moreover, infection of human cell lines with PERV has been confirmed¹⁰³. However, blood samples from 160 humans who had been exposed to living pig tissues were tested for evidence of PERV and were all negative¹⁰⁴. Thus, additional studies are needed to determine the infectivity of PERV and the risk of transmission to humans.

It is also believed that all pig herds are infected with porcine cytomegalovirus. There are serious doubts that a colony negative for this virus can be developed or that the risk management techniques that will be described can prevent infection of source pigs with these viruses. This virus is not believed to infect humans. However, in-vitro co-cultivation studies did not show evidence of infection of human cells by porcine cytomegalovirus¹⁰⁵ though additional studies are needed with this agent. Finally, it should be mentioned that porcine parvovirus is transmitted in utero but that it has been possible to develop negative herds to this virus.

The risk of transmission of the transmissible spongiform encephalopathy (TSE) agent by xenotransplantation has been a concern. The evidence that pigs are resistant to infection is based on the fact that they were fed ruminant-derived meat and bone meal in Great Britain during the Bovine Spongiform Encephalopathy (BSE) outbreak and there have been no cases in pigs. Experimental inoculation of the brains from humans infected with Kuru, a TSE, into pigs by the intracerebral route did not produce disease¹⁰⁵. The brains from BSE-infected cattle were inoculated into ten pigs by the intracerebral route and one developed disease¹⁰⁶. It appears that pigs are refractory to infection and risk management techniques can be adopted to reduce the risk even further.

6.2.2. Risk management for xenotransplant source animals

Risk management techniques have been described in detail in the United Kingdom guidelines¹⁰⁷. The US Public Health Service developed similar guidelines in 1996¹⁰⁸. They have been described in more general terms in other publications^{109,110,111,112,113,114}. The UK guidelines discuss the requirements for production animals, which make up the breeding herd, and source animals, which will provide cells, tissues or organs for xenotransplantation. Introductions into the production herd must be limited and the herd must not include any first generation imported animals. The biosecurity level of the facilities can be less than for source animals, but facilities and handling procedures must be such that the animals are free from the agents being tested. The source animals include the dam at the time of conception through gestation.

The production of source animals under germ-free or gnotobiotic conditions has been proposed, which are hysterotomy derived and raised in isolators under positive pressure. The resulting pigs

should be free from infectious agents other than those transmitted *in utero* and those that are in the germ line, such as PERV. However, there are serious animal welfare implications to these procedures which must be balanced against the need for disease free animals. Pigs for example cannot be reared in gnotobiotic conditions and should not be reared for longer than four weeks in an isolator. The other procedure described and currently used results in the production of qualified pathogen free (QPF) source pigs. These animals are not germ free but meet a microbiological specification, defined by expert risk assessment, as being suitable for use in clinical xenotransplantation. The dams of the source pigs are hysterotomy derived and a QPF breeding colony is established and maintained under strict biosecurity. Using these procedures it should be possible to raise source pigs free from most microbiological agents that pose a threat to xenotransplantation. The risk of introducing microbiological agents can be further reduced by obtaining hysterotomy-derived source pigs, or hysterotomy-derived pigs along with medicated early weaning. However, there are animal welfare concerns with both of these procedures.

Some of the production and all of the source pigs must be raised in a biosecure facility, which will reduce the risk of contamination from the environment. All materials entering the facilities are sterilised if possible or decontaminated. All potential sources of contamination must be controlled, including air, water, feed, supplies, and personnel. The building must be sealed and operated under positive pressure with all air input filtered through HEPA filters. The design of the building must prevent entry of any vermin or insects. Complete operating procedures must be developed to ensure that containment is maintained. The feed must not contain any mammalian protein that could contain the TSE agent. Live vaccines or prophylactic antimicrobials cannot be used on source animals. An approved list of antimicrobials that may be used for treatment of superficial infections can be developed. This list takes into account the risk of development of antimicrobial resistance in the transplant clinic. If other antimicrobials must be used to treat disease, tissues from the animals cannot be used for xenotransplantation. Tissue harvesting procedures must be developed that will ensure that the tissues are not contaminated.

A complete health-monitoring programme must be developed. The screening programme for microbiological agents must be tailored to the particular facility. This will include testing of production animals, source animals and sentinel animals that are housed with the source animals. A complete post-mortem must be done on the source animal after tissues have been collected. The list of agents to be tested, the procedures that will be used and the quality assurance programme that will be followed must be developed. Standard test procedures for the common pig diseases are described in the OIE *Manual of Standards for Diagnostic Tests and Vaccines*¹¹⁵. However, test procedures for most of the other agents must be validated and standardised (which is a potential problem area).

There must also be an occupational surveillance programme developed to ensure that the humans working in the facilities do not introduce infectious agents into the source animals.

A quality assurance, tissue archiving and record keeping programme must be in place. Additional details are outlined in the publications from the UK¹¹⁶. Though the UK standards are very complete; at present no detailed international standards exist. In many countries, the responsibility to ensure that the source animals are free from microbiological agents could fall on the institution that is using the tissues, which would increase the risk.

Procedures should be developed to guarantee that the risk analysis procedure is transparent. This would include procedures to ensure that the risk is communicated. The risk assessment and risk management measures must be documented and be readily available to interested parties.

6.2.3. Conclusion

Risk management schemes have been developed that give some assurance that source animals are free from microbiological agents. However, there is still a need for additional risk assessment and refinement of the risk management procedures. It is important that the risk assessment and management procedures be transparent and be communicated. Additionally, it is important that only pig tissue be used for xenotransplantation which has been obtained using appropriate risk management techniques.

7. CULTURAL, ETHICAL AND RELIGIOUS ASPECTS OF XENOTRANSPLANTATION

In this section some of the cultural, ethical, animal welfare and religious issues raised by xenotransplantation are examined. In particular surveys of attitudes to xenotransplantation in different countries including Australia, Canada, United States, France, Germany, Sweden, and the United Kingdom are studied. The recognition and understanding of these attitudes is an important factor in assessing the public's attitude towards xenotransplantation.

National policies towards xenotransplantation in the United Kingdom, the United States of America, Switzerland, France, Canada, Spain, the Netherlands, and Sweden are considered. The development of such policies has usually included extensive consultation with the community, ranging from public meetings to various pressure groups and advisory bodies. These policy statements offer insight into the wider cultural attitudes and views regarding xenotransplantation as a technology.

Finally, the ethical, animal welfare, and religious issues involved in xenotransplantation are outlined to offer an overall framework for the evaluation of xenotransplantation and the public's response to it.

7.1. Culture

It is hard both to define the nature and content of culture and to assess the cultural attitudes to xenotransplantation. Culture usually refers to the whole way of life of a given society including the mutual, intellectual, ethnic, moral, artistic and spiritual aspects. We are all part of a number of different, often competing cultures, depending on context, background, family, education, experiences and work. In this context it may also be possible to distinguish between the content of such cultures and the carriers of culture and cultural values.

In relation to xenotransplantation, a positive public opinion will be crucial if the technology is to become an accepted part of medical practice. This necessarily involves reflection on moral and cultural concerns raised by xenotransplantation.

The actual content of cultural values relates to a whole spectrum of attitudes towards science, technology, medicine and death. While most people are generally all too ready to accept the benefits of modern science and medicine, there is a growing concern relating to the risks and dangers accompanying biomedical discoveries. Bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease (CJD) and the on-going anxiety over food safety and genetically modified (GM) food have created an atmosphere of distrust of science and scientists. There is a fear that no one is really 'in control' or 'knows what will happen' and doubts exist that government controls will ensure, in this case, food safety without long term negative consequences.

The Environmental and Green lobbies have been particularly effective in harnessing public disquiet and building up the force of the precautionary principle – that we should not introduce new technologies or applied science until and unless we can be sure of the consequences. There is a deep concern about the nature and extent of risks. The more detailed the framework for surveillance of any xenotransplantation experimental procedures, the more people perceive xenotransplantation as a high risk activity and question the wisdom of pursuing such a procedure.

In addition there is a strong interest in and concern about animal rights and animal welfare. Groups concerned to protect the interests of animals and the successful use of publicity by some vocal activists will gather media attention and influence public opinion. Indeed, any group vocally concerned about the issues raised by xenotransplantation (whether for or against the technology)

will play a key role in the battle for public opinion and media attention. Different cultural groups within Europe will reflect different specific attitudes towards science, technology and animals.

Reports have focussed attention on genetic modification in general whether this is of plants, animals, or humans. There is often a negative emotional reaction, frequently referred to as the 'yuck' factor. The potential benefits of xenotransplantation to patients, the continuing shortage of organs in spite of various attempts to increase the number of human organs available and the sensitivities of people in life and death situations, are all part of the wider cultural perspectives that need to be understood when discussing the implications of xenotransplantation.

Underlying these points are the differing cultural attitudes to death and the dying, and to postponing the death of oneself and one's nearest and dearest. Modern medicine in the West is often seen as able to postpone death and enable a good quality of life to be maintained almost indefinitely. Yet many people also fear a 'living death' situation where they are kept alive on machines with little or no quality of life.

It is also a matter of public concern that financial motives may be a key driving force. There is a fear that profit is driving the move to xenotransplantation rather than the genuine benefit or needs of patients. The initial investment that industry needs to make to develop this technology means that there will be little short-term financial benefit, but if it is successful and given the world-wide prospects, the financial returns should be enormous.

Other concerns relate to the fear that some pharmaceutical companies may move their research and experimentation premises to countries where regulations are more permissive.

In the light of the above issues, therefore, the debate over xenotransplantation and the cultural attitudes towards science, technology, medicine and death will be crucial. If the technology is to be accepted and used, there are various questions that must be answered. These include demonstrating that:

- there are no appropriate alternative options which are available;
- there is a genuine medical need for xenotransplantation;
- the technology is efficacious;
- the highest possible levels of safety for patients and the wider human population can be guaranteed;
- all issues of animal husbandry, care, welfare and use can be properly dealt with; and
- there are real benefits for patients, families and society and not just for the commercial companies who stand to make a profit from this procedure.

This provides an agenda for research into cultural attitudes, which needs to be undertaken.

The level of public understanding and the role of the various media in creating a genuine dialogue is critical. Thought needs to be given to the fora and conduct of the public debate as well as by whom and on what basis it will be delivered. For all involved in the xenotransplantation debate, it is vital to examine the grounds in favour of and in opposition to xenotransplantation.

7.2. Attitudes

The following are summaries of surveys on attitudes towards xenotransplantation in Australia, Canada, France, Germany, the United Kingdom, Sweden and the USA.

Australia

The survey was undertaken in Sydney, Australia, on 133 patients – 58 on haem-dialysis, 31 on peritoneal dialysis and 24 who had received human transplants¹¹⁷.

Table 20: Results Australia

	Disagree %	Neutral %	Agree %
If a close relative died, I would agree to the donation of organs	12.4	3.5	81.4
I would accept an organ from a living relative	29.2	8.8	61.1
I would accept an organ from genetically unrelated, but living person e.g. spouse	26.5	11.5	41.6
I would accept an organ from an animal closely related to man e.g. baboon, chimp	45.1	11.5	41.6
I would accept an organ from a species distant to man e.g. pig or sheep	44.2	13.2	41.6
It is appropriate to breed animals to provide organs for transplant	33.6	17.7	47.8

Canada

The survey was administered for Health Canada's Therapeutics Products Programme.¹¹⁸ It surveyed some 2526 Canadians 15 years and older and considered seven questions: two on transplantation and five on xenotransplantation.

Table: 21 Results Canada

Question	% Yes			
	Total	Male	Female	
1. Have you indicated that you are willing to donate an organ or tissue for transplant upon death (for example, on your drivers' license, health insurance card, or notifying your next of kin)?	52%	52%	52%	
2. (If answer was NO in #1) Would you be willing to donate an organ or tissue for transplant upon death?	49%	50%	48%	
3. Have you read or heard about medical researchers proposing to use animal organs for transplant into humans, or are you not sure if you've heard about this?	75%	76%	74%	
4. Have you heard that one of the risks of animal-to-human transplants is the possibility that an unknown and new disease might be transmitted from the animal organ to the person receiving the transplant, or are you not sure if you've heard about this risk?	45%	47%	42%	
5. (If answer was YES in #4) Have you heard about the possibility that, if the person receiving the transplant is infected by a new disease, there is a risk that people who come into contact with that person might also become infected and sick, or are you not sure if you've heard about this risk?	41%	44%	38%	
6. If a human organ were not available, would you consider an animal-to-human transplant for yourself or a member of your family?	54%	63%	45%	
7. In view of the fact that animal-to-human transplants may pose a risk to the general population, what role would you personally want to play in decisions about the acceptability of carrying out this procedure in Canada?	Kept informed	Involved in meetings	Invited to comment	No role
	62%	24%	22%	12%

Those most likely to be aware of xenotransplantation were seniors (87%) and university graduates (85%). By comparison, awareness was lower than average among unskilled workers (61%) and high school and university students (62%).

Based on their current knowledge of potential risks, about half the respondents said they would consider an animal to human transplant for themselves or a member of their family if a human organ were not available. Slightly more than a third (38%) said "Yes" unequivocally and another 16% indicated conditional acceptance. Over a third (39%) said they would refuse a xenotransplant and 8% were undecided. Women (55%) were more likely to say they would refuse a xenotransplant than men (37%).

United States

The following contains the results of two surveys of attitudes towards xenotransplantation. The first survey was conducted by the National Kidney Foundation and polled 1200 randomly selected individuals¹¹⁹. The second by a team from the St. Vincent Medical Centre, Los Angeles California, who surveyed 100 patients regarding their attitudes¹²⁰.

National Kidney Foundation:

Nearly all those surveyed (94%) were aware of the shortage of organs for transplantation and most (62%) accept the concept of xenotransplantation as a viable option. Support for xenotransplantation was with some reservations. Respondents reported concerns over organ compatibility, success rate, and cross-species contamination.

St. Vincent Medical Centre:

Respondents included 65 men, and 35 women. Their ages ranged from 17 to 74 years old and their racial composition was 72 whites, 18 Hispanics, 5 African Americans, and 4 Asian Americans.

Some 80% of patients agreed with xenotransplantation in an emergency situation and ten patients stated they would not accept xenotransplants under any circumstances. In descending order, patients preferred the following organ sources: human (96%), monkey (44%), mechanical (43%), pig (42%), or dog (34%). Twenty-four patients thought that a xenotransplant would change their appearance, personality and eating or sexual habits.

The survey also elicited religious and ethical viewpoints. However, these did not appear to differ on the basis of religion, although within religious groups the cultural or ethnic background did at times seem to play a role in the reasons some animals were viewed as acceptable or unacceptable to donors. For example, Catholics of Mexican-American origin felt that dogs and pigs had poor hygiene and were therefore not acceptable donors.

France

This survey was undertaken in response to a previously published Australian survey (see above)¹²¹. It looked at the attitudes to xenotransplantation by a number of different groups: physicians, nurses, technicians and students. The survey team had a 97.1% response rate, respondents were given full background material on xenotransplantation and those who were unsure were scored as a no. The survey concluded that the more information was given about xenotransplantation, the more acceptable the procedure became.

Table: 22 Results France

	Physicians n=91	Nurses n=128	Technicians n=85	Students n=321
Mean age	42.1	36.7	38.0	23.3
Male	65.9	15.9	29.5	34.6
Believing in God	56.5	59.2	53.7	53.9
Involved in transplantation	41.8	39.1	47.7	0
Definition of xenotransplantation given to participants				
Wish to continue research	94.3	87.2	92.6	90.5
Approve of xenotransplantation	73.1	73.8	68.0	80.3
Accept xenografts in any circumstance	54.9	33.9	41.3	48.3
Only in life or death	69.2	60.8	68.4	72.1
Despite infection risk	42.0	28.9	40.7	23.1
Information given on theoretical infectious risk				
Accept xenografts	85.4	72.2	76.6	74.6
Of all organs and tissues	74.1	71.4	73.9	87.7
Only if vital risk	57.5	50.8	56.4	52.1
Not matter of life of death but handicap	56.8	71.4	71.3	67.5
Information given on choice of pig as source				
Support xenotransplantation	88.1	74.8	85.1	82.3

Germany

The survey was undertaken to assess attitudes towards xenogeneic compared to allogenic organ transplantation.¹²² Detailed questionnaires were given to 1049 patients who either had received transplants or were on waiting lists for various organs. The survey indicates that 77% of patients would accept xenotransplants while 7% would refuse them if results were similar to allotransplantation. If xenotransplantation were associated with increased risks due to more intensive medication 58% would still accept them. Acceptance of xenotransplants was significantly higher in patients who had received transplants and among males. Age, religion, waiting time, and type of organ were not found to influence acceptance rates. Xenotransplants were thought to be associated with considerable or severe emotional stress by 23% of patients, versus 3% for allotransplants. The pig was the preferred source animal and therapeutic genetic manipulation for improved results was accepted by 84%.

Table 23: Results Germany

I would accept a xenograft if transplanted with a similar success as a human graft:	Waiting List Patients (n=327)	Transplanted Patients (n=722)
Yes	55%	53%
Yes, in an urgent situation	16%	30%
Don't know	17%	12%
No	12%	5%
I would accept a xenograft even in case of more immunosuppression and related side effects:	Waiting List Patients (n=327)	Transplanted Patients (n=722)
Yes	12%	10%
Yes, in an urgent situation	26%	57%
Don't know	19%	16%
No	43%	17%
Knowledge about the following characteristics of a transplanted organ would cause severe or considerable emotional stress:	All Patients (n=1049)	
Human graft, donor of opposite sex	1%	
Human graft, donor with criminal history	13%	
Human graft, donor age >65 years	23%	
Xenograft	23%	
I have major concerns about xenotransplantation because of:	All Patients (n=1049)	
Inferior function	60%	
Disease transmission	52%	
Emotional stress	24%	
Animal rights	15%	
Personality change	15%	
Religious reasons	5%	

Sweden

The Department of Public Health and Caring Science/Social Medicine conducted a study on the Swedish people's attitudes concerning the transplantation of organs and tissues from different sources¹²³.

A random sample of 1500 inhabitants, 18-70 years old, in the county of Uppsala, Sweden were sent a questionnaire asking about their opinion on transplantation and transplantation issues. The response rate was 71%. Organs from living donors were preferred (77%), then organs from deceased donors (69%), then artificial organs (63%), and last animal organs (40%).

The United Kingdom

The survey was undertaken by the British Kidney Association¹²⁴. They asked 850 patients known to them how they would respond and why to the offer of a xenotransplant. Respondents were given a full explanation of the procedure of xenotransplantation – the source animal being a transgenic pig. The results were as follows.

- 663 (78%) willing to receive pig kidney
- 144 (17%) were not
- 43 (5%) unsure
- Reasons against included religion and the breeding of pigs specifically as a source of xenotransplants.

7.3. National Policies

Public policies reflect how individual national communities have attempted to address the various cultural, ethical and religious issues that are raised by xenotransplantation, for example issues with respect to the use of animals, safety and risk, and human beings in research. In particular, one issue that is addressed consistently is whether it is permissible to use non-human primates as organ sources for xenotransplantation.

Public policies also reflect cultural attitudes, especially where public consultation has been undertaken.

The following are a synopsis of the national policies of 8 nations: the United Kingdom (UK), the United States (USA), Switzerland, France, Canada, Spain, the Netherlands, and Sweden.

The United Kingdom¹²⁵ (last update: 4 September 2002)

Xenotransplantation is regulated by the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) which is responsible for the development, and implementation of xenotransplantation in the UK. It has produced a number of policy documents including:

- *Draft Guidance Notes on Biosecurity Considerations in Relation to Xenotransplantation*¹²⁶;
- *Draft Report of the Infection Surveillance Steering Group of the UKXIRA*¹²⁷;
- *Guidance on Making Proposals to Conduct Xenotransplantation on Human Subjects*¹²⁸;
- *Infection Risks in Xenotransplantation*¹²⁹.

The Authority met for the first time in 1997 under the chairmanship of Lord Habgood of Calverton. It currently meets several times a year and produces an annual report. Its purpose is to advise the Secretaries of State of the UK Health Departments on what is necessary to regulate xenotransplantation and monitor world-wide developments in xenotransplantation. In particular it advises:

- On safety, efficacy and considerations of animal welfare in liaison with the Home Office, and any other pre-conditions for xenotransplantation for human use, and whether these have been met;
- On research required to assess safety and efficacy factors in xenotransplantation procedures;
- On the acceptability of specific applications to proceed with xenotransplantation on humans; and
- To provide a focal point on xenotransplantation within Government¹³⁰.

Any body or organisation in the UK proposing to do clinical xenotransplantation trials must gain approval from UKXIRA, as well as an independent research ethics committee, and comply with all relevant legislation and regulations such as the Animals (Scientific Procedures) Act 1986, Genetically Modified Organisms regulations, Medicines Act and Related EEC Directives, and EEC Medical Devices Directives.

UKXIRA was established on the recommendation of the Advisory Group on the Ethics of Xenotransplantation. This committee was established in 1996 under the Chairmanship of Professor Ian Kennedy and produced a report *Animal Tissues into Humans*¹³¹ which concluded that xenotransplantation using pigs was ethically acceptable provided certain criteria concerning safety, efficacy and animal welfare could be met. The assessment of the ethical acceptability was regarded as an ongoing rather than a single event and it was suggested that the situation should be subject to regular review.

An independent UK body, the Nuffield Council on Bioethics, also published a report on xenotransplantation: *Animal to Human Transplants: the ethics of xenotransplantation*, which came to similar conclusions¹³².

The UK government's policy on xenotransplantation can be summarised as follows:

Xenotransplantation using pigs as source animals is regarded as acceptable provided issues of safety, efficacy in addition to animal husbandry, care and welfare are adequately addressed and matters relating to privacy, consent and patient surveillance are taken into account. UKXIRA have stated there is a "presumption" against the use of organs from non-human primates. The Authority has developed guidelines on surveillance, infectious risks and biosecurity requirements and a pro-forma form for applicants who want to proceed to clinical trials but, as yet, no such applications have been successful.

***The United States of America*¹³³ (last update: 13 September 2001)**

In 1996 the US Department of Health and Human Services (DHSS) formed an interagency Committee on Xenotransplantation. The committee had representatives from the National Institutes of Health (NIH), the Centres for Disease Control and Prevention (CDC), the Health Resources and Services Administration (HRSA) and the Food and Drug Administration (FDA). Its full working title is the *DHHS Interagency Working Group on Xenotransplantation*.

The FDA is the sole agency responsible for regulating all clinical xenotransplantation in the United States, however the Working Group's purpose is to develop a unified approach to xenotransplantation and to develop policy for the US Secretary of Health and Human Services.

In January 2001, the U.S. Public Health Service published the *Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation*. The Guideline had been published in draft form for public comment in September 1996. It was a product of the Department of Health and Human Services Interagency Working Group on Xenotransplantation which is composed of representatives from the Food and Drug Administration (FDA), National Institutes of Health (NIH), the Centres for Disease Control and Prevention (CDC), the Health Resource Services Administration (HRSA), and staff from the Office of the Assistant Secretary for Planning and Evaluation (OASPE). This document applies established procedures for infectious disease control to xenotransplantation and is aimed at minimising the risks to the public of human disease due to known and new diseases arising from xenotransplantation. It suggests safety measures for the procurement, screening and use of xenotransplantation products as well as clinical care requirements for recipients. It recommends maintaining systematic health records and storage of designated biological specimens from both the source animal and the patient in the event of a public health investigation. The Guideline reiterates an FDA position that nonhuman primates, because of un-addressed safety concerns, should not be used as source animals for xenotransplantation at the current time. FDA has published several guidance documents with recommendations for sponsors of xenotransplantation clinical trials. These documents, which can be found on the internet at <http://www.fda.gov/cber/xap/xap.htm> are still in draft form. They are consistent with the PHS Guideline on Infectious Disease Issues in Xenotransplantation and offer additional recommendations regarding product issues, good manufacturing practices and blood donor deferral in xenotransplantation. Currently limited clinical trials in xenotransplantation are not in progress under FDA regulation.

*Switzerland*¹³⁴ (last update: 1 July 2002)

Parliament has adopted the Federal Decree on the Control of Blood, Blood Products, and Transplants together with an order on xenotransplantation which came into force on the 1st of July 2001^{135,136}.

A decree has the same power as a law but is limited in time (in this case until 31 December 2005). Xenotransplantation is under the authorisation of the Federal Office of Public Health. After the year 2004 (approx.) the federal law on transplantation will also cover xenotransplantation and replace the Federal Decree¹³⁷.

The debate in Switzerland over xenotransplantation has given rise to two technology assessment studies on xenotransplantation. The studies were carried out by the Swiss Technology Assessment Programme under the authority of the Swiss Science Council. It examined clinico-scientific, social, ethical, economic and legal aspects of xenotransplantation as well as assessing the opportunities and risks for those involved in, and affected by, xenotransplantation¹³⁸. Within the first report of this programme which was focussing on xenogenic organ transplants, it was noted that approval for xenotransplantation depended upon the observance of a number of criteria:

- Minimising the risk of infection;
- Resolving the problem of hyper-acute and delayed rejection;
- Guaranteeing the dignity, personality and health of the individual;
- Guaranteeing the protection of animals; and
- Defining the framework (organisational, legal, ethical and social) within which xenotransplantation may take place¹³⁹.

The report of the Assessment programme does not address issues such as biosecurity, surveillance and whether it would be permissible to use non-human primates.

While the first study came to the conclusion that the moment for (organ-)xenotransplantation had not yet arrive since there were too many unanswered scientific and ethical questions, the second and later study¹⁴⁰, focussing on cellular xenotransplantation, concluded that under very restrictive objectives and controlled by governmental authorisation, clinical studies could be envisaged. Basically the risks of xenotransplantation are not different between organs and cells, but for cells there are some strategies (such as encapsulation) which allow to overcome the risks.

France¹⁴¹ (last update: 1 August 2000)

In 1995 the French national transplantation agency, “*Établissement français des greffes*” formed an expert committee on xenotransplantation. This committee produced its first draft document entitled “*Good Practice Guidelines for the Production of Pigs*” in 1996.

In 1998 the French Parliament adopted a draft law on new Health and Safety Regulations¹⁴², which includes a statement on xenotransplantation. It states that research on xenotransplantation will be regulated by existing biomedical research legislation. Following assessment of the applications for clinical trials, the research will need the approval of the Ministry of Health and a newly formed health safety agency, “*Agence française de sécurité sanitaire des produits de santé*” and by the “*Établissement français des greffes*”. Approval of clinical trials is contingent upon the establishment of a national mechanism for long-term epidemiological surveillance.

The French National Consultative Ethics Committee for Health and Life Sciences has produced a report *Ethics and Xenotransplantation*¹⁴³. The general conclusions of the report are as follows: Xenotransplantation is acceptable, in principle, however the report notes that the views of those who consider all life of equal value (thus making animal sacrifice for human survival unacceptable) must be respected. It reports that the questions relating to the risks of xenotransplantation have yet to be ascertained. It notes that any new technique involves risks and that clinical experiments should only begin once the risks have been evaluated and compared to the expected benefits - in conjunction with the consent of fully informed patients.

Canada¹⁴⁴ (last update: 1 August 2000)

Health Canada’s Therapeutic Products Programme (TPP) is responsible for establishing regulatory policy to address the potential use of xenotransplants for transplantation. To date (April 2000), no proposals for clinical trials involving xenotransplantation have yet been submitted to the TPP.

Xenotransplants and therefore xenotransplantation are also subject to the requirements of Canada’s Food and Drugs Act and Regulations.

TPP has supported several consultative and communication actions in order to address the important and complex ethical, legal, social and cultural issues raised by xenotransplantation. These include a National Forum on Xenotransplantation in 1997 and consequent report, and a website to disseminate information. Health Canada has also declared that it will continue to involve Canadians in order to develop public policy relevant to xenotransplantation.

Proposed policy on xenotransplantation has been published in the *Proposed Canadian Standard for Xenotransplantation*, Draft #14: July 1999. This document describes a regulatory framework for the implementation of xenotransplantation, however it notes: “Unlike other areas of clinical practice, xenotransplantation presents issues of ethics which have not been agreed upon by public participation and consultation. It is therefore deemed essential that these aspects be presented to the public for their input. The public are thereby invited to participate in the continuing consideration of these issues.”¹⁴⁵

The Standard also proposes that recipients and their close contacts be monitored, autopsied, and that they never donate blood products, tissues, gametes and other body parts, nor engage in unprotected sex.

***Spain*¹⁴⁶ (last update: 1 August 2000)**

The Permanent Committee on Transplantation of the Inter-territorial Council of the Spanish National Health System in 1997 approved a proposal to form a sub-committee on xenotransplantation. The Ministry of Health appointed experts from different backgrounds to consider within the sub-committee a broad spectrum of issues raised by xenotransplantation. The functions of the sub-committee are summarised below:

- review and monitor research projects involving non-human primates or/and humans;
- review progress in xenotransplantation research;
- develop recommendations for the conduct of research, in particular in relation to infectious disease risk;
- assess health-care systems and other systems involved in xenotransplantation;
- release on a regular basis information on xenotransplantation;
- assess clinical research applications;
- develop and maintain a registry of xenotransplant recipients.

The sub-committee released two documents in 1998: a background document on xenotransplantation and Spanish Guidelines on Xenotransplantation.

The Guidelines require that before human trials can begin, pre-clinical studies must demonstrate a six month survival and function of cells, tissues and organs and the absence of transmission of infectious agents. If transmission is detected the guidelines require that there be no signs of infection for 12 months.

The Guidelines also require that informed consent be gained not only from the organ recipient but also from their family and close contacts. They also recommend lifelong monitoring of the first xenotransplantation subjects.

***The Netherlands*¹⁴⁷ (last update: 27 May 2002)**

In 1996 a Committee on Xenotransplantation of the Health Council was formed. It presented a report on xenotransplantation to the Minister of Health, Welfare and Sport in 1998. The report concludes that xenotransplantation can be an alternative to transplantation assuming the technical difficulties can be overcome. The possible transfer of old or new pathogens to recipients and third parties was a cause of concern and more research was deemed to be necessary. The report suggested that clinical experiments should not be undertaken until rejection problems are similar to those of human transplants and that infection risks can be managed. Only if these concerns are addressed does the report deem that clinical applications be considered ethically acceptable.

With regard to animal issues, the report deemed that the use of animals was ethically acceptable provided that due consideration be given to animal health and welfare. Non-human primates should not be used as source animals, mainly because of high risk of pathogen transfer, but also for ethical reasons. Pigs are deemed as the most suitable source animals.

The report suggests that informed consent be required not only from the organ recipient, but also from their family and close contacts, as well as the need for an extensive monitoring programme aimed at the early detection of possible transferred pathogens.

Finally the report indicated that there were legal loopholes in the xenotransplantation overview and suggested the development of new laws to cover these. It also called for a national ethics committee to have oversight of clinical xenotransplantation experiments as well as the development of international agreements on matters concerning xenotransplantation.

The report of the Health Council and the position on it subsequently formulated by the Dutch Government resulted in many questions of the Parliament on the various problematical aspects of xenotransplantation. The answers of the Government were later the subject of a consultation between the Parliament and the Minister of Health, Welfare and Sports, held in the year 2000. As a result, the Dutch Parliament requested the Dutch Government to prepare a legally binding moratorium on all clinical research and clinical performance of xenotransplantation. In the spring of the year 2002, an act holding a ban on all clinical use of live animal material was gazetted. The act makes it possible however to designate by Order of Council a clinical application that can be performed nevertheless, on the condition that according to the prevailing medical view it is reasonable to assume that unacceptable risks for the patient and society are excluded.

*Sweden*¹⁴⁸ (last update: 1 August 2000)

In 1997 the Swedish Government through the Department of Health and Social Affairs appointed the Committee on Xenotransplantation. This Committee submitted a report entitled '*From One Species to Another*' in 1999 and made proposals concerning ethical, medical, legal and animal welfare issues on xenotransplantation.

In short, the report concludes that, on the basis of current knowledge, there need not be any permanent or temporary prohibition of xenotransplantation. However, the uncertainty about risks require special measures based on the precautionary principle. These would include a system of surveillance. It suggests that additional legislation is required and that a central decision-making body authority should be established. Also, it notes that issues surrounding informed consent require further work. Regarding issues surrounding the use of animals, the report argues that animals must be able to live a good animal life and that the committee considered it unacceptable to use non-human primates as source animals for reasons of high risk of infection, animal protection and ethics.

7.4. International Organisations and Policies

International legal instruments regulating clinical trials and biomedical research in general such as the forthcoming additional Protocol to the Convention on Human Rights and Biomedicine on Biomedical Research of the Council of Europe and the European Union Directive 2001/20/EC¹⁴⁹ could be used, as a first step, to ensure good practice with respect to clinical trials in xenotransplantation.

With respect to specific concerns relating to xenotransplantation, the Council of Europe but also a number of other bodies, national governments and international organisations including the Organisation for Economic Co-operation and Development (OECD), the World Health Organisation (WHO) and the European Union (EU) have been developing a coordinated approach to evaluate, regulate and supervise the new advances in this field. In this respect, the Council of

Europe is preparing, with the help of the aforementioned organisations, a Recommendation on xenotransplantation which will address the safety and efficacy issues of the procedure. Accordingly, the document will respond to specific immunological difficulties, a potential transmission of viruses from the animal source to the recipient or to the general public and issues related to ethics, the quality of the organs and animal welfare.

International organizations have also recognized the need for a mechanism to collate and share research and clinical information between countries. In this respects, there are already some suggestions that a centralized regulatory body should oversee possible procedures to minimize risks¹⁵⁰.

Finally, it has been accepted that a system to respond to possible global threats is required since the transmission of any potential diseases would automatically be considered as an international hazard. All this work is reflected in the following reports :

- Xenotransplantation: Guidance on Infectious Disease Prevention and Management; World Health Organization; Geneva, Switzerland, 1998. WHO/ECM/ZOO/98.1

- Report of WHO on xenotransplantation; World Health Organization; Geneva, Switzerland, 28-30 October 1997. WHO/ECM/ZOO/98.2¹⁵¹

- Xenotransplantation, International Policy Issues; OECD, Paris, France (1999)¹⁵².

- Opinion on the State of the Art concerning Xenotransplantation, European Union, European Commission, Health & Consumer Protection Directorate-General, 1st October 2001.

- OECD/WHO Consultation on Xenotransplantation Surveillance: Summary Report; DSTI/STP/BIO(2001)11/FINAL.

7.5. An Ethical Overview of Clinical Xenotransplantation

Xenotransplantation raises ethical questions concerning humanity, human activity and the status, welfare and use of animals. These will be dealt with in the following separate sections.

7.5.1. “Interfering with nature”

Today’s scientific innovation is often tomorrow’s commonly accepted treatment. The line between what is natural and unnatural is often very difficult to draw. Medicine seems to intervene in order to prevent the ‘natural’ breakdown caused by diseases, while at the same time medicine is trying to restore ‘natural’ functions and well being. In other words, it is extremely difficult to be categorical about what is natural in our modern technological world.

The public has high expectations about what medical technology can and should do. While people are all too delighted to accept the benefits of new medical advances, there is a genuine concern about an interference with nature which is often characterised as ‘playing God’. In reality, the debate over technology is much more a matter of what limits should be set to the use of technology. In relation to xenotransplantation, the kind of limits under discussion cover a wide range of subjects such as the degree of genetic manipulation of animals which may be permitted in order to prevent organ and tissue rejection. In this respect, should species lines be crossed? Should not pigs remain pigs and human beings human beings? Furthermore, animals need to be properly and appropriately protected. The section on the use and the welfare of animals explores this further.

7.5.2. Issues of consent

Given the experimental nature of xenotransplantation, at least initially, and given the unknown consequences of such innovative treatment for human beings, issues of consent are crucial. As in all medical experimental research, patients must be able to make proper decisions, have all necessary information about risks, benefits and likely outcomes and be genuinely free to participate in and withdraw from medical interventions. When a person does not have the capacity to consent, these previous conditions should also be true for his or her representative or an authority or a person or body provided for by law. In xenotransplantation, there are particular problems since the first recipients are likely to be those in fairly extreme situations, whose medical condition, lack of alternative treatments, and level of desperation may make consent a difficult issue.

Because of the potential constraints in xenotransplantation, specifically the long term monitoring and surveillance in addition to the curtailment of activities (including procreation), patients are likely to be asked to consent to a very high level of restriction, monitoring and post mortem examination. Thus, it is difficult to see how the normal right to withdraw from a procedure can be allowed in the case of xenotransplantation. Here, the key term is 'allowed' for it is unlikely that the legal enforcement of such conditions will be possible or acceptable. Those engaged in transplant work stress that the recipients' choice should be well known and therefore it will be easy to assess how willing patients will be to conform (transplant patients are known to be conformists). Legislative steps to be taken are already in place in most countries if a requirement to protect public health exists in the event of serious transmissible diseases developing. Otherwise, it will be extremely important to ensure that patients have been fully informed and have understood the long term implications of being a xenotransplant recipient.

The ability to give consent means that patients may accept risky procedures fully understanding those risks and may set limits to other rights or freedoms to which they are entitled. It is ethically permissible for patients to choose to set aside such human rights such as the right to begin a family or the freedom to donate blood, if there is some overwhelming public good to be attained or public harm avoided.

7.5.3. The effects on others

The risks of xenotransplantation are considered potentially so significant that informed consent should usually be obtained from close contacts such as relatives and family. It is hard to see how such people are able to freely give consent, but it is important that those in close personal relationships with the recipient are as fully informed and educated as possible. Ideally, the informed consent of close relatives and family should be obtained even if this might set aside other moral principles like autonomy, privacy and confidentiality. These principles are never absolute obstacles and communities are entitled to limit them where there is serious harm to the individual concerned or to other people. A high level of risk to public health would be considered an adequate basis for limiting these principles. The morality of including or excluding possible recipients on the grounds of willingness or unwillingness of close relatives to accept responsibility in relation to the recipient is a matter of debate. While the danger is a move away from treating the individual on his or her own terms as an end in him or her self - it is important to recognise that close contacts do have obligations. The medical and nursing staff are well placed to judge the likelihood of a supportive or unsupportive environment and it is in everyone's interest that the recipient be given the necessary support by close contacts. It is vital that clear accessible information is available about the risks, benefits and implications of xenotransplantation for all those involved with and connected to a recipient.

7.5.4. Risk

It is vital that the risks and benefits of xenotransplantation are properly assessed and communicated. To protect patients and medical/nursing participants, the use of external risk and expert assessment would provide assurance that there is no untoward pressure or expectations. Before xenotransplantation takes place, there can be some disagreement concerning the acceptable level of safety and the degree of risk.

Some people argue that the precautionary principle should be the starting point suggesting that because the consequences are likely to be harmful or unknown, we ought not to proceed. If taken literally and to the extreme, then little or no scientific advances would take place. Rather, all those involved in deciding about xenotransplantation must be satisfied that the risks to the individual recipient, the close contacts, the medical and nursing teams, and the general public are minimal and controllable. In the end, there will be no absolute guarantees but it should be clear that, on the basis of data from pre-clinical research and in accordance with internationally accepted standards, it is highly probable that there is no risk to those involved.

Part of such risk assessment recognises that some degree of risk will be acceptable, especially to desperate patients and their families. Likewise, medical research staff may regard a degree of risk to be acceptable in order to establish the validity of a treatment. However, the overwhelming need to gain and maintain public confidence must mean that no xenotransplantation ought to be approved until and unless there is a high level of assurance about safety.

7.5.5. Commercial interests

There are ethical questions raised about the role of commercial companies in all research, both in terms of setting the agenda and controlling the outcomes. Medical research would not proceed without the financial support of commercial companies and it is only fair that they are able to have an appropriate return for their investment. At the same time, all research and commercial activities must be subject to regulation especially in areas like xenotransplantation when humans, animals or the public are at risk and require protection.

7.5.6. The Public

Informed public opinion needs to be given the necessary and appropriate information in order to coordinate a genuine debate about current attitudes towards xenotransplantation, animal issues and human concerns. Openness in the activities of the regulatory bodies, advisory groups and working parties especially in relation to the scientific issues of risks, benefits, safety and monitoring of humans and animals is a vital part of providing a framework within which proper informed debate may take place.

7.6. The Ethical and Welfare Issues relating to the Use of Animals for Xenotransplantation

The next section will explore the many issues concerning the use and welfare of animals. Human beings already use animals for many purposes including for food, clothing, companionship, entertainment, sport and as labour. The application of new technologies such as genetic manipulation to animals and novel uses such as xenotransplantation heightens awareness of questions regarding the status, welfare and limits of use and abuse of animals. The question of the use of non-human primates is a particular issue of concern in this respect. Issues that need to be

addressed include whether animals have certain fundamental rights and interests and what correlative responsibilities should be placed on medical researchers and regulatory bodies. Deeply held ideological and religious views undoubtedly affect our attitudes towards animals and how we relate to and use them.

This section briefly summarises the ethical and welfare concerns regarding the use of animals as organ or tissue sources for xenotransplantation. These issues are an integral part of the ethical debate over the use of animals in xenotransplantation and have been addressed in a number of documents and reports¹⁵³.

There are two key issues in the debate over the use of animals in xenotransplantation.

The first, and most significant, is whether as a matter of principle it is considered to be morally acceptable to use animals as a source of organs and/or tissues. If it is agreed that this is acceptable then there are questions to address regarding the limits that should be imposed on such use and the welfare of animals within any xenotransplantation programme. Such questions include:

- consideration of how far animal welfare is compromised by procedures in, for example, transgenesis;
- the requirements of the source animal breeding programme;
- the husbandry systems imposed by the need to maintain disease free animals; and
- the organ collection process.

In other words it is important to consider whether high standards of humane care from birth to death of the animal can be ensured.

Another important issue concerning xenotransplantation is that it is only a developing technology and a large number of animals are being used in the research and development programme. Indeed, once it has been decided that a goal like xenotransplantation is worth pursuing then this provides the impetus for a vast amount of research involving animals (including studies of efficacy, physiology, immunology, infections risks) to be carried out on an international basis. Animal experimentation is in itself an emotive issue, particularly with respect to the use of non-human primates. This specific issue will need to be addressed as an integral part of any discussion of the ethics of xenotransplantation.

7.6.1. Questions regarding the ethical acceptability of using animals

There are a series of ethical questions to consider: the ethics of animal use *per se*, the ethical acceptability of genetically manipulating animals, the use of particular species and the ethics of experimenting on animals in order to develop the technology.

Within society as a whole there is a spectrum of opinion regarding what is acceptable for animals including whether or not it is morally acceptable to use them as a source of organs or tissues for xenotransplantation. This spectrum of views can be seen in the attitude surveys already noted in this report. It is important to consider all of these opinions, why they are held, and the criteria on which they are based, in determining policy on whether and how xenotransplantation should go ahead.

A logical discussion of the use of animals in xenotransplantation needs to take into account the existing relationship between animals and humans and what is currently considered acceptable in this respect. An animal rights philosophy would not allow the use of animals for any purpose unless it benefited the individual animal concerned. Most people in the world however do accept some use of animals, albeit in some cases limited to uses that do not require the animals' suffering or death.

If some use is accepted, it is then necessary to determine what is, or is not, considered acceptable. People will express a variety of views on this question. Animals are already widely used within human societies for a variety of purposes. These include providing food, clothing, companionship, for entertainment and as ‘tools’ for research in the biomedical sciences. Their tissues, for example pig heart valves and skin, are also used for medical purposes. One view is that a line should be drawn under these current uses (and indeed that these should be further restricted) prohibiting further exploitation. Another view, and one which appears to predominate in the responses to surveys is that uses that may be of direct benefit to humans should be allowed, albeit strictly regulated and controlled with due regard to animal welfare.

There is an additional dimension to xenotransplantation over and above existing uses of animals since many of the animals involved will be genetically engineered. Genetic engineering is different from other breeding technologies and is opposed by some people on moral, social or religious grounds on the principle that humans should not ‘play God’ and manipulate the genes (thus compromising the integrity) of any living organism, animal or plant.

The issue of species is a further topic for debate, i.e. whether it is more acceptable to use some species, such as pigs, as a source of organs rather than others, such as primates. Arguments on this point may be attitudinal and culture based (e.g. it may be considered acceptable to use pigs since they are already farmed for food; conversely it may not be considered acceptable to use primates because they are not used for food in the vast majority of countries). They may also relate to animal welfare, i.e. whether it is considered possible to maintain the species in ‘clean’ disease-free conditions in a laboratory environment without this having a major negative impact on their welfare. There are also separate concerns, discussed in earlier chapters, about the transmission of diseases between the source animal and the human recipient.

The focus of discussion to date has been on the use of pigs and primates as organ and tissue sources because most xenotransplantation research has involved these species. In the USA, it is considered ethically acceptable to use primates as a source of organs although such use is not currently allowed because of concerns relating to the level of disease risk involved. In the UK, both the Nuffield Council of Bioethics and the Kennedy Committee (both of which carried out extensive reviews of the issue which were published in 1996) decided that it is ethically acceptable to use pigs, but not primates, as organ and tissue sources. Several of the other countries in the survey express similar views.

A final issue is that the development of xenotransplantation technology to the point where it can be routinely used involves the use of animals in experiments which cause or have the potential to cause pain, suffering and distress, which in some cases is very severe. The justification for such research will be the perceived life-saving benefit of successful organ transplantation or cell therapy. However, if society as a whole rejects xenotransplantation, or cannot afford to implement it, then it can be argued that the lives of the many animals involved in this research will have been wasted.

7.6.2. Welfare issues for source animals

From the individual source animal’s point of view the first concern is that they will lose their life, which they do not give up voluntarily – hence the almost universal adoption of the term source, as opposed to donor, animal. Aside from this, using animals as organ and tissue sources for xenotransplantation will undoubtedly have a significant impact on their welfare. This mainly arises from the need to maintain animals of a high health status, i.e. in a Specific (or Qualified) Pathogen Free (S/QPF) environment.

The main welfare issues are:

- The manner in which animals are produced and subsequently bred - techniques in transgenesis with associated wastage rates, surgical interventions; hysterotomy derivation, segregated and/or medicated early weaning.
- The husbandry, care and transport of animals; the use of isolators and biocontainment systems.
- Routine procedures carried out on animals, e.g. blood or tissue sampling.
- Harvesting of tissues and organs, and the humane killing of animals.

These concerns will apply to any species used but since discussion to date has focussed on pigs and primates these are the species that will be considered in this document.

7.6.2.1. Techniques in Transgenesis

There are many ethical and welfare problems associated with transgenic and other technologies (such as cloning). With regard to welfare, animals are subjected to hormone treatments to stimulate superovulation and surgery to remove and implant eggs or embryos. Very large numbers of animals are involved and many are 'wasted' because they do not incorporate the relevant gene. These animals - which may amount to thousands - will just be killed.

7.6.2.2. Derivation of 'S/QPF' animals

Animals for xenotransplantation have to be as free from infection as possible and are thus reared and maintained in sterile conditions. S/QPF animals are generally derived by hysterectomy/hysterotomy directly into isolator systems. In some cases, pigs for xenotransplantation research are born naturally, but then transferred to isolators within 3-5 days through the process of segregated or medicated early weaning. Both systems can have a serious detrimental effect on both the sow and her piglets. Where piglets are derived by hysterotomy, the sow is killed. With early weaning, she experiences the distress of early separation from her piglets together with the physical discomfort of milk retention. There is also evidence that early weaning has a detrimental effect on the development of normal behaviour in piglets. Thus in the UK, for example, weaning of farmed pigs before three weeks of age is not allowed by law except in exceptional circumstances, and in Europe a recent expert working party report recommended the minimum weaning age to be 4 weeks¹⁵⁴. The European working party also stressed that segregated early weaning (at 10-14 days) "should be allowed only if it is demonstrated that any welfare advantages to sows and piglets outweigh the disadvantages". Clearly, then, the early weaning required in the derivation of QPF animals is contradictory to the requirements considered essential to safeguard animal welfare by other legislation and guidelines. It is not carried out for the benefit of the sow and/or her piglets and it is extending the limits of allowable suffering beyond that considered by those concerned with the welfare of animals to be acceptable.

7.6.2.3. Husbandry and care

The area where there is probably the potential for most suffering for source animals concerns animal husbandry. Animals for xenotransplantation must be healthy and therefore their physiological requirements are likely to be met. However, physical health is only one component of overall welfare since the social and behavioural needs must also be satisfied. This is where there is a serious conflict of interests between the needs of the animals and the likely requirements of any xenotransplantation breeding and source animal programme. In order to maintain a disease free status, animals will need to be reared in a sterile environment in which it may be difficult, if not impossible, to provide for their behavioural needs. This is a major concern for both primate and pig welfare and is the main reason why the use of primates as source animals was considered unacceptable in the UK.

Pigs are intelligent animals with complex social and behavioural needs which are difficult to satisfy within biocontainment facilities. Indeed the UK has stated definitively that pigs cannot be reared in gnotobiotic conditions and should not be maintained for longer than four weeks in an incubator. This means that the highest standards of pathogen free animals that can be produced are Qualified not Specific Pathogen Free (QPF not SPF).

Welfare standards for pig husbandry in intensive farming systems have recently been developed in Europe (see report of the Scientific Veterinary Committee, 1993). The report of the Veterinary Committee emphasises that pigs need to be kept in social groups with enough living space in which to move around freely, carry out social play and investigative behaviours, and lie down comfortably. Environmental enrichment in the form of materials for manipulation and rooting is stated as essential.

The requirement for S/QPF status, however, immediately imposes limitations on pig husbandry and hence their welfare. Therefore, the provision of appropriate bedding, nesting and rooting material should be taken into account so that animals can have the opportunity, where possible, to perform some of their most important and fundamental natural behaviours. It is clear, however, that the requirements of a xenotransplantation programme may sometimes create a tension with the currently accepted standards necessary to satisfy the requirements of animal welfare.

7.6.2.4. Procedures and collection of organs

Tissue harvest, organ removal and the killing of animals whilst they are anaesthetised if recovery would compromise their subsequent health and welfare all have the potential to cause animal suffering if not carried out humanely by experienced and competent staff. Sequential organ removal, which has been proposed for kidneys and some tissues, would increase the level of suffering and is unacceptable.

7.6.2.5. Welfare issues for animals in research

Many animals of a variety of species, including primates, pigs, dogs, rabbits, and rodents are used in research related to xenotransplantation to 'model' various aspects of serious human medical problems. Where experiments involve organ transplantation this is going to require major surgery which will cause suffering. This is recognised in the UK legislation where protocols that cause a major departure from an animal's usual state of health and well-being (including major surgery) are classified by the national regulatory body as substantial. Tissue rejection and immunosuppressive treatment cause further suffering. However, every effort should be undertaken to control the severity of symptoms. The serious and unpleasant effects of this research on animals described in the scientific literature include haemorrhaging, tremors, weakness, vomiting, and diarrhoea.

There is particular concern about primates as experimental animals and a number of different species are used in xenotransplantation research. All of these animals will suffer to a certain degree. How much they suffer depends on the nature of the research, exactly what is done (whether for example it is studying organ or cell xenotransplantation) - and what is allowed by the legislation in the country where the research is carried out.

With primates there is great potential for both physical and mental suffering, and this is not just confined to experimental procedures. Some of the research involving primates is carried out on animals taken from the wild and these animals will suffer the additional distress of capture and confinement. Macaques and baboons will both have to endure the stress of removal from their social groups, and of long distance transport from their country of origin (e.g. China, Mauritius) with journeys to Europe that can take over 48 hours. They will then be confined in laboratory

caging. In the UK they are usually pair or group housed but in many countries single housing in tiny cages is the norm. When the level of intelligence of these animals, their social and behavioural complexity, and their normal home range in the wild is taken into account, it is obvious that all of these procedures are going to be distressing, and a laboratory can never provide a satisfactory environment for them. Handling of course can also cause suffering if the people concerned do not understand the animals' needs and lack empathy with them. Thus, the necessity and justification for animal use should be critically evaluated, on a case by case basis, with every effort made to avoid and reduce the use of primates.

7.6.3. Licensing and control

If xenotransplantation goes ahead, it is essential that there is a satisfactory system of licensing and control to regulate the process both with respect to animal welfare and to allow for the development of changing ethical perspectives.

The regulatory process must apply to animals used in research, in breeding programmes and as organ or tissue sources together with the organ removal procedures insofar as they affect animals. There needs to be a clear definition of responsibilities in each area. A close liaison between bodies regulating animal research and those setting requirements for pre-clinical data and source animal standards should exist to ensure that the impact on animals of these requirements are recognised and taken into account.

It is important as a first step to identify whether any of the existing European legislation applies to the use of animals for xenotransplantation. The use of animals in research relating to xenotransplantation will come under the provisions of the European Directive and Convention for the protection of vertebrate animals used for experimental and other scientific purposes¹⁵⁵. However, it is not clear whether the rearing of pigs for xenotransplantation and their use as organ sources would be covered by this legislation. This is because both documents regulate experiments carried out for "*experimental or other scientific purposes*". It could be argued that xenotransplantation is a medical rather than a scientific purpose and so is outwith existing legislation. There is a need to clarify this point.

Neither the Directive nor the Convention contain much in the way of guidelines on animal husbandry and care, other than pen dimensions for pigs as farm animals. These guidelines are currently being reviewed. The UK has developed a detailed code of practice¹⁵⁶ for pigs for xenotransplantation under its animal experimentation legislation¹⁵⁷. This aims to provide reasonable standards for animal husbandry and care, taking into account the need for maintenance of a disease-free environment. The United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) has also developed an associated document regarding standards for biosecurity¹⁵⁸. Both these documents should provide useful and detailed models. Other guidance in the USA and Canada focus on the biosecurity issue rather than animal welfare although they are cross-referenced to national documents relating to the care and use of animals in experiments.

The principle of balancing the benefit to humans of a particular use of animals against the harms caused to those research animals which forms the basis of the regulatory system for animal experiments in some EU countries, should also be applied to animals used as organ or tissue sources.

In the race to develop xenotransplantation to a useable technology, there is a large number of research programmes being carried out across the international arena. The Kennedy Committee recognised the impact that this would have for animals and particularly primates used in research and recommended there be a co-ordinated approach to the research effort in the UK. There is a high level of public and political concern within the EU regarding the use of primates in experiments.

The EU has, furthermore, stated its commitment to reducing the overall use of animals in experiments (the 50% reduction target). It would, therefore, seem sensible to seek ways of applying the principle of a co-ordinated approach to xenotransplantation research in the EU as a whole, notwithstanding that this is undoubtedly a difficult thing to do. A move to establish working relationships between the various national licensing authorities could be a first step.

7.6.4. In conclusion

Most of the discussion regarding the acceptability of using animals for xenotransplantation and the related welfare concerns have focussed on the use of animals as a source of organs or tissues rather than their use in research. This reflects the fact that until recently it has been assumed that clinical xenotransplantation of organs was fairly imminent. In fact, in September 1995, the most important company developing the technology envisaged that the first xenotransplants of transgenic pig hearts into human patients would take place the following year. However, this has proved to be very optimistic, and the transplant of whole organs is now recognised as being associated with many clinical challenges.

It is agreed that the acceptability of xenotransplantation depends on the full evaluation of the ‘costs’ and benefits of the technology. The ‘costs’ includes the full impact on animals of the xenotransplant programme. These costs are not generally recognised and understood within the public arena, particularly with respect to the use of animals in the international research effort. This aspect was not therefore fully considered and taken into account in the original consultations and ethical evaluation process. From an animal welfare perspective, it is important for the ethical acceptability of this use of animals to be revisited weighing the real, practical costs for animals, against a critical reassessment of the actual achievements to date in the light of experience and developments.

7.7. Religious faiths and xenotransplantation

The views of different religions concerning xenotransplantation often depend on the manner in which these religions consider animals and how they should be treated. Moreover, because xenotransplantation results in living animal parts being incorporated into the physical body of a human person this may cause some concerns to followers of various religions especially if the animals used are considered by them as impure or having a special status. For some, there may even be problems with respect to the manner in which they consider their new identity after the xenotransplantation. For this reason, the following study has examined the respective views of the major religions concerning xenotransplantation.

Baha’i

Xenotransplantation is acceptable for followers of the Baha’i faith, although unnecessary suffering to the animals should be avoided. The reason behind this view is that human beings are different and of higher order than animals.

Buddhism

In Buddhism, xenotransplantation is unacceptable, although some individual Buddhists may avail themselves of xenotransplantation dependent upon their “stage of perfection.” This is because proper ethical conduct reduces hurt and suffering in both animals and humans since both feel pain in their consciousness.

Christianity

Though a variety of responses toward xenotransplantation exist within Christianity they are, however, generally in agreement with the procedure¹⁵⁹. Christianity would want to minimise the suffering of animals. It is also assumed that human beings have been given authority to rule over creation and over animals. Arguments against include: interfering with creation and playing God.

Hindu

Hindus do not believe in transplantation whether it be allotransplantation or xenotransplantation. For many followers of this faith, the body must remain whole to pass into the next life. However, exceptions might be made to accept an organ and it is recognised that transplantation is a matter for individual choice. In this respect pig and sheep organs would be acceptable, though the cow is sacred to Hindus.

Judaism

Xenotransplantation is acceptable to Judaism because the over-riding value in this religion is to save life. This even over-rides other considerations such as the prohibition on the consumption of pig flesh. In Judaism, concerns are raised over safety, the suffering of the animals, and over interfering with the order of Nature.

Muslim

A diversity of opinion exists for Muslims with respect to xenotransplantation, but generally the procedure is considered as acceptable. Like the Jewish tradition there is an emphasis on the preservation of life.

There are also concerns with respect to the suffering of animals and a general agreement exists that this suffering should be minimised.

Sikh

For Sikhs, the consensus is that xenotransplantation would be acceptable. Again, like other religious traditions the minimisation of animal suffering is a priority.

Native American

Traditional leaders of Native Americans regard any form of transplantation as an unacceptable violation of the integrity of the human body, however the decision whether to accept xenotransplantation is regarded as an individual one.

8. LEGISLATIVE AND REGULATORY FRAMEWORKS

8.1. Surveys

The Working Party on Xenotransplantation, set up within the Council of Europe under the joint responsibility of the Steering Committee on Bioethics (CDBI) and the European Health Committee (CDSP), was instructed to monitor the legal, regulatory and scientific developments in the field of xenotransplantation in member States and in other States.

To this end, a questionnaire prepared by the Working Party on Xenotransplantation was sent at the end of 1999 – beginning of 2000 to each contact person nominated by the States so that he/she could send to the Secretariat a regular up-date of national developments in the field of xenotransplantation. These up-dates concerned the legal-regulatory but also the medico-scientific developments.

This document summarises the answers to the questionnaire received by the Secretariat on 1 April 2000. It concerns the 27 following States: Albania, Andorra, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Denmark, Finland, France, Georgia, Germany, Hungary, Italy, Latvia, Luxembourg, Malta, the Netherlands, Russia, the Slovak Republic, Spain, Sweden, Switzerland, United Kingdom, Turkey, Canada, and the United States of America.

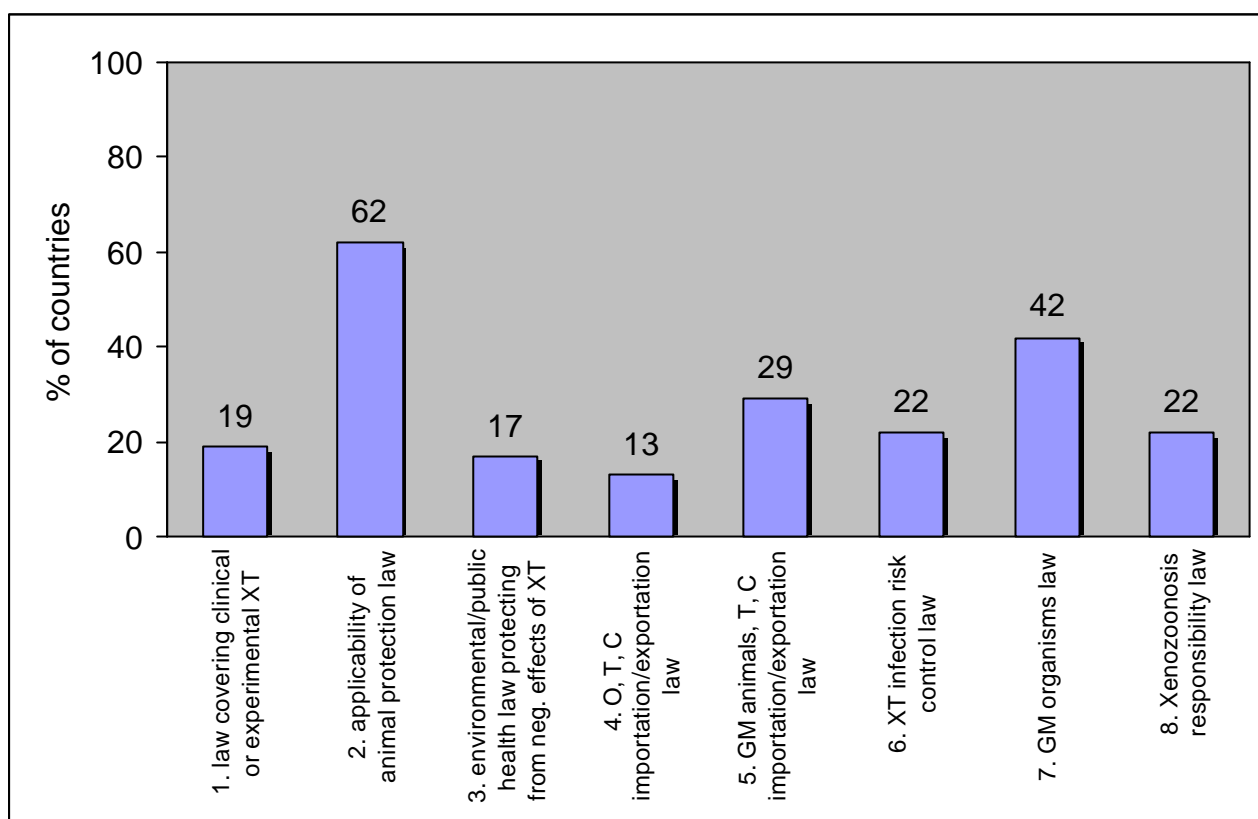
The responses have been analysed with the soft Epi-Info (version 6), produced by the Division for monitoring and epidemiology of the Center of Diseases Control (Atlanta).

Abbreviations used in the tables below:

- XT: Xenotransplantation
- GM: genetically modified
- O, T, C: organs, tissues, cells

Table 24: Existence or applicability of specific laws

This table summarises the percentage of countries in which there are specific laws which exist or are applicable to at least one aspect of xenotransplantation.



Column 1: A total of 5 countries (Denmark, France, the Netherlands, Switzerland and the USA) have legislation currently in place covering clinical or experimental xenotransplantation.

Column 2: A total of 17 countries (Belgium, Canada, the Czech Republic, Denmark, France, Georgia, Germany, Italy, Luxembourg, the Netherlands, Russia, the Slovak Republic, Spain, Sweden, Switzerland, the United Kingdom and the USA) declare that their animal protection laws are applicable in the field of xenotransplantation.

The present Swiss animal protection law is applicable to the field of xenotransplantation, but it is undergoing modifications, as it does not sufficiently cover the breeding of transgenic animals.

Column 3: Only 4 countries (Canada, France, the Netherlands and Switzerland) have legislation covering environmental and public health protection from any possible adverse effects of xenotransplantation (specifically xenozoonosis).

Column 4: Only 3 countries (France, Netherlands and Switzerland) have laws governing importation and exportation of organs, tissues or cells specifically designated for xenotransplantation.

Column 5: A total of 7 countries (Canada, France, Germany, the Netherlands, Russia, Spain and Switzerland) have laws concerning importation and exportation of genetically modified animals, tissues or cells. In Switzerland the law covering importation and exportation (Appendix: Ordinance from the 20th of April 1988 concerning importation, transit and exportation of animals and animal products) is being amended in regard to genetically modified animals.

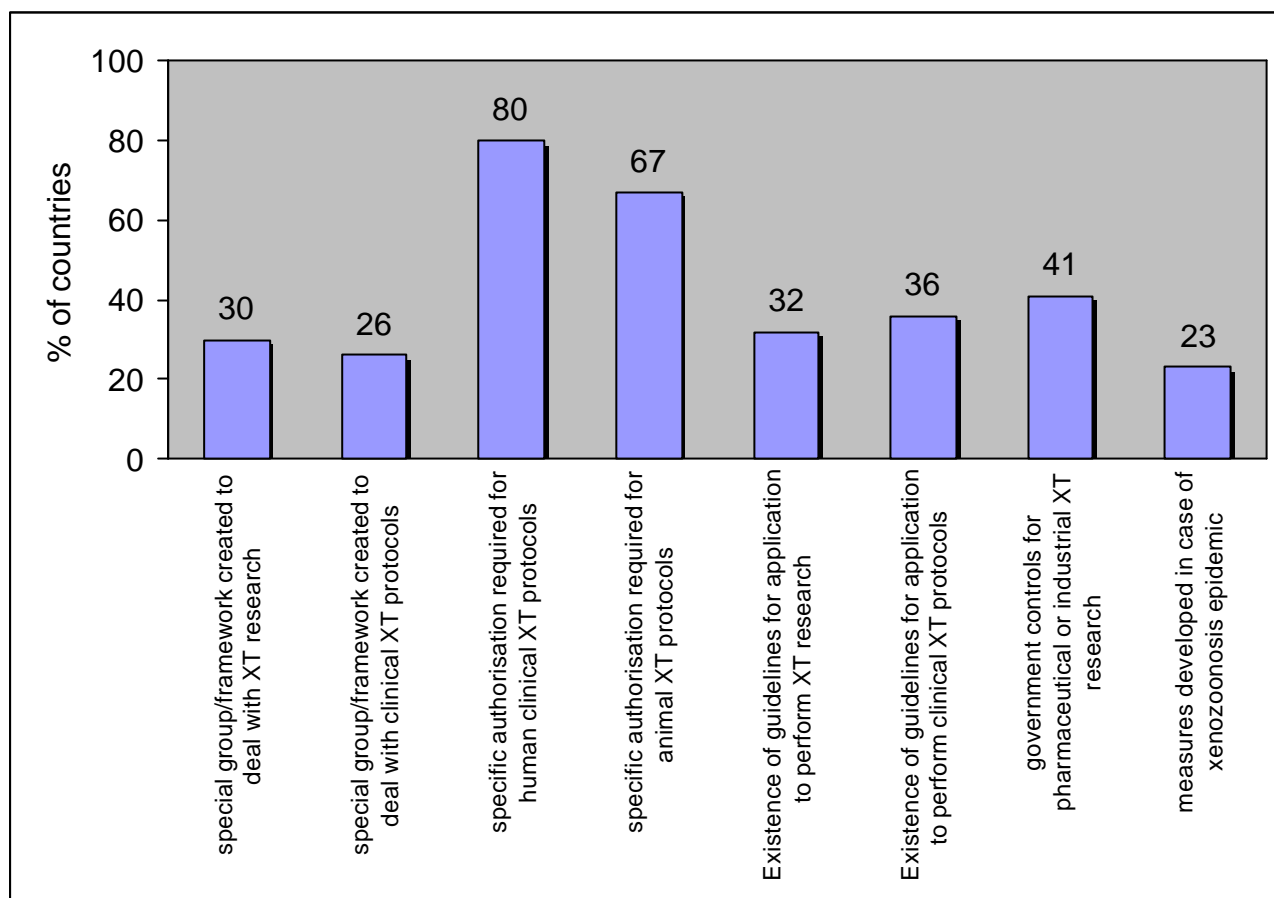
Column 6: A total of 5 countries (France, Germany, the Netherlands, Switzerland and UK) have laws governing cross species (i.e. between different animal species) or xenotransplantation infection risk controls.

Column 7: There is a legislation concerning genetically modified organisms in 10 out of the 24 countries answering this question (Canada, France, Germany, Italy, Luxembourg, the Netherlands, Russia, Spain, Switzerland and the USA). In the United States of America, all animal welfare laws also apply to genetically modified animals.

Column 8: Legislation relating to indemnity, responsibility and compensation rights in the event of a xenozoonosis exists in 5 countries (Denmark, Canada, France, Germany and UK). Financial accountability for any complications arising would fall on the applicant, i.e. the institution, who carried out the research.

Table 25: Regulatory and/or administrative arrangements

This table summarises the percentage of countries in which specific regulatory and/or administrative arrangements related to xenotransplantation exist.



Column 1: A total of 8 countries (France, Germany, Italy, Russia, Spain, Sweden, UK and the USA) have each created a framework to deal with xenotransplantation research; they represent 30 percent of the responding countries.

Column 2: A total of 7 countries (Canada, France, Germany, Spain, Sweden, UK and the USA) have created a special group or framework to deal with clinical xenotransplantation protocols.

Column 3: A total of 20 countries (Bulgaria, Denmark, Canada, Croatia, Cyprus, the Czech Republic, Finland, France, Germany, Georgia, Hungary, Italy, Latvia, Luxembourg, the Netherlands, Russia, Spain, Sweden, UK and the USA), who represent 80 % of the responding countries, specify that xenotransplantation research cannot be carried out without requesting specific authorisation from a regulatory board or government body. The other 20 % of countries do not require specific authorisation.

Column 4: An authorisation is required for xenotransplantation research in the case of animal xenotransplantation protocols in 16 countries (Bulgaria, Canada, Cyprus, the Czech Republic, Denmark, Finland, France, Germany, Georgia, Italy, Latvia, Luxembourg, the Netherlands, the Slovak Republic, Switzerland and UK).

Column 5: A total of 8 countries (Belgium, Bulgaria, Canada, Germany, Russia, Spain, UK and the USA) of the 25 who have responded to the questionnaire have guidelines for submission of an application to perform xenotransplantation research.

Column 6: In addition to the aforementioned countries, France also has guidelines for the submission of an application to perform clinical xenotransplantation protocols. In the countries where guidelines exist, the guidelines are also applicable to independent private actors such as industrial pharmaceutical companies.

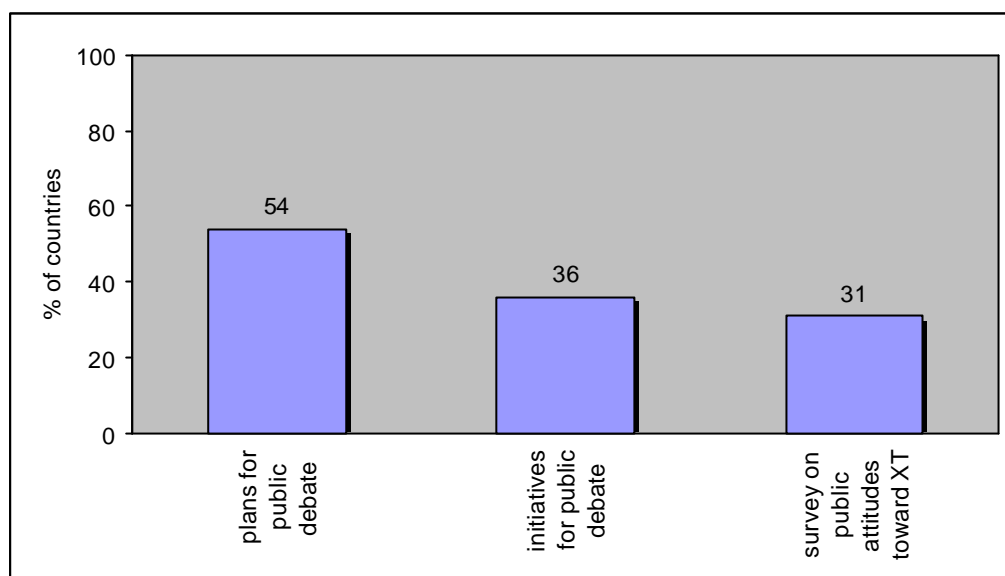
Column 7: There are government controls with respect to pharmaceutical or industrial xenotransplantation research in 9 countries (France, Germany, Latvia, Luxembourg, the Netherlands, Russia, Spain, UK and the USA); in Russia the regulatory body is presently being set-up. Not all of the countries who responded “no” to this question have pharmaceutical industries in their country, as e.g. Andorra.

Column 8: A total of 5 countries (Canada, Germany, the Slovak Republic, UK and the USA) declare that they have developed measures in the event of a cross species infection or a zoonosis epidemic.

A total of 8 countries (Belgium, Canada, Croatia, France, the Netherlands, Spain, UK and the USA) state that xenotransplantation control is developing towards a two tier system (governmental and institutional) of control. In the other four responding countries (the Czech Republic, Finland, Russia and the Slovak Republic) it is developing at a single level, with the Ministry of Health as the regulatory body in Russia.

Table 26: Public involvement

This table summarises the percentage of countries in which public debate or surveys have been performed or are planned in regard to xenotransplantation.



Column 1: In 13 countries (Canada, Denmark, Germany, Italy, Latvia, Malta, the Netherlands, Russia, the Slovak Republic, Spain, Sweden, UK and the USA) there are plans for public debate relating to xenotransplantation.

Column 2: In 9 countries (Canada, Denmark, Germany, Malta, the Netherlands¹⁶⁰, Russia, Sweden, UK and the USA), initiatives for such public debate have already been taken. In the UK, the UKXIRA (UK Xenotransplantation Interim Regulatory Authority) holds an annual open meeting for the public to attend and participate. Furthering public debate will be one of the tasks of the newly formed UK Human Genetics Commission, which will also take an interest in xenotransplantation matters.

Column 3: Surveys relating to public attitudes towards xenotransplantation and its ethical aspects have been performed in 8 out of the 26 responding countries (Canada, France, Germany, Latvia, the Netherlands, Spain, Sweden and UK).

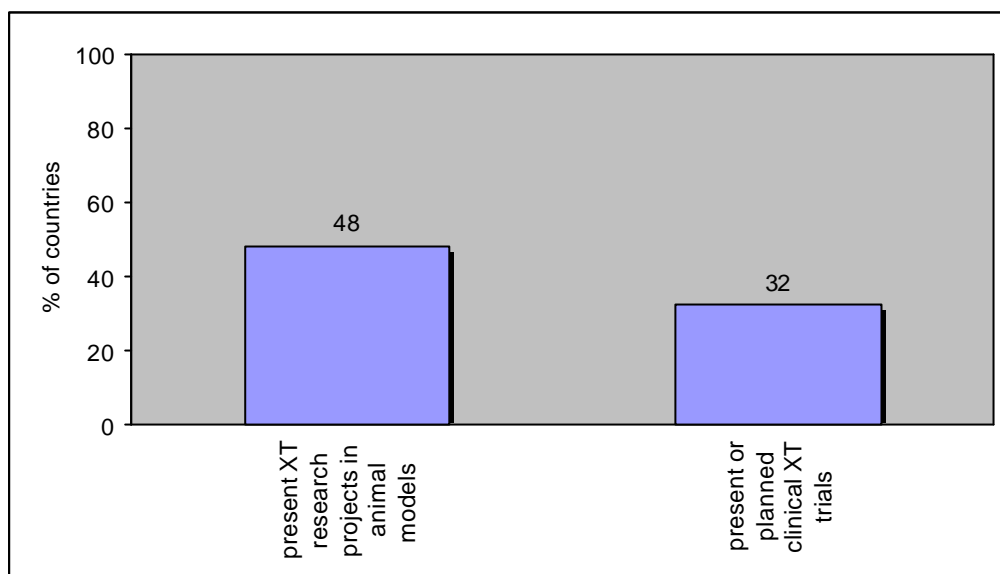
Public information relating to xenotransplantation include television programmes, press articles and websites. Health Canada provides significant documentation on its Website:

<http://www.hc-sc.gc.ca/hpb-dgps/therapeut/htmleng/btox.html>

The UKXIRA also provides the public with information on its Website: www.doh.gov.uk/ukxira.htm

Table 27: Current xenotransplant research

This table summarises the percentage of countries in which xenotransplantation research projects and clinical trials are being undertaken or are planned.

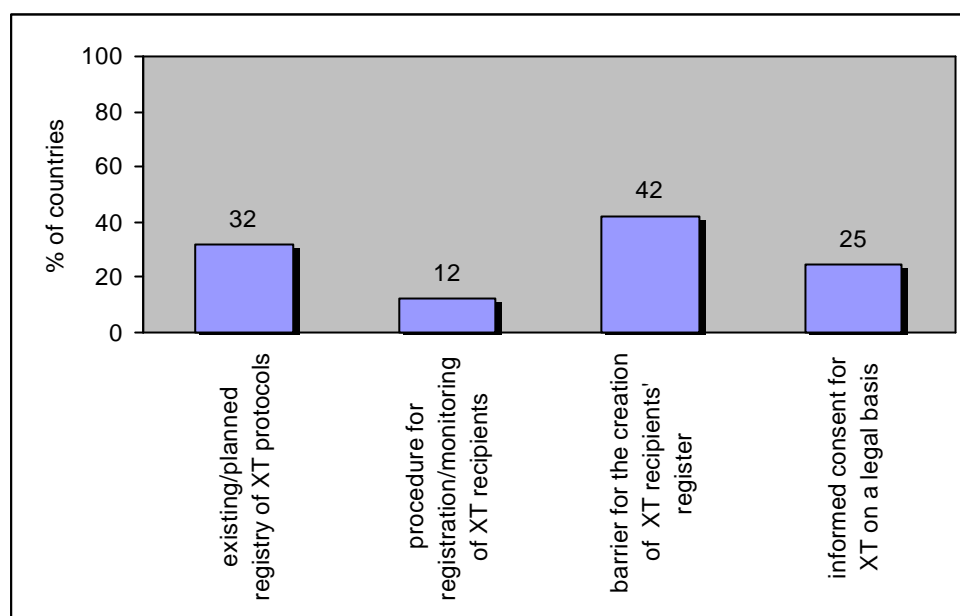


Column 1: There are xenotransplantation research projects on animal models in 12 out of the 26 responding countries (Belgium, Canada, the Czech Republic, France, Germany, Italy, the Netherlands¹⁶¹, Spain, Sweden, Switzerland, UK and the USA). All 12 countries have university based projects; France, Germany, Italy, Switzerland and the USA have industry based projects as well. In Switzerland the basic research projects in the field of rejection are done by the universities, but are often sponsored by industry.

Column 2: Some clinical xenotransplantation trials are being undertaken or are planned in 8 out of the 12 countries listed above (Belgium, Germany, Italy, the Netherlands¹⁶², Russia, Spain, Switzerland and the USA). Germany, Italy and the USA have industry based projects. Germany, Italy, the Netherlands, Russia and Switzerland declare university based projects.

Table 28: Xenotransplant registry for clinical protocols

This table summarises the percentage of countries in which various xenotransplantation registries are planned or exist; it also details some issues with regard to registries and patients' consent.



Column 1: There is an existing or planned registry of xenotransplantation protocols in 8 countries. These countries are Canada, France, the Netherlands, the Slovak Republic, Spain, Sweden, the UK and the USA.

Column 2: A procedure for registration and monitoring of xenotransplantation recipients and their potential immediate contacts has been established at national level in 3 countries (France, UK and the USA).

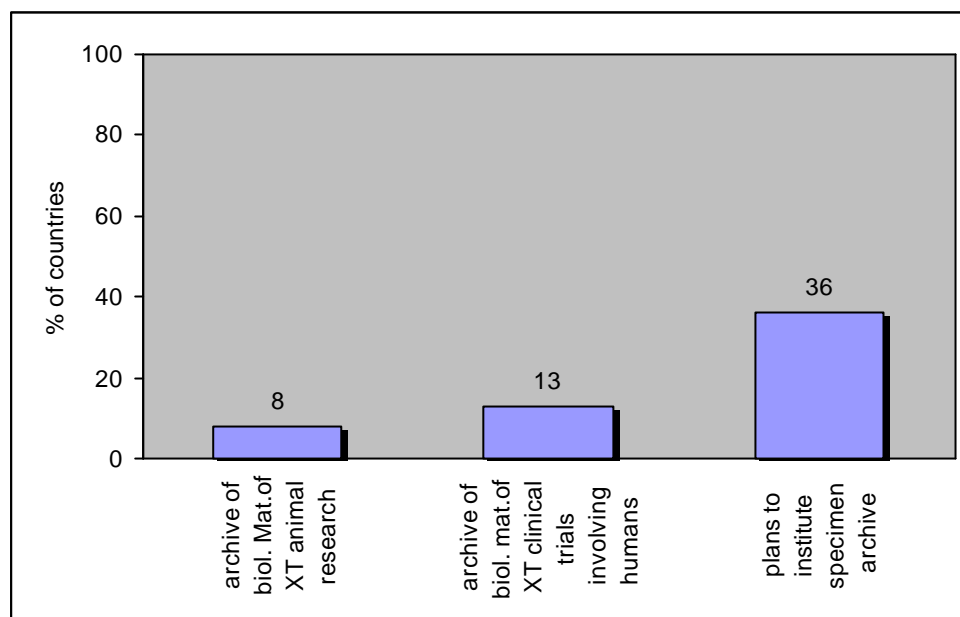
In Albania, Croatia, France and Sweden the legal basis for such a procedure would be a parliamentary act, in the Netherlands and in Latvia a government decision.

Column 3: In 10 countries (Canada, France, Georgia, Italy, Luxembourg, the Netherlands, Spain, Switzerland, UK and the USA) there are some confidentiality rules which could act as a barrier to creating a central register of xenotransplantation recipients and contacts.

Column 4: The issue of 'informed consent' has been addressed in law in 5 countries (Denmark, the Netherlands, France, UK and the USA). The USA declare that their 'informed consent' is not specific to xenotransplantation. The countries having 'informed consent' in law have been asked if 'imposed extended compliance' had been separated from or included in the concept of 'informed consent'. In Denmark, UK and in Canada it has been included in the concept (extended compliance is described in the PCSX - Proposed Canadian Standard for Xenotransplantation - as part of the informed consent; however, 'informed consent' is not regulated, even if a practitioner or institution would be held liable if it was not performed). It is specified that the patient has the right to withdraw consent at any time in France and the USA.

Table 29: Archives

This table summarises the percentage of countries in which archives exist or are planned.



Column 1: There is an archive of biological tissues, cells or fluid specimens kept on xenotransplantation animal research in Spain. All the other countries do not have archives organised at the national level. In the Netherlands, archives should be kept at the local level. In Germany and in the USA some researchers do archive samples for future studies, but the individual researcher determines the quantities and the quality of the archived specimens. In Canada an archive is under consideration.

Column 2: In Belgium and the USA there are archives of biological tissues, cells or fluid specimens kept on clinical trials involving human beings. In the Netherlands there are archives too, but not at a national level. The keeping of an archive is the responsibility of the party performing the research. In Germany it is voluntarily in each clinical centre. In Canada such an archive is under consideration.

Column 3: There are plans to institute biological specimen archives on either research or clinical xenotransplantation protocols in the future in 6 countries (Belgium, Canada, Russia, the Slovak Republic, Spain, UK). The establishment of a national archive is under consideration by the Public Health Service in the United States. In Germany it was under discussion by the Ad Hoc Working Group “Xenotransplantation” of the German Medical Association (Bundesaerztekammer).

8.2. Conclusion

1. This survey collected legal, regulatory and scientific data in the field of xenotransplantation from 27 States (Albania, Andorra, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Denmark, Finland, France, Georgia, Germany, Hungary, Italy, Latvia, Luxembourg, Malta, the Netherlands, Russia, the Slovak Republic, Spain, Sweden, Switzerland, United Kingdom, Turkey, Canada, and the United States of America).
2. The survey shows that a minority of the States which responded has a legal framework specific to xenotransplantation. As a matter of fact, laws pertaining to:
 - i) clinical or experimental xenotransplantation,
 - ii) importation/exportation of organs, tissues or cells specifically designated for xenotransplantation,
 - iii) protection of environmental and public health from possible adverse effects of xenotransplantation,
 - iv) xenotransplantation infection risks control,
 - v) responsibility for indemnity and compensation rights in the event of a xenozoonosis,

are only found in 13% to 22% of these 27 States.

3. As regards regulatory and administrative arrangements, the framework for animal and clinical research in the field of xenotransplantation is much more narrow: 67% of the States require that a specific authorisation is obtained before any animal xenotransplantation research protocol is carried out, and this percentage reaches 80% with respect to human clinical xenotransplantation research protocols. Guidelines to be complied with when submitting an application to perform (a) general xenotransplantation research and (b) clinical xenotransplantation protocols have also been drafted in 32% and 36% of States respectively. One should emphasise that these administrative rules are applicable to research carried out by independent private bodies such as industrial pharmaceutical companies in only 41% of States.
4. Initiatives for public debate on xenotransplantation have already taken place in 36% of States.
5. In the field of registries, 8 States (Canada, France, Netherlands, Slovak Republic, Spain, Sweden, United-Kingdom and United-States) perform – or plan to perform – the registration of all xenotransplantation research protocols; France, United-Kingdom and United-States have already established a national procedure for registering and monitoring xenotransplantation recipients and their close contacts at the national level. It is to be noted that confidentiality rules in several States might act as a barrier to such a procedure.
6. It appears that the archiving of biological samples kept during (a) animal research or (b) clinical xenotransplantation trials is organised in 8% and 13% of States respectively. About 40% of States have plans for archiving research or clinical xenotransplantation protocols in the future, without specifying whether these archives would be organised on a national or local basis.
7. With respect to the scientific perspective, clinical xenotransplantation trials are planned or presently underway in less than one third of the States i.e. 8 countries (Belgium, Germany, Italy, the Netherlands¹⁶³, Russia, Spain, Switzerland and the USA), and close to one-half of the States (i.e. 12 States) indicated that they perform xenotransplantation research projects on animal models.

8. As could be foreseen, one should remember that the performance - or non performance - of research projects and/or clinical trials in a State influences the way a State develops its legal and regulatory framework for xenotransplantation, creates archives and promotes public debate.
9. Furthermore, this survey indicates the fact that the legal and/or regulatory framework specific to xenotransplantation is statistically significantly more developed in States in which research projects and/or xenotransplantation clinical trials are performed in comparison to States where no trials are taking place. Furthermore, even if some States in which clinical xenotransplantation trials are planned or currently under way do not have legislation in place covering clinical or experimental xenotransplantation or do not have legislation covering environmental and public health protection, these States do, on the other hand, have specific regulatory and/or administrative arrangements concerning xenotransplantation. These arrangements prohibit human clinical xenotransplant protocols being carried out without specific authorisation from a regulatory board or government body.
10. In conclusion, this survey shows that the legal and regulatory framework for xenotransplantation is incomplete in many States and pleads in favour of the development of European guidelines with a twofold aim of (a) harmonising regulations in Europe and (b) contributing to the preparation of such a framework in States where no such guidelines exist.

Glossary¹⁶⁴ :

a-gal (galactosyl α -1,3-galactose): a-gal is a sugar molecule found on the surface of pig cells. When pig organs are transplanted into human beings, a -gal acts as an antigen. It is recognised by human antibodies and hyperacute rejection is triggered.

Allotransplant: a graft consisting of live cells, tissues, and/or organs between individuals of the same species.

Antibodies: Antibodies are protein molecules produced by a type of white blood cell called B-cells. Antibodies circulate in the blood and stick to foreign antigens on the cells of foreign organisms or of transplants. This may inactivate the foreign organisms or the transplant directly, or it may enable other white blood cells to destroy them. One important consequence of antibodies sticking to antigens is the activation of a complicated reaction called the complement reaction.

Antigen: An antigen is a molecule found on the outside of a cell that is recognised as foreign by the immune system. Any infectious organisms entering the body, such as bacteria or viruses, have molecules called antigens on their surface. When the antigens are recognised as foreign, an immune response is mounted to protect the body from infection. Unfortunately, an immune response is also induced by transplantation. This is because the cells of organs and tissues also have antigens on their surface.

Cell: The cell is the basic unit of any organism. The human body contains 100 billion cells, each of which is too small to see with the naked eye. Each cell is surrounded by a cell membrane which has, on its surface, protein molecules. Some of these protein molecules are complement regulating proteins. Inside the cell is the nucleus, which contains the genetic material of the cell. Examples of cells are red blood cells, bone marrow cells and pancreatic islet cells. Cells group together to form tissues, and tissues group together to form organs.

Complement regulating proteins: Complement regulating proteins are molecules found on the surface of the body's cells. They prevent complement proteins attacking the body's cells. Examples of complement regulating proteins are DAF, CD59 and MCP.

Complement system: The complement system is a system of twenty complement proteins found in the blood. It is an important element of the immune response to infectious organisms or to a transplanted organ or tissue. The immune response starts when antibodies stick to antigens on the infectious organism or transplant. A reaction is triggered with one complement protein activating the next, and so on. Ultimately, the complement proteins at the end of the chain attack the foreign organisms or the cells of a transplanted organ, punching holes in them and thus destroying them.

Endogenous retroviruses: Retroviruses are RNA viruses that infect cells and then become inserted into the genetic material of the host cells. Some retroviruses will then start to reproduce. Endogenous retroviruses, however, remain in the genetic material of the host cells in a dormant state. If the retrovirus is inserted into the germ cells (eggs or sperm) it may be passed down from parent to offspring. A procedure such as transplantation might reactivate endogenous retroviruses in a pig organ, leading to the production of new retroviruses in the human recipient and their insertion into the genetic material of the human cells.

Good Clinical Practices: A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.

Hyperacute rejection: Hyperacute rejection is the rapid and strong immune response to a transplanted organ from animals, such as pigs, that are only distantly related to human beings. Within minutes the xenograft is reduced to a black, swollen mass. This is because antibodies attack the pig antigen a-gal (galactosyl a-1,3-galactose). Complement is activated and the cells of the transplanted organ are attacked and destroyed.

Immunosuppression: This refers to the inhibition of the immune response in order to prevent organ rejection. It is achieved by the use of immunosuppressive drugs which work in a variety of ways.

Infection Control Program: a systematic activity within a hospital or health care center charged with responsibility for the control and prevention of infections within the hospital or center.

Infectious agents: viruses, bacteria (including the rickettsiae), fungi, parasites, or agents responsible for Transmissible Spongiform Encephalopathies (currently thought to be prions) capable of invading and multiplying within the body.

Nosocomial infection: an infection acquired in a hospital.

Pancreatic islet cells: The islets of Langerhans are groups of cells found in the pancreas which produce the hormones insulin and glucagon that control blood sugar levels. One cause of diabetes is when the islet cells do not make enough insulin, leading to high levels of blood sugar. Human pancreatic islet cells can be transplanted into patients in order to treat diabetes. Unsuccessful attempts have been made to transplant pig fetal pancreatic islets.

Prion: A prion is a small particle made of protein that is thought to cause a type of disease called spongiform encephalopathy. Examples are, in cattle, BSE (bovine spongiform encephalopathy or mad cow disease) and, in human beings, Creutzfeldt-Jakob disease (CJD). The diseases lead to degeneration of the central nervous system. Prions are unusual because they appear to be a unique example of an infectious agent that does not contain genetic material.

Procurement: the process of obtaining or acquiring animals or biological specimens (such as cells, tissues, or organs) from an animal or human for medicinal, research, or archival purposes.

Recipient: a person who receives or who undergoes ex vivo exposure to a xenotransplantation product (as defined in xenotransplantation).

Retrovirus: A type of virus that contains RNA as its genetic material. The HIV virus that causes AIDS is an example of a retrovirus. After a retrovirus has infected a cell, the process of reproduction involves conversion into DNA. The DNA is inserted into the genetic material of the host cell. RNA is then made and used to produce new viruses.

Source animal: an animal from which cells, tissues, and/or organs for xenotransplantation are obtained.

Source animal facility: facility that provides source animals for use in xenotransplantation.

Tissue: A tissue is a collection of similar cells that all perform the same function. An example is the neural tissue of the brain. Bone is a type of tissue where the cells are surrounded by hard deposits. Tissues may group together to form organs.

Transmissible spongiform encephalopathies (TSEs): fatal, subacute, degenerative diseases of humans and animals with characteristic neuropathology (spongiform change and deposition of an abnormal form of a prion protein present in all mammalian brains). TSEs are experimentally transmissible by inoculation or ingestion of diseased tissue, especially central nervous system tissue. The prion protein (intimately associated with transmission and pathological progression) is hypothesized to be the agent of transmission. Alternatively, other unidentified co-factors or an as-yet unidentified viral agent may be necessary for transmission. Creutzfeldt-Jakob disease (CJD) is the most common human TSE.

Virus: A minute infectious organism made of genetic material and protein. It is not normally considered to be a living organism, since it cannot live independently. Instead, viruses must infect living cells and reproduce inside them. New virus particles can then leave the cell. In some viruses, such as the herpes viruses, the genetic material is DNA. In others, such as the HIV virus that causes AIDS, the genetic material is a different type, called RNA.

White blood cells: White blood cells (leucocytes) are the blood cells that enable the body to mount an immune response. They are divided into two main groups: B-cells and T-cells.

Xenogeneic infectious agents: infectious agents that become capable of infecting humans due to the unique facilitating circumstances of xenotransplantation; includes zoonotic infectious agents.

Xenotransplantation: for the purposes of this document, any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (A.) live cells, tissues, or organs from a nonhuman animal source or (B.) human body fluids, cells, tissues or organs that have had ex vivo contact with live nonhuman animal cells, tissues, or organs.

Xenotransplantation Product(s): live cells, tissues or organs used in xenotransplantation (defined above).

Xenotransplantation Product Recipient: a person who receives or who undergoes ex vivo exposure to a xenotransplantation product.

Xenozoonosis: A Zoonosis arising from Xenotransplantation

Zoonosis: A disease of animals that may be transmitted to humans under natural conditions (e.g. brucellosis, rabies).

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¹⁶¹ In the spring of 2002 an act holding a ban on the clinical use of live animal material was gazetted in the Netherlands.

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¹⁶⁴ This glossary was established through a compilation of the glossaries of (1) the PHS Guideline on Infectious Disease Issues in Xenotransplantation, USA (19 January 2001), and (2) the report entitled “Animal-to-Human Transplants, the ethics of xenotransplantation”, Nuffield Council on Bioethics, UK, March 1996.